2016-01-01

Comparative Proteomic Analysis Of Extracellular Vesicles From Prostate Cancer-Derived Cell Lines

Gloria Polanco
University of Texas at El Paso, gpolanco@miners.utep.edu

COMPARATIVE PROTEOMIC ANALYSIS OF EXTRACELLULAR VESICLES FROM PROSTATE CANCER-DERIVED CELL LINES

GLORIA POLANCO

Doctoral Program in Biological Sciences

APPROVED:

Igor Almeida, Ph.D., Chair

Marc Cox, Ph.D., co-chair

Siddhartha Das, Ph.D.

Giulio Francia, Ph.D.

Mahesh Narayan, PhD

Charles Ambler, Ph.D.
Dean of the Graduate School

Dedication

To my family, friends, and mentors.

ISOLATION, CHARACTERIZATION, AND COMPARATIVE PROTEOMIC ANALYSIS OF EXTRACELLULAR VESICLES FROM PROSTATE CANCER-DERIVED CELL LINES

by

GLORIA POLANCO, B.S.

DISSERTATION

Presented to the Faculty of the Graduate School of

The University of Texas at El Paso

in Partial Fulfillment

of the Requirements

for the Degree of

Doctor of Philosophy

Biological Sciences

THE UNIVERSITY OF TEXAS AT EL PASO

August 2016

Acknowledgements

I would first like to state that the completion of this work was a large collaborative effort, and involved the kind dedication of very talented scientists from around the world including: Dr. Hyung Won Choi from National University of Singapore, Dr. Paulo Caravalho from Carlos Chagas Institute, Fiocruz, Paraná, Brazil, Dr. Ernesto Nakayasu of Pacific Northwest National Laboratory, Dr. Lang Ho Lee from Brigham and Women's Hopital, Harvard Medical School, Dr. Shahriar Koochekpour of Roswell Park Cancer Institute, New York, Dr. Valerie Odero-Marah of Clark Atltanta University, Dr. Heinric Williams of Geisinger clinic in Danville, PA, Dr. Achim Treumann of Newcastle University, Dr. Nathan VerBerkmoes, Dr. Yenni Garcia and Dr. Emma Arigi of the University of Texas at El Paso.

I would like to acknowledge my dissertation committee members Dr. Siddhartha Das, Dr. Giulio Francia, Dr. Mahesh Narayan, and Dr. Manuel Miranda for their valuable input in the past four years; which helped form this project into a truly interesting story that I may continue to develop throughout my future career.

Of course, this work would not be possible without my dissertation co-chairs and mentors Dr. Igor Almeida and Dr. Marc Cox. I sincerely cannot put my gratitude into words. Your confidence in me and overwhelming support pushed me further than I thought I could possibly go, and helped shape me into a scientist. I will forever be grateful to have had the opportunity to have worked with you.

I would also like to thank Dr. Renato Aguilera for his tireless efforts in facilitating minority students, such as myself, though the RISE program to pursue careers in science despite the economic and emotional hardships we may face along the way. Dr. Elizabeth Walsh for helping me develop professionally. My first PI, Dr. Rosa Maldonado, for trusting me at a very young age to work in your lab and giving me the confidence to carry-out my own projects. I am also very grateful to Drs. Gustavo Miranda and Susie Krum-Miranda for always motivating me and providing valuable advice on science and life.

Finally, I would like to thank all past and present members of the Almeida and Cox labs including: Ethel Bayer-Santos, Silas Rodrigues, Clemente Aguilar, Carlos Ramon Brito, Aaron M. Valenzuela, Susana Portillo, Nasim G. Salloum, Nasim Karimi Hosseini,

Emanuella F. Fajardo, Brenda Zepeda, Maria Tays Mendes, Trini Ochoa, Uriel Ortega, Veronica Escalante, Igor Estevao da Silva, Mia Swain, Brian Grajeda, Jeff Sivils, and Cheryl Storer. My colleagues and friends Melissa Harris, Diondra Harris, Emma Arigi, Angie Lopez, Zack Martinez, Steven Martinez, Nai Guy, Yenni Garcia, Atasi Chatterjee, Elizabeth Anaya. Gladys Almodovar, Yahaira Santiago-Vasquez, Eva Iniguez, and Elisa Robles for helping me through the cheers and jeers of completing the graduate program. My big loving family, my parents Juan and Leticia Polanco, my brother Sam, and my husband Edgar for being 100% supportive, 100% of the time, putting up with my inconvenient work schedule, always believing in me, and never letting me give up.

Abstract

Prostate cancer (PCa) is the leading non-cutaneous malignancy and the second deadliest among American men. PCa mortality rates among African American men are much higher than any other ethnic group, and the same is true for men of African ancestry world-wide. There is also a lack of reliable diagnostic markers and effective treatment options. Extracellular vesicles (EVs) have been observed to play an important role in cancer processes such as promotion of tumor growth and metastasis. They are also a promising source of diagnostic markers. This study addresses these problems by studying the proteome of EVs derived from PCa cells LNCaP, 22RV1, E006AA-hT, which was derived from an African American PCa patient, and non-cancerous prostate epithelial cell line RWPE1. The enrichment of EVs was conducted using differential centrifugation, where we cleared cell supernatant of cell debris and apoptotic bodies and centrifuged for 2 hours at 100,000xg to pellet EVs (EV2). The supernatant from this spin was subsequently centrifuged for 16 hours to pellet any remaining EVs (EV16). The EV enrichment was deemed successful through transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA); which showed that EV2 and EV16 differ in overall size. Western blotting confirmed the presence of at least one EV marker (ALIX or TSG101) in EV2 and EV16 from all cell lines. Proteomic analysis revealed existing qualitative differences between EV2 and EV16 from the same cell-line. We also observed 67 proteins common only in PCa cell EV2 fractions and 18 in EV16. Each cell-derived EV fraction also had proteins that were exclusive. A differential proteomic analysis using normalized spectral count values revealed cancer-related proteins present across all cell lines that differed in abundance, some of them significantly different when compared to RWPE1. Among the most notable proteins are Vinculin, Thy-1, and multiple subunits of the 26S protease complex, TCP1 containing ring complex (TRiC), and the COP9 signalosome complex. We also observed proteins that were significantly abundant in E006AA-hT EVs which are known to contribute to migration and metastasis, characteristic of E006AA-hT *In vivo*. In summary, our analysis of PCa cell-derived EVs identified proteins in two EV fractions which are significantly abundant in comparison to non-cancerous prostate cell-derived EVs, and have been reported to play a role in cancer. This knowledge provides the basis to further

understand the prostate tumor microenvironment in African American and Caucasian men, and suggests candidates for validation in bio fluids for the development of diagnostic tools.

Table of Contents

List of Tables

List of Figures

Chapter 1: An introduction to Prostate Cancer and Extracellular Vesicles

1.1 Prostate cancer is a devastating disease

Prostate cancer (PCa) is the most common non-cutaneous malignancy and is the second-leading cause of cancer related death in men residing in the United States (American Cancer Society, 2013). Its commonality is echoed internationally as well (International Agency for Research on Cancer, 2012). Timely diagnosis and appropriate treatment are of essence to patient survival. Unfortunately, PCa lacks both an accurate method for diagnosis and a proper treatment post traditional hormone deprivation therapy. The following sections describe the disease pathology, molecular biology, and current treatment and diagnostic strategies for PCa.

Pathology of prostate cancer

The prostate is a chestnut-sized gland of the male reproductive system, located in front of the rectum and bellow the bladder, surrounding the ejaculatory duct. Its biological function is to produce semen necessary for sperm motility (Oh et al., 2003a). The outer regions of the prostate are known as the peripheral zone and the fibromuscular zone, while the inner regions are known as the central zone, transitional zone and periurethral gland region (**Figure 1.1**) (De Marzo et al., 2007). In prostate cancer, the majority of tumors develop in the peripheral zone, less development occurs in the transitional zone, and development has been rarely observed in the central zone

(McNeal et al., 1988).

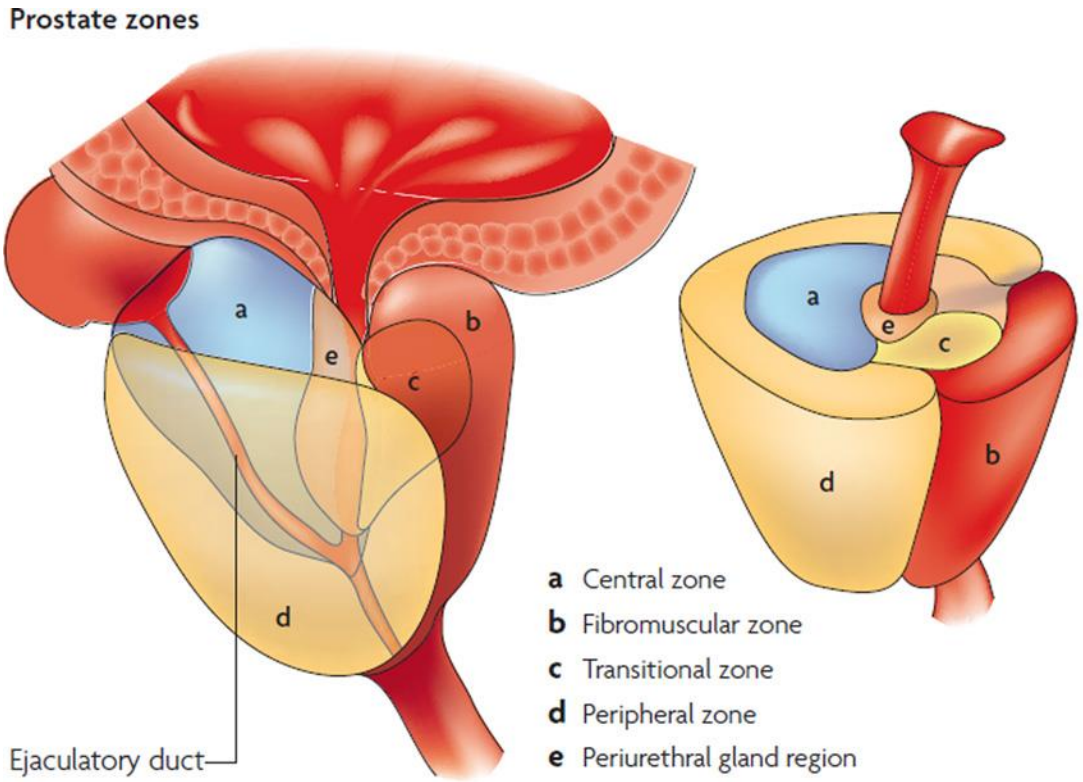


Figure 1.1: Zones of the prostate. The zones of the prostate are labeled **a**-**e**. The peripheral zone (**d**) is the most common site of prostate cancer tumor development. Image adapted from De Marzo *et al.*, Nature Reviews Cancer, 2007.

Cancer progression towards mortality has been described in six hallmarks which consist of: sustainment of proliferative signaling, evading growth suppression, resisting cell death, replicative immortality, induction of angiogenesis, and activation of invasion and metastasis (Hanahan and Weinberg, 2011). When cancer cells undergo uncontrolled proliferation and accumulation, it leads to the development of a solid mass referred to as a malignant tumor (Holland, 2003). A malignant tumor has the ability to invade tissues adjacent to and, potentially, far from the primary site. Its invasive properties are what differentiates this type of tumor from a benign tumor, which remains at its primary site (non-invasive). The migration, colonization, and growth of tumors, also known as metastasis, of cancerous cells onto healthy tissues can cause complete malfunction of vital organs and lead to death (Holland, 2003). In order for metastasis to occur, the tumor goes through several stages which include tumor growth, hypoxia, angiogenesis, intravasation, survival in the circulatory system, extravasation, and colonization (Nguyen et al., 2009; Schilling et al., 2012).

Tumor growth is normally restricted to 1-2 mm in diameter, and its nourishment comes from diffusion of oxygen and other nutrients from nearby blood vessels (Russo et al., 2012). Past its restricted size, tumor core regions have less accessibility to oxygen and nutrients from the surrounding environment, and eventually reach a state known as hypoxia (oxygen deprivation) (Fraga et al., 2015). In order to overcome this, cancer cells secrete signals to recruit the formation of new blood vessels from the host's vascular system, which would provide the nourishment the tumor needs to continue growing; a process known as angiogenesis. Hypoxia is associated with poor prognosis in prostate cancer patients, as it correlates with increased invasiveness, metastasis,

and resistance to treatment (Fraga et al., 2015).

A key player in hypoxia is hypoxia-inducible factor-1α (HIF-1α). HIF-1α mediates the transcription of genes such as vascular endothelial growth factor (VEGF). VEGF regulates angiogenesis by promoting endothelial cell growth and proliferation, leading to the formation of blood vessels (Weis and Cheresh, 2011). Under normal conditions, HIF-1α is located in the cytoplasm and translocates to the nucleus under hypoxic conditions. Here, HIF-1α binds to HIF-1β and induces transcription of effector genes after binding to hypoxia response elements within their promotor regions (Fraga et al., 2015). HIF-1α expression has been observed to be higher in prostate cancer tumors in comparison to benign prostatic hypertrophy (BPH) tumors. Its expression also increases in the presence of androgens, which are very important in prostate cancer tumor development, and decreases after androgen depravation therapy.

Unlike normal vasculature, tumor vessels formed after angiogenesis are irregularly shaped, have chaotic patterns of interconnection, and have branches with uneven diameters. They also have abnormal pericyte coverage, which makes them leak fluid and blood into surrounding tissue (Russo et al., 2012).

In order to proceed towards metastasis, cancer cells must enter the blood stream via blood cells by intravasation (Reymond et al., 2013). The blood stream presents cancer cells with a stressful environment, which includes immune cells that could potentially stop the cancer from spreading. If they survive, PCa cells can extravasate from the blood stream and colonize a new site for tumor development. Each type of cancer seems to have a site of preference for metastasis, an observation that led to the “seed and soil” hypothesis (Talmadge and Fidler, 2010). In PCa, the metastatic site of

preference seems to be the bone (Logothetis and Lin, 2005). The tumors resulting from PCa metastasis are osteoblastic, meaning they form bone and it is thought that this occurs by the secretion of factors that affect osteoblasts, or influencing the bone microenvironment.

Prostate Cancer and the Androgen Receptor Pathway

A crucial target for development of novel PCa drugs is the androgen receptor (AR) pathway (**Figure 1.2a**). AR pathway activation occurs when free testosterone enters prostate cells, and is reduced to dihydrotestosterone (DHT) by 5-α-reductase (SRD5A2). The AR in its basal state is bound to Heat-Shock-Proteins (HSPs) in a conformation that prevents DNA binding. Upon binding of AR with DHT, AR undergoes a conformational change that allows dissociation from heat shock proteins. Then, the ligand-bound AR becomes phosphorylated and forms an AR homodimer complex which translocates to the nucleus and binds to Androgen Response Elements (AREs) in promoter regions of target genes. Once bound to DNA, the AR homodimer complex recruits co-activators or co-repressors, which allow interaction of AR complex with the General Transcription Apparatus (GTA) to stimulate or inhibit gene expression, which can ultimately lead to cell proliferation and apoptosis inhibition of prostate cancer cells (Feldman and Feldman, 2001).

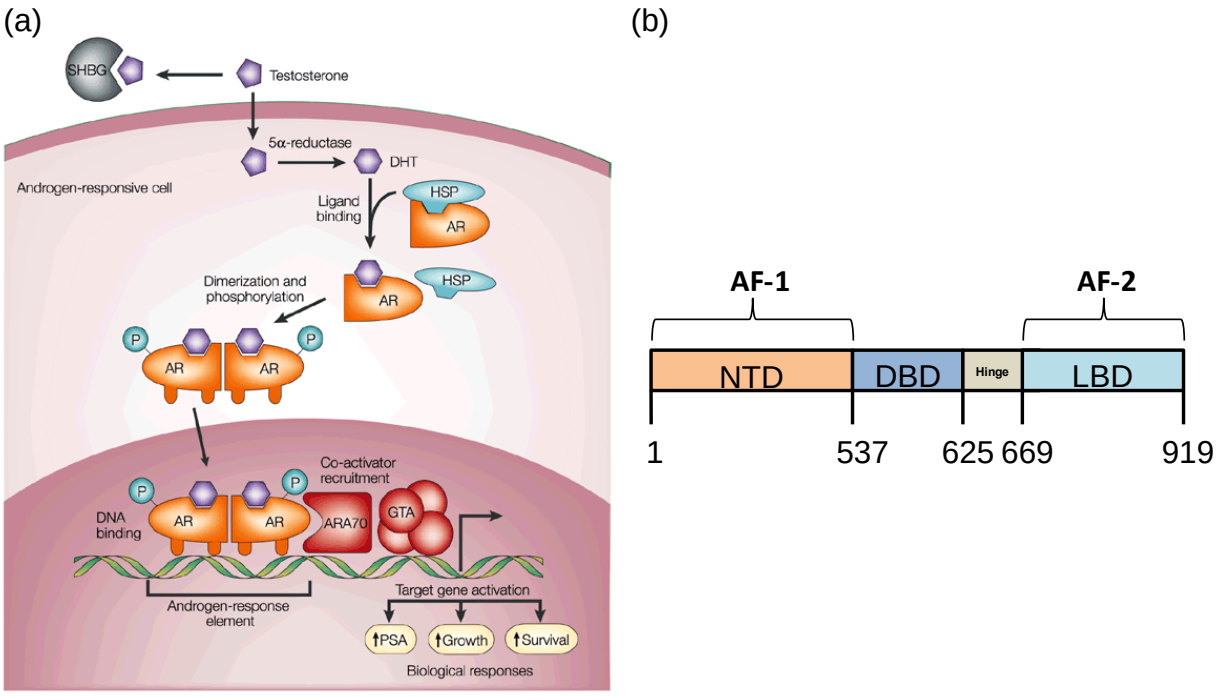


Figure 1.2. The Androgen Receptor. (**a**) The androgen receptor (AR) is activated upon hormone binding which eventually allows translocation to the nucleus. The AR is then able to bind to androgen response elements in DNA, and recruits transcription factors, leading to PCa promoting bioactivity. (**b**) AR domains: N-terminal domain (NTD), DNA binding domain (DBD), hinge region, and ligand-binding domain (LBD). Figure adapted from Feldman and Feldman, Nature Reviews Cancer, 2001.

The wild-type androgen receptor (**Figure 1.2b**) is a 110-KDa protein that consists of an N-terminal domain, DNA-binding domain, hinge region containing a nuclear localization signal, ligand binding domain, and, of course, the C-terminal domain (Marcias et al., 2010). A smaller version of the protein (75-80 KDa) was reported and found to lack the ligand binding domain, which normally binds to androgens in the androgen receptor pathway, leading to the downstream transcription of androgen response elements and subsequent bioactivity such as cell proliferation independent of androgen presence (Tepper et al., 2002).

Treatment of localized prostate cancer can be handled with active surveillance or radiotherapy (Gerritsen and Sharma, 2012). As the disease progresses, patients may require Androgen Deprivation Therapy (ADT); however, tumors can become resistant to primary hormone therapy, and become androgen independent in a median time of 14-30 months (Gerritsen and Sharma, 2012). A treatment option for these advanced stage tumors is docetaxel, an anti-microtubule chemotherapy drug that acts by stabilizing tubulin heterodimers, impairing mitosis and thus cell proliferation in tumors (Hernandez-Vargas et al., 2006). Unfortunately, there are several side effects as well as drug resistance that can occur when using docetaxel (Miller and Ojima, 2001), limiting treatment options. It is therefore imperative that new molecular targets are identified for the development of novel treatments for prostate cancer.

Prostate Cancer Incidence Worldwide and in the United States

Overall, PCa is the fourth leading form of cancer and the second most common cancer in men worldwide with 1,095,000 cases estimated in 2012 (International Agency

for Research on Cancer, 2012). Approximately 70% (759,000) of PCa cases diagnosed in 2012 occurred in more developed regions of the world. The highest incidence rates were observed in Australia/New Zealand (111.6 cases per 100,000), North America (97.2 cases per 100,000), and in Western and Northern Europe. The high incidence in these regions has been attributed to the wide-spread use and availability of Prostate Specific Antigen (PSA) screenings and subsequent biopsies. Incidence in some less developed regions such as the Caribbean, Southern Africa, and South America, is still considered relatively high (79.8, 61.8, 60.1 cases per 100,000, respectively). The lowest incidence rates have been observed in Asian populations (10.5 and 4.5 per 100,000 in eastern and south-central Asia).

In the United States, PCa is the most common non-cutaneous malignancy in men, with 220,800 new cases predicted for 2015 (American Cancer Society, 2015). The states with the highest incidence rates are District of Columbia, Louisiana, and Delaware (157.1, 140.3, 138.8 cases per 100,000 men, respectively). The lowest incidence has been recorded in Arizona, New Mexico, and Hawaii (69.9, 74.0, 84.0 cases per 100,000 men, respectively) (Centers for Disease Control and Prevention, 2015). Incidence rates in the U.S. vary by race and ethnicity, with the highest rates occurring in Blacks (214.5 per 100,000), Non-Hispanic Latinos (141.5 per 100,000), Whites (130.4 per 100,000), and Hispanic Latinos (114.7 per 100,000) (National Cancer Institute Survailence, 2015). As seen in statistics world-wide, Asian/Pacific Islander incidence rates are relatively low in the US (74.0 per 100,000). The population with the lowest PCa incidence rate is American Indian/Alaska Native (67.1 per 100,000). Although PCa incidence rates worldwide vary, less variation is seen in terms of mortality

rates. In addition, the number of deaths is greater in less developed regions of the world (165,000) in comparison to more developed regions (142,000) (International Agency for Research on Cancer, 2012). In general, PCa death rates are higher in black populations (29 per 100,000 in the Caribbean and 19-24 per 100,000 in sub-Saharan Africa). The lowest death rates have been observed in Asian regions of the world (2.9 per 100,000 in South-Central Asia). North America is considered to have an intermediate mortality rate with 6.2-10 deaths occurring per 100,000 men.

Among US men the highest rate of mortality exists in Black men (46.3 per 100,000) (National Cancer Institute Survailence, 2015). Non-Hispanic Latin, White, and Hispanic men have the second, third, and fourth-leading mortality rates (141.5, 130.4, and 114.7 per 100,000). Mortality rates remain low in US Asian populations (74.0 per 100,000) and American Indian/Alaska Native men (67.1 per 100,000). The cause for the differences in incidence and mortality between different racial groups has been largely debated and has been attributed to diet, amount of physical activity, genetic factors, and ability to obtain medical care (Jemal et al., 2010).

Contributing factors to developing PCa development include diet, presence of infection, and genetics (Patel and Klein, 2009). The rates of incidence and mortality of prostate cancer have been correlated with average intake of fats. A study analyzing the risk of prostate cancer development in Japanese immigrants to the US found that diet might influence conversion of latent tumors into clinically significant tumors. In addition, the high consumption of red meat has been classified as a risk factor for prostate cancer. This could be a result of increasing carcinogen levels in meat after cooking at high temperatures, or high consumption of red meat and low consumption of foods that contain

nutrients that may prevent prostate cancer such as fruits and vegetables (Patel and Klein, 2009).

The prostate is exposed to infectious agents via urine and sexual activity, which may lead to the development of prostate cancer (Patel and Klein, 2009). Associations have been made between prostate cancer and seropositivity for cytomegalovirus, herpes simplex virus type 1, and hepatitis B and C antibodies in men 30-49 years of age. In addition, associations have been observed between prostate cancer and seropositivity for human papillomavirus type 16, cytomegalovirus, and hepatitis C in men 50-59 years old. Population studies have also suggested an association between gonorrhea and prostatitis and prostate cancer in African American men.

Diagnosis of Prostate Cancer

There are two common methods used in diagnosing prostate cancer: digital rectal examination (DRE) and screening for prostate specific antigen (PSA). A DRE looks for physical abnormalities in the prostate while a PSA screen looks for high levels of PSA in the patient blood stream , detecting prostate cancer nearly twice as much as DRE would alone (Oh et al., 2003b).

PSA is a glycoprotein with serine protease activity and is located in the cytoplasm of prostate cells whose functional role is to liquefy semen (Stephan et al., 2014). PSA remains mostly within the prostate gland, but may escape in excessive amounts to the blood stream if damage to the basement membrane of prostate epithelial cells occurs. Events that may lead to damage include prostate cancer or benign prostatic tumor development, and physical trauma to the prostate (Stephan et

al., 2014). Thus, high PSA levels are not necessarily indicative of prostate cancer. Normal levels of PSA have been observed to be 0-4 ng/mL of blood. Men whose PSA is in the range of 4.0-10 ng/mL have a possibility of testing positive for prostate cancer (Oh et al., 2003b). While men whose PSA level is above 10 ng/mL are highly likely to be prostate cancer positive. It is important to consider that high levels of PSA may also be attributed to benign prostatic hyperplasia (BPH), or enlargement of the prostate; however, studies have shown that a PSA level above 10ng/mL rarely occurs in men with BPH (2%) (Oh et al., 2003b).

Screening for PSA was approved in 1986 by the FDA to monitor the progression of prostate cancer in men already diagnosed with the disease. In 1993, the FDA approved the use of PSA screens in men asymptomatic for prostate cancer in conjunction with DRE (National Cancer Institute, 2012). After the introduction of PSA screening, the diagnosis of prostate cancer where tumors were still confined to the primary site increased from 50% to 90% of cases diagnosed (Oh et al., 2003b). Although the use of PSA screening improved timely diagnosis of prostate cancer, more recent observations indicate that wide-spread use provides more harms than benefits. In 2008, the U.S. Preventative Services Task Force (USPSTF) issued a recommendation statement on the use of PSA screening for prostate cancer diagnosis (U.S. Preventive Services Task Force, 2008). The report gave PSA screening a grade D recommendation which means that the USPSTF recommended against the use of this service as there is “moderate or high certainty that the service has no net benefit or that the harms outweigh the benefits.” The report cited two major trials as part of the evidence influencing their decision. The first was conducted as part of the U.S. Prostate,

Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial (Andriole et al., 2009), which randomly assigned 76,693 men to receive either annual screening with PSA and DRE or usual care and followed their progress for 7-10 years, tracking number of cancers and deaths as well as causes of death. The study concluded that mortality rate from prostate cancer did not differ significantly from the two study groups. The second trial cited was the European Randomized Study of Screening Prostate Cancer (ERSPC) (Schröder et al., 2009). This study identified 182,000 men age 55-69 from seven European countries and randomly assigned them to a group offering PSA screens once every four years or a group that did not offer screening service. The study initiated in the early 1990s and ended in late 2006. The study found that PSA screening avoided 0.71 death per 1,000 men screened.

In addition to its lack of death prevention , the deficiency in accurate diagnosis of prostate cancer by PSA screens was detected in ERSPC study (Schröder et al., 2009). It was observed that 75.9% of positive PSA test results were false positive according to biopsy results. Aside from the negative psychological effects brought on by false-positive test results, approximately one third of men who undergo biopsy may experience physical harm such as pain, fever, bleeding, infection, and urinary difficulties. Approximately 1% of these men will require hospitalization (Rosario et al., 2012). Considering the low effect PSA screening has in prostate cancer mortality, the deficiency of specificity to diagnose the disease, and the subsequent harms that occur due to unnecessary biopsy, there is an inherent need to develop a more effective method to diagnose prostate cancer.

In 2012, two markers were approved by the US Food and Drug Administration for

the diagnosis of PCa: Progensa ® PC3A assay and Access ® Hybritech p2PSA on the Access Immunoassay Systems, also known as PHI (Prostate Health Index). The PC3A assay requires prostate cells obtained from urine after digital rectal examination, and examines the ratio of PCa gene 3 (PCA3) RNA to PSA RNA to recommend biopsy (Laxman et al., 2008). PHI is an applied formula that requires a serum sample from a patient to measure the presence of total and free PSA (fPSA), as well as isoform p2PSA (Jansen et al., 2010; Loeb and Catalona, 2014). By calculating (p2PSA/fPSA)x√(PSA) the PHI is obtained and physician make recommendations based on the value obtained. Several studies have been conducted to test the accuracy of both markers. One study tested PHI and PCA3 in parallel in blood and urine samples from the same patients, and found that PHI was better at predicting presence of aggressive and more progressed PCa (Cantiello et al., 2015). A separate study found that PCA3 levels did not correlate with Gleason Score; however, MRI outcome did correlate with PCA3 score (Leyten et al., 2013). An existing concern is the cost effectiveness of these tests, and whether that may have an effect on the availability of screening (Nicholson et al., 2015). In addition, more research must be done to assess the usefulness of these markers in PCa diagnosis and to continue to develop alternatives to help enhance or replace current diagnostic options.

1.2 Extracellular Vesicles, Their Role in Cancer Progression and Their Potential as a Source of Diagnostic Markers

Extracellular vesicles (EVs) are cell-derived, spherical, nanoparticles which carry bioactive material such as nucleic acids, proteins, and lipids (Raposo and Stoorvogel, 2013). The study of EVs is relatively new, with the first observations made in 1946 by Chargaff and West when studying coagulation factors in plasma (Chargaff and West, 1946). Although at first considered cell-derived artifacts, we are now understanding that EVs are vehicles for biomolecules, and play an important role in biological processes including immune modulation, coagulation, embryogenesis, and tissue repair among others (Cocucci et al., 2009; Yáñez-Mó et al., 2015). EVs are considered the mediators of the third form of cellular communication; the other two being direct cell-to-cell contact and secretion of molecules (Raposo and Stoorvogel, 2013). The following sections describe EV biogenesis, their importance in cancer progression, and their potential use as a source for diagnostic markers.

EV Biogenesis

"EV" is an umbrella term for an array of extracellular vesicles including exosomes and microvesicles. Current methods of EV enrichment do not allow complete discrimination between exosomes and microvesicles (Raposo and Stoorvogel, 2013). The difference between the two types of EVs is their mechanisms of biogenesis, with exosomes originating from endosomes and microvesicles originating directly from the plasma membrane (Abels and Breakefield, 2016; Nawaz et al., 2014) (**Figure 1.3**).

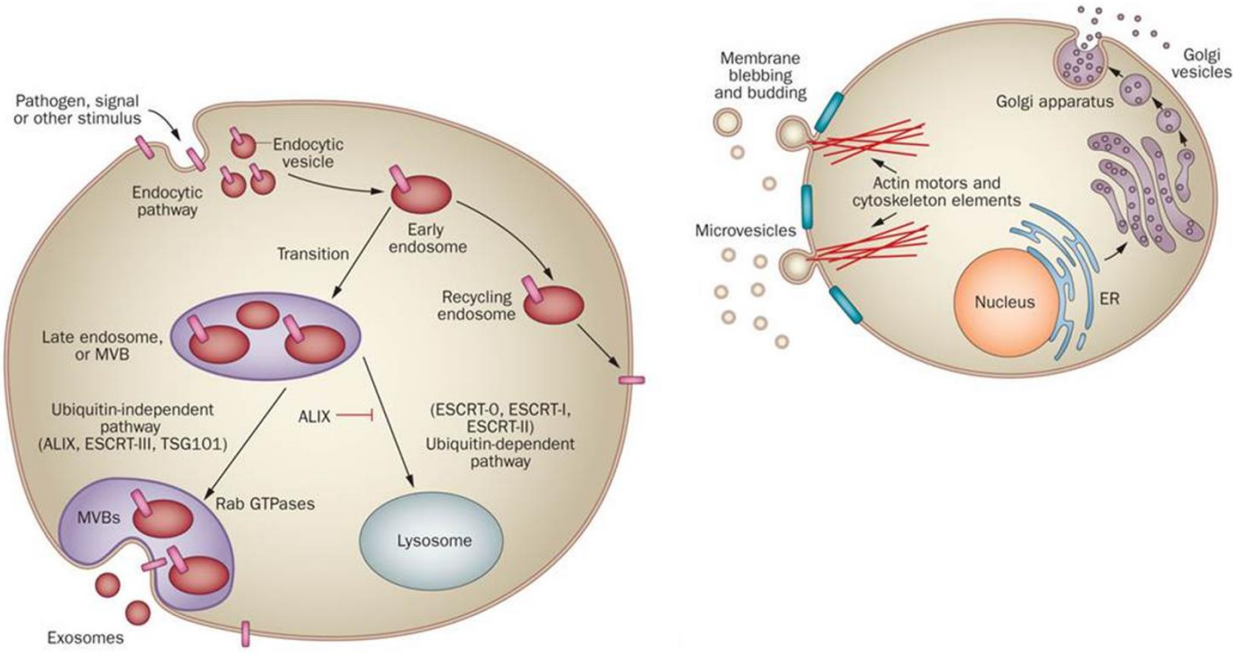


Figure 1.3. Exosomes and microvesicles and their biogenesis. Adapted from Nawaz et al., Nature Reviews Urology, 2014 (Nawaz et al., 2014)

The term “exosome” was coined by Rose Johnstone in 1987 when she observed membrane enclosed vesicles that ranged 30-100 nm in size in the cytoplasm of maturing reticulocytes (Johnstone et al., 1987). We now know that exosomes are formed within the endosomal network; which sorts biological material in large intracellular vesicles known as endosomes for lysosomal degradation, recycling, or exocytosis (Akers et al., 2013). Endosomes can be characterized into three subcategories within their pathway: early endosomes, late endosomes, and recycling endosomes. Early endosomes are first formed by the invagination of the plasma membrane. They can fuse with endocytic vesicles, incorporating their content, which can be destined for recycling (in a recycling endosome); alternatively, the endocytic vesicle can mature into a late endosome. In a late endosome, the biological cargo’s is sorted into 30-100 nm diameter vesicles that are a result of inward budding of the endosomal lumen, forming a multivesicular body (MVB) (Raposo and Stoorvogel, 2013). At this point the fate of the MVB can be lysosomal degradation, or fusion with the plasma membrane, which leads to release of exosomes. Protein ALIX (ALG-2-interacting protein X), can bind to exosomal cargo and prevent lysosomal degradation, allowing release (Nawaz et al., 2014). Microvesicle biogenesis occurs through the direct outward budding of the plasma membrane (Nawaz et al., 2014). This process involves the trafficking of biomolecules to the cell surface, outward budding, and pinching off from the cell body. This process occurs in regions of the plasma membrane where lipid

rafts are located (**Figure 1.3**).

Biological Role of EVs in Cancer

EVs have previously been found to play a role in cancer progression by assisting cancer cells in escaping apoptosis (Mohammed N. Abid Hussein, 2007), metastasis (Janowska-Wieczorek et al., 2005), angiogenesis (Skog et al., 2008), and oncogenic transformation of neighboring cells (Al-Nedawi et al., 2008). The biological cargo (i.e. proteins, lipids, and nucleic acids) seem to be the contributing factors in these activities. EV cargo can interact with the recipient cell by contact between microvesicle trans-membrane proteins and recipient cell receptors, direct fusion of extracellular microvesicle with the recipient cell plasma membrane, or endocytosis of the microvesicle by the recipient cell (Cocucci et al., 2009).

PCa cell-derived EVs have been observed to increase cell proliferation and migration of recipient cells, and increase tumor volume and PSA level in mice administered with PCa EVs intravenously(Hosseini-Beheshti et al., 2016). Another study found that EVs from PCa cells containing protein Ets-1 can be donated to pre-osteoblasts, and induce differentiation(Itoh et al., 2012). This finding suggested that PCa EVs act as a communication tool between cells in osteoblastic metastasis, a symptom commonly observed in prostate cancer progression related to high mortality

and morbidity.

EVs as a source of diagnostic markers

There are several advantages that make EVs a favorable source of diagnostic markers. First, they have been isolated from various biofluids including semen, urine, blood, serum, saliva, amniotic fluid, cerebrospinal fluid, and bile (Raposo and Stoorvogel, 2013). Second, the bioactive cargo they carry is indicative of the pathophysiological conditions of their cell of origin, thus a cell that is cancerous will release different cargo than a cell that is healthy(Lin et al., 2015). For these reasons EVs have been referred to as biomarker “treasure chests(Duijvesz et al., 2011).”

Several studies taking advantage of these properties have already been published. Among them is a study by Eichelser et al., which found that serum levels of EV-derived miR-373 were associated with triple negative and aggressive breast cancer (Eichelser et al., 2014). Alvarez et al., studied the expression levels of neutrophil gelatinase-associated lipocalin (NGAL) as a predictor of kidney dysfunction and found higher expression in patients with delayed kidney graft function in urinary exosomes in comparison to cell fraction from urine (Alvarez et al., 2013).

Project Goal, Hypothesis, and Specific Aims

Goal

The **goal** of this project is to provide further understanding of the prostate tumor microenvironment which may help shed light into biomolecules (proteins) contributing to PCa disease progression, health disparity in PCa positive African American men, and to identify proteins that should be further explored in PCa patient biofluids for use as diagnostic markers.

Hypothesis

The biological properties of EV cargo, such as reflection of cell of origin, and observed contribution to cancer progression, provide basis for us to **hypothesize** that by surveying the proteome of PCa cell-derived EVs, we will be able to identify proteins that may be contributing to disease progression. We also expect to identify proteins that will be differentially expressed when compared to non-cancerous prostate epithelial cell derived EVs; which may serve as a starting point for the development of diagnostic tools for PCa. Finally, we expect to find proteins that may be contributing factors for the aggressive phenotype of African American PCa tumors.

Specific Aims

Specific Aim 1. Isolation and Characterization of Extracellular Vesicles from Prostate Cancer-Derived Cell Lines and Non-Cancerous Prostate Epithelial Cell Line

To address our hypothesis, we will use a cell panel representative of different stages of the disease; which includes: non-cancerous prostate epithelial cell line RWPE1, androgen sensitive PCa cell line metastasized to the lymph node LNCaP, androgen insensitive PCa cell line confined to the prostate 22RV1, and androgen sensitive PCa cell line from a tumor confined to the prostate of an African American patient E006AA-hT.

Our first specific aim will be to optimize a protocol for successful enrichment of EVs from this cell panel. We will assess the effectiveness of our protocol with visualization of our samples using transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA). We will further validate the quality of our EV preparations by blotting for EV markers, TSG101 and ALIX. We will incorporate the use of GM130, a Golgi marker that should be absent in appropriately enriched EV preparations.

Specific Aim 2. Qualitative and Quantitative Proteomic Analysis of Extracellular Vesicles from Prostate Cancer-Derived Cell Lines and Non-Cancerous Prostate Epithelial Cell Line

Our second specific aim is to survey the proteome of EVs derived from the proposed cell lines through the use of liquid chromatography/mass spectrometry (LC-MS/MS) and bioinformatics. We will first conduct a qualitative proteomic analysis, which

will identify proteins exclusively present in PCa cell-derived EVs. Using a label-free quantitative approach, we will then identify proteins with significantly different abundance levels in EVs enriched from PCa and non-cancerous prostate epithelial cells. We will further apply gene ontology and string analysis to determine the known biological functions and possible protein-protein interactions of the identified cargo.

The information obtained from these analyses will provide valuable information on the potential roles of EV cargo in prostate cancer progression, identify proteins that may contribute to health disparity of PCa in African Americans, and propose proteins found to be exclusive or significantly abundant in PCa cell-derived EVs which can be further validated in PCa patient biofluids for the development of diagnostic tools.

Chapter 2: Isolation, Characterization, and Comparative Proteomic Analysis of Extracellular Vesicles from Prostate Cancer-Derived Cell Lines

2.1 Introduction

Prostate cancer is the most common non-cutaneous malignancy in American men and is also the second deadliest (National Cancer Institute Survailence, 2015). There is a disproportionate occurrence of prostate cancer incidence and mortality in men of different race and ethnic backgrounds. In particular, African American men have been reported to have the highest incidence and mortality rate when compared to men of other ethnic backgrounds (DeSantis et al., 2013), and the underlying factors contributing to this disparity need to be investigated further. While the incidence rate of prostate cancer in the United States is among the highest in the world, the mortality rate does not differ much from other regions (International Agency for Research on Cancer, 2012). It has been suggested that the high incidence rate is due to a wide availability for prostate-specific antigen (PSA) screenings (International Agency for Research on Cancer, 2012). Although introduction of PSA screenings was observed to cause a decrease in prostate cancer mortality, recent studies have seen a contradictory trend (Andriole et al., 2009; Schröder et al., 2009). In fact, these studies led to the recommendation against the use of PSA screens by the U.S. Preventive Services Task Force, as the harms of PSA screening outweighed the benefits (U.S. Preventive Services Task Force, 2008). As a result of this recommendation, there is a need for novel, non-invasive, diagnostic markers for prostate cancer.

Extracellular vesicles (EVs) are a promising source of diagnostic markers for several

diseases including breast cancer and kidney disease. Among the characteristics that make them attractive for diagnostic marker identification is the fact that they carry bioactive cargo (i.e., proteins, lipids, and nucleic acids), which reflects the cell of origin. For example, the bioactive cargo from a healthy cell will differ from the cargo of a cancerous cell. In addition, the EV lipid bilayer protects the cargo from the extracellular space, protecting it from being degraded, and allowing it to survive in biological fluids including blood, urine, saliva, and breast milk, among others.

In this study, we sought to identify the protein cargo of prostate cancer cell-derived EVs. We chose to use prostate cancer cell lines LNCaP, 22RV1, E006AA-HT, and non-cancerous prostate epithelial cell-line RWPE1 in our analysis.

The LNCaP cell line was isolated from a metastatic lesion adenocarcinoma located on the supraclavicular of human prostatic lymph node of a Caucasian male (Horoszewicz et al., 1983). In culture, these cells have been observed to be androgen responsive, as proliferation increases depending on the dose of dihydrotestosterone (DHT) that is presented. They maintain malignant properties *in vivo*, forming androgen responsive tumors in mice. LNCaP cells express prostate-specific proteins; including human prostatic acid phosphatase and prostatic antigen.

The 22RV1 cell line is derived from a human prostatic carcinoma xenograft described as CWR22 (Sramkoski et al., 1999). CWR22 xenograft had been reported to form androgen-dependent tumors in mice that secreted PSA into the bloodstream at levels that were related to the severity of the tumor. Castration of mice with tumors formed after introduction of CWR22 resulted in tumor regression, and relapse 3-10 months later (Nagabhushan et al., 1996). The relapsed tumors were then serially

transplanted. 22RV1 is the cell line that was established from these transplanted tumors, and was initially reported as androgen responsive (Sramkoski et al., 1999). Upon further characterization of the 22RV1 androgen receptor, it was reported that 22RV1 expresses two separate AR protein species of 112 and 75-80 KDa (Dehm and Tindall, 2011; Tepper et al., 2002). The wild-type androgen receptor is a 110 kDa protein consisting of an N-terminal domain, DNA-binding domain, hinge region containing a nuclear localization signal, ligand binding domain, and, of course, the C-terminal domain (Marcias et al., 2010). The smaller form of the protein was found to lack the ligand-binding domain, which normally binds to androgens in the androgen receptor pathway, leading to the downstream transcription of androgen response elements and subsequent bioactivity such as cell proliferation. The truncated form of the AR present in 22RV1 was also observed to be constitutively present in the nucleus and could bind to DNA independent of androgens (Tepper et al., 2002). Thus, 22RV1 cells can be used as a model for the androgen independent, therapy resistant, stage of prostate cancer.

E006AA-hT is a subline of E006AA cell line. E006AA is the first immortalized epithelial prostate cancer cell line isolated from a primary site-confined tumor of an African American patient, and is thus representative of the early stages of the disease (Koochekpour et al., 2004). Characterization of the E006AA cell line showed that it is androgen-dependent and is not tumorigenic in nude mice. The E006AA-hT cell line was developed by stably transfecting E006AA parent cells (E006AA-Par) with pcDNA3.1-Neo^R vector and obtaining G418-resistant cells. These cells were introduced into NOG-SCID mice and were found to cause tumor development three weeks after inoculation. The human epithelial cells that were later isolated from these tumors are referred to as

E006AA-hT, due to their high tumorigenic activity (Koochekpour et al., 2014). In comparison to E006AA-Par, E006AA-hT maintained similar morphology and both express high levels of p53; however, E006AA-hT has a higher rate of proliferation and leads to the development of aggressive tumors in mice which were observed to invade lymphovascular channels and infiltrate adjacent adipose tissue, skeletal muscles, bone and cartilage.

RWPE-1 is a non-cancerous cell line derived from a histologically normal prostate of a white male undergoing cystoprostatectomy, which was immortalized using human papilloma virus 18 (HPV18) (Bello et al., 1997). Although immortalized, RWPE-1 cells maintain the characteristics of a normal prostate epithelial cell; such as response to androgens, expression of PSA, normal epithelial morphology, lack of tumorigenicity, and lack of invasive properties. The use of this cell line in our study allows us to have a control in which we can observe changes occurring in cancerous vs. non-cancerous prostate cells.

This panel of cell models are representative of different stages of the disease, including localized and metastasized PCa and we also have also incorporated a cell model for PCa in an African American patient. Cell line RWPE1 was used as a healthy control in order to make a PCa vs. non-PCa prostate epithelial cell comparison. Here, we show that there are considerable proteomic differences in EVs from all cell lines at a qualitative and semi-quantitative level. Using differential centrifugation, we obtained two EV fractions (EV2 and EV16), both of which contained important protein cargo related to cancer. Several proteins were found to be significantly abundant in PCa cell-derived

EVs when compared to RWPE1.

2.2 Experimental procedures

Specific Aim 1. Isolation and Characterization of Extracellular Vesicles from Prostate Cancer-Derived Cell Lines and Non-Cancerous Prostate Epithelial Cell Line

Cell culture

All cell lines were cultured at 37˚C in a 5% CO2 incubator. Prostate cancer cell lines 22RV1 and LNCaP were cultured in RPMI 1640 media supplemented with 10% fetal bovine serum (FBS)(Horoszewicz et al., 1983; Sramkoski et al., 1999). E006AA-hT prostate cancer cells were a kind gift from Dr. Shahriar Koochekpour (University of Roswell Park Cancer Institute, University of Buffalo) (Koochekpour et al., 2014), and were grown in Dulbecco's modified Dulbecco's modified Eagle medium (DMEM) supplemented with 10% FBS. Non-cancerous epithelial cell line, RWPE1, was grown in keratinocyte serum-free medium (KSFM), supplemented with human recombinant Epidermal Growth Factor 1-53 (EGF 1-53) and Bovine Pituitary Extract (BPE)(Bello et al., 1997). In preparation for EV enrichment, cells were cultured under normal conditions for 48 h. After this period, cells were washed with warm (37˚C) PBS three times to remove excess FBS. Supplement-free medium was then added to the cells and incubated for additional 48 h before proceeding to EV enrichment.

EV Enrichment

Cells were incubated in supplement-free medium (RPMI, DMEM, or KSFM) for 48 hours, based on viability assessment by flow cytometry using propidium iodide (data not shown) and previously published articles using the same cell lines (Hosseini-Beheshti et al., 2012) (Sardana et al., 2008). EV enrichment was conducted as described (Bayer-Santos et al., 2013; Théry et al., 2001), with some modifications. Briefly, supernatant was collected and centrifuged for 10 min at 300 x *g*, followed by a 10 min centrifugation at 3,000 x *g* to remove cell debris. The supernatant was then centrifuged for 30 minutes at 10,000 x *g* to remove apoptotic bodies. The resulting supernatant was then centrifuged at 100,000 x *g* for 2 h and the obtained pellet was kept (EV2). The EV2 supernatant was then centrifuged for 16 h and the resulting pellet was also kept (EV16). Both fractions were washed three times with PBS before analysis was conducted. All centrifugation steps were performed at 4˚C.Protein content in whole cell lysate (WCL) or in each EV fraction was quantified by the BCA Protein Assay Kit (Pierce, Thermo Fisher Scientific).

Nanoparticle tracking analysis

Nanoparticle tracking analysis (NTA) was conducted using the NanoSight LM14 system (Malvern Instruments Ltd., Malvern, UK) to determine particle size of prostate cancer cell-derived EVs in EV2 and EV16 fractions. EV samples were diluted in phosphate-buffered saline, pH 7.4 (PBS), and injected into the equipment sample chamber. Tracking was done for 1 min at room temperature, and the data was analyzed using NTA software v2.2.

Transmission Electron Microscopy (TEM)

Enriched EVs were fixed in 1% glutaraldehyde and were allowed to absorb onto glow-discharged formvar/carbon-coated copper grids for 10 min. Grids were washed in dH_2O and stained with 1% aqueous uranyl acetate (Ted Pella Inc., Redding CA) for 1 minute. Excess liquid was gently wicked off and grids were allowed to air dry. Samples were viewed on a JEOL 1200EX transmission electron microscope (JEOL USA, Peabody, MA) equipped with an AMT 8 megapixel digital camera (Advanced Microscopy Techniques, Woburn, MA).

Immunoblotting

Immunoblotting analysis was performed using 4 µg protein of each EV fraction or whole cell lysate (WCL). Protein samples were separated using a Novex Bolt Bis-Tris 10% gel (Life Technologies, Thermo Fisher Scientific). Proteins were then transferred onto a PVDF membrane which was then blocked with 5% non-fat milk. The blot was then washed with 20 mM Tris-buffered saline, 0.1% Tween 20 (TBST), and incubated with monoclonal antibodies (mAbs) conjugated with horseradish peroxidase (HRP) for proteins GM130 (ab52649, Abcam), TSG101 (ab83, Abcam), ALIX (sc-53540, Santa Cruz Biotechnologies). Antigen-antibody complexes were detected using SuperSignal™ West Pico Chemiluminescent Substrate (Thermo Fisher Scientific).

Specific Aim 2. Qualitative and Quantitative Proteomic Analysis of Extracellular Vesicles from Prostate Cancer-Derived Cell Lines and Non-Cancerous Prostate Epithelial Cell Line

Proteomic sample preparation

EV preparations were resuspended in urea to a final concentration of 8M and placed on a Nutating Mixer (product # 05450213, Fisher Scientific) for 30 min at room temperature. Protein concentration was determined using the BCA kit, and 10 µg of EV or WCL were used for digestion. Dithiothreitol (DTT) to a final concentration of 10mM was added to the sample, vortexed briefly, and incubated for 30 min at room temperature on a rocker. A Filter-Aided Sample Preparation (FASP) protein digestion kit (Expedeon Inc., San Diego, CA) was used for tryptic digest of sample proteins with some modifications to manufacturer's protocol. Samples were placed on a 30-kDA spin filter with integrated collection tube and centrifuged at 14.000 x *g* for 15 min, at room temperature. Samples were then alkylated using 100 µL of 500 mM iodoacetamide for 30 min at room temperature in the dark and centrifuged for 10 minutes at 14,000 x *g*. A final wash step with 50 mM ammonium bicarbonate was done, prior to transferring the filter to a new tube, and incubating with 4 µg of trypsin (Sigma Aldrich) overnight at 37˚C. Peptides were eluted from the filter using 200 µL of 0.1% formic acid. Samples were dried to a 50 µL volume and were subjected to liquid chromatography mass spectrometry **(**LC-MS/MS) analysis.

LC-MS/MS Analysis

The resultant complex peptide mixtures from the individual samples were loaded into a Dionex U3000 Autosampler and 5 □L of sample (1 □g each sample) was injected onto a self-packed C18 resolving column (13 cm x 100 µm) with an integrated nanospray

tip (Picofrit packed with Phenomenex Luna C18, New Objective, Woburn, MA). A multistep gradient of increasing acetonitrile (ACN) concentration for 120 min at a flow-rate of 300 nL/minute was performed. The gradient consisted of solvent A (5% ACN, 0.1% formic acid (FA)), and solvent B (80% ACN, 0.1% FA), as follows: 0-10 min: 5% solvent B; 10-95 min: 5-40% B; 95-100 min: 40-95% B; 95% B until for 10 min; 110-120 min: equilibration with 5% B. This was done using an Ultimate 3000 nanoHPLC system coupled to a QExactive mass spectrometer (Thermo Fisher Scientific). An automated 2-h LC-MS/MS run was programmed into Xcalibur software (Thermo Fisher) and each sample analyzed in technical duplicates with two biological replicates. Between samples, two blank runs with 60-min double seesaw washes using 5-80% ACN gradient to optimally clean the resolving column and limit peptide carryover. During each analysis and all sample runs the QExactive was operated in top-ten data-dependent mode, which is 10 data-dependent MS/MS scans for each proceeding full scan. The QExactive settings were as follows: the normalized collision energy for HCD was 28 eV; a full scan resolution of 70,000K from 400-1600 *m/z*; a HCD MS/MS resolution of 17,500 with an isolation width of 3 *m/z*; and the dynamic exclusion was set at 15 seconds. Peptides were not excluded based on charge state and 1 microscan for both full and MS/MS scans were acquired. All MS and MS/MS data were acquired in profile mode.

Bioinformatic analysis of proteomic data

All resultant MS/MS spectra from individual 2-h runs were searched with Proteome Discover 2.0 (Thermo Fisher) and filtered via Percolator with an estimated false-positive rate of 5%. The Proteome Discover settings were as follows: HCD MS/MS; included a

fixed modification for carboxyamidomethylated cysteines; a variable modification methionine oxidation; fully tryptic peptides only; up to 4 missed cleavages; a precursor mass tolerance of 20 ppm; and a fragment mass tolerance of 20 ppm. Tandem MS/MS spectra were searched against a combined protein database of human (GCA_000001405.19, downloaded on August 19th, 2015, from UniProtKB) as well as common contaminants (such as trypsin, keratins and protein lab standards, downloaded from http://compbio.ornl.gov/shewanella_chromium_stress/databases/) were included in this combined database. Spectral counts were normalized using Distributed normalized spectral abundance factor (dNSAF) provided by Zhang *et al.* (Zhang et al., 2010). A similarity matrix was created using R 3.2.1 (http://www.R-project.org/), to assess the correlation between all samples and replicates.

Gene ontology analysis and Venn diagrams were generated using FunRich 2.1.2 (http://www.funrich.org/) (Pathan et al., 2015). The Venn diagram function was also applied to determine which proteins identified in our study had been reported on Vesiclepedia (http://www.microvesicles.org/).

Principal component analysis (PCA) was generated using Perseus 1.5.4.1 (http://www.perseus-framework.org/), based on the normalized spectral count values. Log2 transformation of the data was performed and data points were filtered if they did not appear in three out of the four group replicates (two biological, two technical for each sample type). Remaining missing values were replaced by arbitrary low numbers from normal distribution and Z-score normalization of the obtained values was performed. A heatmap using Perseus 1.5.4.1 was generated for EV2 and EV16 separately with hierarchical clustering using Eucladian distances. Data points were filtered further to

facilitate visualization of abundant protein clusters in each group. The significant difference (q-value ≤ 0.05) in protein abundance was determined using a student's T-test of the mean value of the replicates.

String analysis was performed using String v10.0 with the provided Homo sapiens database (http://www.string-db.org/) (Szklarczyk et al., 2015).

2.3 Results

Specific Aim 1. Isolation and Characterization of Extracellular Vesicles from Prostate Cancer-Derived Cell Lines and Non-Cancerous Prostate Epithelial Cell Line

Isolation and characterization of PCa and non-PCa cell-derived EV2 and EV16

After clearing cell supernatant of cell debris and apoptotic bodies, samples were spun for 2 h at 100,000 x *g* to enrich the first EV pellet (EV2) and the resulting supernatant was spun for 16 h at the same speed to collect any remaining EVs (EV16) (**Figure 2.1**). Both fractions contained vesicles, as shown by TEM imaging (**Figure 2.2**). There is a notable difference in particle size consistent in all cell lines, with EV2 containing larger vesicles in comparison to the EV16 fraction. TEM data was corroborated by NTA results (**Figure 2.3**). Due to the abnormal distribution of the plots, we rely on the calculated mode opposed to the mean in order to obtain the dominant particle size of the sample. In accordance with the TEM images, EV2 fractions were observed to have a higher concentration of vesicles that were over 100 nm in diameter as compared to the corresponding EV16 fraction across all cell lines. EV16, on the other hand, was observed to contain a higher concentration of smaller (<100 nm diameter) particles. An overlap in particle size was also observed between fractions, and more-so can be seen in E006AA-HT EV2 and EV16.

Following characterization of vesicle size, immunoblotting was performed for common EV markers ALIX and TSG101, as well as protein GM130, a Golgi marker which

is expected to be absent in EV preparations (Lötvall et al., 2014). A second gel was loaded with the same samples and ran simultaneously for silver staining. Silver stain of the SDS-PAGE was used as a loading control and to provide a visual of the EV proteome (**Figure 2.4a**). The overall banding pattern showed possible differences in the proteome of EV2 and EV16. There was a complete absence of GM130 in EV preparations, but not in whole cell lysates (WCL) (**Figure 2.4b**). ALIX, on the other hand, was confirmed to be present in all EV2 fractions. Although clear bands corresponding to ALIX appear clearly in EV16 of 22RV1 and E006AA-hT, bands can be seen very faintly in the EV16 fraction of LNCaP and RWPE1. TSG101, another EV marker, was present in variable amounts among different cell lines and EV fractions. It was found to be more abundant in EV2 fractions of LNCaP, 22RV1, and RWPE1, as well as in EV16 fractions of 22RV1 and RWPE. Conversely, this protein could be detected only as faint bands in the blot and could not be identified by proteomic analysis in E006AA-hT WCL and EV fractions and in LNCaP EV16 (**Fig. 2.4c**). Taken together the data suggest that the vesicles observed in out TEM and NTA analyses are positive for two EV markers (ALIX and TSG101) by immunoblotting and are negative for GM130, a Golgi marker. Taken together, our results show that, although TSG101 is commonly used as an EV marker, ALIX proved to be better suitable marker for quality control of EV enrichment, especially in E006AA-hT cells.

Specific Aim 2. Qualitative and Quantitative Proteomic Analysis of Extracellular Vesicles from Prostate Cancer-Derived Cell Lines and Non-Cancerous Prostate Epithelial Cell Line

Qualitative Proteomic Analysis of EV2 and EV16

To study the EV proteome, we employed a slightly modified version of the original FASP protocol, omitting the use of SDS (Wisniewski et al., 2009). The digestion was performed using trypsin and resulting peptides were analyzed using 1D LC-MS/MS. The raw spectra were analyzed using Proteome Discoverer 2.0 with Sequest HT algorithm against a human database containing common contaminants. Two biological replicates, with two technical replicates performed for each sample was used in this analysis. One microgram of protein was used for analysis of each sample. A total of 1,447 proteins were identified across all cell line-derived EVs. From the EV2 fractions of LNCaP, 22RV1, E006AA-hT, and RWPE1 610, 275, 735, and 373 proteins were identified, respectively. For the EV16 fractions of LNCaP, 22RV1, E006AA-hT, and RWPE1 523, 301, 271, and 355 proteins were identified, respectively.

Despite originating from the same cell line, the proteome of EV2 and EV16 differed in all cell lines (**Figures 2.5a-d**). Each EV fraction had a unique set of proteins; however, some overlap existed between the two. The majority of the 22RV1 EV2 and EV16, E006AA-hT EV2, and RWPE1 EV2 was exclusive in comparison to its corresponding fraction; whereas LNCaP EV2 and EV16, E006AA-hT EV16, and RWPE1 EV16 shared most of its proteome with its corresponding fraction.

Using the Vesiclepedia function in FunRich, we compared the proteins identified in this study to those that have been previously published in human EV studies (Kalra et al., 2012). Although the majority of the EV proteins identified from each cell line in this

study have been previously published, some of the proteins identified here have not been reported in the Vesiclepedia database; mainly in the LNCaP and E006AA-hT EV fractions.

In order to compare the EV2 and EV16 proteomes among the four cell lines, a four-way Venn diagram was generated (**Figure 2.5e,f**). Overall, there were 97 proteins shared among EV2 of all cell lines, and 67 proteins common only in prostate cancer (PCa) cell lines. All EV2 fractions had their own exclusive set of proteins, but E006AA-hT EV2 had the highest number with 235 proteins identified exclusively in the EV2 derived from this cell line. There were 109 proteins common among all EV16 fractions, and only 18 common to all prostate cancer cell derived EVs enriched after 16-h centrifugation.

To enhance our understanding of the overall proteomic difference and similarity between all samples, we constructed a principal component (PCA) plot (**Figure 2.6**). Application of PCA reduces the complexity of proteomic data, while maintaining variation, and groups samples with similar data behavior together (Bayer-Santos et al., 2013). Our analysis shows clustering of prostate cancer and non-cancerous cell lines on the left and right side of the plot, respectively. Prostate cancer cell-derived EV2 samples are close together, suggesting similarity in proteomes. Overall, EV2 and EV16 behave differently, with the exception of LNCaP EVs, which cluster close together near prostate cancer cell-derived EV2s. In addition, the plot shows that our technical and biological replicates cluster close together, suggesting that our data is reproducible.

Quantitative Proteomic Analysis of EV2 and EV16

Although proteins exclusive to prostate cancer cell-derived EVs in both fractions were identified, proteins present in low abundance in non-cancerous prostate epithelial

cell-derived EVs in comparison to prostate cancer cell-derived EVs and vice-versa must also be considered. Using a label-free quantitative approach, proteins differentially expressed in EV2 or EV16 were identified (**Figure 2.7 and 2.8**, respectively).

For EV2 and EV16, four and five respective clusters of proteins were found to be differentially present in EVs from each cell line. The clusters were designated a number by the Perseus program. Line plots allowed visualization of the protein abundance trends within each cluster. Based on these trend line plots, we can see that 219 cluster proteins are most abundant in 22RV1 EV2, 222 cluster proteins are most abundant in RWPE1 EV2, 225 cluster proteins are most abundant in LNCaP EV2, 227 cluster proteins are most abundant in E006AA-hT EV2. We applied the same type of clustering for the EV16 heat map and observed that cluster 138 proteins are more abundant in E006AA-hT and RWPE1, cluster 140 proteins are most abundant in RWPE1, cluster 141 proteins are most abundant in 22RV1, and cluster 143 proteins are most abundant in LNCaP.

We proceeded to apply a string analysis of each protein cluster individually, to identify known protein interactions among proteins (**Figure 2.9-2.17**). The biological processes, based on protein gene ontology, and any role in biological complexes were highlighted. Protein complexes identified, at least partially, through string analysis include 26S protease complex, TCP1 Ring Complex (TRiC), and COP9 signalosome. All complexes were identified in clusters 225 and 143, which were most abundant in LNCaP. In other clusters, interacting proteins involved in cell growth, immune response, cell communication, and transport among other functions were observed.

Application of a student's T-test revealed which of these proteins are significantly different in comparison to the corresponding RWPE1 EV fraction. Based on a q-value

cutoff of ≤0.05, we were able to identify 228 and 119 with significantly different abundance in EV2 and EV16 of PCa cells in comparison to non-cancerous prostate epithelial cell line RWPE1 (**Tables 2.1 and 2.2**). We classified these proteins by their corresponding biological process which included protein metabolism, cell growth, transport, metabolism, immune response, cell communication, and regulation of nucleic acids in both EV2 and EV16.

Further specification of reported roles in relevance to cancer, such as overexpression in tumor tissue, promotion of tumorigenesis, metastasis and cell proliferation, was also specified for each protein if available. Identified proteins involved in the progression of PCa included Ras-related protein Rap-2c, which was abundant in all PCa EV2 and is involved in PCa cell growth mediated by AR activation (Bigler et al., 2007). Proteins with reported involvement in PCa cell growth, migration, and/or invasion included guanine nucleotide-binding protein subunit alpha-13 (Rasheed et al., 2013; Zhang et al., 2014b), intercellular adhesion molecule 1 (Conrad et al., 2009), tyrosine-protein kinase Lyn (Goldenberg-Furmanov et al., 2004), and fascin (Fuse, 2011), among others. Identified proteins with reported increased expression in PCa tissue, cells, or biofluids include vinculin (ZHU Li-yong, 2010), Probable ATP-dependent RNA helicase DDX5 (Clark et al., 2008), and basement membrane-specific heparan sulfate proteoglycan core protein(Grindel et al., 2016; Grindel et al., 2014).

Supplementary Tables 1-7 containing the detailed identification of peptides and proteins with all proteomic analysis parameters, as well as the label-free (spectral count) quantitative proteomic data and other files (dNSAF values, similarity matrix, heatmap proteins and clusters, and Venn diagram data) are available to the Dissertation's

Committee members through Dropbox. Raw proteomic data files are available at the PRIDE Archive proteomics data repository (https://www.ebi.ac.uk/pride/archive/).

2.4 Discussion

By studying the proteome of PCa cell-derived EVs, our study sought to establish basic knowledge of potential contributing factors in the disparity of PCa incidence and mortality between African American and Caucasian men, propose proteins for validation in biofluids to determine their use as diagnostic markers, and to further understand the microenvironment of PCa.

Men of African Ancestry worldwide, including southern Africa and the Caribbean, have the highest mortality age-standardized rates (ASR) for PCa (Cooperberg, 2013). In the United States, the mortality ratio between African American and Caucasian men is higher than any other malignancy in men. African American men are found to be at a later stage in the disease than other racial groups at time of diagnosis, and are more likely to have biochemical reoccurrence (Cooperberg, 2013; Das et al., 2016). Because there is a need for more knowledge on the underlying factors that may contribute to racial disparity in PCa incidence, mortality, and severity of disease at the time of diagnosis, it is important to incorporate cell models, such as E006AA-hT, into prostate cancer research.

The E006AA cell line was obtained from a localized PCa tumor from an African American patient; however, characterization of this cell line showed that it was not tumorigenic in nude mice. The E006AA-hT cell line originally came from E006AA, and was altered to allow tumor formation in mice (Koochekpour et al., 2004; Koochekpour et al., 2014). Sub-cutaneous inoculation of E005AA-hT led to contentious aggressive tumor growth in nude mice. Histology showed invasion in lymphovascular channels, adjacent adipose tissues, skeletal muscles, bone, and cartilage had occurred (Koochekpour et al.,

2014). Consistent with these characteristics, our study identified several proteins important for tumor migration that were significantly abundant in E006AA-hT in comparison to RWPE1. These proteins included guanine nucleotide-binding protein subunit alpha-13, ras-related protein Rab-13, and laminin subunit gamma-2. Proteins significantly abundant only in the E006AA-hT cell line included tyrosine-protein kinase Lyn and alpha-enolase. Although these proteins are involved in characteristics of aggressive disease (metastasis and invasion), more studies need to be conducted to determine whether they may be contributing factors to health disparity of PCa in African American men.

Although to our knowledge we are the first group to report the EV proteome of E006AA-hT, Hosseini-Beheshti *et al.* previously explored the EV proteome of LNCaP and RWPE1 qualitatively (Hosseini-Beheshti et al., 2012). This study compared the EV proteome of six different prostate cancer cell lines of distinct AR phenotypes and was able to identify a total of 220 proteins. Compared to our study, Hosseini-Beheshti *et al.* used a different protocol for EV enrichment, which consisted of concentration of conditioned media using a 100-kDa filter followed by ultracentrifugation on a sucrose cushion. A different protocol was also used for the proteomics portion of the study; which consisted of an in-solution digestion of the EV proteins followed by analysis using a QTOF-based mass spectrometer. Differences in methodology might explain why our study yielded a higher number of protein IDs. FASP has been shown to provide better sequence coverage of membrane proteins and increased number of overall protein IDs in comparison to in-solution digestion (Wisniewski et al., 2009). Nonetheless, the two studies complement one another, as proteins such as agrin and serotransferrin were

consistently identified in their corresponding cell lines, proving reproducibility regardless of methodology.

Another previous study by Sardana *et al.* studied the proteome of conditioned media from LNCaP, 22RV1, and PC3 cells (Sardana et al., 2008). This group dialyzed conditioned media using a 3.5-kDa cutoff dialysis tubing and applied in-solution digest of the obtained proteins prior to proteomic analysis using two-dimensional liquid chromatography. Overall, 2,124 proteins were identified among the three cell lines. A major difference between the present study and the work done by Sardana *et al.* is our focus on the EV proteome. However, there is consensus of some proteins in 22RV1 of both studies, such as ADAM10 and Peroxiredoxin-1, 2, and 4.

Our EV enrichment method of choice was classical differential centrifugation, as it successfully allows attainment of vesicles, while avoiding contamination of our samples with materials that affect the proteomic analysis downstream. One artifact that may be obtained by application of differential centrifugation is polyethylene glycol (PEG), which may come from ultracentrifugation or other tubes used in the sample preparation process. The application of FASP during our proteomic sample preparation helped diminish the presence of PEG in our sample (data not shown).

In addition to a two-hour centrifugation, we completed an additional 16-hour centrifugation to analyze remaining material in our sample supernatant. TEM and NTA showed the presence of remaining small vesicles (EV16), with diameter characteristic of exosomes (Lötvall et al., 2014). Qualitative proteomic comparison of EV2 and EV16 fractions showed a difference in the EV proteome despite coming from the same cell-line. Moreover, the comparison of EV2 and EV16 among different cell lines revealed that there

are common and unique proteins within each group. Further comparison of all samples using PCA revealed separate grouping between cancerous and non-cancerous samples, and between EV2 and EV16 with the exception of LNCaP.

Using spectral counts, we were able to determine which proteins differed in abundance and allowed visualization with the construction of a heat map. We identified protein clusters which revealed that each cell-derived EV had a set of abundant proteins. String analysis of these clusters allowed for better visualization of interacting proteins, revealing two (143 and 225) containing 26S protease complex, TCP1 containing ring complex (TRiC), and the COP9 signalosome proteins. Both 225 and 143 clusters show a trend of abundant expression in LNCaP cell-derived EVs. The 26S proteasome complex has been described as an attractive target for cancer therapy due to its proteolytic degradation of key regulatory cellular proteins (Frankland-Searby and Bhaumik, 2012). The implicated role of 26S proteasome in PCa is in promoting AR activity by contributing to the assembly of an AR transcription complex (Reddy et al., 2006; Shen et al., 2014). TRiC is known to assist in folding of cytoskeletal proteins (Gao et al., 1992). TRiC subunits TCP1 and CCT2, also found in our study, were reported to play an important role in breast cancer cell growth, and also predict survival in breast cancer patients (Guest et al., 2015). The COP9 signalosome is a key player in DNA-damage response, cell cycle response, gene expression, and regulates the degradation of polyubiquitinated proteins(Wei et al., 2008). The COPS5 subunit of the COP9 complex has been proposed as potential oncogene in breast cancer (Adler et al., 2008). COPS5 has also been reported alongside COPS4 and CK2 (also identified in our study) to promote cell proliferation and survival of PCa cells (Bhansali and Shemshedini, 2014).

Through statistical testing of our normalized data, we identified a total of 228 and 119 proteins were identified significant in EV2 and EV16, respectively. Several of these proteins have known functions which contribute to disease progression, or have been proposed as biomarkers in various types of cancer. For instance, vinculin was shown to be significantly abundant in E006AA-hT EV2, 22RV1 EV2 and LNCaP EV16 in comparison to corresponding RWPE1 EV2 or EV16. This results is in accordance with other studies which have shown that vinculin is abundant in PCa tissues and PC3 cell-derived exosomes (Kawakami, 2015; Zhu et al., 2010). A qualitative proteomic study of PCa serum-derived exosomes identified vinculin as one of 35 proteins that were commonly expressed in PCa patients of different ethnic backgrounds (African American, Hispanic, and Caucasian) (Turay et al., 2016). Consistent identification of vinculin *in vitro* and *in vivo* regardless of patient ethnic background is suggestive of its use as a non-invasive biomarker. In addition, vinculin has been regularly detected in urinary exosomes (Gonzales et al., 2009; Raj et al., 2012). Comparison of protein abundance levels in PCa and healthy individuals may be of interest.

Another protein of interest for further investigation in regard to its potential as PCa biomarker is Thy-1 (THYmocyte differentiation antigen 1, or THY1), also known as CD90 (Cluster of Differentiation 90) (Herrera-Molina et al., 2013). THY1 is a 25-kDa glycosylphosphatidylinositol (GPI)-anchored membrane glycoprotein, which was identified as the first T-cell marker (Reif and Allen, 1964), although it is expressed in many different cell types, such as neurons, mesenchymal and hematopoietic stem cells, NK cells, renal glomerular mesangial cells, and endothelial cells (Herrera-Molina et al., 2013). In comparison to RWPE1, THY1 was more abundant in 22RV1 and E006AA-hT EV2

fractions. It has been proposed as potential cancer biomarker and has been identified in urinary exosomes (Principe et al., 2013; Shrout et al., 2008; True et al., 2010). True *et al.* (2010) studied the presence of THY1 in prostate cancer (True et al., 2010). Their results showed via immunohistochemistry that THY1 is present in all prostate carcinomas. Proteomic analysis of PCa urine in the same study using ICAT labeling identified THY1, and also noted an absence of this protein in urine from patients who had undergone prostectomy. In a separate study, Principe et al. looked at the proteome of microvesicles from urine of 12 low-grade PCa and 12 healthy participants. THY1 was identified in urinary microvesicles from both PCa and healthy individuals with a ratio of ~0.2680 (PCa:healthy) based on label-free quantification. These results lead to speculation that THY1 identified by True *et al.* (2010) in PCa urine may not be EV-associated; however, a targeted proteomic analysis of EVs in patients with more progressed prostate cancer or from other bio fluids may provide more grounds to use Thy1 as a PCa biomarker.

We compared our PCa EV results to a recent publication by Welton *et al.*, which used size exclusion chromatography to enrich EVs from prostate cancer patient-derived serum and urine samples (**Figure 2.18**) (Welton et al., 2016). We found that the EV2 fractions had a higher percentage of proteins in common with the results from Welton et al. Among the proteins found to be common in the studies are inosine-5'-monophosphate dehydrogenase 2, N-acetylglucosamine-6-sulfatase, and creatine kinase B-type within urine and fibronectin within plasma. However, we found several other proteins that were uniquely upregulated in the EVs of PCa cell lines. The potential of these proteins to be explored as potential biomarkers of PCa needs to be carefully assessed by proteomic analysis of urine samples from PCa patients. To this end, our group has recently

established a collaboration with Dr. Heirinc Williams, a urologic oncologist at Geisinger Medical Center (Danville, PA). We already have an IRB protocol in place and we will start the screening of PCa urine samples soon.

Our results were also compared to a list of 27 PCa biomarker candidate proteins reported by Kim *et al.,* which were obtained by conducting discovery and targeted proteomics on urine obtained from patients with confined and extracapsular PCa *(Kim et al., 2016).* Out of these proteins, we found that at least 4 but no more than 13 were identified in our study. Protein Creatine kinase B-type, was the only protein commonly identified in PCa urine of both published studies and was identified in at least one fraction of 22RV1 and LNCaP, but not in E006AA-hT.

Although working with patient biofluids when identifying biomarker candidates has obvious advantages, our cell-based study is of great importance for multiple reasons. First, we are able to say that the proteins identified in our study came directly from prostate cells; which is not a claim that can be easily made when studying EVs from whole organisms as virtually all cell types in the human body that have been studied are found to secrete EVs (Yáñez-Mó et al., 2015). Second, the list of identified proteins in our study comes from a diversified study representative of different PCa stages and is among the first to describe EVs obtained from a cell line isolated from an African American patient PCa tumor. Epidemiological and pathological studies have suggested that men of African ancestry are frequently diagnosed at later stages of PCa in comparison to men of other ethnic backgrounds (McGinley et al., 2016; Tsivian et al., 2013). Taking these differences into consideration, there is probably not a “one-size fits all” marker for prostate cancer (Shenoy et al., 2016), and investigation of a combination of biomolecules as potential

biomarkers may be advantageous to developing an effective diagnostic tool. Finally, the information provided in our present study provides basis for the development of a targeted proteomic approach when validating these biomarkers in PCa patient biofluids. Due to the high protein complexity of human bio fluids, sensitivity of discovery based studies is hindered when trying to identify differentially expressed proteins (Schiess et al., 2009). With the information provided from our study, we plan on developing a targeted approach for validation of some of our proteins of interest in PCa biofluids. We would also like to narrow our focus to study the post-translational modifications occurring in these proteins as well. Of particular interest is glycosylation, which helps regulate cancer progression (Pinho and Reis, 2015). In particular, GPI-anchored glycoproteins have been found to be elevated in plasma of breast, ovarian, kidney, and brain cancer (Stowell et al., 2015), and is a topic of high interest to our laboratory (Nakayasu et al., 2012; Nakayasu et al., 2009).

In summary, our study analyzed the proteome of three prostate cancer cell lines, one of which was derived from an African American PCa tumor cell line, and a non-cancerous prostate epithelial cell line. Differences were observed qualitatively and differentially among all sample types. Based on previously published works, we were able to confirm that many of the proteins identified play a role in cancer, and have been reported in PCa biofluids and biofluid-derived EVs. We have also identified multiple proteins which have not been reported in these works, and would merit a targeted proteomic analysis in order to validate their presence in PCa patient biofluids.

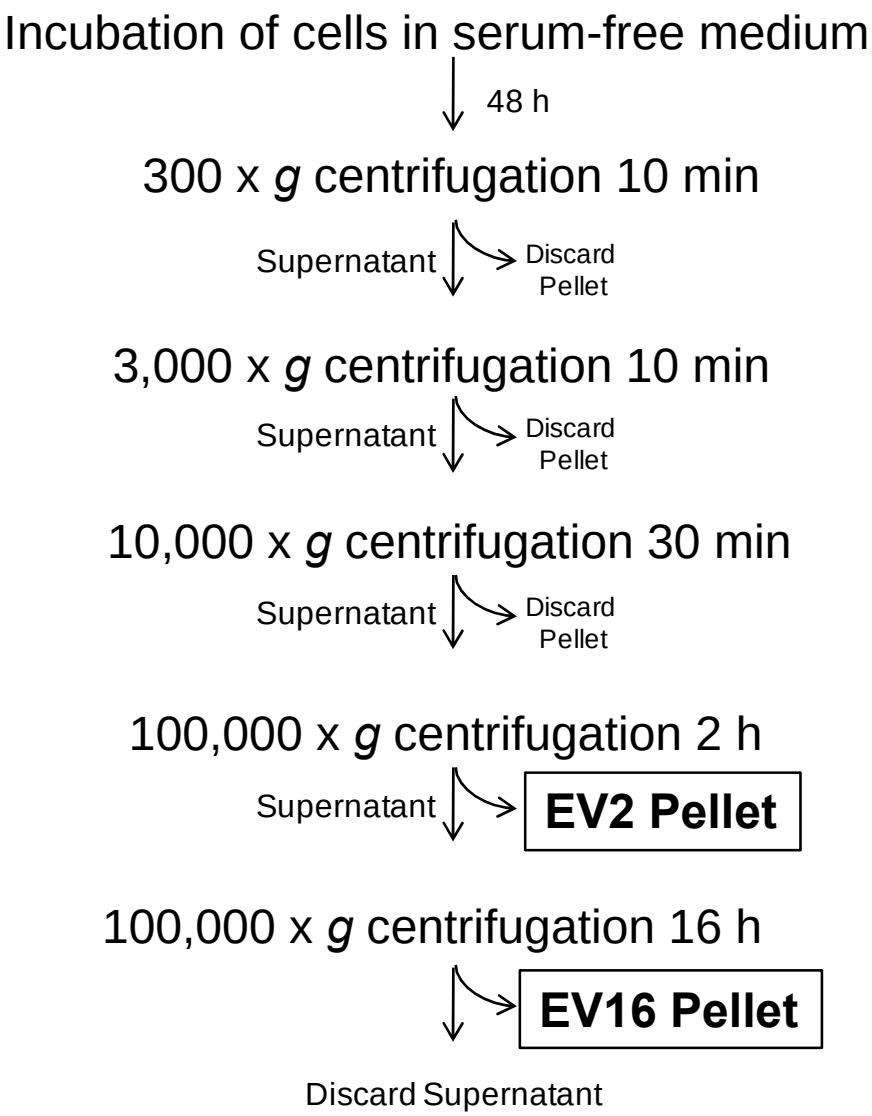


Figure 2.1. Applied methodology for EV enrichment. Cells were incubated in serum-free media for 48 h. Cell debris was removed by centrifugation at 300 and 3,000 x *g*. Apoptotic bodies were removed by a 10,000 x *g* centrifugation. EVs were first enriched by 100,000 x *g* centrifugation for 2 h, resulting in EV enrichment (EV2 pellet). Centrifugation of the EV2 pellet supernatant for 16 h resulted in further EV enrichment (EV16 pellet). Both EV2 and EV16 were characterized by TEM and NTA for all cell lines in this study.

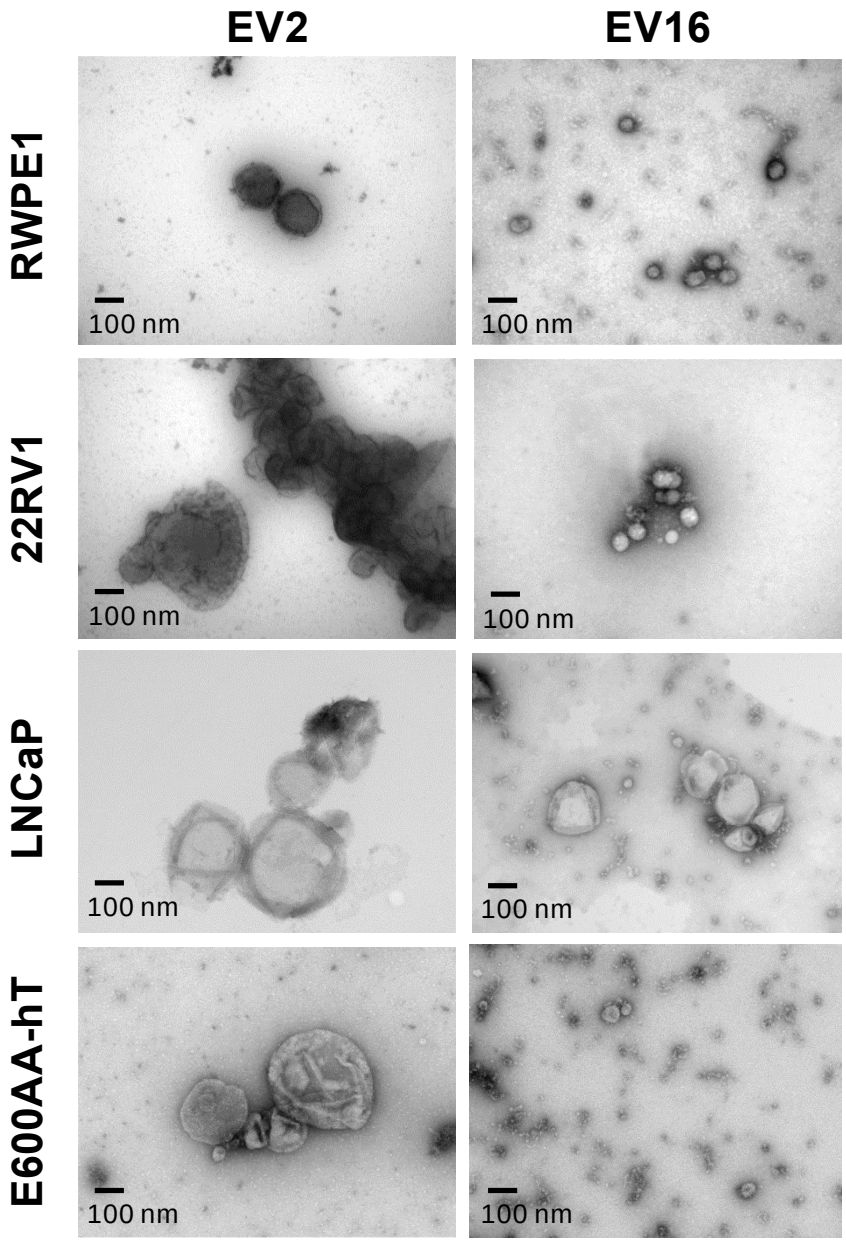


Figure 2.2. TEM Imaging of EV2 and EV16 Fractions. EV2 and EV16 fractions from 22RV1, E006AA-hT, LNCaP prostate cancer cell lines and non-cancerous prostate epithelial cell line RWPE1 were subjected to TEM imaging. All images were taken at 50,000x magnification. Images confirm presence of vesicles in both EV2 and EV16.

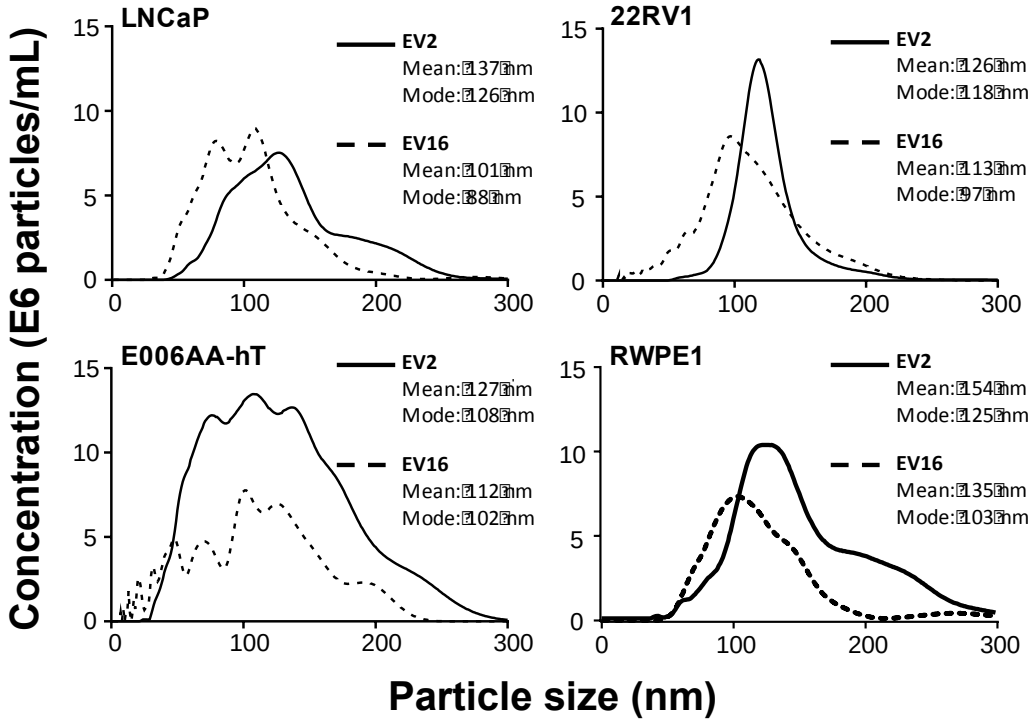
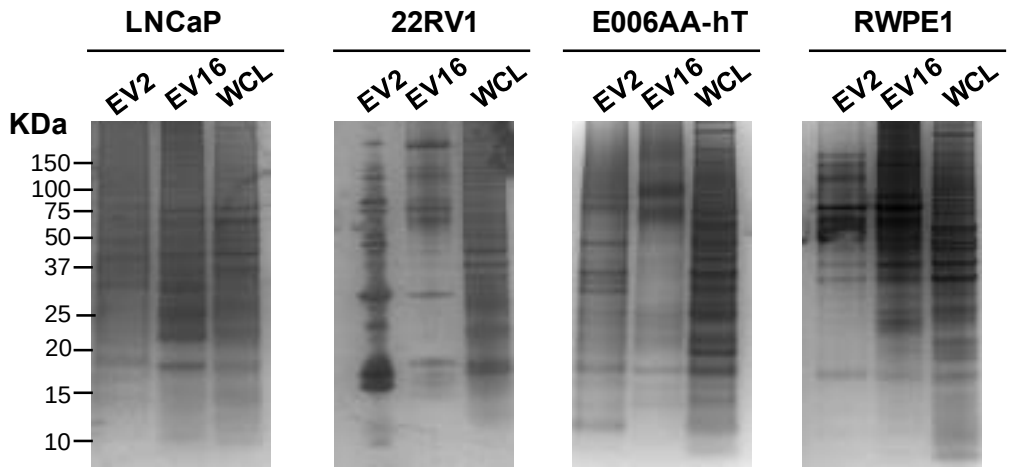
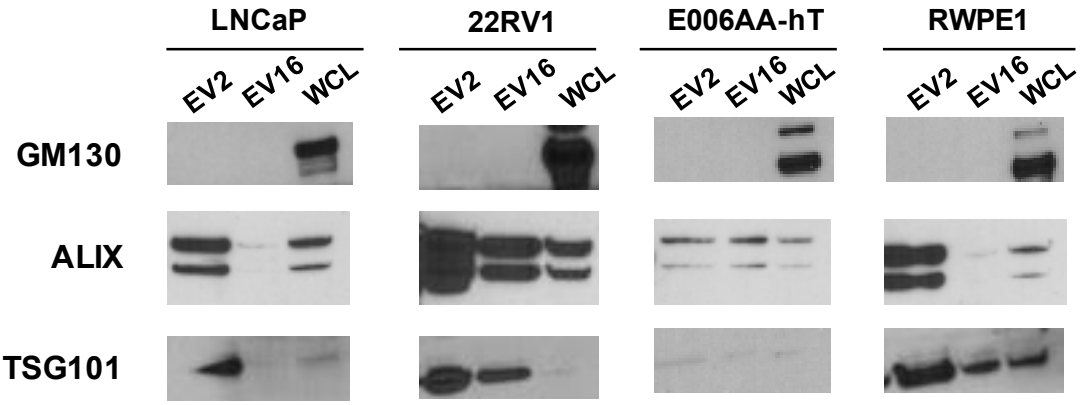


Figure 2.3. NTA of EV2 and EV16 fractions from LNCaP, 22RV1, and E006AA-hT prostate cancer cell lines, and from non-cancerous prostate epithelial cell line RWPE1.

(a)



(b)



(c)

Protein	LNCaP		22RV1		E006AA-hT		RWPE1	
	EV2	EV16	EV2	EV16	EV2	EV16	EV2	EV16
GM130	ND	ND	ND	ND	ND	ND	ND	ND
ALIX	0.0042	0.0009	0.0208	0.0034	0.0021	0.0015	0.0123	0.0054
TSG101	0.0015	ND	0.0105	0.0007	ND	ND	0.0048	0.0017

Figure 2.4. SDS-PAGE/Silver stain of EV fractions and confirmation of EV markers by immunoblotting. (a) Silver stain of SDS-PAGE of EV2, EV16, and whole cell lysate (WCL). (b) Immunoblotting was conducted from the same sample, probing for the presence/absence of the Golgi marker GM130, and EV markers ALIX and TSG101. (c) Distributed normalized spectral

abundance factor (dNSAF) values of blotted proteins obtained from proteomic analysis to confirm presence/absence of protein in the respective EV fraction.

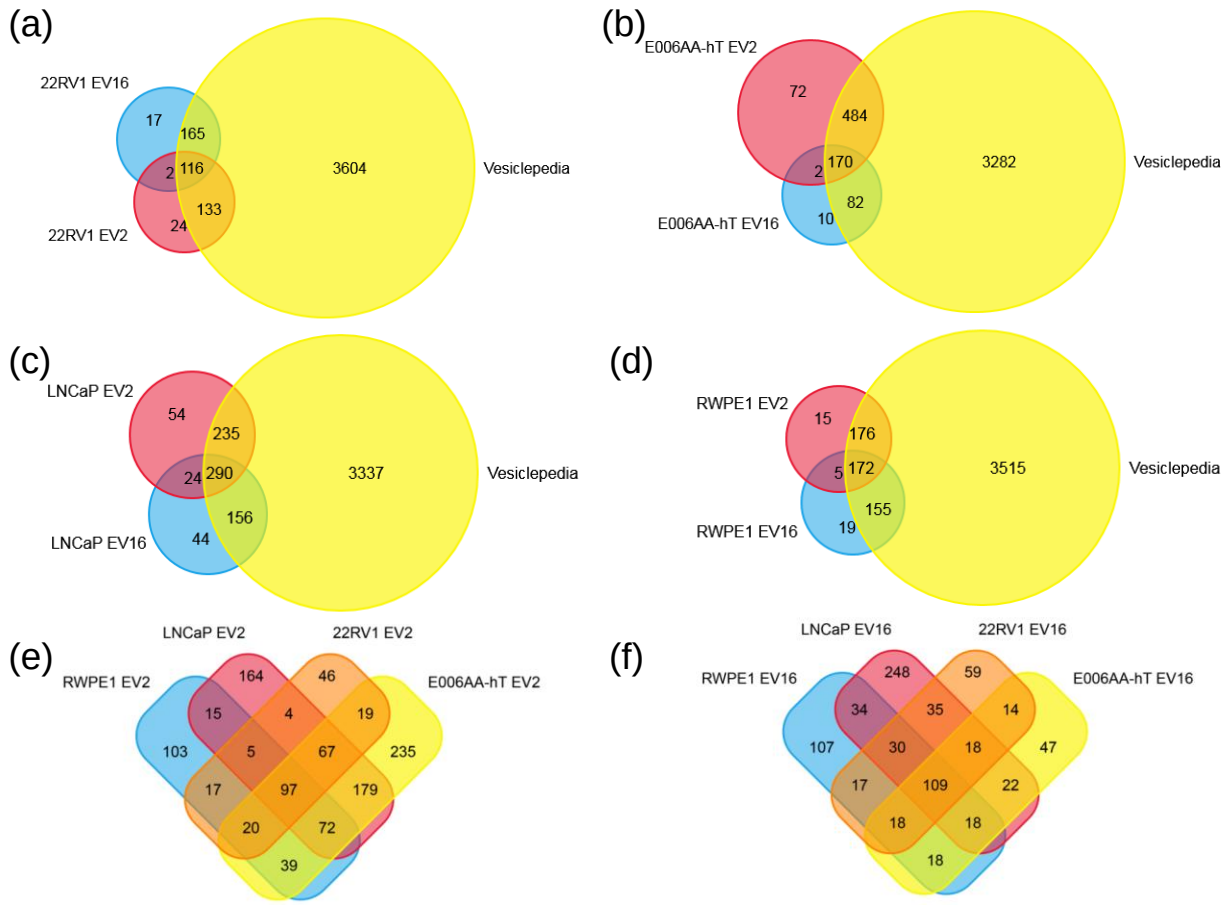


Figure 2.5. Qualitative comparison of EV proteomes of LNCaP, 22RV1, and E006AA-hT prostate cancer cell lines, and from non-cancerous prostate epithelial cell line RWPE1. Venn diagrams were generated to compare proteins identified in EV2 and EV16 for cell lines (a) LNCaP, (b) 22RV1, (c) E006AA-hT, and (d) RWPE1. Identified proteins were also compared to the Vesiclepedia human protein database. The proteome of EV16 (e) and EV2 (f) fractions were also compared.

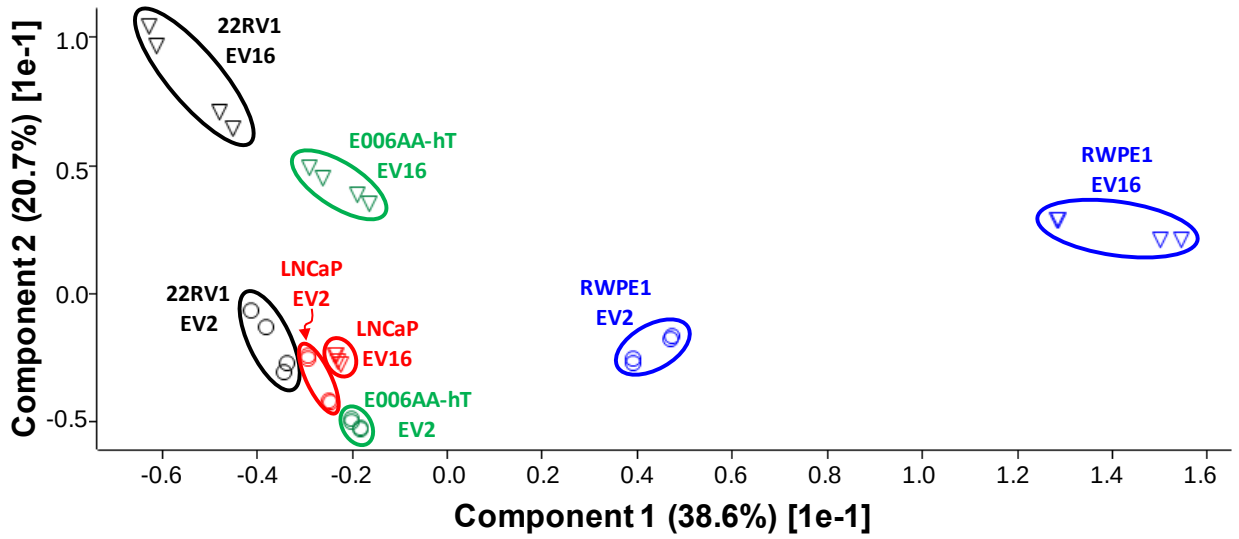


Figure 2.6. Principle component analysis of EV2 and EV16 fractions from LNCaP, 22RV1, E006AA-hT, and RWPE1 cells. EV sample protein IDs with spectral count values normalized by dNSAF were used to construct the PCA plot.

Ocaudillo2

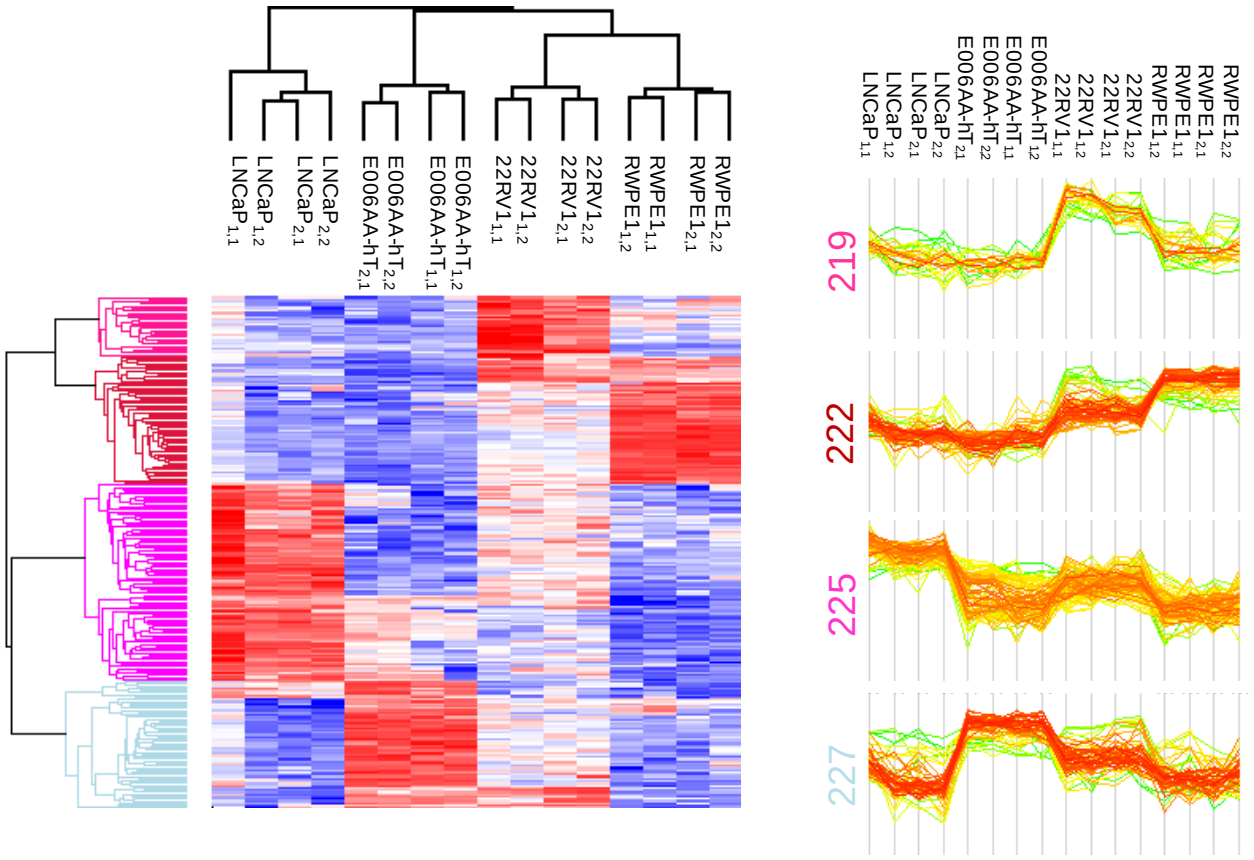


Figure 2.7. Heat map of enriched differentially expressed proteins in EV2 Fractions of LNCaP, E006AA-hT, 22RV1, and RWPE1 cells. Identified proteins were filtered after log transformation and normalization to reveal differentially expressed proteins between cell lines. The sample analyzed is specified on the top of the heatmap, with the first subscript number specifying the biological replicate and the second number specifying the technical replicate number. Outstanding clusters where a cell group or groups contain a higher abundance of protein are visualized in the line plots on the right. The number on the left is the cluster number assigned by the Perseus software.

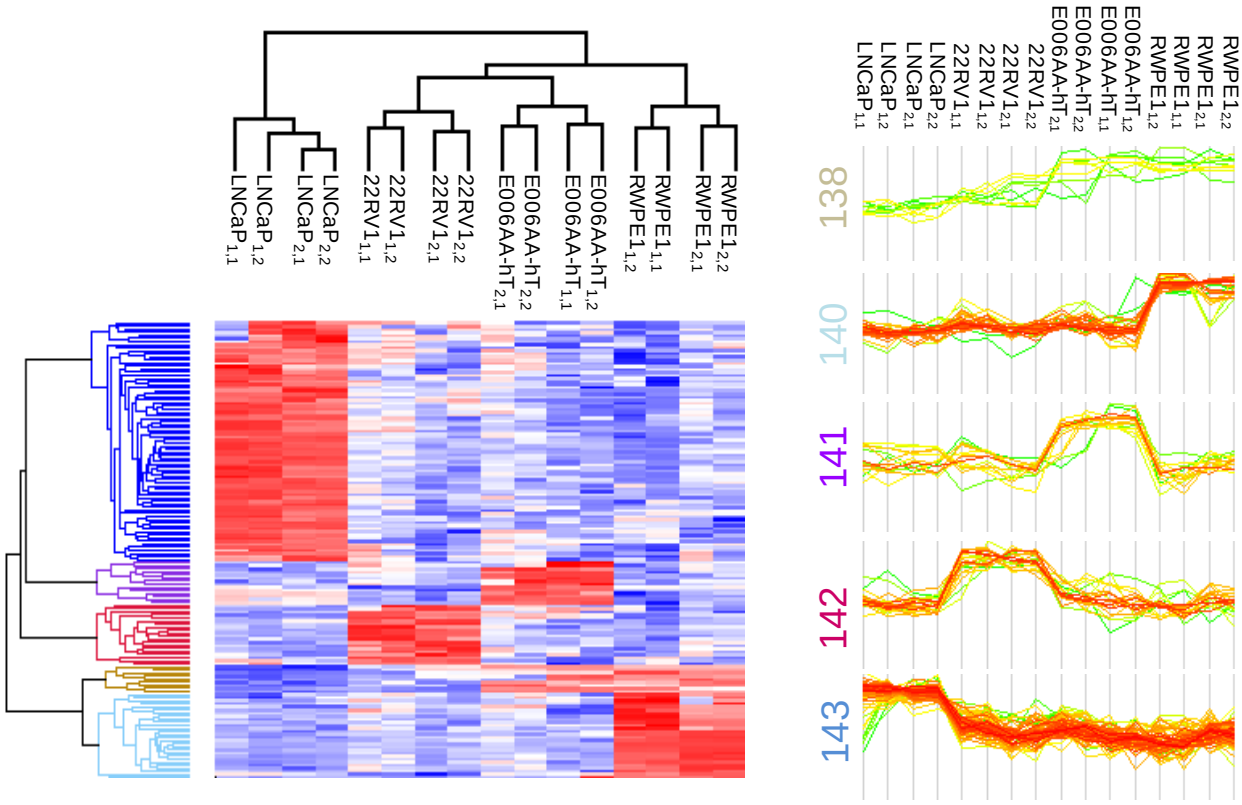


Figure 2.8. Heat map of enriched differentially expressed proteins in EV16 fractions of LNCaP, E006AA-hT, 22RV1, and RWPE1 cells. Identified proteins were filtered after log transformation and normalization to reveal differentially expressed proteins between cell lines. The sample analyzed is specified on the top of the heatmap, with the first subscript number specifying the biological replicate and the second number specifying the technical replicate number. Outstanding clusters where a cell group or groups contain a higher abundance of protein are visualized in the line plots on the right. The number on the left is the cluster number assigned by the Perseus software.

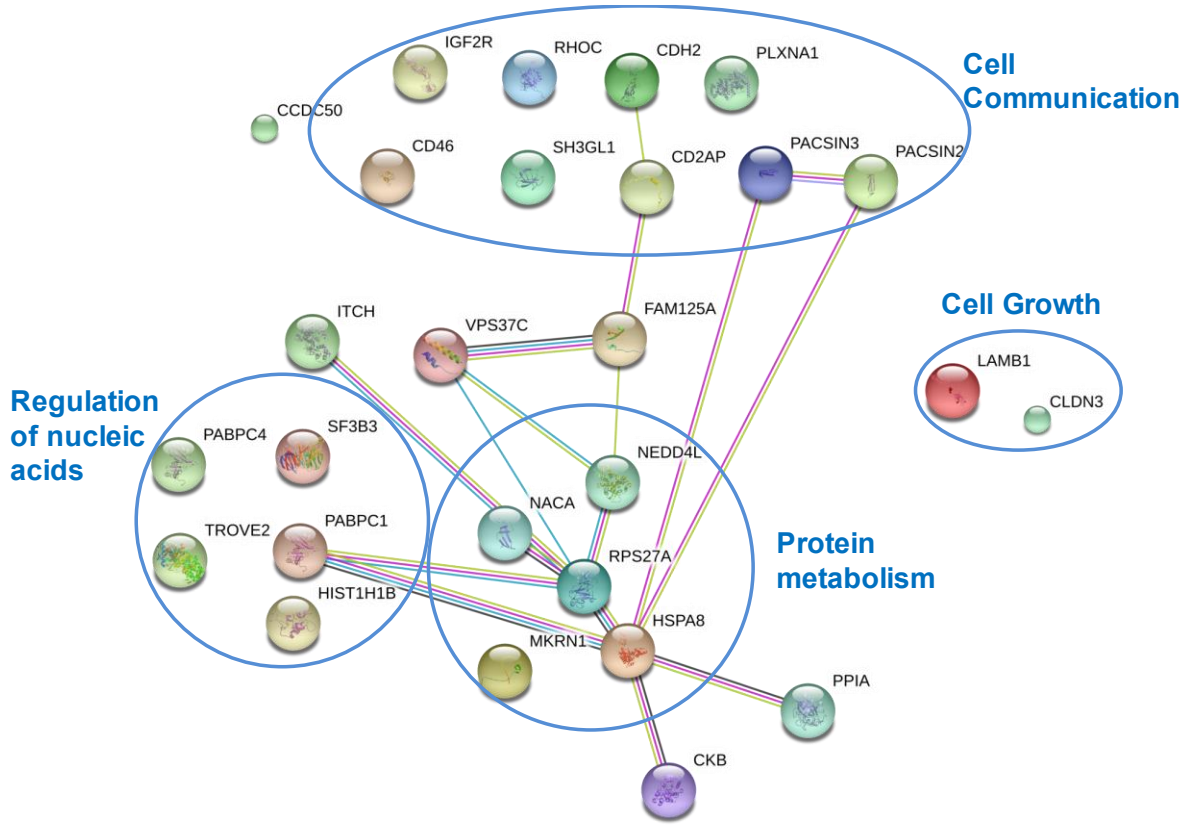


Figure 2.9. String analysis of Cluster 219 from EV2.

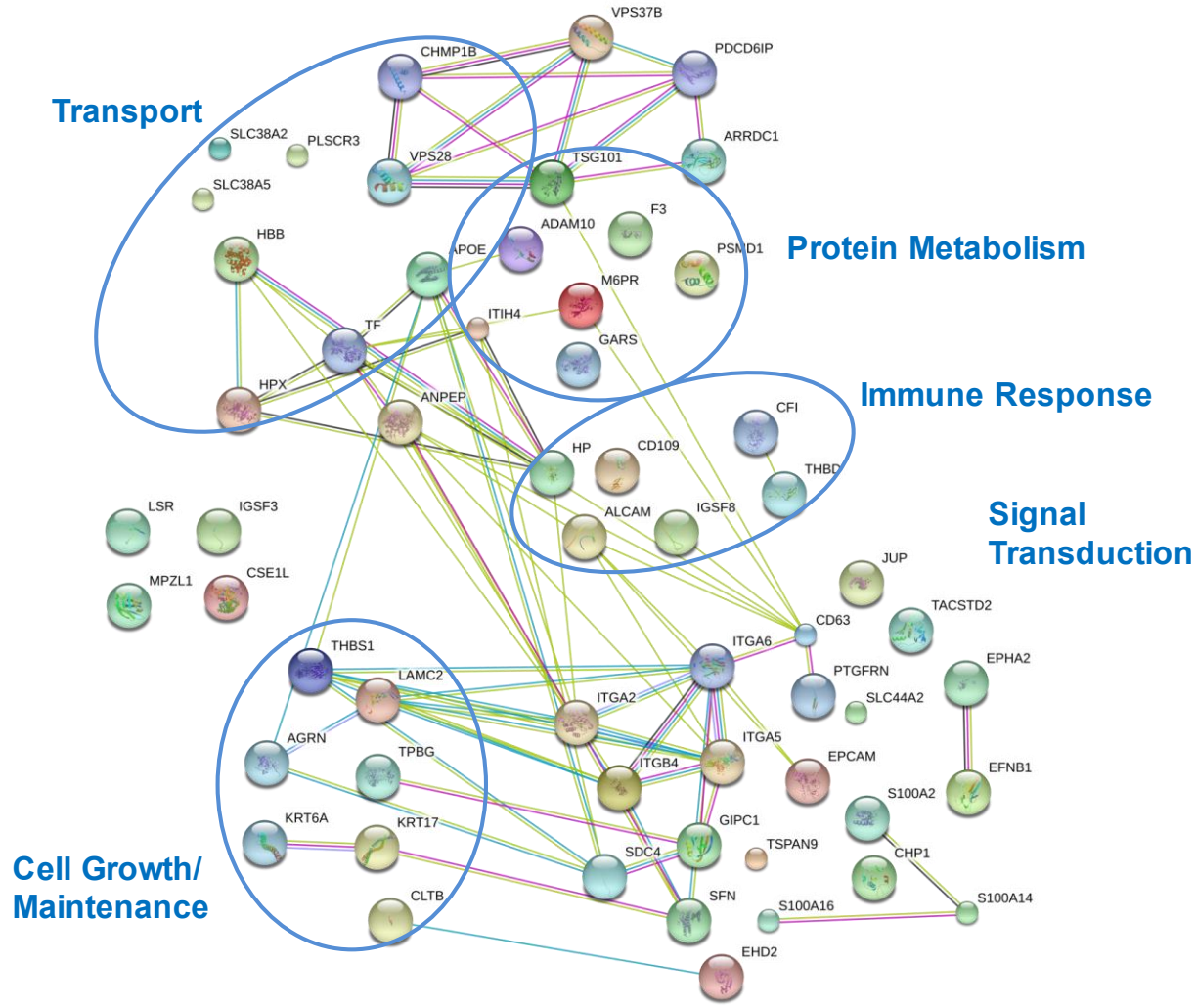


Figure 2.10. String analysis of Cluster 222 from EV2.

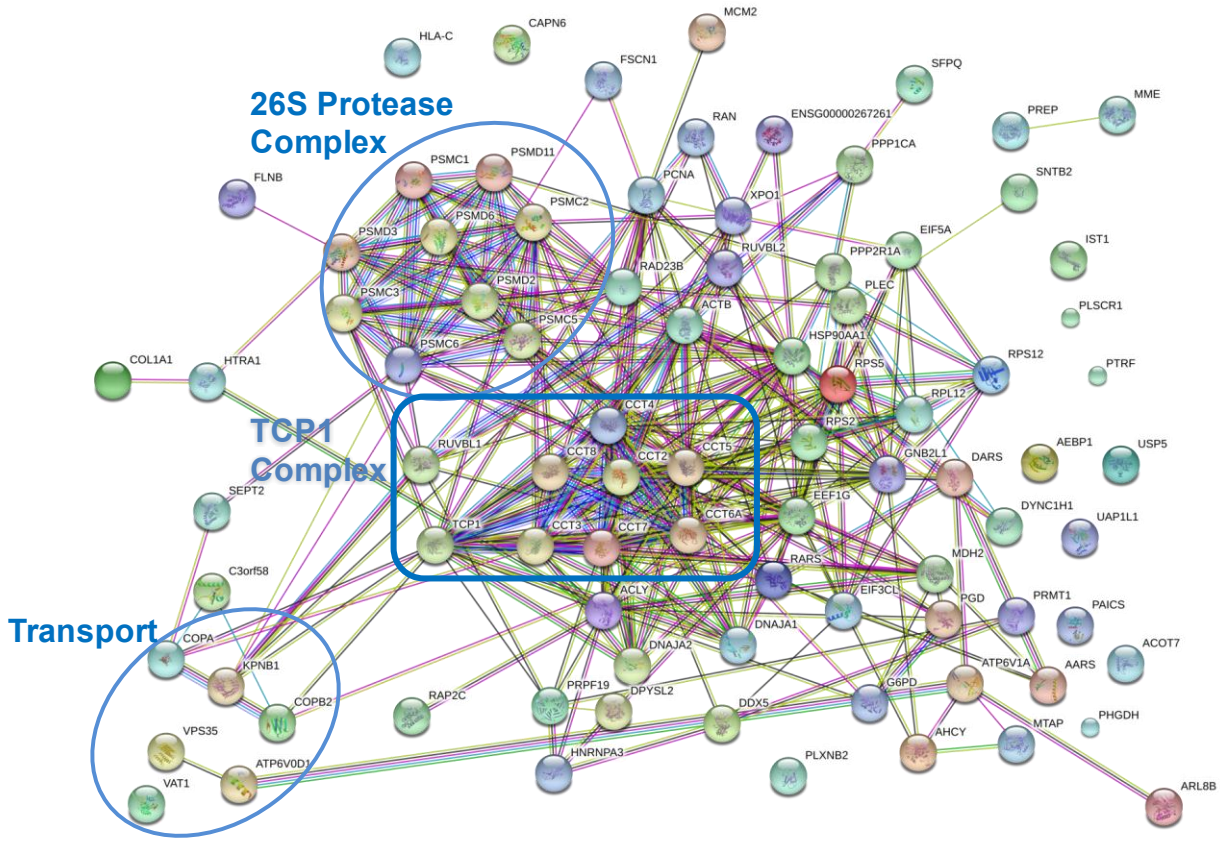


Figure 2.11. String analysis of Cluster 225 from EV2.

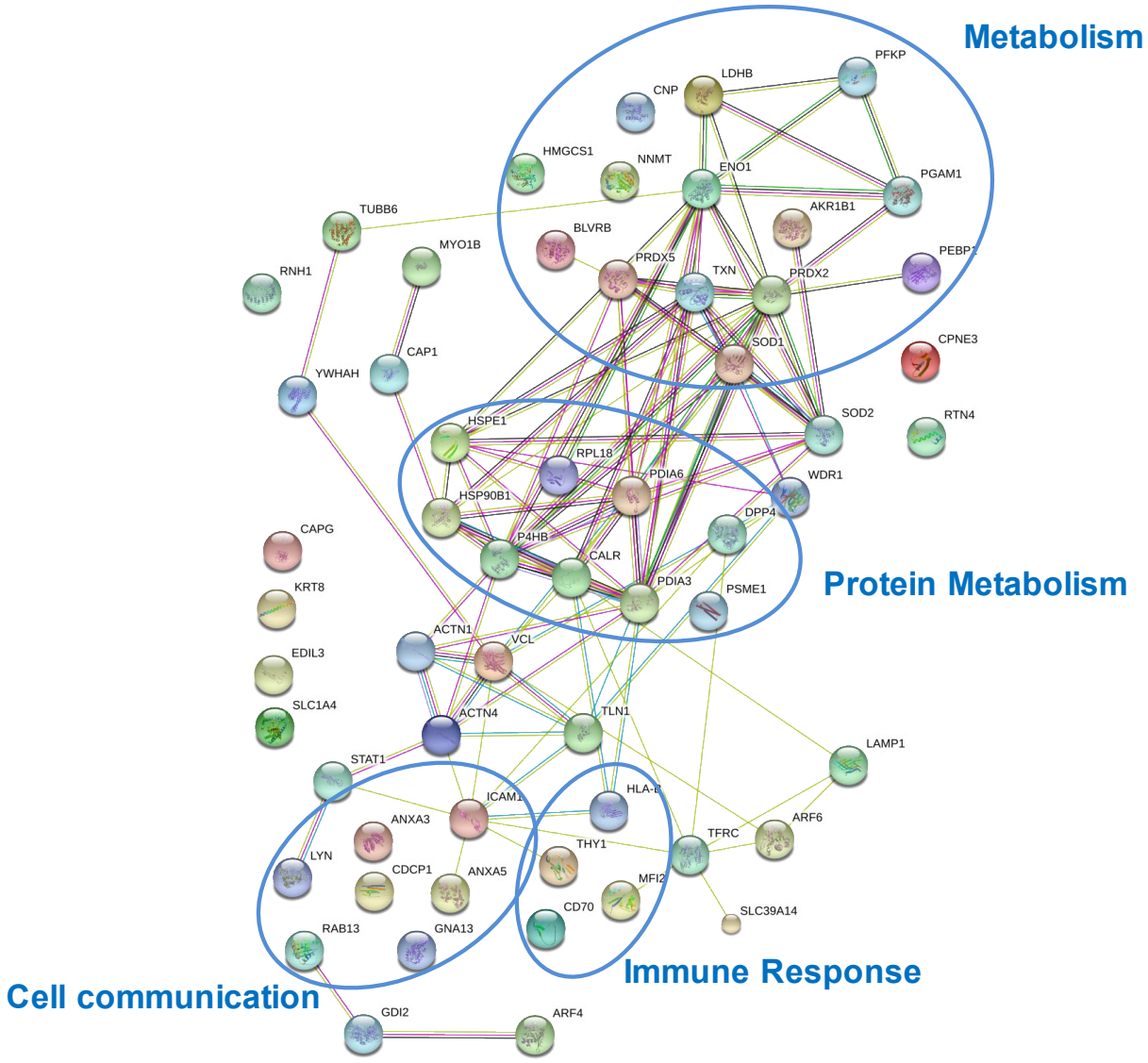


Figure 2.12. String analysis of Cluster 227 from EV2.

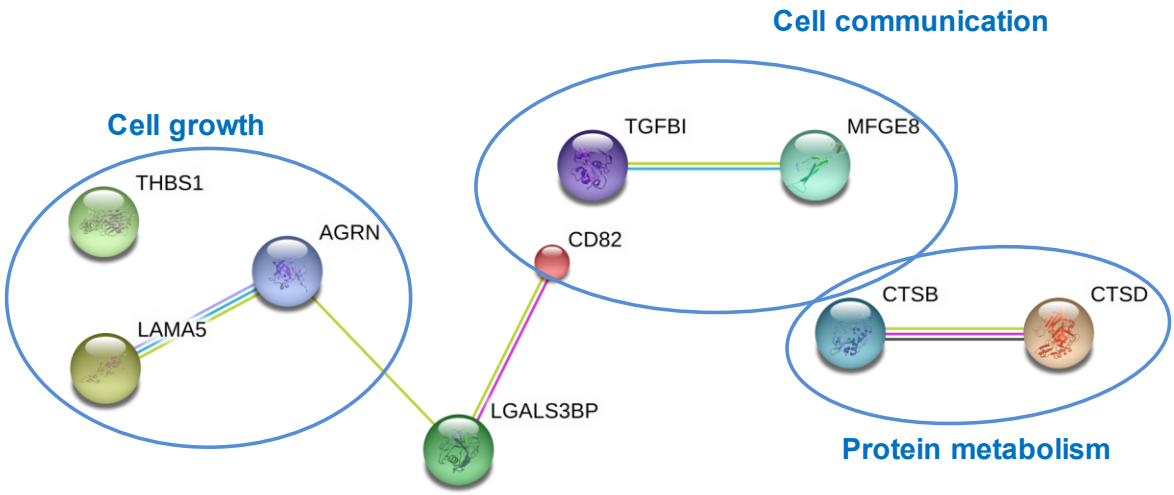


Figure 2.13. String analysis of Cluster 138 from EV16.

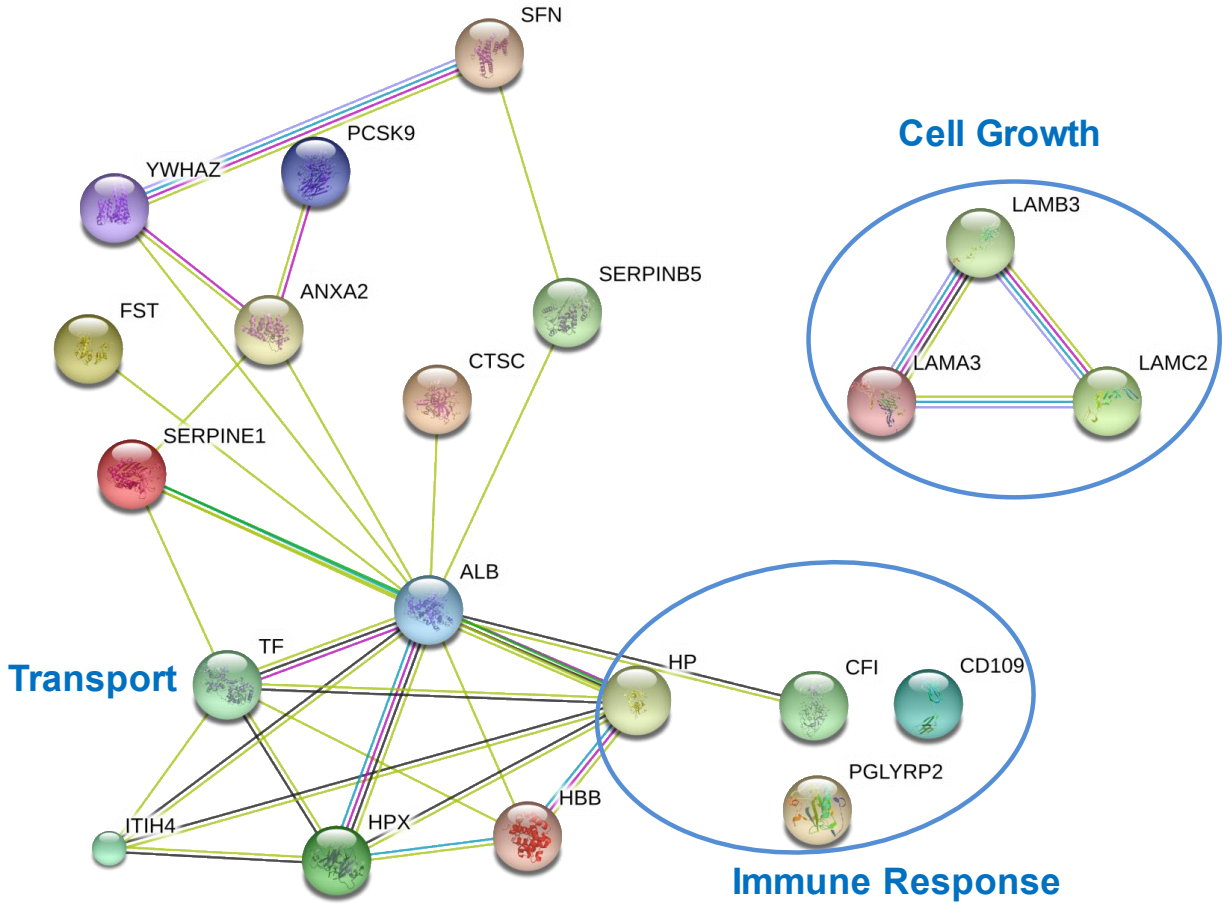


Figure 2.14. String analysis of Cluster 140 from EV16.

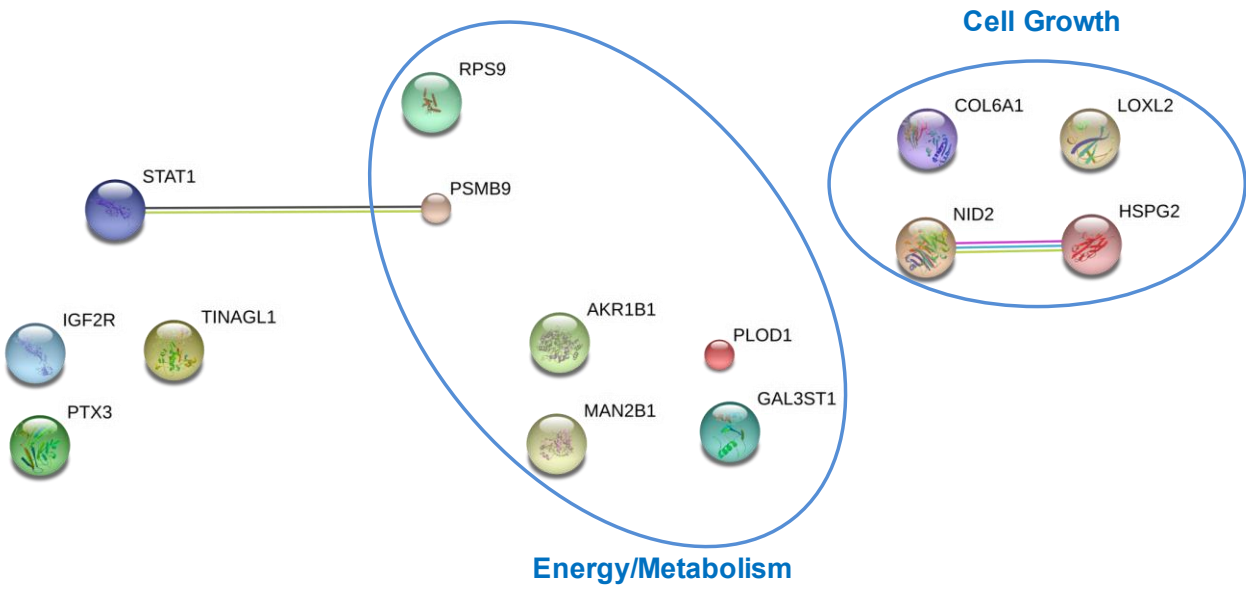


Figure 2.15. String analysis of Cluster 141 from EV16.

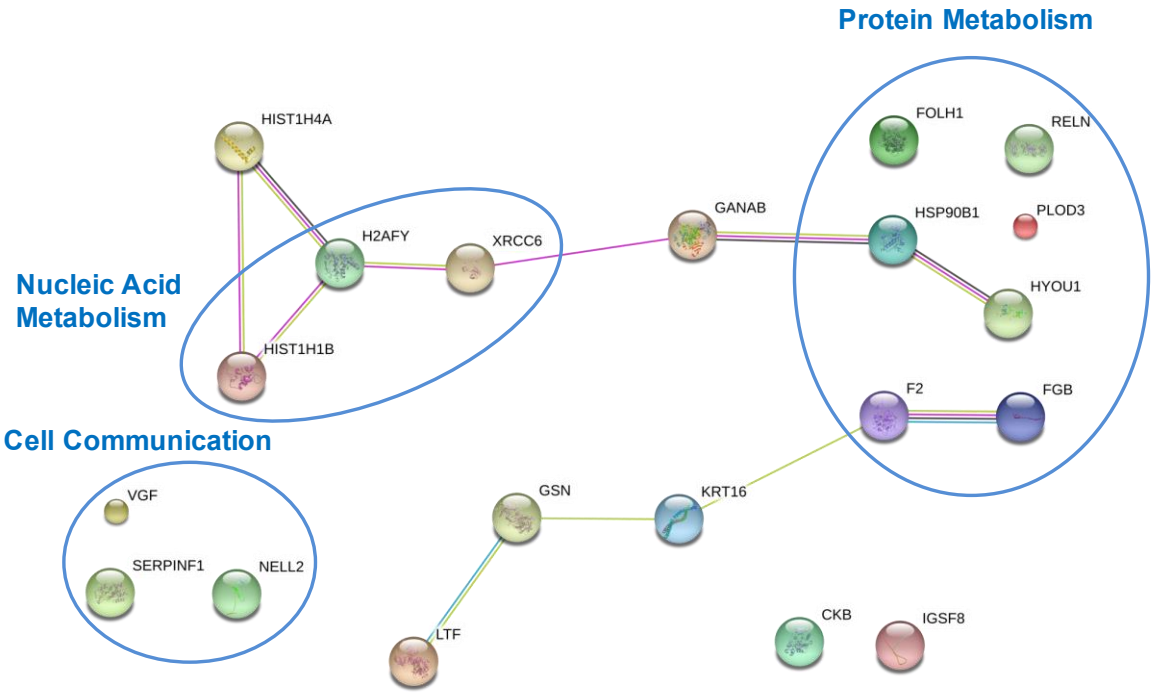


Figure 2.16. String analysis of Cluster 142 from EV16.

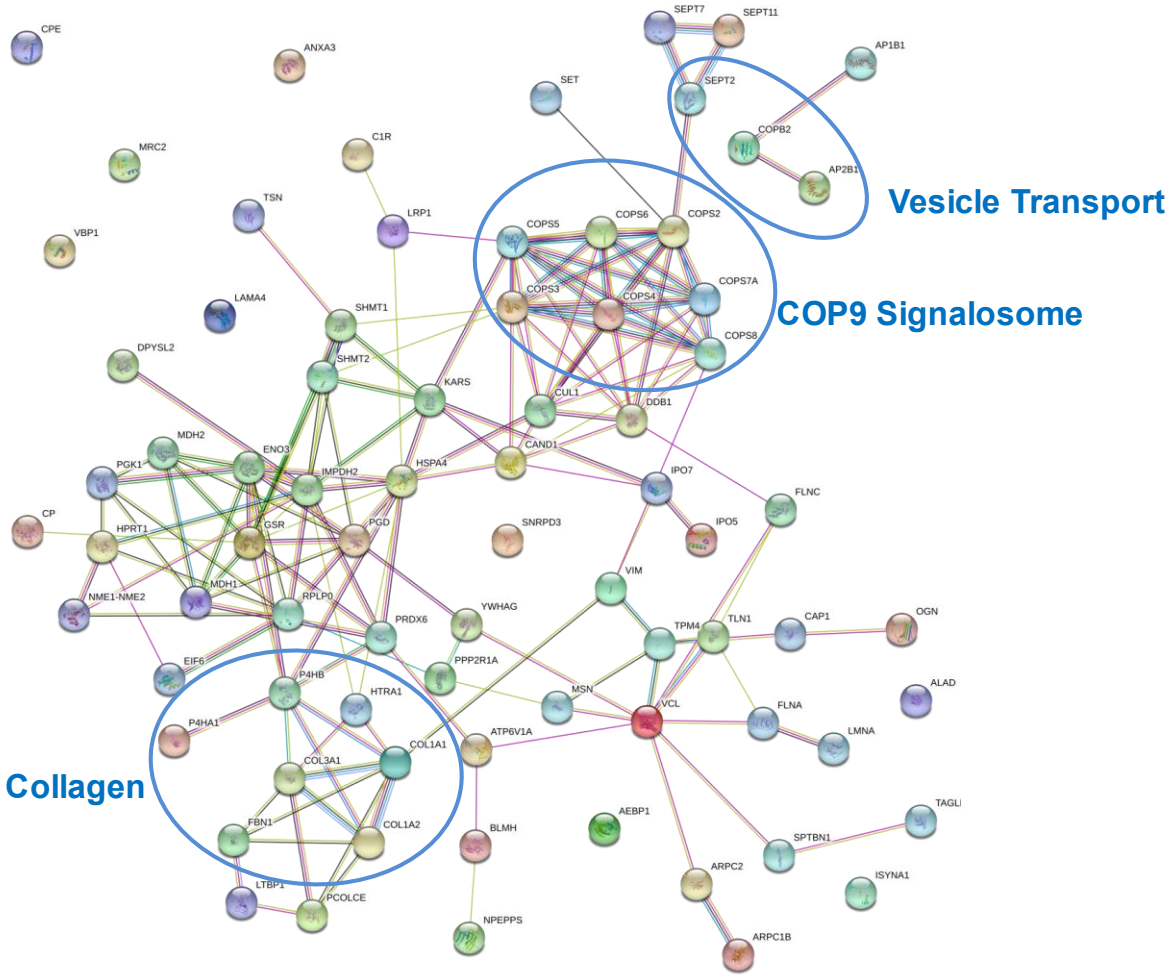


Figure 2.17. String analysis of Cluster 143 from EV16.

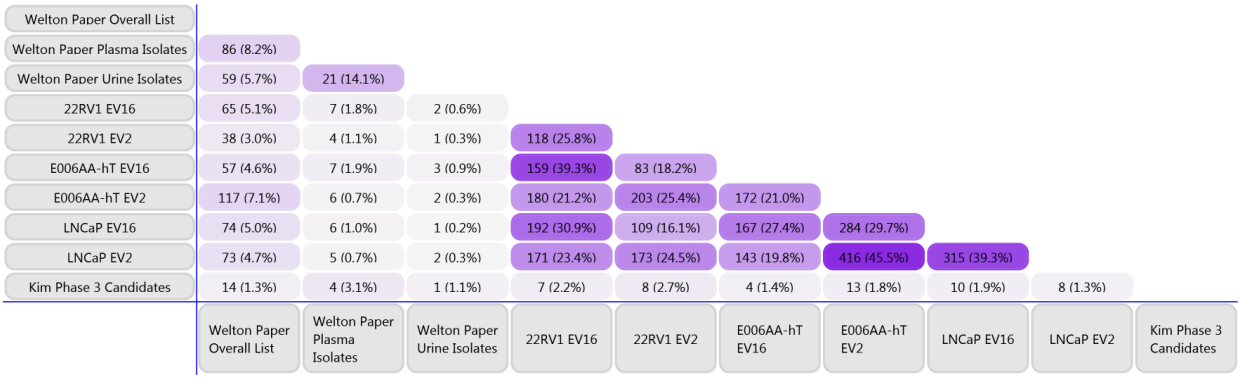


Figure 2.18. Venn diagram comparing our current proteomic data with those from Welton *et al.* (2016) and Kim *et al.* (2016). Welton *et al.* performed proteomic analysis of EVs from urine (n=5) and plasma (n=11) of PCa patients, whereas Kim *et al.* conducted proteomic analysis of urine (n=90) samples from PCa patients. Purple color gradient indicates consensus in protein identifications; a darker color represents a higher consensus between datasets analyzed.

Table 2.1: Proteins with significantly different abundance between PCa and non-cancerous EV2 fractions. The arrow to the left of the q-value signifies that this protein is abundant in PCa EVs. ns, not significant; EMT, epithelial mesenchymal transition.

#	Gene Name (UniProt)	Protein Name	Cluster	Student's T-test q-Value Compared to RWPE1			Vesiclepedia Reported	Cancer-related description	Reference(s)
				LNCaP EV2	22RV1 EV2	E006AA-hT EV2			
Cell communication and/or Signal Transduction									
1	CDH2	Cadherin-2	219	ns	↑0.0089	0.0154	No		
2	IGF2R	Cation-independent mannose-6-phosphate receptor	219	0.0502	↑0.0000	0.0161	Yes		
3	CD2AP	CD2-associated protein	219	ns	↑0.0323	ns	Yes		
4	SH3GL1	Endophilin-A2	219	ns	↑0.0088	ns	No	Tumor formation, progression, and metastasis	(Guan et al., 2016)
5	CD46	Membrane cofactor protein	219	ns	↑0.0358	ns	Yes		
6	PLXNA1	Plexin-A1	219	ns	↑0.0000	0.0477	Yes		
7	PACSIN2	Protein kinase C and casein kinase substrate in neurons protein 2	219	ns	↑0.0000	ns	Yes		
8	PACSIN3	Protein kinase C and casein kinase substrate in neurons protein 3	219	ns	↑0.0085	ns	Yes	Up-regulated in Breast Cancer Cells	(Sharp et al., 2008)
9	RHOC	Rho-related GTP-binding protein RhoC	219	ns	↑0.0407	ns	Yes	Invasion	(Bravo-Cordero et al., 2014)
10	SFN	14-3-3 protein sigma	222	0.0000	0.0000	0.0000	Yes	Lymph Node metastasis-related protein	(Skotheim et al., 2002)
11	CHP1	Calcineurin B homologous protein 1	222	0.0181	0.0000	0.0000	Yes		
12	CD63	CD63 antigen	222	0.0183	0.0000	0.0094	Yes		
13	SLC44A2	Choline transporter-like protein 2	222	0.0179	0.0155	0.0181	Yes		
14	EHD2	EH domain-containing protein 2	222	0.0440	0.0000	0.0202	Yes	Decreased expression in esophageal squamous cell carcinoma	(Li et al., 2013)
15	EPHA2	Ephrin type-A receptor 2	222	0.0277	0.0086	0.0123	Yes	Overexpression in hepatocellular carcinoma	(Fan et al., 2013b)
16	EFNB1	Ephrin-B1	222	0.0198	0.0000	0.0000	Yes	Highly expressed in ovarian and gastric cancer	(Kataoka et al., 2002)
17	EPCAM	Epithelial cell adhesion molecule	222	0.0145	↑0.0138	0.0083	Yes	Overexpressed in local and metastatic PCa	(Massoner et al., 2014)

18	ITGA2	Integrin alpha-2	222	0.0171	0.0000	0.0000	Yes	Suppresses cell migration	(Ding et al., 2015)
19	ITGA5	Integrin alpha-5	222	0.0307	0.0225	0.0071	Yes	Regulation of cell growth in breast cancer	(Fang et al., 2010)
20	ITGA6	Integrin alpha-6	222	0.0000	0.0000	0.0000	Yes	Drug resistance	(Yamakawa et al., 2012)
21	ITGB4	Integrin beta-4	222	0.0357	0.0000	0.0000	Yes	Upregulated in EVs from drug resistant PC-3 cells, associated with poor prognosis, EMT, invasion	(Kawakami, 2015; Masugi et al., 2015)
22	JUP	Junction plakoglobin	222	0.0278	0.0000	0.0000	Yes		
23	GIPC1	PDZ domain-containing protein GIPC1	222	0.0303	0.0147	0.0156	Yes	Migration and invasion	(Wu et al., 2010)
24	PTGFRN	Prostaglandin F2 receptor negative regulator	222	0.0000	0.0148	0.0178	Yes		
25	S100A16	Protein S100-A16	222	0.0000	0.0000	0.0000	Yes	Cell proliferation, EMT, migration, invasion	(Zhou et al., 2014b)
26	S100A2	Protein S100-A2	222	0.0521	ns	0.0168	No	Poor prognosis and metastasis	(Bulk et al., 2008)
27	SDC4	Syndecan-4	222	0.0200	0.0000	0.0000	Yes		
28	TSPAN9	Tetraspanin	222	ns	0.0000	0.0079	Yes		
29	TACSTD2	Tumor-associated calcium signal transducer 2	222	0.0233	0.0000	0.0000	Yes	Poor prognosis and metastasis	(Lin et al., 2013a)
30	MPZL1	Myelin protein zero-like protein 1	222	0.0176	0.0000	0.0183	Yes	Metastasis and migration	(Jia et al., 2014)
31	ARL8B	ADP-ribosylation factor-like protein 8B	225	↑0.0239	↑0.0000	0.0144	Yes		
32	SNTB2	Beta-2-syntrophin	225	↑0.0289	↑0.0235	ns	Yes		
33	DPYSL2	Dihydropyrimidinase-related protein 2	225	↑0.0270	↑0.0000	ns	Yes		
34	XPO1	Exportin-1	225	↑0.0496	↑0.0222	0.0152	Yes	Highly expressed in mantle cell lymphoma, proposed therapeutic target	(Cheng et al., 2014; Yoshimura et al., 2014)
35	RAN	GTP-binding nuclear protein Ran (Fragment)	225	0.0256	0.0409	ns	Yes	Proposed therapeutic target, poor prognosis in colorectal cancer	(Fan et al., 2013a; Yuen et al., 2012)
36	GNB2L1	Guanine nucleotide-binding protein subunit beta-2-like 1	225	↑0.0000	0.0000	0.0165	Yes	Migration	(Wang et al., 2011a)
37	PLSCR1	Phospholipid scramblase 1	225	ns	↑0.0000	0.0176	Yes	Overexpression in colorectal cancer tissue, tumorigenesis, tumor progression	(Cui et al., 2012)
38	PLXNB2	Plexin-B2	225	↑0.0158	↑0.0000	↑0.0129	Yes		
39	RAP2C	Ras-related protein Rap-2c	225	↑0.0173	↑0.0349	0.0147	Yes	Androgen-mediated transcriptional and growth responses of human PCa cells	(Bigler et al., 2007)
40	PPP2R1A	Serine/threonine-protein phosphatase 2A 65 kDa	225	↑0.0221	0.0000	↑0.0164	Yes	Down-regulated in primary and metastatic prostate cancer,	(Bluemn et al., 2013)

		regulatory subunit A alpha isoform						loss associate with PCa mortality	
41	SEPT2	Septin-2	225	↑0.0236	0.0143	↑0.0200	Yes	Cell proliferation, up-regulation hepatoma carcinoma	(Yu et al., 2009)
42	YWHAH	14-3-3 protein eta	227	ns	0.0245	↑0.0193	Yes		
43	ANXA3	Annexin A3	227	↑0.0182	↑0.0206	↑0.0000	Yes	Tumor growth, association with aggressive hepatocellular carcinoma	(Tong et al., 2015)
44	ANXA5	Annexin A5	227	↑0.0238	↑0.0082	↑0.0000	Yes	Poor prognosis in colorectal carcinoma	(Xue et al., 2009)
45	GNA13	Guanine nucleotide-binding protein subunit alpha-13	227	↑0.0499	↑0.0000	↑0.0087	Yes	Invasion and migration in PCa cells	(Rasheed et al., 2013; Zhang et al., 2014b)
46	ICAM1	Intercellular adhesion molecule 1	227	ns	↑0.0090	↑0.0000	Yes	PCa invasion	(Conrad et al., 2009)
47	PEBP1	Phosphatidylethanolamine-binding protein 1	227	↑0.0414	↑0.0000	↑0.0081	Yes	Tumor suppressor and prognostic marker	(Wang et al., 2010)
48	RAB13	Ras-related protein Rab-13	227	ns	↑0.0000	↑0.0160	Yes	Invasion and migration	(Ioannou et al., 2015)
49	CDCP1	CUB domain-containing protein 1	227	0.0493	ns	↑0.0249	Yes	Regulation of invasion and metastasis	(Uekita et al., 2014)
50	LYN	Tyrosine-protein kinase Lyn	227	ns	ns	↑0.0191	Yes	PCa cell proliferation	(Goldenberg-Furmanov et al., 2004)
Cell growth and/or maintenance									
51	CLDN3	Claudin-3	219	ns	↑0.0000	0.0141	Yes	Overexpression in epithelial ovarian cancer	(Heinzelmann-Schwarz et al., 2004)
52	LAMB1	Laminin subunit beta-1	219	0.0511	↑0.0428	0.0169	Yes	Overexpression in ovarian cancer	(Januchowski et al., 2014)
53	AGRN	Agrin	222	0.0333	0.0000	0.0166	Yes	Upregulation in synovial sarcomas	(Fernebro et al., 2006)
54	CLTB	Clathrin light chain B	222	0.0256	0.0366	0.0000	No		
55	KRT17	Keratin, type I cytoskeletal 17	222	0.0170	0.0000	0.0000	Yes	Down regulated in PCa at mRNA level	(Schlomm et al., 2008)
56	KRT6A	Keratin, type II cytoskeletal 6A	222	ns	0.0000	0.0000	Yes		
57	LAMC2	Laminin subunit gamma-2	222	0.0167	0.0000	0.0166	Yes	Migration, invasion, EMT	(Moon et al., 2015)
58	THBS1	Thrombospondin-1	222	0.0000	0.0000	0.0170	Yes	Metastasis, poor prognosis	(Jeanne et al., 2015)
59	TPBG	Trophoblast glycoprotein	222	0.0000	0.0000	0.0163	Yes	Overexpression in cancer, poor prognosis, migration, invasion	(He, 2015)
60	FSCN1	Fascin	225	↑0.0366	ns	ns	Yes	Cell growth, migration, invasion in PCa	(Fuse, 2011)
61	ACTB	Actin, cytoplasmic 1	225	↑0.0385	↑0.0000	ns	Yes	Upregulated in some tumors, abnormal expression associated with metastasis	(Guo et al., 2013a)

62	COL1A1	Collagen alpha-1(I) chain	225	↑0.0198	↑0.0000	ns	Yes		
63	FLNB	Filamin-B	225	↑0.0151	ns	↑0.0140	Yes	Invasion	(Iguchi et al., 2015)
64	PPP1CA	Serine/threonine-protein phosphatase PP1-alpha catalytic subunit	225	↑0.0193	0.0083	↑0.0121	Yes	Proposed biomarker for bladder cancer	(Brems-Eskildsen et al., 2010)
65	CAP1	Adenylyl cyclase-associated protein 1	227	ns	↑0.0237	↑0.0126	Yes	Migration, metastasis, overexpression in ovarian cancer and hepatocellular carcinoma	(Hua et al., 2015; Liu et al., 2014)
66	MYO1B	Unconventional myosin-Ib	227	0.0181	ns	ns	Yes	High mRNA expression in head and neck squamous cell carcinoma	(Ohmura et al., 2015)
67	ACTN1	Alpha-actinin-1	227	ns	ns	↑0.0127	Yes		
68	ACTN4	Alpha-actinin-4	227	↑0.0177	↑0.0445	↑0.0073	Yes	enhance cancer cell motility, invasion, and metastasis	(Fukumoto et al., 2015)
69	EDIL3	EGF-like repeat and discoidin I-like domain-containing protein 3	227	ns	↑0.0219	↑0.0206	Yes	Poor prognosis in Pancreatic ductal adenocarcinoma	(Damhofer et al., 2013)
70	KRT8	Keratin, type II cytoskeletal 8	227	ns	↑0.0226	↑0.0085	Yes		
71	CAPG	Macrophage-capping protein	227	ns	↑0.0000	↑0.0000	Yes	Proposed prognostic marker for breast cancer	(Westbrook et al., 2016)
72	TLN1	Talin-1	227	↑0.0000	0.0000	↑0.0069	Yes	Associated with ovarian serous carcinoma development and progression to metastasis	(Tang, 2013)
73	TUBB6	Tubulin beta-6 chain	227	↑0.0369	ns	↑0.0153	Yes		
74	VCL	Vinculin	227	ns	↑0.0081	↑0.0124	Yes	More abundant in PCa tissue in comparison to BPH, positive correlation with AR presence	(ZHU Li-yong, 2010)
75	SOD2	Superoxide dismutase	227	ns	↑0.0149	↑0.0000	Yes	Tumor progression	(Miar et al., 2015)
Energy pathways and Metabolism									
76	CKB	Creatine kinase B-type	219	ns	↑0.0000	ns	Yes	Proposed marker for lung squamous cell cancer	(Zeng et al., 2012)
77	PGD	6-phosphogluconate dehydrogenase, decarboxylating	225	↑0.0217	↑0.0000	ns	Yes		
78	AHCY	Adenosylhomocysteinase	225	↑0.0263	↑0.0223	↑0.0096	Yes		
79	DARS	Aspartate--tRNA ligase, cytoplasmic	225	↑0.0206	↑0.0151	ns	Yes		
80	ACLY	ATP-citrate synthase	225	↑0.0218	↑0.0000	↑0.0158	Yes	Resistance in colorectal cancer, overexpression in cancer, cell proliferation	(Zaidi et al., 2012; Zhou et al., 2013)
81	DYNC1H1	Cytoplasmic dynein 1 heavy chain 1	225	↑0.0199	↑0.0084	↑0.0139	Yes		

82	PHGDH	D-3-phosphoglycerate dehydrogenase	225	↑0.0161	ns	0.0220	Yes	Overexpression in melanoma, tumorigenesis	(Mullarky et al., 2011)
83	G6PD	Glucose-6-phosphate 1-dehydrogenase	225	↑0.0386	↑0.0384	ns	Yes	Induces tumor growth when glycosylated	(Rao et al., 2015)
84	MDH2	Malate dehydrogenase, mitochondrial	225	↑0.0174	↑0.0087	↑0.0194	Yes		
85	PAICS	Multifunctional protein ADE2	225	↑0.0201	↑0.0139	ns	Yes		
86	PRMT1	Protein arginine N-methyltransferase 1	225	↑0.0156	↑0.0000	ns	Yes	Overexpressed in Non-Small Cell Lung Carcinoma, cell proliferation	(Elakoum et al., 2014)
87	MTAP	S-methyl-5'-thioadenosine phosphorylase	225	↑0.0286	↑0.0081	ns	Yes	Low expression correlates with poor prognosis in non-small cell lung cancer	(Su et al., 2014)
88	UAP1L1	UDP-N-acetylhexosamine pyrophosphorylase-like protein 1	225	↑0.0186	ns	0.0078	No		
89	ATP6V1A	V-type proton ATPase catalytic subunit A	225	↑0.0508	0.0249	ns	Yes		
90	ACOT7	Cytosolic acyl coenzyme A thioester hydrolase	225	↑0.0281	↑0.0045	0.0222	Yes		
91	AKR1B1	Aldose reductase	227	ns	↑0.0000	↑0.0000	Yes	Decreased expression in endometrial cancer, play a protective role against toxic aldehydes	(Hevir et al., 2013; Lefrançois-Martinez et al., 2004)
92	ENO1	Alpha-enolase	227	ns	ns	↑0.0186	Yes	Upregulation in glioma and non-small cell lung cancer, cell proliferation, migration, invasion	(Fu et al., 2015; Song et al., 2014)
93	CNP	2',3'-cyclic-nucleotide 3'-phosphodiesterase	227	0.0292	ns	↑0.0000	Yes	Proposed prognosis marker for Glioblastoma multiforme	(Zorniak et al., 2012)
94	PFKP	ATP-dependent 6-phosphofructokinase, platelet type	227	ns	↑0.0426	↑0.0076	Yes		
95	BLVRB	Flavin reductase (NADPH)	227	ns	ns	↑0.0184	Yes		
96	HMGCS1	Hydroxymethylglutaryl-CoA synthase, cytoplasmic	227	ns	↑0.0000	↑0.0174	No		
97	LDHB	L-lactate dehydrogenase B chain	227	ns	↑0.0000	↑0.0198	Yes	Regulator of cell proliferation	(McCleland et al., 2013)
98	NNMT	Nicotinamide N-methyltransferase	227	ns	↑0.0000	↑0.0000	Yes	Elevated in plasma of kidney cancer patients	(Su Kim et al., 2013)
99	PRDX2	Peroxiredoxin-2	227	0.0283	↑0.0000	↑0.0168	Yes	Increased expression in cisplatin resistant cancer cells	(Kalinina et al., 2012)
100	PRDX5	Peroxiredoxin-5, mitochondrial	227	0.0364	↑0.0220	↑0.0000	Yes		

101	PGAM1	Phosphoglycerate mutase 1	227	↑0.0279	ns	↑0.0097	Yes	Upregulated in hepatocellular carcinoma, cell growth	(Ren et al., 2010)
102	SOD1	Superoxide dismutase [Cu-Zn]	227	ns	↑0.0248	↑0.0000	Yes	Inhibition leads to cell death	(Glasauer et al., 2014)
103	TXN	Thioredoxin	227	ns	↑0.0372	↑0.0000	Yes	Proposed diagnostic marker for gastric cancer	(Lim et al., 2012)
Immune response									
104	CD109	CD109 antigen	222	0.0213	0.0000	0.0068	Yes	Highly expressed in cell secretome and tissue of non-small cell lung cancer	(Zhang et al., 2014a)
105	CFI	Complement factor I	222	0.0231	0.0144	0.0146	Yes	Cell growth, migration, strong association with aggressive cutaneous squamous cell carcinoma tumors	(Riihilä et al., 2015)
106	HP	Haptoglobin	222	0.0225	0.0000	0.0120	Yes	Proposed serum biomarker for pancreatic cancer and lung cancer	(Ayyub, 2015; Haas et al., 2015)
107	IGSF8	Immunoglobulin superfamily member 8	222	0.0144	↑0.0000	0.0204	Yes		
108	THBD	Thrombomodulin	222	0.0184	ns	0.0188	Yes		
109	HLA-C	HLA class I histocompatibility antigen, Cw-17 alpha chain	225	↑0.0182	ns	0.0089	Yes		
110	CD70	CD70 antigen	227	ns	↑0.0045	↑0.0000	Yes	High expression in renal cell carcinoma and gliomas	(Adam et al., 2006; Held-Feindt and Mentlein, 2002)
111	HLA-B	HLA class I histocompatibility antigen, B-7 alpha chain	227	ns	↑0.0000	↑0.0000	Yes		
112	MFI2	Melanotransferrin	227	ns	↑0.0000	↑0.0000	Yes		
113	THY1	Thy-1 membrane glycoprotein (Fragment)	227	ns	↑0.0085	↑0.0072	Yes	Detected in PCa urine and cell supernatant	(True et al., 2010)
Lipoprotein Peptide or Protein Metabolism									
114	PPIA	Peptidyl-prolyl cis-trans isomerase A	219	↑0.0505	↑0.0046	ns	Yes	Cell survival, proposed prognostic marker for colorectal cancer	(Coumans et al., 2014; Peng et al., 2012)
115	MKRN1	E3 ubiquitin-protein ligase makorin-1	219	ns	↑0.0230	ns	No	Tumorigenesis	(Ko et al., 2012)
116	NEDD4L	E3 ubiquitin-protein ligase NEDD4-like	219	ns	↑0.0000	ns	Yes	Decreased expression correlates with poor prognosis in gastric cancer	(Gao et al., 2012)

117	HSPA8	Heat shock cognate 71 kDa protein	219	↑0.0524	↑0.0370	ns	Yes	Overexpression in squamous vaginal and cervical cancer, proposed molecular target for glioblastoma	(Lomnytska et al., 2010; Matsuda et al., 2015)
118	NACA	Nascent polypeptide-associated complex subunit alpha, muscle-specific form	219	ns	↑0.0000	0.0119	Yes		
119	RPS27A	Ubiquitin-40S ribosomal protein S27a	219	0.0000	↑0.0000	0.0139	Yes		
120	ANPEP	Aminopeptidase N	222	0.0000	0.0000	0.0000	Yes	Presence correlates with overall survival in colorectal cancer, downregulated in PCa tissue	(Sanz et al., 2015; Sørensen et al., 2013)
121	M6PR	Cation-dependent mannose-6-phosphate receptor	222	0.0228	0.0000	0.0164	Yes		
122	LSR	Lipolysis-stimulated lipoprotein receptor	222	0.0285	ns	0.0171	Yes		
123	ADAM10	Disintegrin and metalloproteinase domain-containing protein 10	222	0.0391	0.0362	0.0172	Yes	Proliferation, migration, facilitates shedding of ligands important for PCa progression	(Chitadze et al., 2013; Kasina et al., 2009)
124	ITIH4	ITIH4 protein	222	0.0195	0.0000	0.0082	Yes	Peptides significantly higher in breast cancer serum	(van den Broek et al., 2010)
125	F3	Tissue factor	222	0.0244	0.0000	0.0000	Yes	Tumor growth, increased expression correlates with decreased survival in non-small cell lung cancer	(Keshava et al., 2013; Regina et al., 2009)
126	TSG101	Tumor susceptibility gene 101 protein	222	0.0361	ns	0.0000	Yes	Proliferation, migration	(Zhu et al., 2004)
127	VPS37B	Vacuolar protein sorting-associated protein 37B	222	0.0243	ns	0.0075	Yes		
128	PSMC6	26S protease regulatory subunit 10B	225	↑0.0175	0.0152	ns	Yes		
129	PSMC1	26S protease regulatory subunit 4	225	↑0.0233	0.0000	ns	Yes		
130	PSMC3	26S protease regulatory subunit 6A	225	↑0.0215	↑0.0000	↑0.0159	Yes		
131	PSMC2	26S protease regulatory subunit 7	225	0.0168	0.0368	ns	Yes		
132	PSMC5	26S protease regulatory subunit 8	225	0.0345	0.0246	ns	Yes	Overexpression in breast cancer tissue	(Deng et al., 2007)

133	PSMD1	26S proteasome non-ATPase regulatory subunit 1	225	0.0143	0.0000	0.0275	Yes	Overexpression in breast cancer tissue	(Deng et al., 2007)
134	PSMD11	26S proteasome non-ATPase regulatory subunit 11	225	↑0.0232	↑0.0000	0.0000	Yes	Overexpression in breast cancer tissue	(Deng et al., 2007)
135	DNAJA1	DnaJ homolog subfamily A member 1	225	↑0.0515	ns	ns	Yes	downregulated in pancreatic cancer cells	(Stark et al., 2014)
136	RPS5	40S ribosomal protein S5	225	↑0.0163	ns	ns	Yes	Differential expression in colon cancer	(Bandrés, 2007)
137	PSMD2	26S proteasome non-ATPase regulatory subunit 2	225	↑0.0165	↑0.0000	ns	Yes	Overexpression in breast cancer tissue	(Deng et al., 2007)
138	PSMD3	26S proteasome non-ATPase regulatory subunit 3	225	↑0.0155	↑0.0000	ns	Yes		
139	PSMD6	26S proteasome non-ATPase regulatory subunit 6	225	↑0.0000	↑0.0000	ns	Yes	DNA repair, cell survival	(Narayanaswamy et al., 2014)
140	RPS12	40S ribosomal protein S12	225	↑0.0400	↑0.0271	↑0.0170	Yes	Proliferation, migration	(Chen, 2013)
141	RPS2	40S ribosomal protein S2	225	0.0000	↑0.0000	↑0.0158	Yes	Overexpressed in PCa, cell growth and survival	(Wang et al., 2009)
142	RPL12	60S ribosomal protein L12	225	↑0.0148	↑0.0083	↑0.0395	Yes		
143	AARS	Alanine--tRNA ligase, cytoplasmic	225	↑0.0201	↑0.0000	ns	Yes		
144	RARS	Arginine--tRNA ligase, cytoplasmic	225	↑0.0188	↑0.0000	ns	Yes		
145	CAPN6	Calpain-6	225	↑0.0153	↑0.0431	0.0150	No		
146	DNAJA2	DnaJ homolog subfamily A member 2	225	↑0.0212	↑0.0000	ns	Yes		
147	EEF1G	Elongation factor 1-gamma	225	↑0.0164	↑0.0374	0.0163	Yes		
148	EIF3CL	Eukaryotic translation initiation factor 3 subunit C-like protein	225	↑0.0294	↑0.0145	ns	Yes		
149	EIF5A	Eukaryotic translation initiation factor 5A-1	225	↑0.0260	↑0.0091	↑0.0156	Yes	Cell proliferation	(Epis et al., 2012)
150	GARS	Glycine--tRNA ligase	225	↑0.0171	↑0.0000	ns	Yes		
151	HSP90AA1	Heat shock protein HSP 90-alpha	225	↑0.0000	↑0.0000	↑0.0000	Yes	Ovarian cancer cell proliferation and survival	(Chu et al., 2013)
152	PREP	Prolyl endopeptidase	225	↑0.0150	↑0.0000	ns	Yes	High expression in colorectal cancer plasma	(Larrinaga et al., 2014)
153	HTRA1	Serine protease HTRA1	225	↑0.0262	↑0.0142	ns	Yes	Downregulation leads to EMT	(Zhu et al., 2015a)
154	TCP1	T-complex protein 1 subunit alpha	225	↑0.0000	↑0.0000	ns	Yes	Survival	(Guest et al., 2015)
155	CCT2	T-complex protein 1 subunit beta	225	↑0.0000	ns	↑0.0000	Yes	Survival	(Guest et al., 2015)

156	CCT4	T-complex protein 1 subunit delta	225	↑0.0204	↑0.0140	↑0.0142	Yes	Motility in lung adenocarcinoma	(Tano et al., 2010)
157	CCT5	T-complex protein 1 subunit epsilon	225	↑0.0370	↑0.0273	↑0.0000	Yes	Upregulated in tumors with mutated p53, drug resistance in breast cancer	(Ooe et al., 2007)
158	CCT3	T-complex protein 1 subunit gamma	225	↑0.0000	↑0.0092	↑0.0000	Yes		
159	CCT8	T-complex protein 1 subunit theta	225	↑0.0000	↑0.0324	↑0.0000	Yes		
160	CCT6A	T-complex protein 1 subunit zeta	225	↑0.0000	↑0.0000	↑0.0169	Yes		
161	USP5	Ubiquitin carboxyl-terminal hydrolase 5	225	↑0.0203	↑0.0000	0.0148	Yes	Suppresses p53	(Dayal et al., 2009)
162	MME	Neprilysin	225	0.0000	↑0.0000	ns	Yes	Loss in androgen-independent PCa	(Hong et al., 2012)
163	RPL18	60S ribosomal protein L18 (Fragment)	227	ns	↑0.0079	↑0.0247	Yes		
164	CALR	Calreticulin	227	ns	↑0.0328	↑0.0065	Yes	Androgen-regulated, inhibits growth and metastasis in PCa, low expression in PCa tissue	(Alur et al., 2009)
165	DPP4	Dipeptidyl peptidase 4	227	ns	↑0.0000	↑0.0163	Yes	Higher expression in localized PCa in comparison to metastasized tumors	(Nazarian et al., 2014)
166	HSP90B1	Endoplasmin	227	ns	↑0.0326	↑0.0172	Yes	High levels in breast cancer patients who benefit from hormonal treatment, metastasis, poor prognosis	(Cawthorn et al., 2012)
167	PSME1	Proteasome activator complex subunit 1	227	0.0000	ns	↑0.0161	Yes	Elevated in primary and metastasized PCa	(Sánchez-Martín et al., 2013)
168	PDIA3	Protein disulfide-isomerase A3	227	ns	ns	↑0.0162	Yes	Associated with advanced PCa	(Pressinotti et al., 2009)
169	PDIA6	Protein disulfide-isomerase A6	227	ns	↑0.0000	↑0.0000	Yes	Cell survival, drug resistance, overexpression in lung adenocarcinoma	(Tufo et al., 2014)
170	P4HB	Protein disulfide-isomerase	227	↑0.0147	↑0.0000	↑0.0000	Yes	Up-regulated in HER-2/neu-positive breast tumors	(Zhang, 2005)
171	HSPE1	10 kDa heat shock protein, mitochondrial	227	ns	↑0.0467	↑0.0197	Yes	Survival	(Coumans et al., 2014)
Regulation of cell growth, nucleobase, nucleoside, nucleotide, nucleic acid metabolism, or signal transduction									
172	TROVE2	60 kDa SS-A/Ro ribonucleoprotein	219	ns	↑0.0207	ns	No		
173	PABPC1	Polyadenylate-binding protein 1	219	↑0.0185	↑0.0000	↑0.0117	Yes	Expression in cancerous and healthy prostate tissue	(Yang et al., 2013)
174	PABPC4	Polyadenylate-binding protein 4	219	0.0371	↑0.0227	0.0070	No	Positive correlation with survival in colorectal cancer	(Liu et al., 2012)

175	SF3B3	Splicing factor 3B subunit 3	219	ns	↑0.0000	ns	Yes		
176	ITCH	E3 ubiquitin-protein ligase Itchy homolog	219	ns	↑0.0360	ns	No	Cell proliferation and survival	(Ho et al., 2011)
177	AEBP1	Adipocyte enhancer-binding protein 1	225	↑0.0412	↑0.0141	0.0138	Yes	Cell proliferation and survival	(Ladha et al., 2012)
178	PGD	6-phosphogluconate dehydrogenase, decarboxylating	225	↑0.0217	↑0.0000	ns	Yes		
179	MCM2	DNA replication licensing factor MCM2	225	↑0.0203	↑0.0000	0.0143	Yes	Targeted by lovastatin and ciproflaxin, cell growth, survival	(Simon et al., 2013; Zhang, 2015)
180	HNRNPA3	Heterogeneous nuclear ribonucleoprotein A3	225	↑0.0247	↑0.0000	↑0.0066	No		
181	PTRF	Polymerase I and transcript release factor	225	↑0.0141	↑0.0243	↑0.0223	Yes	Inverse correlation with PCa progression	(Meng et al., 2015; Yi et al., 2013)
182	PRPF19	Pre-mRNA-processing factor 19	225	↑0.0294	↑0.0000	0.0160	Yes		
183	DDX5	Probable ATP-dependent RNA helicase DDX5	225	↑0.0160	↑0.0154	ns	Yes	Increased expression in PCa tissue in comparison to benign, AR-interactor	(Clark et al., 2008)
184	RUVBL1	RuvB-like 1	225	↑0.0268	↑0.0000	↑0.0224	Yes	motility and invasiveness of pancreatic cancer cells	(Taniuchi, 2014)
185	SFPQ	Splicing factor, proline- and glutamine-rich	225	↑0.0000	↑0.0146	ns	Yes		
186	RAD23B	UV excision repair protein RAD23 homolog B	225	↑0.0287	↑0.0229	ns	No	downregulated in invasive breast cancer cells	(Linge et al., 2014)
187	RNH1	Ribonuclease inhibitor	227	Ns	↑0.0000	↑0.0000	Yes	Highly expressed in resistant gastric cancer cells	(Zhu et al., 2014)
188	RTN4	Reticulon	227	ns	↑0.0000	↑0.0000	Yes	Suppresses tumor development in some cancers, promotes cell growth in colorectal cancer	(Xue, 2015)
189	STAT1	Signal transducer and activator of transcription 1-alpha/beta	227	0.0518	↑0.0000	↑0.0000	Yes	AR co-regulator, expressed in PCa tissue	(Aoyagi et al., 1998; Urbanucci et al., 2008)
Transport									
190	VPS37C	Vacuolar protein sorting-associated protein 37C	219	ns	↑0.0000	0.0165	Yes		
191	CHMP1B	Charged multivesicular body protein 1b	222	0.0000	0.0080	0.0195	Yes		
192	APOE	Apolipoprotein E	222	0.0196	0.0078	0.0092	Yes	Correlates with advancement of PCa	(Venanzoni, 2003)
193	HBB	Hemoglobin subunit beta	222	0.0167	0.0000	0.0000	Yes	Differentially expressed in ovarian cancer serum	(Huang et al., 2012)
194	HPX	Hemopexin	222	0.0227	0.0000	0.0000	Yes	Differentially expressed in ovarian cancer serum	(Huang et al., 2012)
195	PLSCR3	Phospholipid scramblase 3	222	0.0264	ns	0.0077	Yes		

196	TF	Serotransferrin	222	0.0000	↑0.0000	↑0.0000	Yes	Present in breast cancer tissue	(Korwar et al., 2012)
197	SLC38A2	Sodium-coupled neutral amino acid transporter 2	222	0.0323	0.0000	0.0000	Yes	Tumorigenesis in breast cancer	(Jeon et al., 2015)
198	SLC38A5	Sodium-coupled neutral amino acid transporter 5	222	0.0338	0.0232	0.0189	No		
199	VPS28	Vacuolar protein sorting-associated protein 28 homolog	222	0.0383	ns	0.0000	Yes		
200	COPA	Coatomer subunit alpha	225	↑0.0196	ns	0.0155	Yes		
201	COPB2	Coatomer subunit beta'	225	↑0.0000	↑0.0376	ns	Yes		
202	CSE1L	Exportin-2	225	↑0.0266	↑0.0000	ns	Yes	Tumor growth, cell survival, up-regulated in hepatocellular carcinoma	(Winkler et al., 2014)
203	KPNB1	Importin subunit beta-1	225	↑0.0250	0.0447	0.0145	Yes	malignant peripheral nerve sheath tumor cell, tumorigenesis	(Zhang et al., 2015)
204	VAT1	Synaptic vesicle membrane protein VAT-1 homolog	225	↑0.0252	↑0.0000	ns	Yes	Expression in benign prostatic hyperplasia and prostate cancer cells but not healthy prostate tissue	(Mori et al., 2011)
205	VPS35	Vacuolar protein sorting-associated protein 35	225	↑0.0313	↑0.0000	↑0.0452	Yes		
206	ATP6V0D1	V-type proton ATPase subunit d 1	225	↑0.0192	↑0.0000	ns	Yes		
207	CPNE3	Copine-3	227	↑0.0388	ns	↑0.0171	Yes	Gene possible indicator of PCa susceptibility, invasion, migration	(Lin et al., 2013b; Thomas et al., 2008)
208	SLC1A4	Neutral amino acid transporter A	227	0.0208	ns	↑0.0166	Yes		
209	GDI2	Rab GDP dissociation inhibitor beta	227	ns	↑0.0077	↑0.0000	Yes	Increased levels in pancreatic carcinoma tissue and serum	(Sun et al., 2007)
210	TFRC	Transferrin receptor protein 1	227	ns	↑0.0153	↑0.0067	Yes	Cancer cell growth and tumorigenesis	(O'Donnell et al., 2006)
211	ARF4	ADP-ribosylation factor 4	227	ns	ns	↑0.0165	Yes	Migration, cell survival	(Jang et al., 2012)
212	SLC39A14	Zinc transporter ZIP14	227	0.0275	ns	↑0.0219	Yes	Highly specific to colorectal cancer tissue at transcript level	(Sveen et al., 2012)
Cytoskeletal anchoring									
213	PLEC	Plectin	225	↑0.0186	↑0.0000	↑0.0173	Yes	Upregulation in esophageal squamous cell carcinoma	(Sveen et al., 2012)
Protein folding									

214	CCT7	T-complex protein 1 subunit eta	225	↑0.0222	↑0.0364	ns	Yes		
DNA repair									
215	PCNA	Proliferating cell nuclear antigen	225	↑0.0177	↑0.0233	↑0.0151	Yes	Significantly higher expression prostatic adenocarcinoma than in benign prostatic hyperplasia	(Wang et al., 2011b)
Apoptosis									
216	PDCD6IP	Programmed cell death 6-interacting protein	222	0.0169	ns	0.0086	Yes		
Unknown or Unmapped									
217	CCDC50	Coiled-coil domain-containing protein 50	219	ns	↑0.0000	0.0168	No	Cell survival in chronic lymphocytic leukemia	(Farfsing et al., 2009)
218	S100A14	Protein S100-A14	222	0.0175	0.0000	0.0000	Yes	Correlation with advanced stages of ovarian cancer, proliferation, tumorigenesis, migration, invasion	(Cho et al., 2014; Tanaka et al., 2015)
219	IGSF3	Immunoglobulin superfamily member 3	222	0.0173	ns	0.0074	Yes		
220	IGHA1	Ig alpha-1 chain C region	222	0.0000	0.0000	0.0000	Yes		
221	ARRDC1	Arrestin domain-containing protein 1	222	0.0000	0.0000	0.0000	Yes		
222	IGHG1	Ig gamma-1 chain C region	222	0.0165	0.0000	0.0000	Yes	PCa cell growth and survival	(Pan et al., 2013)
223	IGKC	Ig kappa chain C region	222	0.0209	0.0000	0.0000	Yes	Positive correlation for prolonged disease-free survival in breast and non-small lung cancer	(Chen et al., 2012; Lohr et al., 2013)
224	C3orf58	Deleted in autism protein 1	225	ns	↑0.0424	0.0091	No		
225	IST1	IST1 homolog	225	↑0.0179	↑0.0000	ns	Yes		
226	K7ERQ8	Uncharacterized protein (Fragment)	225	↑0.0179	↑0.0078	ns	No		
227	WDR1	WD repeat-containing protein 1	227	ns	↑0.0079	↑0.0175	Yes	overexpressed in interstitial fluid from ovarian carcinomas,	(Haslene-Hox et al., 2013)
228	LAMP1	Lysosome-associated membrane glycoprotein 1	227	0.0498	ns	ns	Yes	Expression correlates with metastatic potential of melanoma	(Agarwal et al., 2015)

Table 2.2: Proteins with significantly different abundance between PCa and non-cancerous EV16 fractions. The arrow to the left of the q-value signifies that this protein is abundant in PCa EVs. ns, not significant; EMT, epithelial mesenchymal transition.

#	Gene Name (UniProt)	Protein Name	Cluster	Student's T-test q-Value Compared to RWPE1			Vesiclepedia Reported	Cancer Related	Reference(s)
				LNCaP EV16	22RV1 EV16	E006AA-hT EV16			
Cell communication, signal transduction									
1	MFGE8	Lactadherin	138	0.0339	ns	ns	Yes	Promotes bladder tumor development	(Sugano et al., 2011)
2	CD82	CD82 antigen	138	0.0259	ns	ns	Yes	Negatively correlated with metastasis in laryngeal squamous cell carcinoma, cell survival	(Nishioka et al., 2014; Yu et al., 2014)
3	TGFBI	Transforming growth factor-beta-induced protein ig-h3	138	0.0270	ns	ns	Yes	poor prognosis in colorectal cancer patients, tumor suppressor	(Li et al., 2012; Zhu et al., 2015b)
4	SFN	14-3-3 protein sigma	140	0.0394	ns	ns	Yes	Lymph Node metastasis-related protein	(Skotheim et al., 2002)
5	FST	Follistatin	140	0.0209	ns	ns	Yes	Cell proliferation and migration	(Karve et al., 2012)
6	ANXA2	Annexin A2	140	0.0229	ns	ns	Yes	Highly expressed in non-small cell lung cancer, correlation with poor prognosis	(Jia et al., 2013)
7	VGF	Neurosecretory protein VGF	142	↑0.0467	ns	ns	No		
8	ANXA3	Annexin A3	143	↑0.0229	ns	ns	Yes	Tumor growth, association with aggressive Hepatocellular Carcinoma	(Tong et al., 2015)
9	YWHAG	14-3-3 protein gamma	143	↑0.0134	ns	ns	Yes		
10	SEPT7	Septin-7	143	↑0.0000	ns	ns	Yes	Cell proliferation, inhibitis cell migration	(Xu et al., 2010; Yu et al., 2009)
11	COPS6	COP9 signalosome complex subunit 6	143	↑0.0270	ns	ns	No		
12	COPS3	COP9 signalosome complex subunit 3	143	↑0.0285	ns	ns	Yes	metastasis of osteogenic sarcoma cells, genomic instability when highly expressed	(van Dartel and Hulsebos, 2004; Yan et al., 2011)
13	PPP2R1A	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	143	↑0.0000	ns	ns	Yes	down-regulated in primary and metastatic prostate cancer, loss associate with PCa mortality	(Bluemn et al., 2013)

14	DPYSL2	Dihydropyrimidinase-related protein 2	143	↑0.0000	ns	ns	Yes		
15	SEPT2	Septin-2	143	↑0.0317	ns	ns	Yes	Cell proliferation, up-regulation hepatoma carcinoma	(Yu et al., 2009)
16	LRP1	Prolow-density lipoprotein receptor-related protein 1	143	↑0.0351	ns	ns	Yes	Tumor cell adhesion, low expression correlates with poor prognosis in lung cancer	(Meng et al., 2011; Perrot et al., 2012)
Cell growth and/or maintenance									
17	AGRN	Agrin	138	0.0000	ns	ns	Yes	Upregulation in synovial sarcomas	(Fernebro et al., 2006)
18	LAMA5	Laminin subunit alpha-5	138	0.0000	ns	ns	Yes		(Takkunen et al., 2008)
19	THBS1	Thrombospondin-1	138	0.0121	ns	ns	Yes		
20	LAMA3	Laminin subunit alpha-3	140	0.0201	ns	ns	Yes		
21	LAMB3	Laminin subunit beta-3	140	0.0207	ns	ns	Yes		
22	LAMC2	Laminin subunit gamma-2	140	0.0252	ns	ns	Yes		
23	NID2	Nidogen-2	141	↑0.0378	ns	ns	No		
24	HSPG2	Basement membrane-specific heparan sulfate proteoglycan core protein	141	↑0.0219	ns	↑0.0000	Yes	Present in PCa sera, invasion	(Grindel et al., 2016; Grindel et al., 2014)
25	COL6A1	Collagen alpha-1(VI) chain	141	↑0.0308	ns	ns	Yes		
26	ARPC1B	Actin-related protein 2/3 complex subunit 1B	143	↑0.0499	ns	ns	Yes	Used as a potential biomarker for Choroidal malignant melanomas	(Kumagai et al., 2006)
27	TPM4	Tropomyosin alpha-4 chain	143	↑0.0000	ns	ns	Yes	Present in ovarian cancer patient sera at significantly elevated levels.	(Tang et al., 2013)
28	LMNA	Prelamin-A/C	143	↑0.0459	ns	ns	Yes	Expression associated with an increased disease recurrence in colon cancer.	(Belt et al., 2011)
29	FBN1	Fibrillin-1	143	↑0.0358	ns	ns	Yes	Specific and sensitive biomarker candidate for colorectal cancer.	(Guo et al., 2013b)
30	LTBP1	Latent-transforming growth factor beta-binding protein 1	143	↑0.0308	ns	ns	Yes	Levels correlate with grade of malignancy in gliomas	(Tritschler et al., 2009)
31	TLN1	Talin-1	143	0.0281	ns	ns	Yes	Associated with ovarian serous carcinoma development and progression to metastasis	(Tang, 2013)
32	PCOLCE	Procollagen C-endopeptidase enhancer 1	143	↑0.0312	ns	ns	Yes		

33	COL1A1	Collagen alpha-1(I) chain	143	↑0.0000	ns	ns	Yes		
34	FLNC	Filamin-C	143	↑0.0232	ns	ns	Yes	Reduced in many primary and metastasized cancers, silencing improved PCa metastasis	(Qiao et al., 2014)
35	VCL	Vinculin	143	↑0.0000	ns	ns	Yes	More abundant in PCa tissue in comparison to BPH, positive correlation with AR presence	(ZHU Li-yong, 2010)
36	COL1A2	Collagen alpha-2(I) chain	143	↑0.0246	ns	ns	Yes	Methylated form upregulated in bladder cancer	(Mori, 2009)
37	COL3A1	Collagen alpha-1(III) chain	143	↑0.0214	ns	ns	Yes	Upregulated in colorectal cancer	(Wang et al., 2016)
38	LAMA4	Laminin subunit alpha-4	143	↑0.0142	ns	ns	Yes	upregulated in hepatocellular carcinoma, correlation with invasion and metastasis	(Huang et al., 2008)
39	SPTBN1	Spectrin beta chain, non-erythrocytic 1	143	↑0.0210	ns	ns	Yes	Reduced expression correlated with poor prognosis in pancreatic cancer	(Xiaohua Jiang, 2010)
40	MSN	Moesin	143	↑0.0334	ns	ns	Yes	cell proliferation, migration, invasion in head and neck squamous cell carcinoma	(Kinoshita et al., 2012)
41	CAP1	Adenylyl cyclase-associated protein 1	143	↑0.0463	ns	ns	Yes	Migration, metastasis, overexpression in ovarian cancer and hepatocellular carcinoma	(Hua et al., 2015; Liu et al., 2014)
42	FLNA	Filamin-A	143	↑0.0193	ns	ns	Yes	proliferation, invasion in metastatic melanoma and breast cancer	(Zhang et al., 2014c; Zhong et al., 2010)
43	VIM	Vimentin	143	↑0.0262	ns	ns	Yes	Proposed urinary biomarker for bladder cancer	(Costa et al., 2010; Reinert et al., 2012)
44	ARPC2	Actin-related protein 2/3 complex subunit 2	143	↑0.0000	ns	ns	Yes		
Energy pathways, metabolism									
45	PLOD1	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1	141	↑0.0314	ns	ns	Yes		
46	ENO3	Beta-enolase	143	↑0.0212	ns	ns	Yes		
47	PRDX6	Peroxiredoxin-6	143	↑0.0243	ns	ns	Yes	Promotes lung tumor growth and development	(Yun et al., 2014; Yun et al., 2015)
48	SHMT1	Serine hydroxymethyltransferase, cytosolic	143	↑0.0191	ns	ns	Yes	Lung cancer cell survival	(Paone et al., 2014)
49	IMPDH2	Inosine-5'-monophosphate dehydrogenase 2	143	↑0.0202	ns	ns	Yes	Metastasis, advanced tumor progression in PCa, correlation with kidney and	(Zhou et al., 2014a; Zou et al., 2014)

								bladder cancer progression	
50	MDH1	Malate dehydrogenase, cytoplasmic	143	↑0.0199	ns	ns	Yes		
51	GSR	Glutathione reductase, mitochondrial	143	↑0.0429	ns	ns	Yes		
52	PGD	6-phosphogluconate dehydrogenase, decarboxylating	143	↑0.0000	ns	ns	Yes	High expression in cervical cancer tissue	(Lee et al., 2014)
53	ATP6V1A	V-type proton ATPase catalytic subunit A	143	↑0.0323	ns	ns	Yes		
54	BLMH	Bleomycin hydrolase	143	↑0.0206	ns	ns	Yes	Associated with reduced survival and early relapses in testicular cancer	(de Haas et al., 2008)
55	CP	Ceruloplasmin	143	↑0.0294	ns	ns	Yes	Serum levels correlate with advanced progression of PCa	(Rehman et al., 2012)
56	ISYNA1	Inositol-3-phosphate synthase 1	143	↑0.0296	ns	ns	No		
57	ALAD	Delta-aminolevulinic acid dehydratase	143	↑0.0386	ns	ns	Yes	Genetic variation of ALAD between PCa positive black men who have had high lead exposure	(Neslund-Dudas et al., 2014)
58	MDH2	Malate dehydrogenase, mitochondrial	143	↑0.0138	ns	ns	Yes		
59	HPRT1	Hypoxanthine-guanine phosphoribosyltransferase	143	↑0.0205	ns	ns	Yes		
60	PGK1	Phosphoglycerate kinase 1	143	↑0.0000	ns	ns	Yes	Regulates bone formation at the metastatic site when secreted by PCa	(Jung et al., 2009)
61	SHMT2	Serine hydroxymethyltransferase, mitochondrial	143	↑0.0311	ns	ns	Yes	Cell survival	(Kim et al., 2015)
Immune response									
62	LGALS3BP	Galectin-3-binding protein	138	0.0000	0.0000	ns	Yes	Decreased survival and metastasis, immune evasion	(Läubli et al., 2014; Piccolo et al., 2013)
63	CD109	CD109 antigen	140	0.0203	ns	ns	Yes	Highly expressed in cell secretome and tissue of non-small-cell lung cancer	(Zhang et al., 2014a)
64	B2M	Beta-2-microglobulin	140	0.0131	ns	ns	Yes	Indicator of metastasis in colorectal cancer	(Shrout et al., 2008)
65	CFI	Complement factor I	140	0.0000	0.0000	ns	Yes	Cell growth, migration, strong association with aggressive cutaneous squamous cell carcinoma tumors	(Riihilä et al., 2015)
66	HP	Haptoglobin	140	0.0000	0.0000	ns	Yes	Proposed serum biomarker for pancreatic cancer and lung cancer	(Ayyub, 2015; Haas et al., 2015)

67	PGLYRP2	N-acetylmuramoyl-L-alanine amidase	140	0.0115	ns	ns	Yes		
68	PTX3	Pentraxin-related protein PTX3	141	↑0.0216	ns	ns	Yes	positive correlation with tumor grade and severity in glioma, inflammation, metastasis in head and neck squamous cell carcinoma	(Chang et al., 2015; Locatelli et al., 2013)
69	C1R	Complement C1r subcomponent	143	↑0.0303	ns	ns	Yes		
Protein metabolism									
70	CTSD	Cathepsin D	138	0.0000	ns	ns	Yes		
71	CTSB	Cathepsin B	138	0.0000	ns	ns	Yes	Expressed in colon cancer tissue, associated with higher risk of mortality	(Chan et al., 2010)
72	SERPINB5	Serpin B5	140	0.0224	ns	ns	Yes	Increased mRNA levels correlate with metastasis	(Mardin et al., 2010)
73	SERPINE1	Plasminogen activator inhibitor 1	140	0.0237	ns	ns	Yes	Biomarker candidate for colorectal cancer, correlation with poor prognosis	(Cochran et al., 2011; Mazzoccoli et al., 2012)
74	ITIH4	ITIH4 protein	140	0.0000	0.0000	ns	Yes	Peptides significantly higher in breast cancer serum	(van den Broek et al., 2010)
75	CTSC	Dipeptidyl peptidase 1	140	0.0000	ns	ns	Yes		
76	PSMB9	Proteasome subunit beta type-9	141	↑0.0266	ns	ns	Yes		
77	CPE	Carboxypeptidase E	143	↑0.0113	ns	ns	No	Elevated in cancer, cell proliferation and tumorigenicity in colorectal cancer	(Liang et al., 2013)
78	HTRA1	Serine protease HTRA1	143	↑0.0000	ns	ns	Yes	Downregulation leads to EMT	(Zhu et al., 2015a)
79	NPEPPS	Puromycin-sensitive aminopeptidase	143	↑0.0118	ns	ns	Yes		
80	COPS5	COP9 signalosome complex subunit 5	143	↑0.0256	ns	ns	No		
81	COPS4	COP9 signalosome complex subunit 4	143	↑0.0000	ns	ns	Yes		
82	P4HB	Protein disulfide-isomerase	143	↑0.0277	ns	ns	Yes	Up-regulated in HER-2/Neu-positive breast tumors	(Zhang, 2005)
83	HSPA4	Heat shock 70 kDa protein 4	143	↑0.0249	ns	ns	Yes	Poor prognosis in hepatocellular carcinoma	(Yang et al., 2015)
84	CUL1	Cullin-1	143	↑0.0187	ns	ns	No	Poor prognosis in breast cancer	(Bai et al., 2013)
85	VBP1	Prefoldin subunit 3	143	↑0.0221	ns	ns	Yes		

86	P4HA1	Prolyl 4-hydroxylase subunit alpha-1	143	↑0.0240	ns	ns	Yes	Correlation with PCa progression, PCa cell growth and tumor progression	(Chakravarthi et al., 2014)
Cell cycle regulation									
87	YWHAZ	14-3-3 protein zeta/delta (Fragment)	140	0.0000	0.0000	ns	Yes	High expression associated with higher risk of castration resistant PCa and reduced survival	(Ruenauver et al., 2014)
Regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism, signal transduction, translation or tRNA aminoacylation									
88	SET	Protein SET	143	↑0.0197	ns	ns	Yes	Associated with non-small cell lung cancer progression	(Liu et al., 2015)
89	SNRPD3	Small nuclear ribonucleoprotein Sm D3	143	↑0.0364	ns	ns	Yes		
90	DDB1	DNA damage-binding protein 1	143	↑0.0000	ns	ns	Yes	Proposed biomarker of resistance to acyl sulfonamide-based cancer drugs	(Mullenders et al., 2009)
91	TSN	Translin	143	↑0.0345	ns	ns	Yes		
92	COPS2	COP9 signalosome complex subunit 2	143	↑0.0110	ns	ns	No		
93	COPS7A	COP9 signalosome complex subunit 7a	143	↑0.0000	ns	ns	No		
94	AEBP1	Adipocyte enhancer-binding protein 1	143	↑0.0000	ns	ns	Yes	Cell proliferation and survival	(Ladha et al., 2012)
95	CAND1	Cullin-associated NEDD8-dissociated protein 1	143	↑0.0273	ns	ns	Yes	Overexpressed in some PCa tumors	(Korzeniewski et al., 2012)
96	COPS8	COP9 signalosome complex subunit 8	143	↑0.0226	ns	ns	Yes		
97	KARS	Lysine--tRNA ligase	143	↑0.0195	ns	ns	Yes		
98	EIF6	Eukaryotic translation initiation factor 6	143	↑0.0272	ns	ns	Yes		
Transport									
99	TF	Serotransferrin	140	0.0000	0.0000	0.0000	Yes	Expressed on many types of cancer cells including leukemic cells and selectively on angiogenic tumor VECs	(Hu et al., 2010)
100	HPX	Hemopexin	140	0.0000	0.0000	ns	Yes	Differentially expressed in ovarian cancer serum	(Huang et al.)
101	HBB	Hemoglobin subunit beta	140	0.0000	ns	ns	Yes	Differentially expressed in ovarian cancer serum	(Huang et al.)
102	ALB	Serum albumin	140	0.0000	ns	ns	Yes	Low levels pre-operation correlate with surgical complications and mortality in head and neck cancer patients	(Kao et al., 2012)

103	AP1B1	AP-1 complex subunit beta-1	143	↑0.0227	ns	ns	Yes		
104	COPB2	Coatomer subunit beta'	143	↑0.0234	ns	ns	Yes		
105	IPO5	Importin-5 (Fragment)	143	↑0.0124	ns	ns	Yes		
106	IPO7	Importin-7	143	↑0.0298	ns	ns	Yes		
107	SEPT11	Septin-11	143	↑0.0000	ns	ns	Yes		
108	AP2B1	AP-2 complex subunit beta	143	↑0.0328	ns	ns	Yes		
Unknown									
109	SETSIP	Protein SETSIP	140	0.0348	ns	ns	No		
110	IGKC	Ig kappa chain C region	140	0.0189	ns	ns	Yes	Positive correlation for prolonged disease-free survival in breast and non-small lung cancer	(Chen et al., 2012; Lohr et al., 2013)
111	IGHA1	Ig alpha-1 chain C region	140	0.0000	ns	ns	Yes		
112	IGHG4	Ig gamma-4 chain C region (Fragment)	140	0.0000	ns	ns	Yes		
113	IGLC2	Ig lambda-2 chain C regions (Fragment)	140	0.0000	ns	ns	Yes		
114	IGHG2	Ig gamma-2 chain C region	140	0.0000	ns	ns	Yes		
115	B4E1Z4	Uncharacterized protein	140	0.0215	ns	ns	No		
116	TAGLN2	Transgelin-2	143	↑0.0186	ns	ns	Yes	Cell proliferation and invasion in head and neck squamous cell carcinoma, poor prognosis in Barrett's adenocarcinoma	(Elsner et al., 2012; Nohata et al., 2011)
117	MRC2	C-type mannose receptor 2	143	↑0.0289	ns	ns	Yes		
118	NME1-NME2	Nucleoside diphosphate kinase	143	↑0.0127	ns	ns	Yes		
119	DKFZp686J1372	Epididymis luminal protein 189	143	↑0.0371	ns	ns	No		

References

Abels, E.R., and X.O. Breakefield. 2016. Introduction to Extracellular Vesicles: Biogenesis, RNA Cargo Selection, Content, Release, and Uptake. *Cellular and Molecular Neurobiology* 36:301-312.

Adam, P.J., J.A. Terrett, G. Steers, L. Stockwin, J.A. Loader, G.C. Fletcher, L.S. Lu, B.I. Leach, S. Mason, A.C. Stamps, R.S. Boyd, F. Pezzella, K.C. Gatter, and A.L. Harris. 2006. CD70 (TNFSF7) is expressed at high prevalence in renal cell carcinomas and is rapidly internalised on antibody binding. *British Journal of Cancer* 95:298-306.

Adler, A.S., L.E. Littlepage, M. Lin, T.L.A. Kawahara, D.J. Wong, Z. Werb, and H.Y. Chang. 2008. CSN5 Isopeptidase Activity Links COP9 Signalosome Activation to Breast Cancer Progression. *Cancer Research* 68:506-515.

Agarwal, A.K., N. Srinivasan, R. Godbole, S.K. More, S. Budnar, R.P. Gude, and R.D. Kalraiya. 2015. Role of tumor cell surface lysosome-associated membrane protein-1 (LAMP1) and its associated carbohydrates in lung metastasis. *Journal of Cancer Research and Clinical Oncology* 141:1563-1574.

Akers, J.C., D. Gonda, R. Kim, B.S. Carter, and C.C. Chen. 2013. Biogenesis of extracellular vesicles (EV): exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies. *Journal of Neuro-Oncology* 113:1-11.

Al-Nedawi, K., B. Meehan, J. Micallef, V. Lhotak, L. May, A. Guha, and J. Rak. 2008. Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. *Nat Cell Biol* 10:619-624.

Alur, M., M.M. Nguyen, S.E. Eggener, F. Jiang, S.S. Dadras, J. Stern, S. Kimm, K. Roehl, J. Kozlowski, M. Pins, M. Michalak, R. Dhir, and Z. Wang. 2009. Suppressive Roles

of Calreticulin in Prostate Cancer Growth and Metastasis. *The American Journal of Pathology* 175:882-890.

Alvarez, S., C. Suazo, A. Boltansky, M. Ursu, D. Carvajal, G. Innocenti, A. Vukusich, M. Hurtado, S. Villanueva, J.E. Carreño, A. Rogelio, and C.E. Irarrazabal. 2013. Urinary Exosomes as a Source of Kidney Dysfunction Biomarker in Renal Transplantation. *Transplantation Proceedings* 45:3719-3723.

American Cancer Society. 2013. Cancer Facts and Figures 2013, http://www.cancer.org/acs/groups/content/@epidemiologysurveilance/documents/document/acspc-036845.pdf.

American Cancer Society. 2015. What are the Key Statistics About Prostate Cancer? http://www.cancer.org/cancer/prostatecancer/detailedguide/prostatecancer-key-statistics.

Andriole , G.L., E.D. Crawford , R.L.I. Grubb , S.S. Buys , D. Chia , T.R. Church , M.N. Fouad , E.P. Gelmann , P.A. Kvale , D.J. Reding , J.L. Weissfeld , L.A. Yokochi , B. O'Brien , J.D. Clapp , J.M. Rathmell , T.L. Riley , R.B. Hayes , B.S. Kramer , G. Izmirlian , A.B. Miller , P.F. Pinsky , P.C. Prorok , J.K. Gohagan , and C.D. Berg 2009. Mortality Results from a Randomized Prostate-Cancer Screening Trial. *New England Journal of Medicine* 360:1310-1319.

Aoyagi, K., I. Shima, M. Wang, Y. Hu, F.U. Garcia, and M.E. Stearns. 1998. Specific transcription factors prognostic for prostate cancer progression. *American Association for Cancer Research* 4:2153-2160.

Ayyub, A., Saleem, M., Musharraf, S. G., Naz, M., Tariq, A., Hashmi, N. 2015. Mass spectrometric identification, characterization and validation of the haptoglobin β-chain protein as a lung cancer serum biomarker *Molecular Medicine Reports* 12:3755-3762.

Bai, J., H.M. Yong, F.F. Chen, P.J. Mei, H. Liu, C. Li, Z.Q. Pan, Y.P. Wu, and J.N. Zheng. 2013. Cullin1 is a novel marker of poor prognosis and a potential therapeutic target in human breast cancer. *Annals of Oncology* 24:2016-2022.

Bandrés, E., Malumbres, R., Cubedo, E., Honorato, B., Zarate, R., Labarga, A., Gabisu, U., Sola, J. J., García-Foncillas, J. 2007. A gene signature of 8 genes could identify the risk of recurrence and progression in Dukes' B colon cancer patients. *Oncology Reports* 17:1089-1094.

Bayer-Santos, E., C. Aguilar-Bonavides, S.P. Rodrigues, E.M. Cordero, A.F. Marques, A. Varela-Ramirez, H. Choi, N. Yoshida, J.F. da Silveira, and I.C. Almeida. 2013. Proteomic Analysis of Trypanosoma cruzi Secretome: Characterization of Two Populations of Extracellular Vesicles and Soluble Proteins. *Journal of Proteome Research* 12:883-897.

Bello, D., M.M. Webber, H.K. Kleinman, D.D. Wartinger, and J.S. Rhim. 1997. Androgen responsive adult human prostatic epithelial cell lines immortalized by human papillomavirus 18. *Carcinogenesis* 18:1215-1223.

Belt, E.J.T., R.J.A. Fijneman, E.G. van den Berg, H. Bril, P.M. Delis-van Diemen, M. Tijssen, H.F. van Essen, E.S.M. de Lange-de Klerk, J.A.M. Beliën, H.B.A.C. Stockmann, S. Meijer, and G.A. Meijer. 2011. Loss of lamin A/C expression in stage II and III colon cancer is associated with disease recurrence. *European Journal of Cancer* 47:1837-1845.

Bhansali, M., and L. Shemshedini. 2014. COP9 Subunits 4 and 5 Target Soluble Guanylyl Cyclase α1 and p53 in Prostate Cancer Cells. *Molecular Endocrinology* 28:834-845.

Bigler, D., D. Gioeli, M.R. Conaway, M.J. Weber, and D. Theodorescu. 2007. Rap2 regulates androgen sensitivity in human prostate cancer cells. *The Prostate* 67:1590-1599.

Bluemn, E.G., E.S. Spencer, B. Mecham, R.R. Gordon, I. Coleman, D. Lewinshtein, E. Mostaghel, X. Zhang, J. Annis, C. Grandori, C. Porter, and P.S. Nelson. 2013. PPP2R2C loss promotes castration-resistance and is associated with increased prostate cancer-specific mortality. *Molecular cancer research : MCR* 11:568-578.

Bravo-Cordero, J.J., L. Hodgson, and J.S. Condeelis. 2014. Spatial regulation of tumor cell protrusions by RhoC. *Cell Adhesion & Migration* 8:263-267.

Brems-Eskildsen, A.S., K. Zieger, H. Toldbod, C. Holcomb, R. Higuchi, F. Mansilla, P.P. Munksgaard, M. Borre, T.F. Ørntoft, and L. Dyrskjøt. 2010. Prediction and diagnosis of bladder cancer recurrence based on urinary content of hTERT, SENP1, PPP1CA, and MCM5 transcripts. *BMC Cancer* 10:646-646.

Bulk, E., B. Sargin, U. Krug, A. Hascher, Y. Jun, M. Knop, C. Kerkhoff, V. Gerke, R. Liersch, R.M. Mesters, M. Hotfilder, A. Marra, S. Koschmieder, M. Dugas, W.E. Berdel, H. Serve, and C. Müller-Tidow. 2008. S100A2 Induces Metastasis in Non–Small Cell Lung Cancer. *American Association for Cancer Research* 15:22-29.

Cantiello, F., G.I. Russo, M. Ferro, A. Cicione, S. Cimino, V. Favilla, S. Perdonà, D. Bottero, D. Terracciano, O. De Cobelli, G. Morgia, and R. Damiano. 2015. Prognostic accuracy of Prostate Health Index and urinary Prostate Cancer Antigen 3 in predicting pathologic features after radical prostatectomy. *Urologic Oncology: Seminars and Original Investigations* 33:163.e115-163.e123.

Cawthorn, T.R., J.C. Moreno, M. Dharsee, D. Tran-Thanh, S. Ackloo, P.H. Zhu, G. Sardana, J. Chen, P. Kupchak, L.M. Jacks, N.A. Miller, B.J. Youngson, V. Iakovlev, C.J. Guidos, K.A. Vallis, K.R. Evans, D. McCready, W.L. Leong, and S.J. Done. 2012. Proteomic Analyses Reveal High Expression of Decorin and Endoplasmin (HSP90B1) Are Associated with Breast Cancer Metastasis and Decreased Survival. *PloS one* 7:e30992.

Centers for Disease Control and Prevention. 2015. Prostate Cancer Rates by State. In C.f.D.C.a. Prevention, editor.

Chakravarthi, B.V.S.K., S.S. Pathi, M.T. Goswami, M. Cieślik, H. Zheng, S. Nallasivam, S.R. Arekapudi, X. Jing, J. Siddiqui, J. Athanikar, S.L. Carskadon, R.J. Lonigro, L.P. Kunju, A.M. Chinnaiyan, N. Palanisamy, and S. Varambally. 2014. The miR-124-Prolyl Hydroxylase P4HA1-MMP1 axis plays a critical role in prostate cancer progression.

Chan, A.T., Y. Baba, K. Shima, K. Nosho, D.C. Chung, K.E. Hung, U. Mahmood, K. Madden, K. Poss, A. Ranieri, D. Shue, R. Kucherlapati, C.S. Fuchs, and S. Ogino. 2010. Cathepsin B Expression and Survival in Colon Cancer: Implications for Molecular Detection of Neoplasia. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 19:2777-2785.

Chang, W.-C., S.-L. Wu, W.-C. Huang, J.-Y. Hsu, S.-H. Chan, J.-M. Wang, J.-P. Tsai, and B.-K. Chen. 2015. PTX3 gene activation in EGF-induced head and neck cancer cell metastasis. *Oncotarget* 6:7741-7757.

Chargaff, E., and R. West. 1946. The biological significance of thromboplastic protein of blood. *J Biol Chem* 166:189-197.

Chen, D., Zhang, R., Shen, W., Fu, H., Liu, S., Sun, K., Sun, X. 2013. RPS12-specific shRNA inhibits the proliferation, migration of BGC823 gastric cancer cells with S100A4 as a downstream effector. *International Journal of Oncology* 42:1763-1769.

Chen, Z., A. Gerhold-Ay, S. Gebhard, D. Boehm, C. Solbach, A. Lebrecht, M. Battista, I. Sicking, C. Cotarelo, C. Cadenas, R. Marchan, J.D. Stewart, M. Gehrmann, H. Koelbl, J.G. Hengstler, and M. Schmidt. 2012. Immunoglobulin Kappa C Predicts Overall Survival in Node-Negative Breast Cancer. *PloS one* 7:e44741.

Cheng, Y., M.P. Holloway, K. Nguyen, D. McCauley, Y. Landesman, M.G. Kauffman, S. Shacham, and R.A. Altura. 2014. XPO1 (CRM1) inhibition represses STAT3 activation to drive a survivin-dependent oncogenic switch in triple negative breast cancer. *Molecular cancer therapeutics* 13:675-686.

Chitadze, G., M. Lettau, J. Bhat, D. Wesch, A. Steinle, D. Fürst, J. Mytilineos, H. Kalthoff, O. Janssen, H.-H. Oberg, and D. Kabelitz. 2013. Shedding of endogenous MHC class I-related chain molecules A and B from different human tumor entities: Heterogeneous involvement of the “a disintegrin and metalloproteases” 10 and 17. *International Journal of Cancer* 133:1557-1566.

Cho, H., H.Y. Shin, S. Kim, J.S. Kim, J.Y. Chung, E.J. Chung, K.H. Chun, S.M. Hewitt, and J.H. Kim. 2014. The role of S100A14 in epithelial ovarian tumors. *Oncotarget* 5:3482-3496.

Chu, S.-h., Y.-w. Liu, L. Zhang, B. Liu, L. Li, and J.-z. Shi. 2013. Regulation of survival and chemoresistance by HSP90AA1 in ovarian cancer SKOV3 cells. *Molecular Biology Reports* 40:1-6.

Clark, E.L., A. Coulson, C. Dalgliesh, P. Rajan, S.M. Nicol, S. Fleming, R. Heer, L. Gaughan, H.Y. Leung, D.J. Elliott, F.V. Fuller-Pace, and C.N. Robson. 2008. The RNA Helicase p68 Is a Novel Androgen Receptor Coactivator Involved in Splicing and Is Overexpressed in Prostate Cancer. *Cancer Research* 68:7938-7946.

Cochran, B.J., D.R. Croucher, S. Lobov, D.N. Saunders, and M. Ranson. 2011. Dependence on Endocytic Receptor Binding via a Minimal Binding Motif Underlies the Differential Prognostic Profiles of SerpinE1 and SerpinB2 in Cancer. *The Journal of Biological Chemistry* 286:24467-24475.

Cocucci, E., G. Racchetti, and J. Meldolesi. 2009. Shedding microvesicles: artefacts no more. *Trends Cell Biol* 19:43-51.

Conrad, F., X. Zhu, X. Zhang, R.J. Chalkley, A.L. Burlingame, J.D. Marks, and B. Liu. 2009. Human antibodies targeting cell surface antigens overexpressed by the hormone refractory metastatic prostate cancer cells: ICAM-1 is a tumor antigen that mediates prostate cancer cell invasion. *Journal of Molecular Medicine (Berlin, Germany)* 87:507-514.

Cooperberg, M.R. 2013. Re-Examining Racial Disparities in Prostate Cancer Outcomes. *Journal of Clinical Oncology* 31:2979-2980.

Costa, V.L., R. Henrique, S.A. Danielsen, S. Duarte-Pereira, M. Eknaes, R.I. Skotheim, Â. Rodrigues, J.S. Magalhães, J. Oliveira, R.A. Lothe, M.R. Teixeira, C. Jerónimo, and G.E. Lind. 2010. Three Epigenetic Biomarkers, GDF15, TMEFF2, and VIM, Accurately Predict Bladder Cancer from DNA-Based Analyses of Urine Samples. *American Association for Cancer Research* 16:5842-5851.

Coumans, J.V.F., D. Gau, A. Poljak, V. Wasinger, P. Roy, and P.D.J. Moens. 2014. Profilin-1 Overexpression in MDA-MB-231 Breast Cancer Cells Is Associated with Alterations in Proteomics Biomarkers of Cell Proliferation, Survival, and Motility as Revealed by Global Proteomics Analyses. *OMICS : a Journal of Integrative Biology* 18:778-791.

Cui, W., S.Y. Li, J.F. Du, Z.M. Zhu, and P. An. 2012. Silencing phospholipid scramblase 1 expression by RNA interference in colorectal cancer and metastatic liver cancer. *Hepatobiliary Pancreat Dis Int* 11:393-400.

Damhofer, H., J.P. Medema, V.L. Veenstra, L. Badea, I. Popescu, H. Roelink, and M.F. Bijlsma. 2013. Assessment of the stromal contribution to Sonic Hedgehog-dependent pancreatic adenocarcinoma. *Molecular oncology* 7:10.1016/j.molonc.2013.1008.1004.

Das, D.K., J.R. Osborne, H.-Y. Lin, J.Y. Park, and O.O. Ogunwobi. 2016. miR-1207-3p Is a Novel Prognostic Biomarker of Prostate Cancer(). *Translational Oncology* 9:236-241.

Dayal, S., A. Sparks, J. Jacob, N. Allende-Vega, D.P. Lane, and M.K. Saville. 2009. Suppression of the Deubiquitinating Enzyme USP5 Causes the Accumulation of Unanchored Polyubiquitin and the Activation of p53. *Journal of Biological Chemistry* 284:5030-5041.

de Haas, E.C., N. Zwart, C. Meijer, J. Nuver, H.M. Boezen, A.J.H. Suurmeijer, H.J. Hoekstra, G. van der Steege, D.T. Sleijfer, and J.A. Gietema. 2008. Variation in Bleomycin Hydrolase Gene Is Associated With Reduced Survival After Chemotherapy for Testicular Germ Cell Cancer. *Journal of Clinical Oncology* 26:1817-1823.

De Marzo, A.M., E.A. Platz, S. Sutcliffe, J. Xu, H. Gronberg, C.G. Drake, Y. Nakai, W.B. Isaacs, and W.G. Nelson. 2007. Inflammation in prostate carcinogenesis. *Nat Rev Cancer* 7:256-269.

Dehm, S.M., and D.J. Tindall. 2011. Alternatively Spliced Androgen Receptor Variants. *Endocrine-related cancer* 18:R183-R196.

Deng, S., H. Zhou, R. Xiong, Y. Lu, D. Yan, T. Xing, L. Dong, E. Tang, and H. Yang. 2007. Over-expression of genes and proteins of ubiquitin specific peptidases (USPs) and proteasome subunits (PSs) in breast cancer tissue observed by the methods of RFDD-PCR and proteomics. *Breast Cancer Research and Treatment* 104:21-30.

DeSantis, C., D. Naishadham, and A. Jemal. 2013. Cancer statistics for African Americans, 2013. *CA: A Cancer Journal for Clinicians* 63:151-166.

Ding, W., X.-L. Fan, X. Xu, J.-Z. Huang, S.-H. Xu, Q. Geng, R. Li, D. Chen, and G.-R. Yan. 2015. Epigenetic Silencing of ITGA2 by MiR-373 Promotes Cell Migration in Breast Cancer. *PloS one* 10:e0135128.

Duijvesz, D., T. Luider, C.H. Bangma, and G. Jenster. 2011. Exosomes as Biomarker Treasure Chests for Prostate Cancer. *European Urology* 59:823-831.

Eichelser, C., I. Stückrath, V. Müller, K. Milde-Langosch, H. Wikman, K. Pantel, and H. Schwarzenbach. 2014. Increased serum levels of circulating exosomal microRNA-373 in receptor-negative breast cancer patients. *Oncotarget* 5:9650-9663.

Elakoum, R., G. Gauchotte, A. Oussalah, M.-P. Wissler, C. Clément-Duchêne, J.-M. Vignaud, J.-L. Guéant, and F. Namour. 2014. CARM1 and PRMT1 are dysregulated in lung cancer without hierarchical features. *Biochimie* 97:210-218.

Elsner, M., S. Rauser, S. Maier, C. Schöne, B. Balluff, S. Meding, G. Jung, M. Nipp, H. Sarioglu, G. Maccarrone, M. Aichler, A. Feuchtinger, R. Langer, U. Jütting, M. Feith, B. Küster, M. Ueffing, H. Zitzelsberger, H. Höfler, and A. Walch. 2012. MALDI imaging mass spectrometry reveals COX7A2, TAGLN2 and S100-A10 as novel prognostic markers in Barrett's adenocarcinoma. *Journal of Proteomics* 75:4693-4704.

Epis, M.R., K.M. Giles, F.C. Kalinowski, A. Barker, R.J. Cohen, and P.J. Leedman. 2012. Regulation of Expression of Deoxyhypusine Hydroxylase (DOHH), the Enzyme That Catalyzes the Activation of eIF5A, by miR-331-3p and miR-642-5p in Prostate Cancer Cells. *Journal of Biological Chemistry* 287:35251-35259.

Fan, H., Y. Lu, H. Qin, Y. Zhou, Y. Gu, J. Zhou, X. Wang, and D. Fan. 2013a. High Ran level is correlated with poor prognosis in patients with colorectal cancer. *International Journal of Clinical Oncology* 18:856-863.

Fan, M., Y. Liu, F. Xia, Z. Wang, Y. Huang, J. Li, Z. Wang, and X. Li. 2013b. Increased expression of EphA2 and E-N cadherin switch in primary hepatocellular carcinoma. 99:689.

Fang, Z., W. Yao, Y. Xiong, J. Zhang, L. Liu, J. Li, C. Zhang, and J. Wan. 2010. Functional elucidation and methylation-mediated downregulation of ITGA5 gene in breast cancer cell line MDA-MB-468. *Journal of Cellular Biochemistry* 110:1130-1141.

Farfsing, A., F. Engel, M. Seiffert, E. Hartmann, G. Ott, A. Rosenwald, S. Stilgenbauer, H. Dohner, M. Boutros, P. Lichter, and A. Pscherer. 2009. Gene knockdown studies revealed CCDC50 as a candidate gene in mantle cell lymphoma and chronic lymphocytic leukemia. *Leukemia* 23:2018-2026.

Feldman, B.J., and D. Feldman. 2001. The development of androgen-independent prostate cancer. *Nat Rev Cancer* 1:34-45.

Fernebro, J., P. Francis, P. Edén, Å. Borg, I. Panagopoulos, F. Mertens, J. Vallon-Christersson, M. Åkerman, A. Rydholm, H.C.F. Bauer, N. Mandahl, and M. Nilbert. 2006. Gene expression profiles relate to SS18/SSX fusion type in synovial sarcoma. *International Journal of Cancer* 118:1165-1172.

Fraga, A., R. Ribeiro, P. Príncipe, C. Lopes, and R. Medeiros. 2015. Hypoxia and Prostate Cancer Aggressiveness: A Tale With Many Endings. *Clinical Genitourinary Cancer*

Frankland-Searby, S., and S.R. Bhaumik. 2012. The 26S Proteasome Complex: An Attractive Target for Cancer Therapy. *Biochimica et biophysica acta* 1825:64-76.

Fu, Q.-F., Y. Liu, Y. Fan, S.-N. Hua, H.-Y. Qu, S.-W. Dong, R.-L. Li, M.-Y. Zhao, Y. Zhen, X.-L. Yu, Y.-Y. Chen, R.-C. Luo, R. Li, L.-B. Li, X.-J. Deng, W.-Y. Fang, Z. Liu, and X. Song. 2015. Alpha-enolase promotes cell glycolysis, growth, migration, and invasion in non-small cell lung cancer through FAK-mediated PI3K/AKT pathway. *Journal of Hematology & Oncology* 8:22.

Fukumoto, M., S. Kurisu, T. Yamada, and T. Takenawa. 2015. α-Actinin-4 Enhances Colorectal Cancer Cell Invasion by Suppressing Focal Adhesion Maturation. *PloS one* 10:e0120616.

Fuse, M., Nohata, N., Kojima, S., Sakamoto, S., Chiyomaru, T., Kawakami, K., Enokida, H., Nakagawa, M., Naya, Y., Ichikawa, T., Seki, N. 2011. Restoration of miR-145 expression suppresses cell proliferation, migration and invasion in prostate cancer by targeting FSCN1. *International Journal of Oncology* 38:1093-1101.

Gao, C., L. Pang, C. Ren, and T. Ma. 2012. Decreased expression of Nedd4L correlates with poor prognosis in gastric cancer patient. *Medical Oncology* 29:1733-1738.

Gao, Y., J.O. Thomas, R.L. Chow, G.-H. Lee, and N.J. Cowan. 1992. A cytoplasmic chaperonin that catalyzes β-actin folding. *Cell* 69:1043-1050.

Gerritsen, W., and P. Sharma. 2012. Current and Emerging Treatment Options for Castration-Resistant Prostate Cancer: A Focus on Immunotherapy. *Journal of Clinical Immunology* 32:25-35.

Glasauer, A., L.A. Sena, L.P. Diebold, A.P. Mazar, and N.S. Chandel. 2014. Targeting SOD1 reduces experimental non–small-cell lung cancer. *The Journal of Clinical Investigation* 124:117-128.

Goldenberg-Furmanov, M., I. Stein, E. Pikarsky, H. Rubin, S. Kasem, M. Wygoda, I. Weinstein, H. Reuveni, and S.A. Ben-Sasson. 2004. Lyn Is a Target Gene for Prostate Cancer. *Cancer Research* 64:1058-1066.

Gonzales, P.A., T. Pisitkun, J.D. Hoffert, D. Tchapyjnikov, R.A. Star, R. Kleta, N.S. Wang, and M.A. Knepper. 2009. Large-Scale Proteomics and Phosphoproteomics of Urinary Exosomes. *Journal of the American Society of Nephrology* 20:363-379.

Grindel, B., Q. Li, R. Arnold, J. Petros, M. Zayzafoon, M. Muldoon, J. Stave, L.W.K. Chung, and M.C. Farach-Carson. 2016. Perlecan/HSPG2 and matrilysin/MMP-7 as indices of tissue invasion: tissue localization and circulating perlecan fragments in a cohort of 288 radical prostatectomy patients.

Grindel, B.J., J.R. Martinez, C.L. Pennington, M. Muldoon, J. Stave, L.W. Chung, and M.C. Farach-Carson. 2014. Matrilysin/matrix metalloproteinase-7(MMP7) cleavage of perlecan/HSPG2 creates a molecular switch to alter prostate cancer cell behavior. *Matrix Biology* 36:64-76.

Guan, H., P. Zhao, Z. Dai, X. Liu, and X. Wang. 2016. SH3GL1 inhibition reverses multidrug resistance in colorectal cancer cells by downregulation of MDR1/P-glycoprotein via EGFR/ERK/AP-1 pathway. *Tumor Biology* 1-8.

Guest, S.T., Z.R. Kratche, A. Bollig-Fischer, R. Haddad, and S.P. Ethier. 2015. Two members of the TRiC chaperonin complex, CCT2 and TCP1 are essential for survival of breast cancer cells and are linked to driving oncogenes. *Experimental Cell Research* 332:223-235.

Guo, C., S. Liu, J. Wang, M.-Z. Sun, and F.T. Greenaway. 2013a. ACTB in cancer. *Clinica Chimica Acta* 417:39-44.

Guo, Q., Y. Song, H. Zhang, X. Wu, P. Xia, and C. Dang. 2013b. Detection of hypermethylated fibrillin-1 in the stool samples of colorectal cancer patients. *Medical Oncology* 30:1-5.

Haas, M., C. Kern, S. Kruger, M. Michl, D.P. Modest, C. Giessen, C. Schulz, J.C. von Einem, S. Ormanns, R.P. Laubender, S. Holdenrieder, V. Heinemann, and S. Boeck. 2015. Assessing novel prognostic serum biomarkers in advanced pancreatic cancer: the role of CYFRA 21-1, serum amyloid A, haptoglobin, and 25-OH vitamin D3. *Tumor Biology* 36:2631-2640.

Hanahan, D., and Robert A. Weinberg. 2011. Hallmarks of Cancer: The Next Generation. *Cell* 144:646-674.

Haslene-Hox, H., E. Oveland, K. Woie, H.B. Salvesen, H. Wiig, and O. Tenstad. 2013. Increased WD-repeat containing protein 1 in interstitial fluid from ovarian carcinomas shown by comparative proteomic analysis of malignant and healthy gynecological tissue. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* 1834:2347-2359.

He, P., Jiang, S., Ma, M., Wang, Y., Li, R., Fang, F., Tian, G., Zhang, Z. 2015. Trophoblast glycoprotein promotes pancreatic ductal adenocarcinoma cell metastasis through Wnt/planar cell polarity signaling. *Molecular Medicine Reports* 12:503-509.

Heinzelmann-Schwarz, V.A., M. Gardiner-Garden, S.M. Henshall, J. Scurry, R.A. Scolyer, M.J. Davies, M. Heinzelmann, L.H. Kalish, A. Bali, J.G. Kench, L.S. Edwards, P.M.V. Bergh, N.F. Hacker, R.L. Sutherland, and P.M. O'Brien. 2004. Overexpression of the Cell Adhesion Molecules DDR1, Claudin 3, and Ep-CAM in Metaplastic Ovarian Epithelium and Ovarian Cancer. *American Association for Cancer Research* 10:4427-4436.

Held-Feindt, J., and R. Mentlein. 2002. CD70/CD27 ligand, a member of the TNF family, is expressed in human brain tumors. *International Journal of Cancer* 98:352-356.

Hernandez-Vargas, H., J. Palacios, and G. Moreno-Bueno. 2006. Molecular profiling of docetaxel cytotoxicity in breast cancer cells: uncoupling of aberrant mitosis and apoptosis. *Oncogene* 26:2902-2913.

Herrera-Molina, R., A. Valdivia, M. Kong, A. Alvarez, A. Cardenas, A.F. Quest, and L. Leyton. 2013. Thy-1-interacting molecules and cellular signaling in cis and trans. *Int Rev Cell Mol Biol* 305:163-216.

Hevir, N., J. Šinkovec, and T. Lanišnik Rižner. 2013. Decreased levels of AKR1B1 and AKR1B10 in cancerous endometrium compared to adjacent non-cancerous tissue. *Chemico-Biological Interactions* 202:226-233.

Ho, K.C., Z. Zhou, Y.-M. She, A. Chun, T.D. Cyr, and X. Yang. 2011. Itch E3 ubiquitin ligase regulates large tumor suppressor 1 stability. *Proceedings of the National Academy of Sciences of the United States of America* 108:4870-4875.

Holland, J.F. 2003. Cardinal Manifestations of Cancer. In Holland-Frei Cancer Medicine R.E.P. Donald W Kufe, Ralph R Weichselbaum, Robert C Bast, Ted S Gansler, James F Holland, MD, and Emil Frei III, editor BC Decker, Hamilton, ON.

Hong, Y., C. Beckett, N.D. Belyaev, and A.J. Turner. 2012. The impact of amyloid precursor protein signalling and histone deacetylase inhibition on neprilysin expression in human prostate cells. *International Journal of Cancer* 130:775-786.

Horoszewicz, J.S., S.S. Leong, E. Kawinski, J.P. Karr, H. Rosenthal, T.M. Chu, E.A. Mirand, and G.P. Murphy. 1983. LNCaP Model of Human Prostatic Carcinoma. *Cancer Research* 43:1809-1818.

Hosseini-Beheshti, E., W. Choi, L.-B. Weiswald, G. Kharmate, M. Ghaffari, M. Roshan-Moniri, M.D. Hassona, L. Chan, M.Y. Chin, I.T. Tai, P.S. Rennie, L. Fazli, and E.S.T. Guns. 2016. Exosomes confer pro-survival signals to alter the phenotype of prostate cells in their surrounding environment. *Oncotarget* 7:14639-14658.

Hosseini-Beheshti, E., S. Pham, H. Adomat, N. Li, and E.S. Guns. 2012. Exosomes as Biomarker Enriched Microvesicles: Characterization of Exosomal Proteins derived from a Panel of Prostate Cell Lines with distinct AR phenotypes. *Molecular & Cellular Proteomics*

Hu, Z., B. Rao, S. Chen, and J. Duanmu. 2010. Targeting tissue factor on tumour cells and angiogenic vascular endothelial cells by factor VII-targeted verteporfin

photodynamic therapy for breast cancer in vitro and in vivo in mice. *BMC Cancer* 10:235-235.

Hua, M., S. Yan, Y.A.N. Deng, Q. Xi, R. Liu, S. Yang, J. Liu, C. Tang, Y. Wang, and J. Zhong. 2015. CAP1 is overexpressed in human epithelial ovarian cancer and promotes cell proliferation. *International Journal of Molecular Medicine* 35:941-949.

Huang, X., G. Ji, Y. Wu, B. Wan, and L. Yu. 2008. LAMA4, highly expressed in human hepatocellular carcinoma from Chinese patients, is a novel marker of tumor invasion and metastasis. *Journal of Cancer Research and Clinical Oncology* 134:705-714.

Huang, Y., X. Zhang, W. Jiang, Y. Wang, H. Jin, X. Liu, and C. Xu. Discovery of serum biomarkers implicated in the onset and progression of serous ovarian cancer in a rat model using iTRAQ technique. *European Journal of Obstetrics and Gynecology and Reproductive Biology* 165:96-103.

Huang, Y., X. Zhang, W. Jiang, Y. Wang, H. Jin, X. Liu, and C. Xu. 2012. Discovery of serum biomarkers implicated in the onset and progression of serous ovarian cancer in a rat model using iTRAQ technique. *Eur J Obstet Gynecol Reprod Biol* 165:96-103.

Iguchi, Y., S. Ishihara, Y. Uchida, K. Tajima, T. Mizutani, K. Kawabata, and H. Haga. 2015. Filamin B Enhances the Invasiveness of Cancer Cells into 3D Collagen Matrices. *Cell Structure and Function* 40:61-67.

International Agency for Research on Cancer. 2012. GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevelance Worldwide in 2012. In W.H. Organization, editor Lyon.

Ioannou, M.S., E.S. Bell, M. Girard, M. Chaineau, J.N.R. Hamlin, M. Daubaras, A. Monast, M. Park, L. Hodgson, and P.S. McPherson. 2015. DENND2B activates Rab13 at the leading edge of migrating cells and promotes metastatic behavior. *The Journal of Cell Biology* 208:629-648.

Itoh, T., Y. Ito, Y. Ohtsuki, M. Ando, Y. Tsukamasa, N. Yamada, T. Naoe, and Y. Akao. 2012. Microvesicles released from hormone-refractory prostate cancer cells facilitate mouse pre-osteoblast differentiation. *J Mol Hist* 43:509-515.

Jang, S.Y., S.W. Jang, and J. Ko. 2012. Regulation of ADP-ribosylation factor 4 expression by small leucine zipper protein and involvement in breast cancer cell migration. *Cancer Letters* 314:185-197.

Janowska-Wieczorek, A., M. Wysoczynski, J. Kijowski, L. Marquez-Curtis, B. Machalinski, J. Ratajczak, and M.Z. Ratajczak. 2005. Microvesicles derived from activated platelets induce metastasis and angiogenesis in lung cancer. *International Journal of Cancer* 113:752-760.

Jansen, F.H., R.H.N. van Schaik, J. Kurstjens, W. Horninger, H. Klocker, J. Bektic, M.F. Wildhagen, M.J. Roobol, C.H. Bangma, and G. Bartsch. 2010. Prostate-Specific Antigen (PSA) Isoform p2PSA in Combination with Total PSA and Free PSA Improves Diagnostic Accuracy in Prostate Cancer Detection. *European Urology* 57:921-927.

Januchowski, R., P. Zawierucha, M. Ruciński, M. Nowicki, and M. Zabel. 2014. Extracellular Matrix Proteins Expression Profiling in Chemoresistant Variants of the A2780 Ovarian Cancer Cell Line. *BioMed Research International* 2014:365867.

Jeanne, A., C. Schneider, L. Martiny, and S. Dedieu. 2015. Original insights on thrombospondin-1-related antireceptor strategies in cancer. *Frontiers in Pharmacology* 6:252.

Jemal, A., M.M. Center, C. DeSantis, and E.M. Ward. 2010. Global Patterns of Cancer Incidence and Mortality Rates and Trends. *Cancer Epidemiology Biomarkers & Prevention* 19:1893-1907.

Jeon, Y.J., S. Khelifa, B. Ratnikov, D.A. Scott, Y. Feng, F. Parisi, C. Ruller, E. Lau, H. Kim, L.M. Brill, T. Jiang, D.L. Rimm, R.D. Cardiff, G.B. Mills, J.W. Smith, A.L. Osterman, Y. Kluger, and Z.A. Ronai. 2015. Regulation of Glutamine Carrier Proteins by RNF5 Determines Breast Cancer Response to ER Stress-Inducing Chemotherapies. *Cancer Cell* 27:354-369.

Jia, D., Y. Jing, Z. Zhang, L. Liu, J. Ding, F. Zhao, C. Ge, Q. Wang, T. Chen, M. Yao, J. Li, J. Gu, and X. He. 2014. Amplification of MPZL1/PZR promotes tumor cell migration through Src-mediated phosphorylation of cortactin in hepatocellular carcinoma. *Cell Res* 24:204-217.

Jia, J.-W., K.-L. Li, J.-X. Wu, and S.-L. Guo. 2013. Clinical significance of annexin II expression in human non-small cell lung cancer. *Tumor Biology* 34:1767-1771.

Johnstone, R.M., M. Adam, J.R. Hammond, L. Orr, and C. Turbide. 1987. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). *Journal of Biological Chemistry* 262:9412-9420.

Jung, Y., Y. Shiozawa, J. Wang, J. Wang, Z. Wang, E.A. Pedersen, C.H. Lee, C.L. Hall, P.J. Hogg, P.H. Krebsbach, E.T. Keller, and R.S. Taichman. 2009. Expression of PGK1 by Prostate Cancer Cells Induces Bone Formation. *American Association for Cancer Research* 7:1595-1604.

Kalinina, E.V., T.T. Berezov, A.A. Shtil', N.N. Chernov, V.A. Glazunova, M.D. Novichkova, and N.K. Nurmuradov. 2012. Expression of Peroxiredoxin 1, 2, 3, and 6 Genes in Cancer Cells during Drug Resistance Formation. *Bulletin of Experimental Biology and Medicine* 153:879-882.

Kalra, H., R.J. Simpson, H. Ji, E. Aikawa, P. Altevogt, P. Askenase, V.C. Bond, F.E. Borras, X. Breakefield, V. Budnik, E. Buzas, G. Camussi, A. Clayton, E. Cocucci, J.M. Falcon-Perez, S. Gabrielsson, Y.S. Gho, D. Gupta, H.C. Harsha, A. Hendrix, A.F. Hill, J.M. Inal, G. Jenster, E.M. Kramer-Albers, S.K. Lim, A. Llorente, J. Lotvall, A. Marcilla, L. Mincheva-Nilsson, I. Nazarenko, R. Nieuwland, E.N. Nolte-'t Hoen, A. Pandey, T. Patel, M.G. Piper, S. Pluchino, T.S. Prasad, L. Rajendran, G. Raposo, M. Record, G.E. Reid, F. Sanchez-Madrid, R.M. Schiffelers, P. Siljander, A. Stensballe, W. Stoorvogel, D. Taylor, C. Thery, H. Valadi, B.W. van Balkom, J. Vazquez, M. Vidal, M.H. Wauben, M. Yanez-Mo, M. Zoeller, and S. Mathivanan. 2012. Vesiclepedia: a compendium for extracellular vesicles with continuous community annotation. *PLoS Biol* 10:e1001450.

Kao, H.-K., W.F. Chen, C.-H. Chen, V.B.-H. Shyu, M.-H. Cheng, and K.-P. Chang. 2012. The Roles of Albumin Levels in Head and Neck Cancer Patients with Liver Cirrhosis Undergoing Tumor Ablation and Microsurgical Free Tissue Transfer. *PLoS ONE* 7:e52678.

Karve, T.M., A. Preet, R. Sneed, C. Salamanca, X. Li, J. Xu, D. Kumar, E.M. Rosen, and T. Saha. 2012. BRCA1 Regulates Follistatin Function in Ovarian Cancer and Human Ovarian Surface Epithelial Cells. *PLoS ONE* 7:e37697.

Kasina, S., P.A. Scherle, C.L. Hall, and J.A. Macoska. 2009. ADAM-mediated amphiregulin shedding and EGFR transactivation. *Cell Proliferation* 42:799-812.

Kataoka, H., M. Tanaka, M. Kanamori, S. Yoshii, M. Ihara, Y.-J. Wang, J.-P. Song, Z.-Y. Li, H. Arai, Y. Otsuki, T. Kobayashi, H. Konno, H. Hanai, and H. Sugimura. 2002. Expression profile of EFNB1, EFNB2, two ligands of EPHB2 in human gastric cancer. *Journal of Cancer Research and Clinical Oncology* 128:343-348.

Kawakami, K., Fujita, Y., Kato, T., Mizutani, K., Kameyama, K., Tsumoto, H., Miura, Y., Deguchi, T., Ito, M. 2015. Integrin β4 and vinculin contained in exosomes are

potential markers for progression of prostate cancer associated with taxane-resistance. *International Journal of Oncology* 47:384-390.

Keshava, S., S. Sahoo, T.A. Tucker, S. Idell, L.V.M. Rao, and U.R. Pendurthi. 2013. Endothelial Cell Protein C Receptor Opposes Mesothelioma Growth Driven by Tissue Factor. *Cancer Research* 73:3963-3973.

Kim, D., B.P. Fiske, K. Birsoy, E. Freinkman, K. Kami, R.L. Possemato, Y. Chudnovsky, M.E. Pacold, W.W. Chen, J.R. Cantor, L.M. Shelton, D.Y. Gui, M. Kwon, S.H. Ramkissoon, K.L. Ligon, S.W. Kang, M. Snuderl, M.G. Vander Heiden, and D.M. Sabatini. 2015. SHMT2 drives glioma cell survival in ischaemia but imposes a dependence on glycine clearance. *Nature* 520:363-367.

Kim, Y., J. Jeon, S. Mejia, C.Q. Yao, V. Ignatchenko, J.O. Nyalwidhe, A.O. Gramolini, R.S. Lance, D.A. Troyer, R.R. Drake, P.C. Boutros, O.J. Semmes, and T. Kislinger. 2016. Targeted proteomics identifies liquid-biopsy signatures for extracapsular prostate cancer. *Nat Commun* 7:

Kinoshita, T., N. Nohata, M. Fuse, T. Hanazawa, N. Kikkawa, L. Fujimura, H. Watanabe-Takano, Y. Yamada, H. Yoshino, H. Enokida, M. Nakagawa, Y. Okamoto, and N. Seki. 2012. Tumor suppressive microRNA-133a regulates novel targets: Moesin contributes to cancer cell proliferation and invasion in head and neck squamous cell carcinoma. *Biochemical and Biophysical Research Communications* 418:378-383.

Ko, A., J.-Y. Shin, J. Seo, K.-D. Lee, E.-W. Lee, M.-S. Lee, H.-W. Lee, I.-J. Choi, J.S. Jeong, K.-H. Chun, and J. Song. 2012. Acceleration of Gastric Tumorigenesis Through MKRN1-Mediated Posttranslational Regulation of p14ARF. *JNCI Journal of the National Cancer Institute* 104:1660-1672.

Koochekpour, S., G.A. Maresh, A. Katner, K. Parker-Johnson, T.-J. Lee, F.E. Hebert, Y.S. Kao, J. Skinner, and W. Rayford. 2004. Establishment and characterization of a

primary androgen-responsive African-American prostate cancer cell line, E006AA. *The Prostate* 60:141-152.

Koochekpour, S., S.S. Willard, M. Shourideh, S. Ali, C. Liu, G. Azabdaftari, M. Saleem, and K. Attwood. 2014. Establishment and Characterization of a Highly Tumorigenic African American Prostate Cancer Cell Line, E006AA-hT. *International Journal of Biological Sciences* 10:834-845.

Korwar, A.M., H.S. Bhonsle, A.D. Chougale, S.S. Kote, K.R. Gawai, V.S. Ghole, C.B. Koppikar, and M.J. Kulkarni. 2012. Analysis of AGE modified proteins and RAGE expression in HER2/neu negative invasive ductal carcinoma. *Biochemical and Biophysical Research Communications* 419:490-494.

Korzeniewski, N., M. Hohenfellner, and S. Duensing. 2012. CAND1 Promotes PLK4-Mediated Centriole Overduplication and Is Frequently Disrupted in Prostate Cancer. *Neoplasia (New York, N.Y.)* 14:799-806.

Kumagai, K., Y. Nimura, A. Mizota, N. Miyahara, M. Aoki, Y. Furusawa, M. Takiguchi, S. Yamamoto, and N. Seki. 2006. Arpc1b Gene Is a Candidate Prediction Marker for Choroidal Malignant Melanomas Sensitive to Radiotherapy. *Investigative Ophthalmology & Visual Science* 47:2300-2304.

Ladha, J., S. Sinha, V. Bhat, S. Donakonda, and S.M.R. Rao. 2012. Identification of Genomic Targets of Transcription Factor Aebp1 and its role in Survival of Glioma Cells. *American Association for Cancer Research* 10:1039-1051.

Larrinaga, G., I. Perez, L. Blanco, B. Sanz, P. Errarte, M. Beitia, M.C. Etxezarraga, A. Loizate, J. Gil, J. Irazusta, and J.I. López. 2014. Prolyl Endopeptidase Activity Is Correlated with Colorectal Cancer Prognosis. *International Journal of Medical Sciences* 11:199-208.

Läubli, H., F. Alisson-Silva, M.A. Stanczak, S.S. Siddiqui, L. Deng, A. Verhagen, N. Varki, and A. Varki. 2014. Lectin Galactoside-binding Soluble 3 Binding Protein (LGALS3BP) Is a Tumor-associated Immunomodulatory Ligand for CD33-related Siglecs. *The Journal of Biological Chemistry* 289:33481-33491.

Laxman, B., D.S. Morris, J. Yu, J. Siddiqui, J. Cao, R. Mehra, R.J. Lonigro, A. Tsodikov, J.T. Wei, S.A. Tomlins, and A.M. Chinnaiyan. 2008. A First-Generation Multiplex Biomarker Analysis of Urine for the Early Detection of Prostate Cancer. *Cancer Research* 68:645-649.

Lee, M., E.S. Nam, S.-H. Jung, S.Y. Kim, S.-J. Lee, J.H. Yoon, N.W. Lee, S. Jeon, J.S. Choi, C.-H. Cho, Y. Moon, Y.-J. Chung, and Y. Kwon. 2014. 1p36.22 region containing PGD gene is frequently gained in human cervical cancer. *Journal of Obstetrics and Gynaecology Research* 40:545-553.

Lefrançois-Martinez, A.-M., J. Bertherat, P. Val, C. Tournaire, N. Gallo-Payet, D. Hyndman, G. Veyssière, X. Bertagna, C. Jean, and A. Martinez. 2004. Decreased Expression of Cyclic Adenosine Monophosphate-Regulated Aldose Reductase (AKR1B1) Is Associated with Malignancy in Human Sporadic Adrenocortical Tumors. *The Journal of Clinical Endocrinology & Metabolism* 89:3010-3019.

Leyten, G.H.J.M., E.A. Wierenga, J.P. Michiel Sedelaar, I.M. van Oort, J.J. Futterer, J.O. Barentsz, J.A. Schalken, and P.F.A. Mulders. 2013. Value of PCA3 to Predict Biopsy Outcome and Its Potential Role in Selecting Patients for Multiparametric MRI. *International Journal of Molecular Sciences* 14:11347-11355.

Li, B., G. Wen, Y. Zhao, J. Tong, and T.K. Hei. 2012. The role of TGFBI in mesothelioma and breast cancer: association with tumor suppression. *BMC Cancer* 12:239-239.

Li, M., X. Yang, J. Zhang, H. Shi, Q. Hang, X. Huang, G. Liu, J. Zhu, S. He, and H. Wang. 2013. Effects of EHD2 interference on migration of esophageal squamous cell carcinoma. *Medical Oncology (Northwood, London, England)* 30:396.

Liang, X.-H., L.-l. Li, G.-G. Wu, Y.-C. Xie, G.-X. Zhang, W. Chen, H.-F. Yang, Q.-L. Liu, W.-H. Li, W.-g. He, Y.-N. Huang, and X.-C. Zeng. 2013. Upregulation of CPE promotes cell proliferation and tumorigenicity in colorectal cancer. *BMC Cancer* 13:1-9.

Lim, J.Y., S.O. Yoon, S.W. Hong, J.W. Kim, S.H. Choi, and J.Y. Cho. 2012. Thioredoxin and thioredoxin-interacting protein as prognostic markers for gastric cancer recurrence. *World Journal of Gastroenterology : WJG* 18:5581-5588.

Lin, H., J.-F. Huang, J.-R. Qiu, H.-L. Zhang, X.-J. Tang, H. Li, C.-J. Wang, Z.-C. Wang, Z.-Q. Feng, and J. Zhu. 2013a. Significantly upregulated TACSTD2 and Cyclin D1 correlate with poor prognosis of invasive ductal breast cancer. *Experimental and Molecular Pathology* 94:73-78.

Lin, H.-c., F.-l. Zhang, Q. Geng, T. Yu, Y.-q. Cui, X.-h. Liu, J. Li, M.-x. Yan, L. Liu, X.-h. He, J.-j. Li, and M. Yao. 2013b. Quantitative Proteomic Analysis Identifies CPNE3 as a Novel Metastasis-promoting Gene in NSCLC. *Journal of Proteome Research* 12:3423-3433.

Lin, J., J. Li, B. Huang, J. Liu, X. Chen, X.-M. Chen, Y.-M. Xu, L.-F. Huang, and X.-Z. Wang. 2015. Exosomes: Novel Biomarkers for Clinical Diagnosis. *The Scientific World Journal* 2015:8.

Linge, A., P. Maurya, K. Friedrich, G.B. Baretton, S. Kelly, M. Henry, M. Clynes, A. Larkin, and P. Meleady. 2014. Identification and Functional Validation of RAD23B as a Potential Protein in Human Breast Cancer Progression. *Journal of Proteome Research* 13:3212-3222.

Liu, D., B. Yin, Q. Wang, W. Ju, Y. Chen, H. Qiu, J. Li, X. Peng, and C. Lu. 2012. Cytoplasmic Poly(A) Binding Protein 4 Is Highly Expressed in Human Colorectal Cancer and Correlates with Better Prognosis. *Journal of Genetics and Genomics* 39:369-374.

Liu, H., Y. Gu, H. Wang, J. Yin, G. Zheng, Z. Zhang, M. Lu, C. Wang, and Z. He. 2015. Overexpression of PP2A inhibitor SET oncoprotein is associated with tumor progression and poor prognosis in human non-small cell lung cancer.

Liu, Y., X. Cui, B. Hu, C. Lu, X. Huang, J. Cai, S. He, L. Lv, X. Cong, G. Liu, Y. Zhang, and R. Ni. 2014. Upregulated expression of CAP1 is associated with tumor migration and metastasis in hepatocellular carcinoma. *Pathology - Research and Practice* 210:169-175.

Locatelli, M., S. Ferrero, F. Martinelli Boneschi, L. Boiocchi, M. Zavanone, S. Maria Gaini, L. Bello, S. Valentino, E. Barbati, M. Nebuloni, A. Mantovani, and C. Garlanda. 2013. The long pentraxin PTX3 as a correlate of cancer-related inflammation and prognosis of malignancy in gliomas. *Journal of Neuroimmunology* 260:99-106.

Loeb, S., and W.J. Catalona. 2014. The Prostate Health Index: a new test for the detection of prostate cancer. *Therapeutic Advances in Urology* 6:74-77.

Logothetis, C.J., and S.-H. Lin. 2005. Osteoblasts in prostate cancer metastasis to bone. *Nat Rev Cancer* 5:21-28.

Lohr, M., K. Edlund, J. Botling, S. Hammad, B. Hellwig, A. Othman, A. Berglund, M. Lambe, L. Holmberg, S. Ekman, M. Bergqvist, F. Ponten, C. Cadenas, R. Marchan, J.G. Hengstler, J. Rahnenfuhrer, and P. Micke. 2013. The prognostic relevance of tumour-infiltrating plasma cells and immunoglobulin kappa C indicates an important role of the humoral immune response in non-small cell lung cancer. *Cancer Letters* 333:222-228.

Lomnytska, M.I., S. Becker, K. Hellman, A.-C. Hellström, S. Souchelnytskyi, M. Mints, U. Hellman, S. Andersson, and G. Auer. 2010. Diagnostic protein marker patterns in squamous cervical cancer. *PROTEOMICS – Clinical Applications* 4:17-31.

Lötvall, J., A.F. Hill, F. Hochberg, E.I. Buzás, D. Di Vizio, C. Gardiner, Y.S. Gho, I.V. Kurochkin, S. Mathivanan, P. Quesenberry, S. Sahoo, H. Tahara, M.H. Wauben, K.W. Witwer, and C. Théry. 2014. Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles. *2014*

Marcias, G., E. Erdmann, G. Lapouge, C. Siebert, P. Barthélémy, B. Duclos, J.-P. Bergerat, J. Céraline, and J.-E. Kurtz. 2010. Identification of novel truncated androgen receptor (AR) mutants including unreported pre-mRNA splicing variants in the 22Rv1 hormone-refractory prostate cancer (PCa) cell line. *Human Mutation* 31:74-80.

Mardin, W.A., K.O. Petrov, A. Enns, N. Senninger, J. Haier, and S.T. Mees. 2010. SERPINB5 and AKAP12 -- Expression and promoter methylation of metastasis suppressor genes in pancreatic ductal adenocarcinoma. *BMC Cancer* 10:549-549.

Massoner, P., T. Thomm, B. Mack, G. Untergasser, A. Martowicz, K. Bobowski, H. Klocker, O. Gires, and M. Puhr. 2014. EpCAM is overexpressed in local and metastatic prostate cancer, suppressed by chemotherapy and modulated by MET-associated miRNA-200c/205. *British Journal of Cancer* 111:955-964.

Masugi, Y., K. Yamazaki, K. Emoto, K. Effendi, H. Tsujikawa, M. Kitago, O. Itano, Y. Kitagawa, and M. Sakamoto. 2015. Upregulation of integrin [beta]4 promotes epithelial-mesenchymal transition and is a novel prognostic marker in pancreatic ductal adenocarcinoma. *Lab Invest* 95:308-319.

Matsuda, Y., T. Ishiwata, H. Yoshimura, M. Hagio, and T. Arai. 2015. Inhibition of nestin suppresses stem cell phenotype of glioblastomas through the alteration of post-translational modification of heat shock protein HSPA8/HSC71. *Cancer Letters* 357:602-611.

Mazzoccoli, G., V. Pazienza, A. Panza, M.R. Valvano, G. Benegiamo, M. Vinciguerra, A. Andriulli, and A. Piepoli. 2012. ARNTL2 and SERPINE1: potential biomarkers for tumor aggressiveness in colorectal cancer. *Journal of Cancer Research and Clinical Oncology* 138:501-511.

McCleland, M.L., A.S. Adler, L. Deming, E. Cosino, L. Lee, E.M. Blackwood, M. Solon, J. Tao, L. Li, D. Shames, E. Jackson, W.F. Forrest, and R. Firestein. 2013. Lactate Dehydrogenase B Is Required for the Growth of KRAS-Dependent Lung Adenocarcinomas. *American Association for Cancer Research* 19:773-784.

McGinley, K.F., K.J. Tay, and J.W. Moul. 2016. Prostate cancer in men of African origin. *Nat Rev Urol* 13:99-107.

McNeal, J.E., E.A. Redwine, F.S. Freiha, and T.A. Stamey. 1988. Zonal Distribution of Prostatic Adenocarcinoma: Correlation with Histologic Pattern and Direction of Spread. *The American Journal of Surgical Pathology* 12:897-906.

Meng, F., B. Joshi, and I.R. Nabi. 2015. Galectin-3 Overrides PTRF/Cavin-1 Reduction of PC3 Prostate Cancer Cell Migration. *PloS one* 10:e0126056.

Meng, H., G. Chen, X. Zhang, Z. Wang, D.G. Thomas, T.J. Giordano, D.G. Beer, and M.M. Wang. 2011. Stromal LRP1 in Lung Adenocarcinoma Predicts Clinical Outcome. *American Association for Cancer Research* 17:2426-2433.

Miar, A., D. Hevia, H. Muñoz-Cimadevilla, A. Astudillo, J. Velasco, R.M. Sainz, and J.C. Mayo. 2015. Manganese superoxide dismutase (SOD2/MnSOD)/catalase and SOD2/GPx1 ratios as biomarkers for tumor progression and metastasis in prostate, colon, and lung cancer. *Free Radical Biology and Medicine* 85:45-55.

Miller, M.L., and I. Ojima. 2001. Chemistry and chemical biology of taxane anticancer agents. *The Chemical Record* 1:195-211.

Mohammed N. Abid Hussein, A.N.B., Augueste Sturk, Chi M. Hau, Rienk Nieuwland 2007. Inhibition of microparticle release triggers endothelial cell apoptosis and detachment *Thromb Haemost* 98:1096-10107.

Moon, Y.W., G. Rao, J.J. Kim, H.S. Shim, K.S. Park, S.S. An, B. Kim, P.S. Steeg, S. Sarfaraz, L. Changwoo Lee, D. Voeller, E.Y. Choi, J. Luo, D. Palmieri, H.C. Chung, J.H. Kim, Y. Wang, and G. Giaccone. 2015. LAMC2 enhances the metastatic potential of lung adenocarcinoma. *Cell Death Differ* 22:1341-1352.

Mori, F., K. Tanigawa, K. Endo, K. Minamiguchi, M. Abe, S. Yamada, and K. Miyoshi. 2011. VAT-1 is a novel pathogenic factor of progressive benign prostatic hyperplasia. *The Prostate* 71:1579-1586.

Mori, K., Enokida, H., Kagara, I., Kawakami, K., Chiyomaru, T., Tatarano, S., Kawahara, K., Nishiyama, K., Seki, N., Nakagawa, M. 2009. CpG hypermethylation of collagen type I α 2 contributes to proliferation and migration activity of human bladder cancer. *International Journal of Oncology* 36:1593-1602.

Mullarky, E., K.R. Mattaini, M.G. Vander Heiden, L.C. Cantley, and J.W. Locasale. 2011. PHGDH amplification and altered glucose metabolism in human melanoma. *Pigment Cell & Melanoma Research* 24:1112-1115.

Mullenders, J., W. von der Saal, M.M.W. van Dongen, U. Reiff, R. van Willigen, R.L. Beijersbergen, G. Tiefenthaler, C. Klein, and R. Bernards. 2009. Candidate Biomarkers of Response to an Experimental Cancer Drug Identified through a Large-scale RNA Interference Genetic Screen. *American Association for Cancer Research* 15:5811-5819.

Nagabhushan, M., C.M. Miller, T.P. Pretlow, J.M. Giaconia, N.L. Edgehouse, S. Schwartz, H.-J. Kung, R.W. de Vere White, P.H. Gumerlock, M.I. Resnick, S.B. Amini, and T.G. Pretlow. 1996. CWR22: The First Human Prostate Cancer

Xenograft with Strongly Androgen-dependent and Relapsed Strains Both in Vivo and in Soft Agar. *Cancer Research* 56:3042-3046.

Nakayasu, E.S., T.J.P. Sobreira, R. Torres, L. Ganiko, P.S.L. Oliveira, A.F. Marques, and I.C. Almeida. 2012. Improved proteomic approach for the discovery of potential vaccine targets in Trypanosoma cruzi. *Journal of Proteome Research* 11:237-246.

Nakayasu, E.S., D.V. Yashunsky, L.L. Nohara, A.C.T. Torrecilhas, A.V. Nikolaev, and I.C. Almeida. 2009. GPIomics: global analysis of glycosylphosphatidylinositol-anchored molecules of Trypanosoma cruzi. *Molecular Systems Biology* 5:261-261.

Narayanaswamy, P.B., M. Hodjat, H. Haller, I. Dumler, and Y. Kiyan. 2014. Loss of Urokinase Receptor Sensitizes Cells to DNA Damage and Delays DNA Repair. *PloS one* 9:e101529.

National Cancer Institute. 2012. Prostate-Specific Antigen (PSA) Test. In Prostate Cancer. National Cancer Institute.

National Cancer Institute Survailence, E., and End Results Program. 2015. SEER Stat Fact Sheets: Prostate Cancer. In.

Nawaz, M., G. Camussi, H. Valadi, I. Nazarenko, K. Ekstrom, X. Wang, S. Principe, N. Shah, N.M. Ashraf, F. Fatima, L. Neder, and T. Kislinger. 2014. The emerging role of extracellular vesicles as biomarkers for urogenital cancers. *Nat Rev Urol* 11:688-701.

Nazarian, A., K. Lawlor, S.S. Yi, J. Philip, M. Ghosh, M. Yaneva, J. Villanueva, A. Saghatelian, M. Assel, A.J. Vickers, J.A. Eastham, H.I. Scher, B.S. Carver, H. Lilja, and P. Tempst. 2014. Inhibition of Circulating Dipeptidyl Peptidase 4 Activity in Patients with Metastatic Prostate Cancer. *Molecular & Cellular Proteomics* 13:3082-3096.

Neslund-Dudas, C., A.M. Levin, A. Rundle, J. Beebe-Dimmer, C.H. Bock, N.L. Nock, M. Jankowski, I. Datta, R. Krajenta, Q.P. Dou, B. Mitra, D. Tang, and B.A. Rybicki. 2014. Case-only gene–environment interaction between ALAD tagSNPs and occupational lead exposure in prostate cancer. *The Prostate* 74:637-646.

Nguyen, D.X., P.D. Bos, and J. Massague. 2009. Metastasis: from dissemination to organ-specific colonization. *Nat Rev Cancer* 9:274-284.

Nicholson, A., J. Mahon, and A. Boland. 2015. The clinical effectiveness and cost-effectiveness of the PROGENSA® prostate cancer antigen 3 assay and the Prostate Health Index in the diagnosis of prostate cancer: a systematic review and economic evaluation. In NIHR Journals Library Southampton (UK): .

Nishioka, C., T. Ikezoe, J. Yang, A. Nobumoto, S. Kataoka, M. Tsuda, K. Udaka, and A. Yokoyama. 2014. CD82 regulates STAT5/IL-10 and supports survival of acute myelogenous leukemia cells. *International Journal of Cancer* 134:55-64.

Nohata, N., Y. Sone, T. Hanazawa, M. Fuse, N. Kikkawa, H. Yoshino, T. Chiyomaru, K. Kawakami, H. Enokida, M. Nakagawa, M. Shozu, Y. Okamoto, and N. Seki. 2011. miR-1 as a tumor suppressive microRNA targeting TAGLN2 in head and neck squamous cell carcinoma.

O'Donnell, K.A., D. Yu, K.I. Zeller, J.-w. Kim, F. Racke, A. Thomas-Tikhonenko, and C.V. Dang. 2006. Activation of Transferrin Receptor 1 by c-Myc Enhances Cellular Proliferation and Tumorigenesis. *Molecular and Cellular Biology* 26:2373-2386.

Oh, W.K., M. Hurwitz, A.V. D'Amico, J.P. Richie, and P.W. Kantoff. 2003a. Biology of Prostate Cancer. In Holland-Frei Cancer Medicine. R.E.P. Donald W Kufe, Ralph R Weichselbaum, Robert C Bast, Jr, Ted S Gansler, James F Holland, Emil Frei, editor BC Decker, Hamilton (ON).

Oh, W.K., M. Hurwitz, A.V. D'Amico, J.P. Richie, and P.W. Kantoff. 2003b. Screening for Prostate Cancer. In Holland-Frei Cancer Medicine. R.E.P. Donald W Kufe, Ralph R Weichselbaum, Robert C Bast, Jr, Ted S Gansler, James F Holland, Emil Frei, editor BC Decker, Hamilton (ON).

Ohmura, G., T. Tsujikawa, T. Yaguchi, N. Kawamura, S. Mikami, J. Sugiyama, K. Nakamura, A. Kobayashi, T. Iwata, H. Nakano, T. Shimada, Y. Hisa, and Y. Kawakami. 2015. Aberrant Myosin 1b Expression Promotes Cell Migration and Lymph Node Metastasis of HNSCC. *American Association for Cancer Research* 13:721-731.

Ooe, A., K. Kato, and S. Noguchi. 2007. Possible involvement of CCT5, RGS3, and YKT6 genes up-regulated in p53-mutated tumors in resistance to docetaxel in human breast cancers. *Breast Cancer Research and Treatment* 101:305-315.

Pan, B., S. Zheng, C. Liu, and Y. Xu. 2013. Suppression of IGHG1 gene expression by siRNA leads to growth inhibition and apoptosis induction in human prostate cancer cell. *Molecular Biology Reports* 40:27-33.

Paone, A., M. Marani, A. Fiascarelli, S. Rinaldo, G. Giardina, R. Contestabile, A. Paiardini, and F. Cutruzzolà. 2014. SHMT1 knockdown induces apoptosis in lung cancer cells by causing uracil misincorporation. *Cell Death & Disease* 5:e1525.

Patel, A.R., and E.A. Klein. 2009. Risk factors for prostate cancer. *Nat Clin Pract Urol* 6:87-95.

Pathan, M., S. Keerthikumar, C.-S. Ang, L. Gangoda, C.Y.J. Quek, N.A. Williamson, D. Mouradov, O.M. Sieber, R.J. Simpson, A. Salim, A. Bacic, A.F. Hill, D.A. Stroud, M.T. Ryan, J.I. Agbinya, J.M. Mariadason, A.W. Burgess, and S. Mathivanan. 2015. FunRich: An open access standalone functional enrichment and interaction network analysis tool. *PROTEOMICS* 15:2597-2601.

Peng, Y., X. Li, M. Wu, J. Yang, M. Liu, W. Zhang, B. Xiang, X. Wang, X. Li, G. Li, and S. Shen. 2012. New prognosis biomarkers identified by dynamic proteomic analysis of colorectal cancer. *Molecular BioSystems* 8:3077-3088.

Perrot, G., B. Langlois, J. Devy, A. Jeanne, L. Verzeaux, S. Almagro, H. Sartelet, C. Hachet, C. Schneider, E. Sick, M. David, M. Khrestchatisky, H. Emonard, L. Martiny, and S. Dedieu. 2012. LRP-1–CD44, a New Cell Surface Complex Regulating Tumor Cell Adhesion. *Molecular and Cellular Biology* 32:3293-3307.

Piccolo, E., N. Tinari, D. Semeraro, S. Traini, I. Fichera, A. Cumashi, R. La Sorda, F. Spinella, A. Bagnato, R. Lattanzio, M. D'Egidio, A. Di Risio, P. Stampolidis, M. Piantelli, C. Natoli, A. Ullrich, and S. Iacobelli. 2013. LGALS3BP, lectin galactoside-binding soluble 3 binding protein, induces vascular endothelial growth factor in human breast cancer cells and promotes angiogenesis. *Journal of Molecular Medicine* 91:83-94.

Pinho, S.S., and C.A. Reis. 2015. Glycosylation in cancer: mechanisms and clinical implications. *Nat Rev Cancer* 15:540-555.

Pressinotti, N.C., H. Klocker, G. Schäfer, V.-D. Luu, M. Ruschhaupt, R. Kuner, E. Steiner, A. Poustka, G. Bartsch, and H. Sültmann. 2009. Differential expression of apoptotic genes PDIA3 and MAP3K5 distinguishes between low- and high-risk prostate cancer. *Molecular Cancer* 8:130-130.

Principe, S., E.E. Jones, Y. Kim, A. Sinha, J.O. Nyalwidhe, J. Brooks, O.J. Semmes, D.A. Troyer, R.S. Lance, T. Kislinger, and R.R. Drake. 2013. In-depth proteomic analyses of exosomes isolated from expressed prostatic secretions in urine. *PROTEOMICS* 13:1667-1671.

Qiao, J., S.-J. Cui, L.-L. Xu, S.-J. Chen, J. Yao, Y.-H. Jiang, G. Peng, C.-Y. Fang, P.-Y. Yang, and F. Liu. 2014. Filamin C, a dysregulated protein in cancer revealed by label-free quantitative proteomic analyses of human gastric cancer cells.

Raj, D.A.A., I. Fiume, G. Capasso, and G. Pocsfalvi. 2012. A multiplex quantitative proteomics strategy for protein biomarker studies in urinary exosomes. *Kidney International* 81:1263-1272.

Rao, X., X. Duan, W. Mao, X. Li, Z. Li, Q. Li, Z. Zheng, H. Xu, M. Chen, P.G. Wang, Y. Wang, B. Shen, and W. Yi. 2015. O-GlcNAcylation of G6PD promotes the pentose phosphate pathway and tumor growth. *Nat Commun* 6:

Raposo, G., and W. Stoorvogel. 2013. Extracellular vesicles: Exosomes, microvesicles, and friends. *The Journal of cell biology* 200:373-383.

Rasheed, S.A.K., C.R. Teo, E.J. Beillard, P.M. Voorhoeve, and P.J. Casey. 2013. MicroRNA-182 and MicroRNA-200a Control G-protein Subunit α-13 (GNA13) Expression and Cell Invasion Synergistically in Prostate Cancer Cells. *Journal of Biological Chemistry* 288:7986-7995.

Reddy, G.P.V., E.R. Barrack, Q.P. Dou, M. Menon, R. Pelley, F.H. Sarkar, and S. Sheng. 2006. Regulatory processes affecting androgen receptor expression, stability, and function: Potential targets to treat hormone-refractory prostate cancer. *Journal of Cellular Biochemistry* 98:1408-1423.

Regina, S., J.-B. Valentin, S. Lachot, E. Lemarié, J. Rollin, and Y. Gruel. 2009. Increased Tissue Factor Expression Is Associated with Reduced Survival in Non–Small Cell Lung Cancer and with Mutations of TP53 and PTEN. *Clinical Chemistry* 55:1834-1842.

Rehman, I., C.A. Evans, A. Glen, S.S. Cross, C.L. Eaton, J. Down, G. Pesce, J.T. Phillips, O.S. Yen, G.N. Thalmann, P.C. Wright, and F.C. Hamdy. 2012. iTRAQ Identification of Candidate Serum Biomarkers Associated with Metastatic Progression of Human Prostate Cancer. *PloS one* 7:e30885.

Reif, A.E., and J.M. Allen. 1964. The Akr Thymic Antigen and Its Distribution in Leukemias and Nervous Tissues. *J Exp Med* 120:413-433.

Reinert, T., M. Borre, A. Christiansen, G.G. Hermann, T.F. Ørntoft, and L. Dyrskjøt. 2012. Diagnosis of Bladder Cancer Recurrence Based on Urinary Levels of EOMES, HOXA9, POU4F2, TWIST1, VIM, and ZNF154 Hypermethylation. *PLoS ONE* 7:e46297.

Ren, F., H. Wu, Y. Lei, H. Zhang, R. Liu, Y. Zhao, X. Chen, D. Zeng, A. Tong, L. Chen, Y. Wei, and C. Huang. 2010. Quantitative proteomics identification of phosphoglycerate mutase 1 as a novel therapeutic target in hepatocellular carcinoma. *Molecular Cancer* 9:81-81.

Reymond, N., B.B. d'Agua, and A.J. Ridley. 2013. Crossing the endothelial barrier during metastasis. *Nat Rev Cancer* 13:858-870.

Riihilä, P., L. Nissinen, M. Farshchian, A. Kivisaari, R. Ala-aho, M. Kallajoki, R. Grénman, S. Meri, S. Peltonen, J. Peltonen, and V.-M. Kähäri. 2015. Complement Factor I Promotes Progression of Cutaneous Squamous Cell Carcinoma. *Journal of Investigative Dermatology* 135:579-588.

Rosario, D.J., J.A. Lane, C. Metcalfe, J.L. Donovan, A. Doble, L. Goodwin, M. Davis, J.W.F. Catto, K. Avery, D.E. Neal, and F.C. Hamdy. 2012. Short term outcomes of prostate biopsy in men tested for cancer by prostate specific antigen: prospective evaluation within ProtecT study. *BMJ* 344:

Ruenauver, K., R. Menon, M.A. Svensson, J. Carlsson, W. Vogel, O. Andren, M. Nowak, and S. Perner. 2014. Prognostic significance of YWHAZ expression in localized prostate cancer. *Prostate Cancer Prostatic Dis* 17:310-314.

Russo, G., M. Mischi, W. Scheepens, J.J. De la Rosette, and H. Wijkstra. 2012. Angiogenesis in prostate cancer: onset, progression and imaging. *BJU International* 110:E794-E808.

Sánchez-Martín, D., J. Martínez-Torrecuadrada, T. Teesalu, K.N. Sugahara, A. Alvarez-Cienfuegos, P. Ximénez-Embún, R. Fernández-Periáñez, M.T. Martín, I. Molina-Privado, I. Ruppen-Cañás, A. Blanco-Toribio, M. Cañamero, Á.M. Cuesta, M. Compte, L. Kremer, C. Bellas, V. Alonso-Camino, I. Guijarro-Muñoz, L. Sanz, E. Ruoslahti, and L. Alvarez-Vallina. 2013. Proteasome activator complex PA28 identified as an accessible target in prostate cancer by in vivo selection of human antibodies. *Proceedings of the National Academy of Sciences of the United States of America* 110:13791-13796.

Sanz, B., I. Perez, M. Beitia, P. Errarte, A. Fernández, L. Blanco, I. Estalella, A. Loizate, J. Irazusta, J.I. López, and G. Larrinaga. 2015. Aminopeptidase N Activity Predicts 5-Year Survival in Colorectal Cancer Patients. *Journal of Investigative Medicine* 63:740-746.

Sardana, G., K. Jung, C. Stephan, and E.P. Diamandis. 2008. Proteomic Analysis of Conditioned Media from the PC3, LNCaP, and 22Rv1 Prostate Cancer Cell Lines: Discovery and Validation of Candidate Prostate Cancer Biomarkers. *Journal of Proteome Research* 7:3329-3338.

Schiess, R., B. Wollscheid, and R. Aebersold. 2009. Targeted Proteomic Strategy for Clinical Biomarker Discovery. *Molecular oncology* 3:33-44.

Schilling, D., T. Todenhofer, J. Hennenlotter, C. Schwentner, T. Fehm, and A. Stenzl. 2012. Isolated, disseminated and circulating tumour cells in prostate cancer. *Nat Rev Urol* 9:448-463.

Schlomm, T., E. Näkel, A. Lübke, A. Buness, F.K.H. Chun, T. Steuber, M. Graefen, R. Simon, G. Sauter, A. Poustka, H. Huland, A. Erbersdobler, H. Sültmann, and

O.J.C. Hellwinkel. 2008. Marked Gene Transcript Level Alterations Occur Early During Radical Prostatectomy. *European Urology* 53:333-346.

Schröder , F.H., J. Hugosson , M.J. Roobol , T.L.J. Tammela , S. Ciatto , V. Nelen , M. Kwiatkowski , M. Lujan , H. Lilja , M. Zappa , L.J. Denis , F. Recker , A. Berenguer , L. Määttänen , C.H. Bangma , G. Aus , A. Villers , X. Rebillard , T. van der Kwast , B.G. Blijenberg , S.M. Moss , H.J. de Koning , and A. Auvinen 2009. Screening and Prostate-Cancer Mortality in a Randomized European Study. *New England Journal of Medicine* 360:1320-1328.

Sharp, J.A., S.L. Mailer, P.C. Thomson, C. Lefèvre, and K.R. Nicholas. 2008. Identification and transcript analysis of a novel wallaby (Macropus eugenii) basal-like breast cancer cell line. *Molecular Cancer* 7:1-1.

Shen, H.C., L. Jia, and G.A. Coetzee. 2014. Involvement of the 26S proteasome in androgen receptor-mediated transactivation reveals mechanistic differences between ligand-dependent and ligand-independent signaling. *Cancer Research* 66:404-404.

Shenoy, D., S. Packianathan, A.M. Chen, and S. Vijayakumar. 2016. Do African-American men need separate prostate cancer screening guidelines? *BMC Urology* 16:19.

Shrout, J., M. Yousefzadeh, A. Dodd, K. Kirven, C. Blum, A. Graham, K. Benjamin, R. Hoda, M. Krishna, M. Romano, M. Wallace, E. Garrett-Mayer, and M. Mitas. 2008. β(2)microglobulin mRNA expression levels are prognostic for lymph node metastasis in colorectal cancer patients. *British Journal of Cancer* 98:1999-2005.

Simon, N., Matthew L. Bochman, S. Seguin, Jeffrey L. Brodsky, William L. Seibel, and A. Schwacha. 2013. Ciprofloxacin is an inhibitor of the Mcm2-7 replicative helicase. *Bioscience Reports* 33:e00072.

Skog, J., T. Wurdinger, S. van Rijn, D.H. Meijer, L. Gainche, W.T. Curry, B.S. Carter, A.M. Krichevsky, and X.O. Breakefield. 2008. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol* 10:1470-1476.

Skotheim, R.I., O. Monni, S. Mousses, S.D. Fosså, O.-P. Kallioniemi, R.A. Lothe, and A. Kallioniemi. 2002. New Insights into Testicular Germ Cell Tumorigenesis from Gene Expression Profiling. *Cancer Research* 62:2359-2364.

Song, Y., Q. Luo, H. Long, Z. Hu, T. Que, X.a. Zhang, Z. Li, G. Wang, L. Yi, Z. Liu, W. Fang, and S. Qi. 2014. Alpha-enolase as a potential cancer prognostic marker promotes cell growth, migration, and invasion in glioma. *Molecular Cancer* 13:65-65.

Sørensen, K.D., M.O. Abildgaard, C. Haldrup, B.P. Ulhøi, H. Kristensen, S. Strand, C. Parker, S. Høyer, M. Borre, and T.F. Ørntoft. 2013. Prognostic significance of aberrantly silenced ANPEP expression in prostate cancer. *British Journal of Cancer* 108:420-428.

Sramkoski, R.M., T.G. Pretlow, J.M. Giaconia, T.P. Pretlow, S. Schwartz, M.-S. Sy, S.R. Marengo, J.S. Rhim, D. Zhang, and J.W. Jacobberger. 1999. A new human prostate carcinoma cell line, 22Rv1. *In Vitro Cellular & Developmental Biology - Animal* 35:403-409.

Stark, J.L., K. Mehla, N. Chaika, T.B. Acton, R. Xiao, P.K. Singh, G.T. Montelione, and R. Powers. 2014. Structure and Function of Human DnaJ Homologue Subfamily A Member 1 (DNAJA1) and Its Relationship to Pancreatic Cancer. *Biochemistry* 53:1360-1372.

Stephan, C., B. Ralla, and K. Jung. 2014. Prostate-specific antigen and other serum and urine markers in prostate cancer. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer* 1846:99-112.

Stowell, S.R., T. Ju, and R.D. Cummings. 2015. Protein Glycosylation in Cancer. *Annual review of pathology* 10:473-510.

Su, C.Y., Y.C. Chang, Y.C. Chan, T.C. Lin, M.S. Huang, C.J. Yang, and M. Hsiao. 2014. MTAP is an independent prognosis marker and the concordant loss of MTAP and p16 expression predicts short survival in non-small cell lung cancer patients. *European Journal of Surgical Oncology* 40:1143-1150.

Su Kim, D., Y.D. Choi, M. Moon, S. Kang, J.-B. Lim, K.M. Kim, K.M. Park, and N.H. Cho. 2013. Composite Three-Marker Assay for Early Detection of Kidney Cancer. *Cancer Epidemiology Biomarkers & Prevention* 22:390-398.

Sugano, G., I. Bernard-Pierrot, M. Lae, C. Battail, Y. Allory, N. Stransky, S. Krumeich, M.L. Lepage, P. Maille, M.H. Donnadieu, C.C. Abbou, S. Benhamou, T. Lebret, X. Sastre-Garau, S. Amigorena, F. Radvanyi, and C. Thery. 2011. Milk fat globule[mdash]epidermal growth factor[mdash]factor VIII (MFGE8)/lactadherin promotes bladder tumor development. *Oncogene* 30:642-653.

Sun, Z.-L., Y. Zhu, F.-Q. Wang, R. Chen, T. Peng, Z.-N. Fan, Z.-K. Xu, and Y. Miao. 2007. Serum proteomic-based analysis of pancreatic carcinoma for the identification of potential cancer biomarkers. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* 1774:764-771.

Sveen, A., A.C. Bakken, T.H. Ågesen, G.E. Lind, A. Nesbakken, O. Nordgård, S. Brackmann, T.O. Rognum, R.A. Lothe, and R.I. Skotheim. 2012. The exon-level biomarker SLC39A14 has organ-confined cancer-specificity in colorectal cancer. *International Journal of Cancer* 131:1479-1485.

Szklarczyk, D., A. Franceschini, S. Wyder, K. Forslund, D. Heller, J. Huerta-Cepas, M. Simonovic, A. Roth, A. Santos, K.P. Tsafou, M. Kuhn, P. Bork, L.J. Jensen, and C. von Mering. 2015. STRING v10: protein–protein interaction networks, integrated over the tree of life. *Nucleic Acids Research* 43:D447-D452.

Takkunen, M., M. Ainola, N. Vainionpää, R. Grenman, M. Patarroyo, A. García de Herreros, Y.T. Konttinen, and I. Virtanen. 2008. Epithelial-mesenchymal transition downregulates laminin α5 chain and upregulates laminin α4 chain in oral squamous carcinoma cells. *Histochemistry and Cell Biology* 130:509-525.

Talmadge, J.E., and I.J. Fidler. 2010. AACR Centennial Series: The Biology of Cancer Metastasis: Historical Perspective. *Cancer Research* 70:5649-5669.

Tanaka, M., N. Ichikawa-Tomikawa, N. Shishito, K. Nishiura, T. Miura, A. Hozumi, H. Chiba, S. Yoshida, T. Ohtake, and T. Sugino. 2015. Co-expression of S100A14 and S100A16 correlates with a poor prognosis in human breast cancer and promotes cancer cell invasion. *BMC Cancer* 15:53.

Tang, H., Yao, L., Tao, X., Yu, Y., Chen, M., Zhang, R., Xu, C. 2013. miR-9 functions as a tumor suppressor in ovarian serous carcinoma by targeting TLN1. *International Journal of Molecular Medicine* 32:381-388.

Tang, H.-Y., L.A. Beer, J.L. Tanyi, R. Zhang, Q. Liu, and D.W. Speicher. 2013. Protein isoform-specific validation defines multiple chloride intracellular channel and tropomyosin isoforms as serological biomarkers of ovarian cancer. *Journal of Proteomics* 89:165-178.

Taniuchi, K., Furihata, M., Iwasaki, S., Tanaka, K., Shimizu, T., Saito, M., Saibara, T. 2014 RUVBL1 directly binds actin filaments and induces formation of cell protrusions to promote pancreatic cancer cell invasion. *International Journal of Oncology* 44:1945-1954.

Tano, K., R. Mizuno, T. Okada, R. Rakwal, J. Shibato, Y. Masuo, K. Ijiri, and N. Akimitsu. 2010. MALAT-1 enhances cell motility of lung adenocarcinoma cells by influencing the expression of motility-related genes. *FEBS Letters* 584:4575-4580.

Tepper, C.G., D.L. Boucher, P.E. Ryan, A.-H. Ma, L. Xia, L.-F. Lee, T.G. Pretlow, and H.-J. Kung. 2002. Characterization of a Novel Androgen Receptor Mutation in a Relapsed CWR22 Prostate Cancer Xenograft and Cell Line. *Cancer Research* 62:6606-6614.

Théry, C., S. Amigorena, G. Raposo, and A. Clayton. 2001. Isolation and Characterization of Exosomes from Cell Culture Supernatants and Biological Fluids. In Current Protocols in Cell Biology. John Wiley & Sons, Inc.,

Thomas, G., K.B. Jacobs, M. Yeager, P. Kraft, S. Wacholder, N. Orr, K. Yu, N. Chatterjee, R. Welch, A. Hutchinson, A. Crenshaw, G. Cancel-Tassin, B.J. Staats, Z. Wang, J. Gonzalez-Bosquet, J. Fang, X. Deng, S.I. Berndt, E.E. Calle, H.S. Feigelson, M.J. Thun, C. Rodriguez, D. Albanes, J. Virtamo, S. Weinstein, F.R. Schumacher, E. Giovannucci, W.C. Willett, O. Cussenot, A. Valeri, G.L. Andriole, E.D. Crawford, M. Tucker, D.S. Gerhard, J.F. Fraumeni, R. Hoover, R.B. Hayes, D.J. Hunter, and S.J. Chanock. 2008. Multiple loci identified in a genome-wide association study of prostate cancer. *Nat Genet* 40:310-315.

Tong, M., T.-M. Fung, Steve T. Luk, K.-Y. Ng, Terence K. Lee, C.-H. Lin, Judy W. Yam, Kwok W. Chan, F. Ng, B.-J. Zheng, Y.-F. Yuan, D. Xie, C.-M. Lo, K. Man, X.-Y. Guan, and S. Ma. 2015. ANXA3/JNK Signaling Promotes Self-Renewal and Tumor Growth, and Its Blockade Provides a Therapeutic Target for Hepatocellular Carcinoma. *Stem Cell Reports* 5:45-59.

Tritschler, I., D. Gramatzki, D. Capper, M. Mittelbronn, R. Meyermann, J. Saharinen, W. Wick, J. Keski-Oja, and M. Weller. 2009. Modulation of TGF-β activity by latent TGF-β-binding protein 1 in human malignant glioma cells. *International Journal of Cancer* 125:530-540.

True, L.D., H. Zhang, M. Ye, C.-Y. Huang, P.S. Nelson, P.D. von Haller, L.W. Tjoelker, J.-S. Kim, W.-J. Qian, R.D. Smith, W.J. Ellis, E.S. Liebeskind, and A.Y. Liu. 2010.

CD90/THY1 is overexpressed in prostate cancer-associated fibroblasts and could serve as a cancer biomarker. *Mod Pathol* 23:1346-1356.

Tsivian, M., L.L. Banez, C.J. Keto, M.R. Abern, P. Qi, L. Gerber, J.W. Moul, and T.J. Polascik. 2013. African-American men with low-grade prostate cancer have higher tumor burdens: Results from the Duke Prostate Center. *Prostate Cancer Prostatic Dis* 16:91-94.

Tufo, G., A.W.E. Jones, Z. Wang, J. Hamelin, N. Tajeddine, D.D. Esposti, C. Martel, C. Boursier, C. Gallerne, C. Migdal, C. Lemaire, G. Szabadkai, A. Lemoine, G. Kroemer, and C. Brenner. 2014. The protein disulfide isomerases PDIA4 and PDIA6 mediate resistance to cisplatin-induced cell death in lung adenocarcinoma. *Cell Death Differ* 21:685-695.

Turay, D., S. Khan, C.J.D. Osterman, M.P. Curtis, B. Khaira, J.W. Neidigh, S. Mirshahidi, C.A. Casiano, and N.R. Wall. 2016. Proteomic Profiling of Serum-Derived Exosomes from Ethnically Diverse Prostate Cancer Patients. *Cancer investigation* 34:1-11.

U.S. Preventive Services Task Force. 2008. Screening for Prostate Cancer: U.S. Preventive Services Task Force Recommendation Statement. *Annals of Internal Medicine* 149:185-191.

Uekita, T., S. Fujii, Y. Miyazawa, R. Iwakawa, M. Narisawa-Saito, K. Nakashima, K. Tsuta, H. Tsuda, T. Kiyono, J. Yokota, and R. Sakai. 2014. Oncogenic Ras/ERK Signaling Activates CDCP1 to Promote Tumor Invasion and Metastasis. *American Association for Cancer Research* 12:1449-1459.

Urbanucci, A., K.K. Waltering, H.E. Suikki, M.A. Helenius, and T. Visakorpi. 2008. Androgen regulation of the androgen receptor coregulators. *BMC Cancer* 8:219-219.

van Dartel, M., and T.J.M. Hulsebos. 2004. Amplification and overexpression of genes in17p11.2~p12 in osteosarcoma. *Cancer Genetics and Cytogenetics* 153:77-80.

van den Broek, I., R.W. Sparidans, A.W.J. van Winden, M.-C.W. Gast, E.J. van Dulken, J.H.M. Schellens, and J.H. Beijnen. 2010. The absolute quantification of eight inter-α-trypsin inhibitor heavy chain 4 (ITIH4)-derived peptides in serum from breast cancer patients. *PROTEOMICS – Clinical Applications* 4:931-939.

Venanzoni, M.C., Giunta, S., Muraro, G. B., Storari, L., Crescini, C., Mazzucchelli, R., Montironi, R., Seth, A. 2003. Apolipoprotein E expression in localized prostate cancers. *International Journal of Oncology* 22:779-786.

Wang, F., M. Yamauchi, M. Muramatsu, T. Osawa, R. Tsuchida, and M. Shibuya. 2011a. RACK1 Regulates VEGF/Flt1-mediated Cell Migration via Activation of a PI3K/Akt Pathway. *The Journal of biological chemistry* 286:9097-9106.

Wang, M., Y. Hu, and M.E. Stearns. 2009. RPS2: a novel therapeutic target in prostate cancer. *Journal of Experimental & Clinical Cancer Research : CR* 28:6-6.

Wang, X., R.J. Hickey, L.H. Malkas, M.O. Koch, L. Li, S. Zhang, G.E. Sandusky, D.J. Grignon, J.N. Eble, and L. Cheng. 2011b. Elevated expression of cancer-associated proliferating cell nuclear antigen in high-grade prostatic intraepithelial neoplasia and prostate cancer. *The Prostate* 71:748-754.

Wang, X., S. Wang, X. Tang, A. Zhang, T. Grabinski, Z. Guo, E. Hudson, B. Berghuis, C. Webb, P. Zhao, and B. Cao. 2010. Development and evaluation of monoclonal antibodies against phosphatidylethanolamine binding protein 1 in pancreatic cancer patients. *Journal of Immunological Methods* 362:151-160.

Wang, X.-Q., Z.-X. Tang, D. Yu, S.-J. Cui, Y.-H. Jiang, Q. Zhang, J. Wang, P.-Y. Yang, and F. Liu. 2016. Epithelial but not stromal expression of collagen alpha-1(III) is a

diagnostic and prognostic indicator of colorectal carcinoma. *Oncotarget* 7:8823-8838.

Wei, N., G. Serino, and X.-W. Deng. 2008. The COP9 signalosome: more than a protease. *Trends in Biochemical Sciences* 33:592-600.

Weis, S.M., and D.A. Cheresh. 2011. Tumor angiogenesis: molecular pathways and therapeutic targets. *Nat Med* 17:1359-1370.

Welton, J.L., P. Brennan, M. Gurney, J.P. Webber, L.K. Spary, D.G. Carton, J.M. Falcón-Pérez, S.P. Walton, M.D. Mason, Z. Tabi, and A. Clayton. 2016. Proteomics analysis of vesicles isolated from plasma and urine of prostate cancer patients using a multiplex, aptamer-based protein array. *2016*

Westbrook, J.A., D.A. Cairns, J. Peng, V. Speirs, A.M. Hanby, I. Holen, S.L. Wood, P.D. Ottewell, H. Marshall, R.E. Banks, P.J. Selby, R.E. Coleman, and J.E. Brown. 2016. CAPG and GIPC1: Breast Cancer Biomarkers for Bone Metastasis Development and Treatment. *JNCI Journal of the National Cancer Institute* 108:djv360.

Winkler, J., A. Ori, K. Holzer, C. Sticht, D. Dauch, E.M. Eiteneuer, F. Pinna, R. Geffers, V. Ehemann, A. Andres-Pons, K. Breuhahn, T. Longerich, J.L. Bermejo, N. Gretz, L. Zender, P. Schirmacher, M. Beck, and S. Singer. 2014. Prosurvival function of the cellular apoptosis susceptibility/importin-α1 transport cycle is repressed by p53 in liver cancer. *Hepatology* 60:884-895.

Wisniewski, J.R., A. Zougman, N. Nagaraj, and M. Mann. 2009. Universal sample preparation method for proteome analysis. *Nat Meth* 6:359-362.

Wu, D., A. Haruta, and Q. Wei. 2010. GIPC1 Interacts with MyoGEF and Promotes MDA-MB-231 Breast Cancer Cell Invasion. *The Journal of biological chemistry* 285:28643-28650.

Xiaohua Jiang, S.G., Irene Esposito, Nathalia A. Giese, Christoph W. Michalski, Helmut Friess, Jörg Kleeff. 2010. Reduced expression of the membrane skeleton protein beta1-spectrin (SPTBN1) is associated with worsened prognosis in pancreatic cancer. *Histol Histopathol* 25:1497-1506.

Xu, S., Z.-F. Jia, C. Kang, Q. Huang, G. Wang, X. Liu, X. Zhou, P. Xu, and P. Pu. 2010. Upregulation of SEPT7 Gene Inhibits Invasion of Human Glioma Cells. *Cancer Investigation* 28:248-258.

Xue, G., L.-Q. Hao, F.-X. Ding, Q. Mei, J.-J. Huang, C.-G. Fu, H.-L. Yan, and S.-H. Sun. 2009. Expression of Annexin A5 Is Associated With Higher Tumor Stage and Poor Prognosis in Colorectal Adenocarcinomas. *Journal of Clinical Gastroenterology* 43:831-837.

Xue, H., Wang, Z., Chen, J., Yang, Z., Tang, J. 2015. Knockdown of reticulon 4C by lentivirus inhibits human colorectal cancer cell growth. *Molecular Medicine Reports* 12:2063-2067.

Yamakawa, N., K. Kaneda, Y. Saito, E. Ichihara, and K. Morishita. 2012. The Increased Expression of Integrin α6 (ITGA6) Enhances Drug Resistance in EVI1(high) Leukemia. *PloS one* 7:e30706.

Yan, T., G. Tang, T. Ren, D. Shen, K. Sun, W. Liang, and W. Guo. 2011. RNAi-mediated COPS3 gene silencing inhibits metastasis of osteogenic sarcoma cells. *Cancer Gene Ther* 18:450-456.

Yáñez-Mó, M., P.R.-M. Siljander, Z. Andreu, A.B. Zavec, F.E. Borràs, E.I. Buzas, K. Buzas, E. Casal, F. Cappello, J. Carvalho, E. Colás, A. Cordeiro-da Silva, S. Fais, J.M. Falcon-Perez, I.M. Ghobrial, B. Giebel, M. Gimona, M. Graner, I. Gursel, M. Gursel, N.H.H. Heegaard, A. Hendrix, P. Kierulf, K. Kokubun, M. Kosanovic, V. Kralj-Iglic, E.-M. Krämer-Albers, S. Laitinen, C. Lässer, T. Lener, E. Ligeti, A. Linē, G. Lipps, A. Llorente, J. Lötvall, M. Manček-Keber, A. Marcilla, M. Mittelbrunn, I.

Nazarenko, E.N.M. Nolte-'t Hoen, T.A. Nyman, #039, L. Driscoll, M. Olivan, C. Oliveira, É. Pállinger, H.A. del Portillo, J. Reventós, M. Rigau, E. Rohde, M. Sammar, F. Sánchez-Madrid, N. Santarém, K. Schallmoser, M. Stampe Ostenfeld, W. Stoorvogel, R. Stukelj, S.G. Van der Grein, M.H. Vasconcelos, M.H.M. Wauben, and O. De Wever. 2015. Biological properties of extracellular vesicles and their physiological functions. *2015*

Yang, C., P. Ströbel, A. Marx, and I. Hofmann. 2013. Plakophilin-associated RNA-binding proteins in prostate cancer and their implications in tumor progression and metastasis. *Virchows Archiv* 463:379-390.

Yang, Z., L. Zhuang, P. Szatmary, L. Wen, H. Sun, Y. Lu, Q. Xu, and X. Chen. 2015. Upregulation of Heat Shock Proteins (HSPA12A, HSP90B1, HSPA4, HSPA5 and HSPA6) in Tumour Tissues Is Associated with Poor Outcomes from HBV-Related Early-Stage Hepatocellular Carcinoma. *International Journal of Medical Sciences* 12:256-263.

Yi, J.-S., D.-G. Mun, H. Lee, J.-s. Park, J.-W. Lee, J.-S. Lee, S.-J. Kim, B.-R. Cho, S.-W. Lee, and Y.-G. Ko. 2013. PTRF/Cavin-1 is Essential for Multidrug Resistance in Cancer Cells. *Journal of Proteome Research* 12:605-614.

Yoshimura, M., J. Ishizawa, V. Ruvolo, A. Dilip, A. Quintás-Cardama, T.J. McDonnell, S.S. Neelapu, L.W. Kwak, S. Shacham, M. Kauffman, Y. Tabe, M. Yokoo, S. Kimura, M. Andreeff, and K. Kojima. 2014. Induction of p53-mediated transcription and apoptosis by exportin-1 (XPO1) inhibition in mantle cell lymphoma. *Cancer Science* 105:795-801.

Yu, L., L. Zhou, S. Wu, X. Gong, Z. Feng, L. Ma, B. Zhu, N. Yao, D. Wang, and H. Dong. 2014. Clinicopathological significance of cancer stem cells marked by CD133 and KAI1/CD82 expression in laryngeal squamous cell carcinoma. *World Journal of Surgical Oncology* 12:118-118.

Yu, W., X. Ding, F. Chen, M. Liu, S. Shen, X. Gu, and L. Yu. 2009. The phosphorylation of SEPT2 on Ser218 by casein kinase 2 is important to hepatoma carcinoma cell proliferation. *Molecular and Cellular Biochemistry* 325:61-67.

Yuen, H.-F., K.-K. Chan, C. Grills, J.T. Murray, A. Platt-Higgins, O.S. Eldin, K. O'Byrne, P. Janne, D.A. Fennell, P.G. Johnston, P.S. Rudland, and M. El-Tanani. 2012. Ran Is a Potential Therapeutic Target for Cancer Cells with Molecular Changes Associated with Activation of the PI3K/Akt/mTORC1 and Ras/MEK/ERK Pathways. *American Association for Cancer Research* 18:380-391.

Yun, H.-M., K.-R. Park, H.P. Lee, D.H. Lee, M. Jo, D.H. Shin, D.-Y. Yoon, S.B. Han, and J.T. Hong. 2014. PRDX6 promotes lung tumor progression via its GPx and iPLA2 activities. *Free Radical Biology and Medicine* 69:367-376.

Yun, H.-M., K.-R. Park, M.H. Park, D.H. Kim, M.R. Jo, J.Y. Kim, E.-C. Kim, D.Y. Yoon, S.B. Han, and J.T. Hong. 2015. PRDX6 promotes tumor development via the JAK2/STAT3 pathway in a urethane-induced lung tumor model. *Free Radical Biology and Medicine* 80:136-144.

Zaidi, N., J.V. Swinnen, and K. Smans. 2012. ATP-Citrate Lyase: A Key Player in Cancer Metabolism. *Cancer Research* 72:3709-3714.

Zeng, G.-Q., P.-F. Zhang, X. Deng, F.-L. Yu, C. Li, Y. Xu, H. Yi, M.-Y. Li, R. Hu, J.-H. Zuo, X.-H. Li, X.-X. Wan, J.-Q. Qu, Q.-Y. He, J.-H. Li, X. Ye, Y. Chen, J.-Y. Li, and Z.-Q. Xiao. 2012. Identification of Candidate Biomarkers for Early Detection of Human Lung Squamous Cell Cancer by Quantitative Proteomics. *Molecular & Cellular Proteomics* 11:

Zhang, D.a.T., Lee Kian and Wong, Lee Lee and Chiu, Lily-Lily and Sethi, Sunil K. and Koay, Evelyn S. C. 2005. Proteomic Study Reveals That Proteins Involved in Metabolic and Detoxification Pathways Are Highly Expressed in HER-2/neu-positive Breast Cancer. *Molecular & Cellular Proteomics* 4:1686-1696.

Zhang, F., H. Lin, A. Gu, J. Li, L. Liu, T. Yu, Y. Cui, W. Deng, M. Yan, J. Li, and M. Yao. 2014a. SWATH™- and iTRAQ-based quantitative proteomic analyses reveal an overexpression and biological relevance of CD109 in advanced NSCLC. *Journal of Proteomics* 102:125-136.

Zhang, J.X., S.J. Mai, X.X. Huang, F.W. Wang, Y.J. Liao, M.C. Lin, H.F. Kung, Y.X. Zeng, and D. Xie. 2014b. MiR-29c mediates epithelial-to-mesenchymal transition in human colorectal carcinoma metastasis via PTP4A and GNA13 regulation of β-catenin signaling. *Annals of Oncology* 25:2196-2204.

Zhang, K., T. Zhu, D. Gao, Y. Zhang, Q. Zhao, S. Liu, T. Su, M. Bernier, and R. Zhao. 2014c. Filamin A expression correlates with proliferation and invasive properties of human metastatic melanoma tumors: implications for survival in patients. *Journal of Cancer Research and Clinical Oncology* 140:1913-1926.

Zhang, P., X. Yang, X. Ma, D.R. Ingram, A.J. Lazar, K.E. Torres, and R.E. Pollock. 2015. Antitumor effects of pharmacological EZH2 inhibition on malignant peripheral nerve sheath tumor through the miR-30a and KPNB1 pathway. *Molecular Cancer* 14:55.

Zhang, X., Teng, Y., Yang, F., Wang, M., Hong, X., Ye, L., Gao, Y., Chen, G. 2015. MCM2 is a therapeutic target of lovastatin in human non-small cell lung carcinomas. *Oncology Reports* 33:2599-2605.

Zhang, Y., Z. Wen, M.P. Washburn, and L. Florens. 2010. Refinements to Label Free Proteome Quantitation: How to Deal with Peptides Shared by Multiple Proteins. *Analytical Chemistry* 82:2272-2281.

Zhong, Z., W.-S. Yeow, C. Zou, R. Wassell, C. Wang, R.G. Pestell, J.N. Quong, and A.A. Quong. 2010. Cyclin D1/cyclin dependent kinase 4 interacts with filamin A and affects the migration and invasion potential of breast cancer cells. *Cancer research* 70:2105-2114.

Zhou, L., D. Xia, J. Zhu, Y. Chen, G. Chen, R. Mo, Y. Zeng, Q. Dai, H. He, Y. Liang, F. Jiang, and W. Zhong. 2014a. Enhanced expression of IMPDH2 promotes metastasis and advanced tumor progression in patients with prostate cancer. *Clinical and Translational Oncology* 16:906-913.

Zhou, W., H. Pan, T. Xia, J. Xue, L. Cheng, P. Fan, Y. Zhang, W. Zhu, Y. Xue, X. Liu, Q. Ding, Y. Liu, and S. Wang. 2014b. Up-regulation of S100A16 expression promotes epithelial-mesenchymal transition via Notch1 pathway in breast cancer. *Journal of Biomedical Science* 21:97.

Zhou, Y., L.R. Bollu, F. Tozzi, X. Ye, R. Bhattacharya, G. Gao, E. Dupre, L. Xia, J. Lu, F. Fan, S. Bellister, L.M. Ellis, and Z. Weihua. 2013. ATP Citrate Lyase Mediates Resistance of Colorectal Cancer Cells to SN38. *American Association for Cancer Research* 12:2782-2791.

Zhu, F., Y.-F. Duan, W.-Y. Bao, W.-S. Liu, Y. Yang, and H.-H. Cai. 2015a. HtrA1 regulates epithelial–mesenchymal transition in hepatocellular carcinoma. *Biochemical and Biophysical Research Communications* 467:589-594.

Zhu, G., R. Gilchrist, N. Borley, H.W. Chng, M. Morgan, J.F. Marshall, R.S. Camplejohn, G.H. Muir, and I.R. Hart. 2004. Reduction of TSG101 protein has a negative impact on tumor cell growth. *International Journal of Cancer* 109:541-547.

Zhu, J., X. Chen, Z. Liao, C. He, and X. Hu. 2015b. TGFBI protein high expression predicts poor prognosis in colorectal cancer patients. *International Journal of Clinical and Experimental Pathology* 8:702-710.

Zhu, L.-y., K.-b. Zhong, S.-x. Lu, and L.-y. He. 2010. Vinculin and the androgen receptor in prostate cancer: expressions and correlations *National Journal of Andrology* 794-798.

ZHU Li-yong, Z.K.-b., LU Shen-xiu, HE Le-ye. 2010. Vinculin and the androgen receptor in prostate cancer: expressions and correlations *National Journal of Andrology* 794-798.

Zhu, Y., K. Das, J. Wu, M.H. Lee, and P. Tan. 2014. RNH1 regulation of reactive oxygen species contributes to histone deacetylase inhibitor resistance in gastric cancer cells. *Oncogene* 33:1527-1537.

Zorniak, M., P.A. Clark, H.E. Leeper, M.D. Tipping, D.M. Francis, K.R. Kozak, M.S. Salamat, and J.S. Kuo. 2012. Differential Expression of 2′,3′-Cyclic-Nucleotide 3′-Phosphodiesterase and Neural Lineage Markers Correlate with Glioblastoma Xenograft Infiltration and Patient Survival. *American Association for Cancer Research* 18:3628-3636.

Zou, J., Z. Han, L. Zhou, C. Cai, H. Luo, Y. Huang, Y. Liang, H. He, F. Jiang, C. Wang, and W. Zhong. 2014. Elevated expression of IMPDH2 is associated with progression of kidney and bladder cancer. *Medical Oncology* 32:1-6.

Vita

Gloria Polanco began her scientific career as a Research Experience for Undergraduates (REU) scholar at the University of Texas at El Paso (UTEP) in 2007. She studied the biocidal effects of organotin compounds on *Trypanosoma cruzi* with the goal of identifying therapeutic agents under the direction of Dr. Rosa Maldonado. Later that year, she was awarded a Research Initiative for Scientific Enhancement (RISE) undergraduate fellowship; which allowed her to continue her research until the completion of her B.S. in Microbiology in 2009. Her work was presented at local and national conferences, and was awarded best undergraduate poster presentation in 2009 at the Rio Grande chapter American Society for Microbiology meeting.

After graduation, Gloria was awarded a Post baccalaureate Research Education Program fellowship at the University of California, Los Angeles (UCLA) from 2009 to 2010. There, she studied the role of estrogen in maintenance of bone integrity under the direction of Dr. Susan Krum-Miranda. Her work in this lab led to a publication listing her as co-author in the Journal of Bone and Mineral Research.

In 2010 Gloria was accepted into the PhD program at UTEP, where she was also awarded a RISE graduate fellowship. Her work focused on studying the proteome of extracellular vesicles derived from prostate cancer cell lines. Gloria was given the opportunity to complete part of her training at the Cold Spring Harbor Course on Proteomics. In addition, Gloria was awarded the Dodson grant from the UTEP graduate school to fund her research project. She also presented her research at international and national meetings, and was selected to give an oral presentation of her work at the Society for the Advancement of Chicanos and Native Americans in Science (SACNAS) national conference in Los Angeles, CA. Gloria will go-on to begin her post-doctoral training at Washington University in St. Louis, studying *Leishmania major* under the direction of Dr. Stephen Beverley.

Contact Information: gpolanco@miners.utep.edu

This thesis/dissertation was typed by Gloria Polanco.