3-12-2021

Expression and Function of Transcription Factors FOXA1, HOXB13 and CDX2 in Prostate Cancer

Hamsat B. Anwar
Portland State University

Expression and Function of Transcription Factors FOXA1, HOXB13 and CDX2 In Prostate Cancer.

by

Hamsat Anwar

An undergraduate honors thesis submitted in partial fulfillment

of the requirements for the degree of Bachelor of Science in

University Honors and Biology

Thesis Advisor

Sebnem Ece Eksi

Portland State University

2021

ABSTRACT

Prostate cancer (PC) is the second leading cause of death in men, affecting around 190 thousand patients in 2020 alone (National Institute of Cancer, 2020). Much of the current research focuses on treatments for advanced PC, which is problematic due to the rapid evolution of tumors and development of treatment resistance. Cancer early detection presents a better approach to control and treat patients before they acquire an incurable disease. Earlier diagnosis of patients leads to better prognosis and a more comfortable treatment process (Etzioni et al., 2003). However, we currently lack molecular markers to stratify PC during its early stages. This study reviews the function and expression level of three DNA-binding transcription factors, FOXA1, HOXB13, and CDX2, in different grades of localized PC. Our investigation shows a grade-dependent increase in FOXA1 expression in prostate tumors, such that high-grade tumors have the highest levels of expression. In contrast, HOXB13 expression does not change in different grades of localized PC. Interestingly, CDX2 has an inverse correlation with tumor grade, such that high-grade tumors have the lowest level of CDX2 expression. We found that these markers exhibit various functions in different tissues and tumors. Future research investigating the function of the combinatory expression of FOXA1, HOXB13, and CDX2 in localized PC is necessary to gain an in-depth insight into how these transcription factors work together, which may potentially lead to an efficient PC early detection and treatment method.

1. Background

Cancer is the leading cause of death worldwide, responsible for 9.6 million deaths in 2018 alone, as estimated by the world health organization (World Health Organization, 2018). In the US, prostate cancer is the second most common type of cancer detected in men, following skin cancer (CDC Division of Cancer Prevention and Control, 2020). In terms of mortality, prostate cancer remains the second leading cause of death in American men, following lung cancer (American Cancer Society, 2020). Most prostate cancers are adenocarcinomas, but other types exist, such as transitional cell carcinomas, small cell carcinomas, and neuroendocrine tumors (Markman, 2020). Although the majority of the diagnosed prostate adenocarcinomas are localized to the prostate gland, some become aggressive, such as castration-resistant prostate cancers (CRPC) (Heidegger et al., 2019). It is essential to identify these tumors before they present themselves as CRPCs to save patients' lives. However, it is not possible to identify which localized prostate tumors would develop to become a CRPC, which is essential for saving many patients' lives.

Many factors increase the risk of prostate cancer. For instance, familial history is one of the most significant known risk factors. Having a first-degree relative with prostate cancer increases a patient's risk by two-fold, which increases to 4-folds when the relative is diagnosed before the age of 60 years old (Attard et al., 2016). Epigenetic factors, DNA replication, and immune response are some of the other factors that contribute to prostate cancer progression (Attard et al., 2016).

Molecular biomarkers hold a promise for the stratification of prostate cancer patients with localized tumors. It is essential to understand the gene regulatory networks that consist of *cis*- and trans-regulatory elements to identify the molecular biomarkers of aggressive prostate cancer.

Here, we review the expression and function of three essential transcription factors, FOXA1, HOXB13, and CDX2, that control the expression of several downstream targets that may be responsible for switching prostate tumors to an aggressive state. This paper focuses explicitly on the trans-regulatory changes in high-grade versus low-grade localized prostate cancers. Specifically, we aim to understand the expression and function of transcription factors FOXA1, HOXB13, and CDX2 in different grades of prostate tumors, as well as in other cancer types. Our overarching goal is to identify molecular biomarkers that can be potentially used as markers for early detection of aggressive disease to prevent prostate cancer-related deaths.

1.1 Grading and stages of prostate cancer

Prostate cancer is identified and reported through a system called the Gleason scoring system. Gleason scoring is based on assigning a composite score to prostate tumors through examining histopathology images and their Gleason grades. To determine the Gleason grade, pathologists review Hematoxylin and Eosin (H&E) stains of prostate tumor sections and assign grades ranging from 1 to 5. Gleason grades are assigned based on the differentiation states of cells in the tumor. A low Gleason grade indicates high similarity to normal prostate tissue and thus a less aggressive tumor. Clinically, Gleason grades 1 and 2 are considered insignificant. In comparison, Gleason grades 4 and 5 are associated with poorly differentiated cells, and thus much worse patient outcomes (Prostate Cancer - Stages and Grades, 2019).

Prostate tumors are very heterogeneous. As a result, each prostate tumor foci often consist of separate Gleason grades. The two Gleason grades identified in a prostate tumor with the highest percentages are added based on their relative percentage to calculate an overall Gleason score. For instance, if a prostate tumor consists predominantly of Gleason grade 3 and a

low percentage of Gleason grade 4, a Gleason score of 3+4 is assigned to the tumor. As a result, Gleason scores for clinically significant prostate tumors can vary from 6 (3+3) to 10 (5+5). Prostate cancers with a score of 6 (3+3) often indicate low-grade cancer, 7 (4+3 or 3+4) medium-grade cancer, and 8 (4+4) or higher indicate high-grade cancer, depending on other variables (Prostate Cancer - Stages and Grades, 2019).

There is a system to group prostate cancers with different Gleason scores into different grades of cancer. Grade 1 group cancers consist of Gleason score 6 (3+3), grade 2 group cancers consist of Gleason score 7 (3+4), grade 3 group cancers consist of Gleason score 7 (4+3), grade 4 cancers consist of Gleason score 8 (4+4), and finally grade 5 cancers consist of Gleason score 9 and 10 (Prostate Cancer - Stages and Grades, 2019). These grades are used to eliminate deficiencies in the Gleason scoring system (Epstein et al., 2016). Patients often have hard time understanding the composite Gleason score (Epstein et al., 2016). One way that Gleason scoring system can create confusion for patients, is a Gleason score of 4+3 has a worse prognosis than a score of 3+4 even though both of these cases have a Gleason score of 7 (Epstein et al., 2016).

Another way of classifying prostate cancer is through the TNM stages, which considers the location, lymph node involvement, and metastatic status of the tumor (The American Cancer Society, 2019). Five distinct prognostic stages, 0 and I to IV, are proposed that incorporate results from diagnostic tests such as the digital rectal exam (DRE) and the measurement of prostate antigen level (PSA). A stage I prostate cancer is early prostate cancer with slow growth. Tumors at this TNM stage cannot be felt in a DRE, have a low PSA score (<10ng/mL), and the cells are usually well differentiated. During stage II prostate cancer, the patients' PSA levels are medium ($10ng/mL<x\leq20ng/mL$), and the tumor is small. At this stage, the tumor has a high risk of growing and spreading to other parts of the body. There are three subdivisions in this stage:

IIA, where the tumor is confined to the prostate; IIB, where the tumor is large enough to be felt in a DRE; and IIC, where the cells are moderately or poorly differentiated. Stage III prostate cancer is marked by elevated PSA levels, fast-growing tumors, and a high Gleason score. While stage III tumors are localized to the prostate gland, they have a high possibility of aggressive growth and metastasis. There are three subdivisions of stage III cancers: subdivision A that is identified by spread to the outer layer of the prostate and possibly to the seminal vesicles; subdivision B that is identified by tumor invasion to nearby structures such as the bladder; and subdivision C that is identified by further metastasis, poor differentiation of cells, and a high Gleason score. Finally, stage IV prostate tumors show metastasis to other organs: subdivision IVA tumors metastasize to a lymph node, and subdivision IVB tumors metastasize to distant organs such as the brain (Prostate Cancer - Stages and Grades, 2019).

1.2 The significance of early detection of cancer

Most cancer studies focus on therapies for advanced cancers. This is because most patients are diagnosed when their disease has already taken a toll and spread from its origin. In addition, many cancers develop resistance to therapy, and most of the advanced cancer therapies are not the cure to all cancer types as they tend to focus on a specific type of cancer (Etzioni et al., 2003).

It is well known that when cancer is detected early, patients have a better chance of survival. Cancer early detection offers an alternative that not only helps lessen the burden on patients, families, and the economy but also eliminates the possibility of cancer invasiveness and spread, allowing earlier treatment and care (Etzioni et al., 2003).

Early detection screenings provide a mechanism for disease control when the method screens for common diseases with high rates of mortality and morbidity. Detection of cancer at its early stages improves prognosis assuming that treatment is available for the early stages. Effective early detection screening methods can accurately identify cancer patients and can be used to prevent the progression of these cancers to an advanced state. In addition, an effective early detection screening can differentiate between aggressive and benign tumors, is inexpensive, and does not cause discomfort to patients. On the other hand, early detection screenings can lead to overdiagnosis, which is an essential consideration in cancer early detection research (Etzioni et al., 2003).

In prostate cancer specifically, early detection is extremely important. Prostate cancer risk increases significantly after the age of 65 years (St. Anthony Blogs, 2019). It is known that having routine checkups starting at age 40 significantly aids in improvement for patients of African American descent and those with a family history of prostate cancer (Prostate Cancer Foundation, 2019). Prostate cancer screening usually includes a PSA level test, which can be further supplemented with additional testing such as a DRE and a needle biopsy (Prostate Cancer Foundation, 2019). However, many patients are misdiagnosed and mistreated every year in the clinic due to the high number of false positives with PSA scores and under-sampling of the prostate tissue in regular biopsies. In other words, there is an urgent clinical need to identify precise markers to improve early detection screening tools as well as to develop early treatment options for prostate cancer patients.

1.3 Gene regulation and transcription factors

The central dogma in biology describes the process of information transformed from DNA to protein: DNA is transcribed into mRNA molecules, which are then translated into proteins in the ribosomes (Black et al., 2016). Gene transcription in eukaryotes involves the use of RNA polymerase and consists of three stages: initiation, elongation, and termination (Black et al., 2016). During initiation, RNA polymerase binds to a specific region in the DNA known as the promoter sequence (Black et al., 2016). In this stage, the double-helical DNA is unzipped and separated into a template strand and a coding strand. During elongation, RNA polymerase uses the template strand of DNA to create a transcript that is complementary to the coding strand (Black et al., 2016). Finally, during termination, RNA polymerase arrives at a specific sequence that signals the end of transcription (Black et al., 2016). This process creates a pre-mRNA, which is then modified, exported out of the nucleus, and spliced into mRNA (Black et al., 2016). mRNA molecules are then translated into proteins by the ribosomes located in the cytoplasm (Black et al., 2016). Ribosomes, made of tRNAs, read the mRNA sequence in codons (three adjacent nucleotides in the sequence) and match each codon with a corresponding amino acid (Black et al., 2016). The amino acids are linked in a sequence by peptide bonds to make the primary structure of a protein (Tymoczko et al., 2015). The protein then acquires a secondary structure through a hydrogen bond linking an amino group of one amino acid to a carboxylic acid group of another amino acid (Tymoczko et al., 2015). There are two types of secondary structures depending on the pattern of hydrogen bonding. The first is in the form of an alpha helix where a hydrogen bond exists between an amino group of one amino acid and the carboxylic acid group of the fourth amino acid following it so that a hydrogen bond exists every four amino acids (Tymoczko et al., 2015). The second type is the beta-sheet, which has a

hydrogen bond between an amino group of one amino acid on one strand and the carboxylic acid group of an opposing amino acid on another strand (Tymoczko et al., 2015). In the tertiary structure, R-group interactions lead the protein to fold into a more functional 3D shape (Tymoczko et al., 2015). Tertiary structure interactions include hydrophobic and hydrophilic interactions, Van der Waal's interactions, disulfide bridges, hydrogen bonds, and ionic interactions (Tymoczko et al., 2015). Multiple tertiary protein units can then be linked to form a quaternary structured protein composed of two or more subunits (Tymoczko et al., 2015).

The process of gene regulation is crucial for cell specification and function during development. The types and expression levels of genes in distinct cells directly determine the type, state, and function of those cells. As a result, it is a tightly controlled system at different levels of the central dogma: both at the transcriptional and at the protein level. In multicellular organisms, gene expression is controlled both spatially and temporally across multiple cell types. Mistakes in gene regulation can have detrimental consequences associated with developmental diseases and even cancer. For instance, transcription can be regulated in different ways by modifying the chromatin structure. Translation can also be regulated by factors that modify mRNA to an unstable form preventing it from being exported out of the nucleus. It is also possible to regulate protein expression via post-protein modifications, such as adding specific tags to the protein to mark it for degradation.

Transcription factors (TFs) are proteins that regulate gene expression by directly binding to the regulatory sequences of a gene (Black et al., 2016). Each TF recognizes and binds to specific nucleotide sequences in the DNA, governing cell-specific gene expression programs (Labbe & Brown, 2018). There are two major types of TFs: activators and repressors. Transcriptional activators increase the transcription of the genes they regulate, whereas

transcriptional repressors decrease or inhibit the transcription of their target genes. The portions of DNA that TFs bind to are called regulatory DNA sequences, and these include promoters, enhancers, and silencers. These non-coding regulatory sequences can be 1) distal to the transcription initiation site, 2) downstream of the gene body, or 3) intronic. TFs have DNA binding domains that recognize and bind to single or double-stranded DNA. In comparison to DNA binding domains, activator domains of TFs do not directly interact with the DNA. Activator domains are responsible for mediating the physical interaction of TFs with co-factors and co-regulators that can change the binding specificity and the function of TFs.

In prostate cancer, there are several known TFs that are important for the initiation and progression of the disease. One well-known example is the androgen receptor (AR). AR is a ligand-dependent nuclear TF, and this ligand can be testosterone or dihydrotestosterone (DHT) (Dai et al., 2017). In the absence of a ligand, AR is located in the cytoplasm bound to chaperon and heat shock proteins. Once AR binds to a ligand, it undergoes a conformational change that causes it to translocate to the nucleus, turning on its transcription activation function. The conformational change of AR enhances its ability to homodimerize, stabilizes the binding of its ligands, and stimulates the recruitment of AR's co-regulators (Dai et al., 2017). The homodimer AR inside the nucleus works as a TF that recognizes and binds to specific DNA regions, known as Androgen Recognition Elements (AREs). AREs are usually located in the promoter or enhancer regions of the downstream target genes of AR. Upon binding to AREs, AR recruits co-regulators, other TFs, and RNA polymerases to begin transcription. AR's ligand-binding site is an important site where any changes, such as a deletion or a mutation occurring in this site, renders AR inactive. A common chromosomal rearrangement in prostate cancer that leads to

fusion of TMPRSS2 promoter and oncogenic ERG increase AR activity, which is associated with aggressive disease (Dai et al., 2017).

2. Methods

A variety of research and review articles were reviewed to collect and summarize findings on FOXA1, HOXB13, and CDX2. The articles chosen dealt explicitly with the TFs studied. They were grouped based on the TF they discussed. These groups were further divided into two divisions; one included normal functioning of the TF, and the other included the specific TF and its role in prostate cancer. This review included articles over the past two decades with a focus on the most recent findings. The human protein atlas was used to analyze expression levels and study prostate tumor slides (Figure 1). JASPAR was used to find TF binding motifs (Figure 2) (JASPAR, 2020). Finally, the UCSC genome browser was used to investigate the conversation of TF binding sites across different species of mammals (UCSC Genome Browser).

3. Trans-regulators of Prostate Cancers

3.1 FOXA1

3.1.a Structure and Binding

FOXA1 is one of the three TFs in the FOX family. This family of TFs is known to have a unique forkhead domain that binds to DNA monomers (Hankey et al., 2020). As a pioneer factor, FOXA1 binds to nucleosomes and repositions them by moving histones and counteracting areas in the condensed chromatin region, upregulating transcription (Hankey et al., 2020). It is important to note that FOXA1 binds to condensed euchromatin and not heterochromatin (Hankey

et al., 2020). FOXA1 was found to bind to sites in the genome that match the sequence 5'-T[G/A]TT[T/G][A/G][C/T][T/A/C][T/C][T/A][G/T/C]-3' (JASPAR, 2020) (Figure 2).

3.1.b Expression

Due to the variety of functions that FOXA1 has, it is expressed in many organs. FOXA1 is expressed in the pancreas, specifically at the glucagon expressing pancreatic alpha islet cells (Bernardo & Keri, 2012). It is also expressed in Type II alveolar cells, including both the lungs' epithelial and secretory cells (Bernardo & Keri, 2012). FOXA1 is expressed at the notochord and floor plate of the fetal brain (Bernardo & Keri, 2012). Expression of FOXA1 is also found in the epithelial cells of the gastrointestinal (GI) tract. In the mammary glands, the expression of FOXA1 is correlated with both ER and GATA3, contributing to the development of mammary glands (Bernardo & Keri, 2012).

Research studies so far show that both low-level expression and overexpression of FOXA1 can contribute to cancer. In certain types of cancers such as Acute Myeloid Leukemia, anaplastic thyroid carcinoma, along with subsets of esophageal and lung cancers, overexpression of FOXA1 was noted (Bernardo & Keri, 2012).

3.1.c Function

FOXA1 has a critical role in embryonic development and in regulating nuclear receptor signaling systems, including estrogen receptor (ER), glucocorticoid receptor (GR), and androgen receptor (AR) (Hankey et al., 2020).

FOXA1 is required for the development of a normal pancreas morphology and liver (Hankey et al., 2020) (Bernardo & Keri, 2012). Interestingly, mice that lost FOXA1 have normal

liver and pancreas development (Bernardo & Keri, 2012). This is hypothesized to be due to compensation by FOXA2 (Bernardo & Keri, 2012). However, these mice maintained a high postnatal fatal rate even with normally developed organs (Bernardo & Keri, 2012). Loss of both FOXA1 and FOXA2 led to bile duct hyperplasia and fibrosis in adult mice, while liver development was unaffected (Bernardo & Keri, 2012).

In the mammary glands, the expression of FOXA1 is required for the full activity and expression of ER and, thus, mammary gland morphogenesis (Bernardo & Keri, 2012). FOXA1 mainly functions to promote the morphogenesis of the mammary ducts and inhibits terminal differentiation of the alveoli (Bernardo & Keri, 2012).

In breast cancer and pancreatic cancer, where AR is not expressed, FOXA1 works as a tumor suppressor (Hankey et al., 2020). A study showed that the loss of FOXA1 and FOXA2 led to an increase in the epithelial-mesenchymal transitions in pancreatic cancer cells, indicating a metastatic suppressive role of FOXA1 in that case (Bernardo & Keri, 2012).

In lung development, FOXA1 plays an indispensable role. Experiments with FOXA1 null mice showed delayed lung development and decreased expression of the markers required for lung differentiation during embryogenesis (Bernardo & Keri, 2012). Similarly, in lung tissue, FOXA2 can compensate for the loss of FOXA1. Mice that lack both FOXA1 and FOXA2 had a drastically impaired differentiation of epithelial lung cells and a decreased mRNA expression of SHH, typically required for the differentiation of lung cells (Bernardo & Keri, 2012). FOXA1 may have a specifically crucial function in the lung tissue due to its role in alveolar type II apoptosis and hydrogen peroxide-induced apoptosis (Bernardo & Keri, 2012).

In the brain tissue, FOXA1 helps in the maturation of midbrain dopaminergic neurons (Bernardo & Keri, 2012). FOXA2 can compensate for the loss of FOXA1 in the brain, similarly to the lung, liver, and pancreas (Bernardo & Keri, 2012).

In the GI tract, loss of FOXA1 resulted in viable mice with slow growth and low mass (Bernardo & Keri, 2012). Although the intestinal morphology was normal, the intestinal tracts' secretory functions were reduced (Bernardo & Keri, 2012). FOXA1 was shown to bind to the promoter of the Muc2 gene, which encodes for mucin (Bernardo & Keri, 2012). When FOXA1 is absent, this protein is not produced (Bernardo & Keri, 2012). This highlights the importance of FOXA1 in the secretory function of the GI tract. Overall, loss of FOXA1 in the intestinal tract led to a diminished form of the goblet cells and reduced differentiation of D-cells and L-cells, essential for intestinal hormones (Bernardo & Keri, 2012). Loss of FOXA1 was also seen to decrease cellular invasion and metastasis in some types of esophageal cancers (Bernardo & Keri, 2012).

FOXA1 has many other essential functions, such as activating the transcriptional enhancers via H3K4me2. H3K4 recruits FOXA1, which then recruits MLL3, leading to a methylation switch from H3K4 to H3K4me2 (Hankey et al., 2020). This works in a positive feedback loop since methylated H3K4 recruits, even more, FOXA1 (Hankey et al., 2020).

3.1.d FOXA1 expression and function in prostate cancer

FOXA1 is uniquely expressed in both the epithelial cells and the stromal cells of the prostate. FOXA1 is responsible for the prostate morphogenesis in the glandular epithelial cells and has secretory functions in the tumor stroma. The expression of FOXA1 in the prostate starts during embryonic development and is maintained throughout adulthood (Bernardo & Keri,

2012). Although FOXA1 has an essential role in liver development, the prostate, seminal vesicles, and bladder tissues have higher mRNA levels than the liver (Hankey et al., 2020). The expression level of FOXA1 is critical to the prostate since its overexpression can easily lead to reprogramming of the AR cistrome and thus promote prostate tissue tumorigenesis (Labbe & Brown, 2020).

FOXA1 is vital for proper luminal secretions in the prostate (Bernardo & Keri, 2012). FOXA2 cannot compensate for the loss of FOXA1 in the prostate epithelial cells (Bernardo & Keri, 2012). When FOXA1 is lost, FOXA2 is upregulated, which triggers AR to function in a manner that is dependent on androgen, resulting in hyperproliferation (Bernardo & Keri, 2012). Loss of FOXA1 can lead to prostate cancer in two ways. The first is via the inhibition of the tumor suppressor NKX3 gene (Bernardo & Keri, 2012). The second way is by increasing the SHH signaling (Bernardo & Keri, 2012). Our analysis using the human protein atlas shows that FOXA1 is strongly expressed in low-, medium-, and high-grade prostate cancers with an increased level of expression in medium- and high-grades (Figure 1).

Loss of FOXA1 in the prostate leads to cancer by increased hyperproliferation of epithelial cells, increased SHH oncogenic signaling, and decreased suppressor activity of NKX3 (Bernardo & Keri, 2012). This indicates that FOXA1 has a tumor-suppressive role in the prostate's epithelium (Bernardo & Keri, 2012). This role changes in the absence of androgens, where FOXA1 is shown to promote growth (Bernardo & Keri, 2012). In this case, a high expression level of FOXA1 is correlated with a poorer prognosis for prostate cancer patients and more metastasis of the tumor (Bernardo & Keri, 2012). Besides, the co-expression of FOXA1 and AR led to even worse outcomes and prognoses in the absence of androgen (Bernardo &

Keri, 2012). FOXA1 was also shown to positively regulate AGR2, which is a metastasis inducer contributing to prostate cancer (Bernardo & Keri, 2012).

FOXA1 has a role in establishing the AR transcriptional program of the prostate cells by recruiting AR to half-AREs with low affinity (Labbe & Brown, 2020). Increased levels of FOXA1 lead to an increase in the binding of AR to the open regions of chromatin. This results in AR being forced to areas of AREs that would not be accessible under normal cellular conditions (Labbe & Brown, 2020). This means FOXA1 expands the binding of AR to less available AR binding sites on the chromatin, allowing for increased control over the transcription of genes (Hankey et al., 2020). However, when FOXA1 is low, AR binding to AREs diminishes, which triggers corrupted transcriptional programs in the cell (Labbe & Brown, 2020). This shows the important role FOXA1 plays along with AR in promoting prostate differentiation during development (Bernardo & Keri, 2012) (Labbe & Brown, 2020).

Since FOXA1 is involved in AR signaling, an increase in FOXA1 can lead to oncogenic transcription driven by increased AR binding (Hankey et al., 2020). The overexpression of FOXA1 reprograms the AR cistrome leading to tumorigenesis in the prostate (Bernardo & Keri, 2012). Many prostate cancer patients with mutated FOXA1 have a high transcriptional activity of AR and hypermethylation of the genome, which aligns well with the known functions of FOXA1 in the prostate (Hankey et al., 2020). It is also well established that FOXA1 can work independently of AR. In these cases, FOXA1 has been shown to function as a repressor and a barrier to the epithelial-mesenchymal transition (Hankey et al., 2020). FOXA1 works as a tumor suppressor in the absence of androgen by inhibiting the progression of the cell cycle, preventing AR binding, decreasing cell motility, and decreasing the epithelial-mesenchymal transition (Hankey et al., 2020).

3.2 HOXB13

3.2.a Structure and binding

HOXB13 is another pioneer factor in the HOX group, which belongs to the homeobox TF family (Hankey et al., 2020). It contains a homeobox DNA binding domain that recognizes and binds to A/T rich sequences (Hankey et al., 2020). HOXB13 works closely with the cofactor MEIS1, which is part of the TALES family (Hankey et al., 2020). Upon MEIS1 binding, HOXB13 acquires a stronger DNA binding ability to specific target sequences (Hankey et al., 2020). There are two sites within the activator domain of HOXB13, which are responsible for the physical interaction of HOXB13 with MEIS1 (Hankey et al., 2020). HOXB13 binding motif often matches the following sequence 5'-[C/G/A][C/T][A/C/T]AT[A/T]AA[A/C/T][C/A/T]-3' (JASPAR, 2020) (Figure 2).

The mutation of HOXB13 at its interaction domain with MEIS1, G84E mutation, was found to increase the risk of prostate cancer development via mechanisms that are currently unknown (Hankey et al., 2020). This mutation was associated with an earlier onset of prostate cancer, higher PSA levels, and higher Gleason scores in patients (Hankey et al., 2020). MEIS1 expression is known to decrease gradually during prostate carcinogenesis (Hankey et al., 2020). However, MEIS1 silencing was shown in one study to inhibit cell proliferation. These findings regarding the G84E mutation indicate a complex mechanistic control of HOXB13-MEIS1 interaction over prostate cancer progression (Hankey et al., 2020).

3.2.b Expression

HOXB13 is expressed in the posterior region of human and mice embryos, and it controls the anterior/posterior patterning of the prostate gland (Hankey et al., 2020). The highest level of expression of HOXB13 occurs during the development of the body's posterior segments (Decker & Ostrander, 2014). This expression then persists at lower levels through adulthood, such as in the colon (Decker & Ostrander, 2014).

3.2.c Function

HOXB13 mainly regulates the development of anterior/posterior patterning in vertebrates (Hankey et al., 2020). Another function of HOXB13 is to promote cells' differentiation into luminal epithelial fate (Hankey et al., 2020).

Working independently of MEIS1, HOXB13 is able to repress the transcription of specific genes (Hankey et al., 2020). One example of this is when HOXB13 inhibits the transcription of CBP, or P300, by repressing the activity of the histone acetyltransferases associated with them (Hankey et al., 2020). HOXB13 can also function independently of its DNA binding activity by using other TFs to repress the transcription of downstream target genes (Hankey et al., 2020).

There are many contradicting findings regarding HOXB13 and the role it plays in cancer. The overexpression of HOXB13 was found to inhibit growth in colorectal cancers, while its decreased expression was shown to reduce tumor invasion in ovarian cancer (Hankey et al., 2020). Along with that, other studies have also found that HOXB13 expression inhibits the growth of colon cancer (Decker & Ostrander, 2014). In renal carcinomas, HOXB13 was found to suppress tumor growth (Decker & Ostrander, 2014).

3.2.d HOXB13 expression and function in prostate cancer

HOXB13 is expressed in the luminal epithelial cells in developing prostate and adult prostate and contributes to its secretory function (Hankey et al., 2020). Its expression is imperative to develop the prostate and the luminal epithelial cells of the prostatic ducts (Decker & Ostrander, 2014). More specifically, HOXB13 is important for the differentiation of the ventral prostate, which contains the secretory cells (Decker & Ostrander, 2014).

Mutations in the HOXB13 gene lead to defective morphology of the ventral prostate due to the function of HOXB13 in anterior/posterior development. HOXB13 mutations also lead to loss of differentiation of the luminal epithelial cells and can cause a complete absence of the prostate's secretory function (Hankey et al., 2020). HOXB13 knockout mice exhibit a cuboidal morphology instead of the normal columnar prostate epithelial cell morphology (Decker & Ostrander, 2014).

In prostate cancer, HOXB13 was found to have contradicting roles depending on the expression of other factors in the same gene network. However, it is found to be mainly oncogenic when overexpressed (Hankey et al., 2020). Our analysis using the human protein atlas shows that HOXB13 is expressed in low-grade and high-grade prostate cancer (Figure 1). However, no data was found on medium-grade prostate cancer (Figure 1). HOXB13 interacts with AR and, as a result of this interaction, can both enhance or repress AR transcription of shared target genes, similar to FOXA1 (Hankey et al., 2020). Even though HOXB13 represses the transcription of some AR-regulated genes, it acts as an obligate coactivator of other AR genes at the same time (Decker & Ostrander, 2014). This shows how HOXB13 has the function of fine-tuning AR transcription in androgen-dependent prostate cancer (ADPC) (Hankey et al., 2020).

HOXB13 has different roles in Androgen-Dependent Prostate Cancer (ADPC) in the absence versus presence of AR. It has been shown that AR expression results in HOXB13 controlled repression of the cell cycle, and the absence of AR results in HOXB13 controlled stimulation of the cell cycle (Decker & Ostrander, 2014). In ADPC, HOXB13 was found to have a tumor-suppressive role in the presence of AR (Hankey et al., 2020). HOXB13 inhibited T-cell factors, precisely T-cell factor 4 when overexpressed and caused the prostate cell to be arrested at the G1 phase (Decker & Ostrander, 2014). In agreement with this study, another study found that the knockdown of HOXB13 led the cells to grow more rapidly (Decker & Ostrander, 2014). It has also been found that the overexpression of HOXB13 inhibited the growth of prostate cancer cell lines (Hankey et al., 2020). On the other hand, HOXB13 overexpression was shown to inhibit P21, an important tumor suppressor, and promote cellular growth in AR negative cells (Decker & Ostrander, 2014). Further investigations showed that overexpression of HOXB13 suppressed the formation of colonies in LNCaP cells, which was overcame by an increase in AR (Hankey et al., 2020). In comparison, HOXB13 was shown to promote prostate cancer growth in hormone-independent tumors (Hankey et al., 2020). Tumor invasion and metastasis were stimulated by the expression of HOXB13 in androgen-independent prostate cancer cell lines (Decker & Ostrander, 2014).

The comparison of HOXB13 expression levels in ADPC versus CRPC showed that HOXB13 was overexpressed in CRPC relative to ADPC (Hankey et al., 2020). One study found that the silencing of HOXB13 led to the inhibition of CRPC cell growth (Hankey et al., 2020). Generally, HOXB13 is overexpressed in prostate tumor tissue and specifically overexpressed in CRPC tumors (Decker & Ostrander, 2014). A positive correlation between HOXB13 expression with both PSA levels and Gleason score was reported in patients (Decker & Ostrander, 2014).

In knockdown studies, loss of HOXB13 decreased the ability of androgen treatment to stimulate proliferation and migration in prostate cells (Hankey et al., 2020). In another study, knockdown of HOXB13 inhibited the growth of prostate cancer cell lines (Decker & Ostrander, 2014). Both of these studies support the aforementioned findings in that HOXB13 overexpression led to prostate cancer through the disruption of cell proliferation pathways.

The co-expression of HOXB13 and FOXA1 was shown to change AR cistrome and transform normal prostate epithelial tissue into a tumor-like state (Hankey et al., 2020). This is very likely since HOXB13 also regulates AR transcription, as mentioned earlier, just like FOXA1. The binding sites of HOXB13 on AR are enriched in AR regions that are tumor-specific, indicating a significance of HOXB13 to prostate cancer (Hankey et al., 2020).

3.3 CDX2

3.3.a Structure and Binding

CDX2 is a TF that belongs to the Homeobox family as part of the non-clustered genes (Herawi et al., 2006). CDX2 interacts with HOX genes and greatly affects their function by either repressing or activating them (Herawi et al., 2006). This regulation is directly related to HOXB13 expression in the prostate tissue since the promoter region of HOXB13 contains multiple CDX2 binding sites. In addition, the gene that codes for vitamin D receptor has a binding site for CDX2, which indicates that CDX2 can affect Vitamin D binding. It was shown that a higher expression of calcium channel proteins is accompanied by higher CDX2 binding (Herawi et al., 2006). CDX2 binding motif often matches the following sequence 5’-[A/T/G]G[C/T]AATAAA[A/T/C]-3’ (JASPAR, 2020) (Figure 2).

3.3.b Expression

CDX2 is expressed in the epithelium of the intestinal tract during development (Moskaluk et al., 2003). More specifically, CDX2 is expressed in the epithelial lining of the duodenum, ileum, appendix, colon, and rectum (Moskaluk et al., 2003). One study confirmed that CDX2 is strongly expressed in the epithelial cells of the small bowel and the colon (Kaimaktchiev et al., 2004). In comparison, CDX2 is not expressed in the epithelium of the most distal portion of the GI tract (Moskaluk et al., 2003).

CDX2 is also expressed in normal pancreatic tissue, specifically in the small pancreatic ducts, epithelial lining of the ducts, and centroclinal cells of the pancreas (Moskaluk et al., 2003). CDX2 expression in the pancreas is weaker than the expression in the epithelial cells of the GI tract and is more prominent in areas with pancreatitis (Kaimaktchiev et al., 2004). CDX2 expression was not detected in the pancreatic acinar epithelium or the epithelium of the islets (Moskaluk et al., 2003). Additionally, CDX2 is not expressed in the main pancreatic duct or the distal common bile duct (Kaimaktchiev et al., 2004).

CDX2 expression was not observed in any other normal tissues, including the prostate tissue, even though there have been reports of CDX2 in normal prostate tissue (Moskaluk et al., 2003) (Kaimaktchiev et al., 2004).

Multiple studies found that CDX2 is strongly expressed in both benign and malignant epithelium of the intestine (Herawi et al., 2006) and GI tumors (Dennis et al., 2005). This aligns well with CDX2's expression pattern in normal tissue, which is the strongest in the gut and the GI tract (Dennis et al., 2005).

On the other hand, it has been reported that CDX2 is expressed only in colon cancer and is not expressed in gastric cancer (Dennis et al., 2005). Intense staining of CDX2 of more than

50% was found in about 90% of colorectal cancers indicating high CDX2 expression in colon carcinomas (Moskaluk et al., 2003). Another study by Kaimaktchiev et al. found nuclear expression of CDX2 in 97% of colon adenocarcinomas. They also found cytoplasmic expression of CDX2 in 85.7% of colorectal adenocarcinomas (Kaimaktchiev et al., 2004). Moskaluk et al. did not agree with Dennis et al. since they found CDX2 to be expressed in 89% of midgut carcinoids and 44% of hindgut carcinomas (Moskaluk et al., 2003). In contrast, this same expression pattern of CDX2 was found at much lower percentages in other cancers. For example, CDX2 was expressed in 20% of gastroesophageal adenocarcinomas, 20% of mucinous adenocarcinomas, and 4.3% of endometrioid adenocarcinomas (Moskaluk et al., 2003).

CDX2 is also expressed in other carcinomas, including urinary bladder and ovary, specifically in ovarian mucinous tumors (Herawi et al., 2006). In the ovary, 10.5% of mucinous adenocarcinomas had positive CDX2 expression while only 9.3% of endometrioid adenocarcinoma had CDX2 expression (Kaimaktchiev et al., 2004).

CDX2 expression was found to be lower in stomach and pancreas cancers (Dennis et al., 2005). A positive CDX2 expression was found in 28.9% of intestinal-type stomach adenocarcinomas (Kaimaktchiev et al., 2004). Moskaluk et al. also found that CDX2 is expressed in 20-30% of stomach adenocarcinomas. Although CDX2 is expressed in the normal pancreatic tissue, no CDX2 expression was found in ductal pancreatic adenocarcinomas (Moskaluk et al., 2003) (Dennis et al., 2005). In addition, the absence of CDX2 expression in any other pancreatic adenocarcinomas is reported (Dennis et al., 2005). In stomach and pancreas cancers, CDX2 expression was often accompanied by the expression of CK20, mesohaline, and/or CA125 (Dennis et al., 2005).

Less than 1% of the samples with other types of cancer had a strong expression of CDX2 (Moskaluk et al., 2003). Kaimaktchiev et al. found that CDX2 expression decreased in poorly differentiated cancers. They also found an inverse relation between CDX2 expression and tumor grade: a higher grade showed a decreased CDX2 expression. A high expression of CDX2 was found in neuroendocrine tumors derived from the intestinal epithelium (Moskaluk et al., 2003).

3.3.c Function

The primary function of CDX2 is to regulate the axial development of embryos. It also has an essential function in cell proliferation and differentiation of epithelial cells, specifically in the GI tract (Herawi et al., 2006) (Moskaluk et al., 2003). Due to this function, CDX2 is often used as a marker for colon cancers (Moskaluk et al., 2003) and adenocarcinomas of the GI tract in general (Kaimaktchiev et al., 2004). CDX2 can also increase the expression of many genes involved in developing the epithelium of the intestine (Moskaluk et al., 2003).

Some studies have found that CDX2 may act as a tumor suppressor (Moskaluk et al., 2003). CDX2 arrested the proliferation of undifferentiated intestinal cells when its expression was induced in these cells (Moskaluk et al., 2003). However, this finding was skeptical since CDX2 is well overexpressed in the GI tract and adenocarcinomas in that area, making its function as a tumor suppressor unlikely (Moskaluk et al., 2003).

CDX2 expression can also act as a predictor of primary cancers originating from the GI tract (Moskaluk et al., 2003). Tumors that metastasized from the GI tract tend to have high levels of CDX2 expression compared to tumors that metastasized from other areas such as the pancreas, stomach, or lung, which tend to have very low levels of CDX2 expression (Moskaluk et al., 2003).

3.3.d CDX2 expression and function in prostate cancer

CDX2 expression in prostate cancer varies among different studies depending on the subtypes of prostate cancer samples used (Herawi et al., 2006). CDX2 was shown to be weakly expressed in 5.7% of prostate cancers with no dependence on the cancer stage (Herawi et al., 2006). When radical prostatectomies of benign prostate tissues were analyzed for CDX2 expression, 11.7% of cases showed CDX2 expression (Herawi et al., 2006). This same study found no CDX2 expression in metastatic prostate carcinomas (Herawi et al., 2006). Werling et al. found that CDX2 was expressed in only 3.7% of prostate tumors, while Moskaluk et al. only had 2% expression of CDX2 in prostate tumors (Werling et al., 2003). Our analysis using the human protein atlas shows that CDX2 is expressed at higher levels in low-grade prostate cancer as compared to high-grade prostate cancer (Figure 1).

4. Conclusion

Prostate cancer is the second common cause of death for men in America. Most of the research around prostate cancer focuses on finding treatment for advanced stages since patients are often diagnosed only after their prostate cancer has progressed. Early detection screenings can help by diagnosing patients earlier, reducing poor prognoses, and allowing earlier treatment. Because cancer results from abnormal genetic changes that lead to hyperproliferation of cells, regulators of gene expression, such as TFs, present an ideal target for early detection of cancers. We reviewed the expression level and function of three crucial TFs involved in prostate cancer, which can be used to develop a prostate cancer early detection method.

The detailed research studies on FOXA1, HOXB13, and CDX2 provide information on these TFs in prostate cancer and other cancer types. However, the combinatory expression and

function of these three TFs have not been addressed before. From the information we reviewed here and expression data we collected from the Human Protein Atlas as seen in Figure 1, we observe that: FOXA1 expression increases with the tumor grade, HOXB13 seems to be moderately expressed regardless of grade, and CDX2 expression is inversely related to tumor grade in prostate cancer. We observe patchy stains for CDX2 in low-grade, weaker staining in medium-grade, and no expression of CDX2 in high-grade prostate adenocarcinomas. These results indicate that all three TFs are combinatorically expressed in many localized prostate tumors with various grades. Additionally, the interaction of FOXA1 and HOXB13 leading to prostate carcinogenesis and potential CDX2 regulation of HOXB13 in the prostate tissue indicate that these three TFs may have a synergistic function in aggressive prostate cancer. We speculate that combinatory expression of FOXA1, HOXB13, and CDX2 in the early stages of prostate tumors may mark "low-grade" prostate tumors with a high potential of transforming into aggressive prostate cancer. Further research needs to be done analyzing expression levels of FOXA1, HOXB13, and CDX2 using immunofluorescent microscopy to analyze biopsy samples from patients who started with a low-grade tumor that progressed to a medium- or high-grade prostate tumor.

Studying the levels and combinatory function of FOXA1, HOXB13, and CDX2 will ultimately help generate methods for prostate cancer early detection that use these TFs as target markers. As discussed earlier, TFs regulate gene expression in various ways, and many of them have the ability to induce cellular hyperproliferation and uncontrolled division. These TFs often tend to vary in expression levels in healthy and cancer cells. An early detection screening test can measure the levels of the three TFs reviewed here and, based on a decrease or an increase,

can then be used to make an early diagnosis of patients with prostate cancer. As explained previously, with early diagnosis, patients can have improved prognoses and outcomes.

5. Acknowledgment

I would like to thank my thesis advisor Dr. Sebnem Ece Eksi for her mentorship, guidance, and supervision throughout the research process. Her work in prostate cancer early detection and wide knowledge in tumor evolution and heterogeneity continue to inspire me to be involved in research and make an impact in the field.

Figure 1. The protein expression patterns of FOXA1, HOXB13, and CDX2 in low-, medium- and high-grade prostate cancer collected from the human protein atlas (The Human Protein Atlas, 2020).

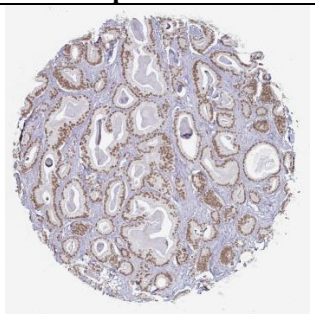
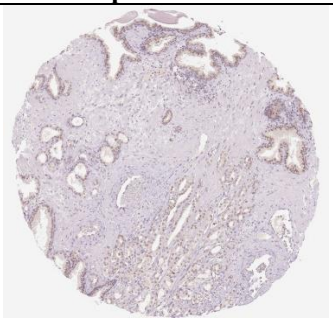
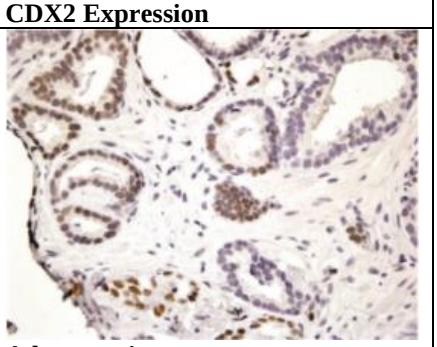
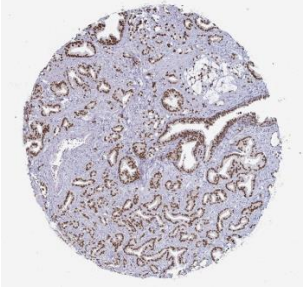
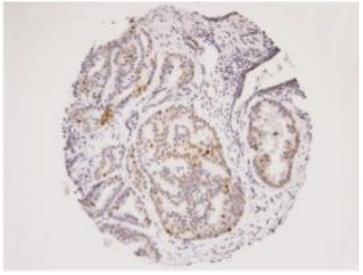
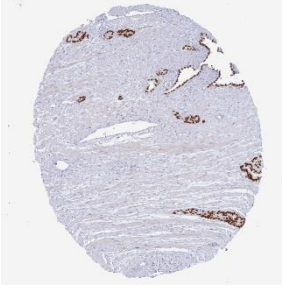
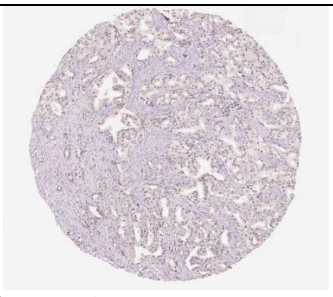
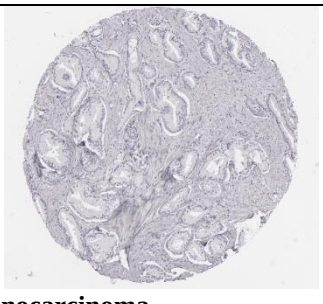
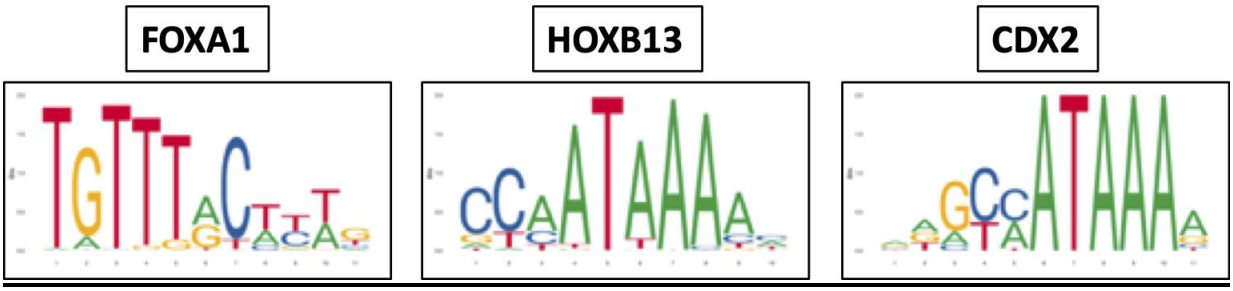
Prostate cancer Grade	FOXA1 Expression	HOXB13 Expression	CDX2 Expression
Low Grade	 **Adenocarcinoma** **Moderate Intensity** **>75%** **Nuclear** **PT ID: 4519** **HPA050505**	 **Adenocarcinoma** **Moderate Intensity** **>75%** **Nuclear** **PT ID: 4359** **HPA062852**	 **Adenocarcinoma** **3+3 =6 low grade** **Patchy staining**
Medium Grade	 **Adenocarcinoma** **Strong Intensity** **>75%** **Nuclear** **PT ID: 3454** **HPA050505**	**No Data Available**	 **Adenocarcinoma** **4+3 = 7 medium grade** **Patchy cdx2 staining**
High Grade	 **Adenocarcinoma** **Strong Intensity** **>75%** **Nuclear** **PT ID: 4337** **HPA050505**	 **Adenocarcinoma** **Moderate Intensity** **>75%** **Nuclear** **PT ID: 2812** **HPA062852**	 **Adenocarcinoma** **Negative Intensity** **0%** **No location** **PT ID: 689** **CAB002221**

Figure 2. The DNA binding sequences of FOXA1, HOXB13, and CDX2, shown as position weight matrices from the JASPAR database (JASPAR, 2020).



References

American Cancer Society. (2020, January 8). Key Statistics for Prostate Cancer: Prostate Cancer Facts. Retrieved November 08, 2020, from https://www.cancer.org/cancer/prostate-cancer/about/key-statistics.html

Attard, G., Parker, C., Eeles, R. A., Schröder, F., Tomlins, S. A., Tannock, I., Drake, C. G., & De Bono, J. S. (2016). Prostate Cancer. The Lancet, 387(10013), 70-82.

Bernardo, G. M., & Keri, R. A. (2012). FOXA1: A Transcription Factor with Parallel Functions In Development And Cancer. Bioscience Reports, 32(2), 113-130.

Bhatlekar, S., Fields, J. Z., & Boman, B. M. (2014). HOX Genes and Their Role In The Development Of Human Cancers. Journal of Molecular Medicine (Berlin, Germany), 92(8), 811-823. https://doi.org/0.1007/s00109-014-1181-y

Black, M., Podgorski, G., Quillin, K., Carmichal, J., Burggren, W., Allsion, L., Taylor, E. & Freeman, S. (2016). *Biological Science*, (6). Pearson.

Cancer.net Editorial Board. (2019, December 12). Prostate Cancer - Stages and Grades. Retrieved November 28, 2020, from https://www.cancer.net/cancer-types/prostate-cancer/stages-and-grades

Dai, C., Heemers, H. & Sharifi, N. (2017). Androgen Signaling in Prostate Cancer. Cold Spring Harbor Perspectives in Medicine, 7(9), a030452. https://doi.org/10.1101/cshperspect.a030452

Decker, B., & Ostrander, E. A. (2014). Dysregulation of The Homeobox Transcription Factor Gene HOXB13: Role In Prostate Cancer. *Pharmacogenomics and Personalized Medicine, 7,* 193-201. https://doi.org/10.2147/PGPM.S38117

Dennis, J.L., Hvidsten, T. R., Wit, E. C., Komorowski, J., Bell, A. K., Downie, I., Mooney, J., Verbeke, C., Bellamy, C., Keith, W. N., & Oien, K. A. (2005). Markers of Adenocarcinoma Characteristic of the Site of Origin: Development of a Diagnostic Algorithm. *Clinical Cancer Research*, *11*(10) 3766-3772 https://doi.org/10.1158/1078-0432.CCR-04-2236

Division of Cancer Prevention and Control, Centers for Disease Control and Prevention. (2020, June 08). Prostate Cancer Statistics. Retrieved November 08, 2020, from https://www.cdc.gov/cancer/prostate/statistics/index.htm

Epstein, J. I., Egevad, L., Amin, M. B., Delahunt, B., Srigley, J. R., Humphrey, P. A., & Grading Committee (2016). The 2014 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma: Definition of Grading Patterns and Proposal for a New Grading System. *The American journal of surgical pathology*, *40*(2), 244–252. https://doi.org/10.1097/PAS.0000000000000530

Etzioni, R., Urban, N., Ramsey, S., McIntosh, M., Schwartz, S., Reid, B., Radich, J., Anderson, G., Hartwell, L. (2003). The Case for Early Detection. *Nature Reviews*, *3*(), 243-252. https://doi.org/10.1038/nrc1041

Ewing, C.M., Ray, A.M., Lange, E.M., Zuhlke, K.A., Robbins, C.M., Tembe, W.D., Wiley, K.E., Isaacs, S.D., Johng, D., Wang, Y., Bizon, C., Yan, G., Gielzak, M., Partin, A.W., Shanmugam, V., Izatt, T., Sinari, S., Craig, D.W., Zheng, S.L., Walsh, P.C., Montie, J.E., Xu, J., Carpten, J.D., Isaacs, W.B., & Cooney, K.A. (2012). Germline Mutations in HOXB13 and Prostate-Cancer Risk. *The New England Journal of Medicine*, *366*(2), 141–149. https://doi.org/10.1056/nejmoa1110000

Foley, C., & Mitsiades, N. (2016). Moving Beyond the Androgen Receptor (AR): Targeting AR-Interacting Proteins to Treat Prostate Cancer. *Hormones & Cancer, 7*(2), 84-103. https://doi.org/10.1007/s12672-015-0239-9

Hankey, W., Chen, Z., & Wang, Q. (2020). Shaping Chromatin States in Prostate Cancer by Pioneer Transcription Factors. Cancer Research, 80(12), 2427-2436.

Herawi, M., De Marzo, A., Kristiansen, G., & Epstein, J. I. (2007). Expression of CDX2 In Benign Tissue and Adenocarcinoma of The Prostate. Human Pathology, 38, 72-78.

Heidegger, I., Tsaur, I., Borgmann, H., Surcel, C., Kretschmer, A., Mathieu, R., Visschere, P., Valerio, M., Van Den Bergh, R.C.N., Ost, P., Tilki, D., Gandaglia, G., Ploussard, G.; EAU-YAU Prostate Cancer Working Party. Hereditary Prostate Cancer - Primetime For Genetic Testing? Cancer Treatment Reviews, 81, 101927-101927. https://doi.org/10.1016/j.ctrv.2019.101927

JASPAR 2020: An open-access database of transcription factor binding profiles. (n.d.). Retrieved November 30, 2020, from http://jaspar.genereg.net/

Kaimaktchiev, V., Terracciano, L., Tornillo, L. *et al.* (2004). The homeobox intestinal differentiation factor CDX2 is selectively expressed in gastrointestinal adenocarcinomas. *Mod Pathol 17***,** 1392–1399. https://doi.org/10.1038/modpathol.3800205

Labbe, D. P., & Brown, M. (2018). Transcriptional Regulation in Prostate Cancer. Cold Spring Harb Perspect Med, 8(11), a030437. https://doi.org/10.1101/cshperspect.a030437

Larnaudi, L., Comperat, E., Conort, P., & Varinot, J. (2017). HOXB13 A Useful Marker In Pleomorphic Giant Cell Adenocarcinoma Of The Prostate: A Case Report And Review

Of The Literature. *Virchows Arch, 471*, 133-136. https://doi.org/10.1007/s00428-017-2134-y

Li, Q. L., Yang, Z. L., Liu, J. Q., & Miao, X. Y. (2011). Expression of CDX2 and hepatocyte antigen in benign and malignant lesions of gallbladder and its correlation with histopathologic type and clinical outcome. *Pathology oncology research: POR, 17*(3), 561–568. https://doi.org/10.1007/s12253-010-9346-7

Markman, M. (2020, November 05). Types of Prostate Cancer: Common, Rare and More. Retrieved November 08, 2020, from https://www.cancercenter.com/cancer-types/prostate-cancer/types

Moskaluk, C., Zhang, H., Powell, S. et al. (2003). Cdx2 Protein Expression in Normal and Malignant Human Tissues: An Immunohistochemical Survey Using Tissue Microarrays. *Mod Pathol, 16*, 913–919. https://doi.org/10.1097/01.MP.0000086073.92773.55

Murillo-Garzon, V., & Kypta, R. (2017). Wnt Signalling in Prostate Cancer. *Nature Reviews Urology, 14*, 683-696. https://doi.org/10.1038/nrurol.2017.144

National Institute of Cancer. (2020). Cancer of the prostate - cancer stat facts. Retrieved February 16, 2021, from https://seer.cancer.gov/statfacts/html/prost.html

Prostate Cancer Foundation. (2019, December 26). Prostate Cancer Screenting and Early Detection. Retrieved November 30, 2020, from https://www.pcf.org/about-prostate-cancer/diagnosis-staging-prostate-cancer/screening-early-detection/

Sen, A., Mitra, S., Das, R. N., Dasgupta, S., Saha, K., Chatterjee, U., Mukherjee, K., Datta, C., & Chattopadhyay, B. K. (2015). Expression of CDX-2 and Ki-67 in different grades of

colorectal adenocarcinomas. *Indian journal of pathology & microbiology*, *58*(2), 158–162. https://doi.org/10.4103/0377-4929.155304

St. Anothony Blogs. (2019, October 29). Home. Retrieved November 30, 2020, from https://www.stanthonyhospital.org/news/blogs-from-stanthony/the-importance-of-early-detection-for-prostate-cancer/

Takakura, Y., Hinoi, T., Oue, N., Sasada, T., Kawaguchi, Y., Okajima, M., Akyol, A., Fearon, E. R., Yasui, W., and Ohdan, H. (2010). CDX2 Regulates Multidrug Resistance 1 Gene Expression in Malignant Intestinal Epithelium. *Cancer Research, 70* (17), 6767-6778. https://doi.org/10.1158/0008-5472.CAN-09-4701

The American Cancer Society Medical and editorial content team. (2019, August 1). Prostate Cancer Stages and Other Ways to Assess Risk. November 08, 2020, from https://www.cancer.org/cancer/prostate-cancer/detection-diagnosis-staging/staging.html

The Human Protein Atlas. (n.d.). Retrieved November 30, 2020, from https://www.proteinatlas.org/

Tymoczko, J. L., Berg, J. M., & Stryer, L. (2015). *Biochemistry: A Short Course,* (3). W. H. Freeman.

UCSC Genome Browser Home. (n.d.). Retrieved November 30, 2020, from https://genome.ucsc.edu/

Werling RW, Yaziji H, Bacchi CE, et al. CDX2, a highly sensitive and specific marker of adenocarcinomas of intestinal origin: an immunonhistochemical survey of 476 primary and metastatic carcinomas. (2003). The American journal of surgical pathology, 27(3), 1532-0979. https://doi.org/10.1097/00000478-200303000-00003

World Health Organization. (2018, September 12). Cancer. Retrieved November 08, 2020, from https://www.who.int/news-room/fact-sheets/detail/cancer

Yuan, J., Yin, Z., Tao, K., Wang, G., & Gao, J. (2017). Homeobox protein CDX2 as a prognostic biomarker in solid malignancies: a meta-analysis. *Oncotarget*, *8*(51), 89160–89172. https://doi.org/10.18632/oncotarget.20808