Bachelor's Thesis

Role of human HOX-proteins in different cancer types

Vladislav Iaroshenko

Oulun yliopisto
Biokemian ja molekyylilääketieteen tiedekunta
2019

Used contractions:

ALDH – aldehyde dehydrogenase

Bcl-2 - B-cell lymphoma 2

BTM – basal transcription machinery

CBP - CREB-binding protein

CDCA3 - Cell division associated protein-3

EGFR – epidermal growth factor receptor

EMT - epithelial-mesenchymal transition

EOC - Epithelial ovarian cancer

ER - estrogen receptor

FADD - Fas-associated protein with death domain

GTF - general transcription factor

H3K27me3 – trimethylation to the lysine 27 on the histone H3

H3K4me3 – trimethylation to the lysine 4 on the histone H3

HAT – histone acetyltransferase

HOX - Homeotic complex

Hs578T – Homo Sapiens 578T

ICAM-1 – intercellular adhesion molecule 1

IGF – insulin-like growth factor

IGFBP - insulin-like growth factor binding protein

ISL1 - Insulin gene enhancer protein

MBIC - muscle invasive bladder cancer

MCF-7 - Michigan Cancer Foundation-7

MEIS – myeloid ecotropic integration site

NEP – neural endopeptidase

NF-kB - nuclear factor kappa-light-chain-enhancer of activated B cells

NMBIC - non-muscle invasive bladder cancer

NSLMC – non-small-cell lung carcinoma

OSE - ovarian surface epithelium

PAX8 - paired box gene 8

PBC - Pre-cell B leukaemia transcription factor

PIC – preinitiation complex

RAR - retinoic acid receptor

RARE - retinoic acid responsive element

RBX – RING-box

RKO – colorectal carcinoma

SCF – Skp, Cullin, F-box

SLMC - small-cell lung carcinoma

SNP – single nucleotide polymorphism

SRC-3 - steroid receptor coactivator-3

TAD – topologically associating domain

TALE - three amino acid loop extension

TBP – TATA-binding protein

TF – transcription factor

TNF – tumor necrosis factor

TNFR - tumor necrosis factor receptor

VLA – very late antigen

Wnt – Wingless/Integrated

Wt1 - Wilms tumor protein

Table of contents

1. Introduction

Genes are stretches of DNA containing specific instructions to form proteins. Proteins are synthesized according to this DNA code every day during embryogenesis as well as in adult organism. To develop a functional organism from one single cell and to maintain its homeostasis when it is mature, very precise regulation is needed. Every cell contains a copy of the same DNA sequence or, in other words, all the possible genes for that particular organism. But only a certain part of them is used for protein synthesis and even this protein coding part is regulated by different stimuli such as hormones, for example.

Hormones may act through nuclear or cell membrane receptors, depending on their solubility. Hormone binding to the receptor leads to activation of certain transcription factors needed for the initiation of a process known as transcription or production of mRNA from the DNA. Different transcription factors have different targets that are activated or inactivated by them, also they may be activated differently. In this thesis I will discuss the role of HOX-family transcription factors in different cancer types.

1.1. Protein synthesis and transcription

To create an mRNA copy of a gene coding a certain protein, a particular region of the DNA is used as a template to produce a complementary mRNA. Process of mRNA synthesis is done by RNA-polymerase that binds to a promoter region of genes. To direct the binding of the RNA-polymerase to a particular promoter region a group of different proteins is needed. There are many supportive proteins and transcription factors that belong to this group. Thus, initiation of transcription is the process where transcription factors significance is highlighted. Therefore, this process should be described more in detail to uncover the role of transcription factors. Since the thesis is focused on human HOX-proteins we will only describe eukaryotic transcription.

Basal transcription machinery (BTM) contains fundamental elements needed for transcription in general which can be affected or directed by other transcription factors for more specificity, for example by HOX-proteins.

Main components of the BTM - 1) RNA-polymerase (II), 2) transcription factor (TF) complexes - TFIIB, TFIID, TFIIE, TFIIF, TFIIH and TFIIA, 3) Mediator-complex of proteins.

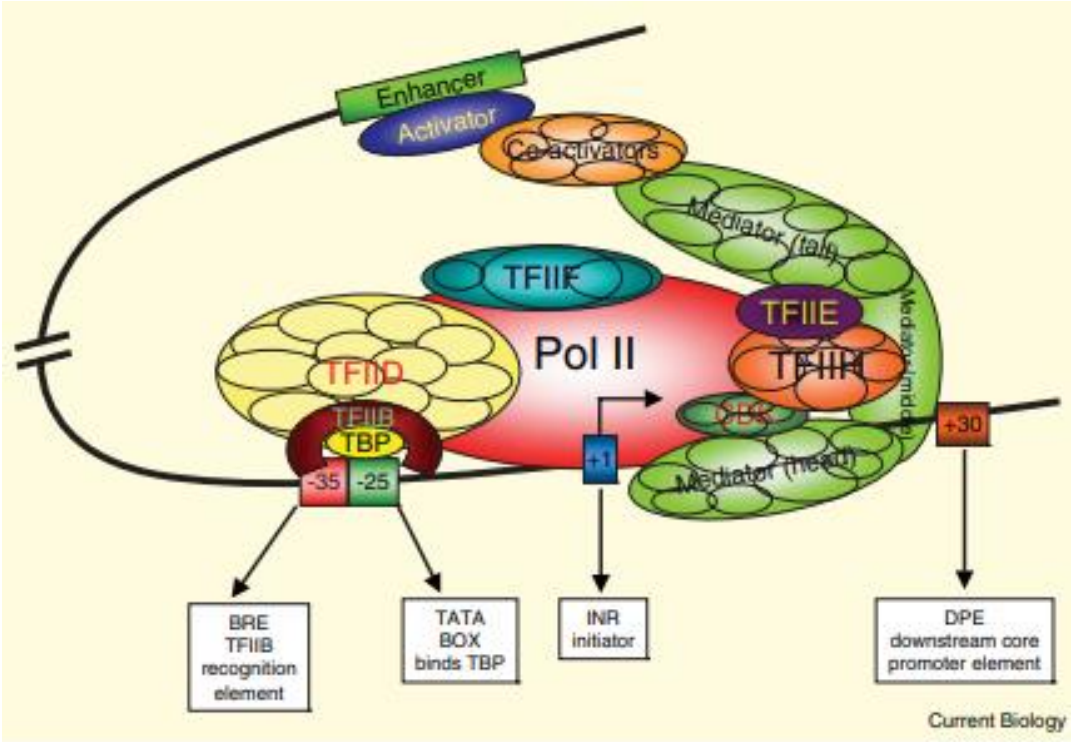

Figure 1. Schematic summary of a Pol II transcription preinitiation complex (PIC). Core promoter elements are depicted at positions –35, –25, +1 (start site of transcription) and +30. PIC assembly is nucleated by binding of the TBP subunit of TFIID to the TATA box, followed by stepwise binding of the general transcription factors, TFIIB, Pol II/TFIIF, TFIIE and TFIIH. (Figure and text from Krishnamurthy-Hampsey 2008)

To start describing the initiation of transcription, the structure of the gene is also to be determined. Gene consists of the following main regions: enhancer region, promoter region, and coding region. Promoter region includes a very important consensus motif called TATA-box. TATA indicates here a repeating pattern of thymine and adenine nucleotides. TFIID starts the formation of the preinitiation complex (PIC) by specifically binding to the TATA-box with help of its TBP (TATA-binding protein) subunit. This binding promotes structural changes strongly bending the DNA. The enhancer region may be located after the promoter region in the direction to 5' end of the DNA or in the 3' direction. The role of the enhancer is to bind transcription factors, for example, HOX-proteins.

TFIIB is responsible for the next step of PIC formation. This transcription complex is able to bind TBP of the TFIID and it also binds DNA with its helix-turn-helix motif (Reese 2003). Apart from TFIIB transcription complex TFIIA also interacts with TBP thus stabilizing the PIC.

TFIIH has helicase and ATP-hydrolase activity, so it plays a role in DNA unwinding that is critical for transcription initiation. It has also kinase activity to phosphorylate the CTD domain of the RNA-polymerase. CTD phosphorylation is needed for initiation and processivity of the RNA polymerization activity of the RNA polymerase.

TFIIE ensures the binding of TFIIH to the PIC (Okamoto *et al* 1998) and possibly has other roles. TFIIF does not play a role in the transcription initiation as such, but it was observed to stabilize TFIIB after transcription initiation (Cabart *et al* 2011).

The binding of inactive RNA-polymerase to the complex happens via the TBP domain of TFIID.

The next level of the transcription is a big protein complex called Mediator-complex. It has many functions one which is signal transduction from the activators or repressors bound to enhancer region. For example, Med19 a member of the Mediator-complex was found to bind Hox-protein in *Drosophila melanogaster* and thereby access RNA polymerase II machinery (Boube *et al* 2014). Mediators or cofactors are essential for relaying the stimulus from specific transcription factors (like, for example, HOX-protein) to the general transcription machinery (Figure 2).

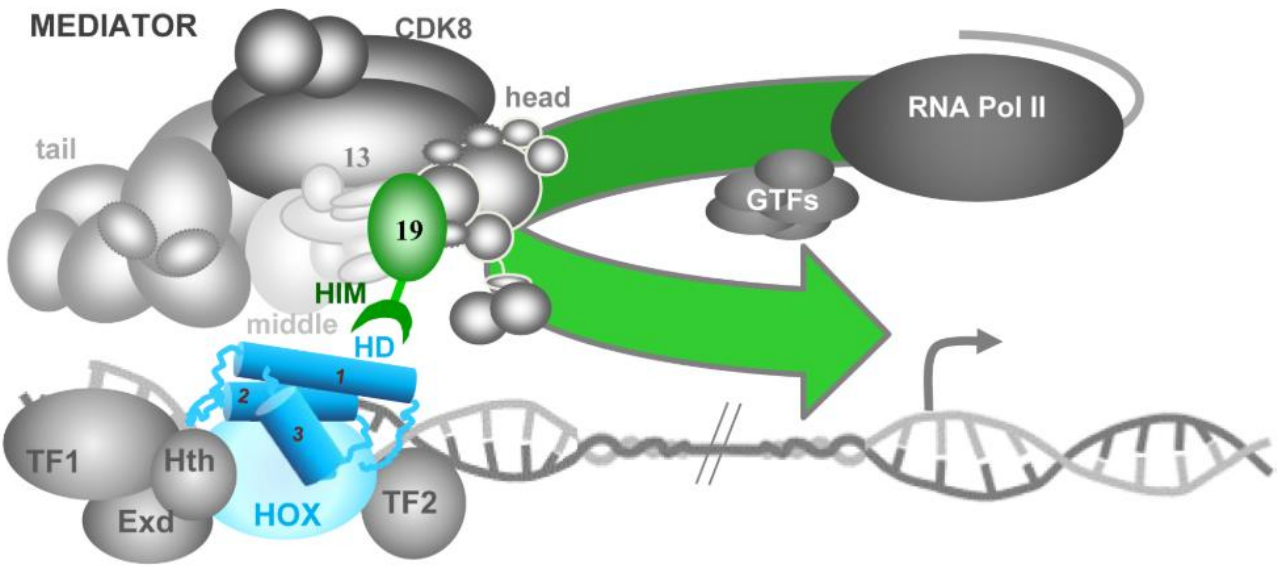

Figure 2. The Mediator complex, composed of four modules – tail, middle, head and CDK8 –, binds physically to PolII, principally through its head module. Hox transcription factors (HD in blue, its three α-helices indicated as cylinders) bind to regulatory DNA sequences distant from the transcription start site (grey arrow), together with unknown numbers of other TFs (here, Hox co-factors Exd and Hth plus cell-specific factors TF1 and TF2). We propose that the DNA-bound Hox homeodomain serves to recruit MED directly through Med19 HIM (green hook). This Hox-MED association then permits the general PolII transcription machinery (PolII+GTF) to be recruited to the Hox target promoter. Text and figure from Boube *et al* 2014.

2. HOX-protein family and their structure

In human, HOX-proteins can be divided into 13 paralog groups that can be further divided into 4 groups from A to D based on their genomic location. HOX paralogs within each cluster are located in a single locus in the 3' to 5' direction starting from the 1st to the 13th paralog respectively. The closer the gene is to the 3' end of the HOX locus the more anterior part of the body is mapped by respective HOX protein during the embryogenesis. Therefore, HOX proteins can also be divided into 3 following groups: anterior (paralogues 1-4), central (paralogues 5-8), posterior (paralogues 9-13).

HOX proteins contain two main parts: a hexapeptide motif and a homeodomain. The latter is closer to the N-terminus and is responsible for DNA binding. It contains three helices with loops between them (helix-loop-helix motif). The first two helices lie in the minor groove of the DNA while the third helix makes specific contacts with base pairs of the major groove. However, sequences recognized by the third helix are just 4-6 base pairs long, therefore, there is a big possibility for such sequences to be repeated several times in different parts

of DNA and these sequences are not enough to create specificity for such transcription factors as HOX proteins. So it is logical that there must be a way to increase the specificity. Pre-B cell leukaemia transcription factor (PBC) play an important role in conveying specificity to HOX DNA-binding.

PBC proteins also contain a homeodomain but their homeodomains belong to three amino acid loop extension (TALE) class. Homeodomains of HOX and PBC proteins both contain the homeobox sequence found in different eukaryotes. Both HOX and TALE domains contain a helix-turn-helix motif but in TALE proteins there are three extra amino acids between two first helices. The homeodomain of HOX proteins contains 60 amino acids and the homeodomain of PBC proteins contains 63 amino acids. HOX and PBC proteins form heterodimers through the hexapeptide motif of HOX proteins (will be discussed later). The consensus sequence in the enhancer region recognized by HOX/PBC heterodimer is 5'-TGATTNAT-3', where TGAT (1-4 base pairs) is recognized by PBC homeodomain and TNAT (5-8 base pairs) by HOX homeodomain. The 6^{th} base (depicted as N) varies in different HOX proteins and determines their binding specificity. However, this specificity was observed for single HOX proteins, whereas HOX-PBC heterodimers got more flexibility at position 6.

Hexapeptide has the following consensus sequence: hydrophobic residue-Y/F-P-W-M-K/R (Piper *et al* 1999). In contrast, another study reported great variation in the hexapeptide sequence in HOX proteins and suggested that only the tryptophan is common to all the different variants (LaRonde-LeBlanc-Wolberger 2003). Hexapeptide motif forms a 3_{10} helix the function of which is to insert the very conserved tryptophan residue in the hexapeptide-binding pocket of the PBC proteins (Piper *et al* 1999). 3_{10} helix is a subtype of α-helix, but 3_{10} helices are usually short and they are triangular in a cross-section.

3. Function of HOX proteins.

3.1. As transcription factors in embryogenesis.

The main role of HOX proteins is to direct the transcription during embryogenesis. It is a complicated process and its mechanism still contains many unknown details. However, there are many different hypotheses about different parts of this regulation that provide together an interesting picture of the patterning along the anterior-posterior axis during embryonic development.

Vertebrates have an exceptional organization of HOX-genes in the genome comparing to other species. In vertebrates, HOX genes are densely clustered in 4 different groups on four different chromosomes. During the evolution, the organization of HOX-genes developed from dispersed to clustered in the vertebrates. These four clusters are A, B, C and D that can all be divided into 13 paralogs. Clusters contain different amounts of paralogs, often missing a few. But in the vertebrates, it is never more than 13 paralogs per each cluster. Moreover, the direction of gene transcription is the same for each paralog inside the cluster in vertebrates.

The expression of HOX-proteins starts in the posterior-to-anterior direction of the epiblast and is collinear with the organization of HOX-clusters. HOX-genes that are closer to 3' end of the DNA are expressed more anteriorly and vice versa. This is called spatial collinearity and it is directly connected to the second important feature of HOX-patterning – temporal collinearity. Temporal colinearity means that more anterior parts along the embryonic axis are developed earlier than more posterior ones.

The precise mechanism of the HOX-gene establishment during embryonic development is unknown, but there are several interesting hypotheses. Two first hypotheses are based on the different sensitivity of enhancers of HOX to the inducer (the possible inducer will be discussed later). It was hypothesized that more anterior HOX-genes are more sensitive to the inducer and thus the gradient of the spreading inducer would determine the individual HOX-expression for different regions of embryonic cells. In the first hypothesis, the constant spreading of the inducer is required, whereas only a short-time exposure is required according to the second hypothesis.

The most preferred model, however, is based on histones instead of enhancer sensitivity. The three-step model is based on the fact that the inducer is spread starting from the more posterior part. So it affects more posterior cells earlier, in which it is believed to affect histone binding at the HOX-cluster region. Depending on its duration of effect it manages to "open" the HOX-cluster to a different extent in different parts of the epiblast. So, in more posterior parts, the "opening" would progress starting from more anterior cluster to more posterior, whereas in more anterior parts of the epiblast only more anterior clusters would be ready for the transcription. After exposure to the inducer, each region of cells would acquire an individual HOX-expression program in which only more posterior parts of the epiblast would express the posterior clusters (Gaunt-Strachan 1996).

3.1.1. Genetic regulation of HOXA-cluster as an example

Although HOX genes are master regulators, they, like other genes, also need to be expressed with the help of certain activators. Still, the precise regulation of the initiation of HOX-genes' transcription is not entirely clear.

The putative HOX inducer in the posterior part of the epiblast is Wnt3 that was found to be expressed in the proximo-posterior epiblast before the gastrulation (Rivera *et al* 2005).

To explain the proposed effect of the Wnt-signal on expression some terminology has to be revised. H3K4me3 is a three-methylated modification of histone H3 that is known as an activating or a state of the histone in which it is not tightly bound to the DNA thereby allowing transcription. On the opposite, H3K27me3, another three-methylated modification of the histone H3, is associated with the downregulation of gene transcription or, in other words, a state where histone is bound to the DNA.

The correlation between the time of Wnt-induction and the amount of the two above-listed types of histones was studied in epiblast tissues. It was observed that the longer the time of Wnt-induction the more H3K4me3 and less H3K27me3 modifications we seen across the HOXA cluster. So the exposure to Wnt opens the DNA in the region of the HOXA cluster making it accessible for further transcription.

Like other genes, HOXA-cluster requires enhancer regions to transmit the signal from the inducer molecule. Although in the transcription model regulatory region is usually located next to the gene in the 3' side direction, it is not necessarily structured this way. Regulatory regions of a given gene may be placed relatively far, moreover, the same "regulatory cluster" may be responsible for more than one gene as in the case with HOXA-cluster. Areas of the chromatin where the self-interaction is most abundant are called topologically associating domains (TAD). So these are areas of the chromatin where the interactions between the particular gene and its regulatory elements occur.

The big area called 3'subTAD and also a part of 5'TAD are parts of chromatin that contain the highest amount of interactions with the HOXA cluster and its inducer. HOXA cluster lies between two TADs: 3'TAD and 5'TAD, where region 3'subTAD is a part of 3'TAD that also contains the HOXA cluster itself. Most genes of the HOXA cluster have interactions with 3'subTAD, whereas the HOXA13 is the only one to interact with the 5'TAD.

3.1.2. Cis-regulation of HOXA-genes

In this subchapter, the structure of the 3'subTAD will be discussed to show possible enhancers and other regulatory parts that are observed to be involved in the regulation of HOX-genes' transcription.

Wnt-pathway is the signal transduction leading to the stabilization of the β-catenin that is transported to the nucleus and acts as transcription factor. In the case of the HOXA-cluster, binding sites of the β-catenin are hypothesized to be possible enhancer regions required for the transcription initiation.

Elements of the 3'subTAD that are observed to regulate expression of the early HOXA genes are called HOXA developmental early side (Ades). There are six Ades proteins. Ades1 is involved in the expression of HOXA1 member of HOXA-cluster, so the initial signal from the Wnt3 activates the transcription of the HOXA1 gene through Ades1 regulatory element. Ades2-Ades6 were observed to interact with 3' and middle HOXA genes. β-catenin binding sites overlap with all the six Ades regions (Neijts *et al* 2016).

The first enhancers becoming active are Ades1 and Ades2 that become acetylated after the exposure to Wnt3. This leads to the initial expression of the HOXA1 in the posterior-anterior gradient direction of the spreading Wnt3 along the epiblast. It was also noticed that Ades regions 3-6 may already interact with β-catenin in an uninduced epiblast, whereas Ades1 and Ades2 are opened only upon exposure to Wnt3. When all the enhancers are acetylated together with the gradual HOXA cluster opening the expression continues from HOXA1 towards 5' neighbors.

In the case where Ades1-6 enhancer region was deleted only the response of HOXA1-HOXA5 was decreased, while 5' neighbors HOXA7 and HOXA9 were not affected. Wnt signaling through Ades enhancers is important for the transcription initiation of more anterior HOXA genes in the very beginning of the gastrulation.

The expression of the HOXA-cluster middle part was observed to require Cdx2 that is needed for opening the DNA region of middle HOXA genes. Interestingly the expression of Cdx2 is regulated by the Wnt3 signal, such that it gets expressed in the epiblast only after exposure to Wnt3 (Neijts *et al* 2017).

The last member of the cluster to be expressed is HOXA13, but its expression is probably regulated by other enhancers located in the 5'TAD. HOXA13 acts as a transcription factor and stops the axial elongation, and regulates limb development.

4. HOX - proteins and carcinogenesis

Due to the fundamental role of HOX-family proteins in embryogenesis, alterations of HOX expression are known to be connected with different cancer types. Furthermore, not only

the up- or downregulation may play a crucial role, but also the type of tissue affects the final result of HOX-protein expression. In this chapter different types of cancer will be discussed with the focus on the role of HOX-proteins in carcinogenesis.

4.1. Prostate cancer

Prostate cancer is the second most common cause of death amongst males in the world, therefore the understanding of the origin of this disease is of high importance (Zhao *et al* 2016). There are various correlations that can be observed for HOX-proteins expression in prostate cancer cases, but the interpretation is uniformly nuanced**.**

4.1.1. HOXC8 and HOXB13 as a source of androgen insensitivity

The expression of HOXC8 correlates with the Gleason grades of prostate tumors. The more aggressive the cancer, the higher the HOXC8 expression (Waltregny *et al* 2002). In benign cells, HOXC8 proteins are almost absent or totally absent, however, in prostate cancer cells its production increases significantly (Waltregny *et al* 2002). HOXC8 is a transcription factor of the HOXC subfamily that is suggested to inhibit CBP (or CREBBP) histone acetyltransferase activity (Shen-Krishnan *et al* 2001), although other sources suggest that it is rather a nuclear receptor coactivator (SRC-3) protein than CBP the activity of which is inhibited by HOXC8 (Axlund *et al* 2010). CBP and p300 are coactivators needed for androgen receptor to function as a transcription factor inside the nucleus of the cell, these proteins contain the histone acetyltransferase domain (HAT) that is needed to perform acetylation of lysine residues in histone. The acetylation of the lysine residues leads to DNA and histone unbinding by neutralizing positive charges of lysine residues (Liu *et al* 2008). As for SRC-3, it is also the transcriptional coactivator with the HAT activity needed for DNA and histones unbinding. In the presence of overexpressed HOXC8 prostate cancer cells become resistant to androgen stimulation (Miller *et al* 2003). CBP HAT activity inhibition is also a possible outcome of HOXB13 protein action (Kim *et al* 2010).

The fact that the androgen activated pathway is inhibited in prostate cancer cells seems to be counterintuitive because androgen mediates growth-stimulating signals. But there is an assumption that it may be explained by the need for cancer cells to be resistant to androgen action because the latter is able to inhibit HOXC8 expression. At early stages, cancer cells may get prepared for the androgen action by preliminary inhibition of its receptor expression (Miller *et al* 2003).

4.1.2. HOXC6 as a possible inhibitor of apoptotic pathways; activator of proliferation

Another possible mechanism of HOX-proteins' action is connected to the expression of neutral endopeptidase (NEP) and insulin-like growth factor binding protein (IGFBP-3). The actual mechanism is not yet known so this information is based on the expression correlation between HOXC6 and the two above-listed proteins. It was observed that the expression of HOXC6 is significantly increased in malignant cells when compared with benign cells. Together with the upregulation of HOXC6 downregulation of NEP and IGFBP-3 was observed. Both of these proteins relate to the group of proapoptotic genes, but these proteins use different mechanisms of switching on the controlled cell death.

NEP causes the proteolysis of neuropeptides as endothelin-1 and bombesin (Sumitomo *et al* 2001). Endothelin-1 and bombesin proteins are small peptides needed for activation of insulin-like growth factor receptors-1 (IGF-1) and the Akt kinase thus increasing growth and proliferation of cells. Besides this mechanism, NEP also acts as an inhibitor of c-SRC kinase the active state of which is required for cell proliferation. c-SRC is usually activated with bombesin and endothelin-1. Also, NEP is able to inhibit protein kinase C degradation thus inducing the apoptosis in the presence of phorbol-esters (Sumitomo *et al* 2000). It is

important to mention that all above-listed mechanisms can take place only in androgen-sensitive prostate cancer cells because the expression of NEP is regulated by androgen receptor that is present in required amount only in androgen-sensitive cells (Shen *et al* 2000).

IGFBP-3 is similarly involved in IGF-1 signaling but as an antagonist of IGF-1 receptor preventing activation of the metabolic pathway by IGF-1 and inhibiting cell proliferation (Thelen *et al* 2004). Furthermore, the action of IGFBP-3 is also connected with transforming growth factor (TGF-β) signaling; TGF-β inhibits growth and activates apoptosis through TGF-β receptors. Although it is not clear whether there is any direct interaction between the TGF-β receptor or not it is known that in the presence of IGFBP-3 the sensitivity of TGF-β growth inhibitory signaling gets increased. (Fanayan *et al* 2000). Therefore, it can be concluded that cell proliferation is activated in androgen-insensitive prostate cancer cells with elevated HOXC6 expression. Additionally, apoptotic pathways are inhibited by possible downregulation of NEP and IGFBP-3.

4.1.3. HOXB3 may possibly transactivate expression of CDCA3

Cell division associated protein-3 (CDCA3) belongs to the F-box-like proteins group, it means that it is a part of SCF complex belonging to E3 ligases the function of which is ubiquitination. SCF complexes consist of the following parts: F-box protein, Skp1, cullin, and RBX1. F-box part of the complex is the specificity determining part; Skp1 and cullin are structural parts of the complex linking the F-box protein and RBX-1 protein the second of which serves the enzymatic activity transferring the ubiquitin to lysine residues of the target protein (Nakayama *et al* 2005).

The role of the whole SCF complex is the recruitment of proteins to proteasomes for proteolysis. In the case of CDCA3 at the position of F-box protein in the complex, the target protein for degradation is Wee1 kinase. Wee1 kinase functions as an inhibitor of cyclin-dependent kinase 1 (cdk1) that activates cell proliferation when it is phosphorylated (Nakayama *et al* 2005).

It was suggested that HOXB3 binds to the CDCA3 promoter region thus activating the expression of the CDCA3. In case of elevated expression of HOXB3, the following upregulation of CDCA3 leads to increased cell proliferation rate (Chen *et al* 2013).

4.1.4. Downregulation of HOX-co-activators Pbx1 and Meis1, Meis2

Pbx and Meis proteins are known to interact with HOX proteins. Pbx proteins contain a TALE motif that is able to bind the hexapeptide motif of HOX proteins (Piper *et al* 1999). There is also an α-helix (α4, HCM) domain that binds to the homeobox pattern of DNA (Piper *et al* 1999). Furthermore, PBC-A and -B domains in Pbx proteins bind to Meis proteins (Rhyoo *et al* 1999). As for Meis proteins they contain HM1 and HM2 domains required to interact with Pbx proteins (Rhyoo *et al* 1999, Burglin 1997, Burglin 1998).

HOX, Meis, and Pbx form a protein complex. The role of this complex is not yet understood and it is not clear whether these interactions affect the specificity or activity of HOX-proteins. But there is a piece of evidence suggesting that downregulation of Pbx1, Meis1, and Meis2 during the progression of prostate oncogenesis may be connected with alterations in HOX-protein activity (Chen *et al* 2012). Also, it has been reported that this interaction does affect the target genes of HOX-proteins. A particular mutation in the Meis interaction domain of HOXB13 protein is connected with prostate cancer (Ewing *et al* 2012). This data suggests that dysregulation of Meis-HOX interaction may lead to the development of prostate cancer. Also, Meis1 and Meis2 and Pbx1 proteins may function as possible tumor suppressors targets for prostate cancer diagnosis or therapy (Chen *et al* 2012).

4.1.5. SNP inside the RFX6 gene a possible predisposition to increased HOXB13 activity

Single nucleotide polymorphism (SNP) is a single nucleotide variation in the genome of different individuals. SNPs are mostly found outside the coding regions of genes. Most SNPs do not influence the protein sequence, but some do. rs339331 is one of the many SNPs within an intron of RFX6 gene. rs339331 has either C or T nucleotide at position 500 of the RFX6 gene. The gene itself is located in chromosome 6 at locus 6q22.

It was observed that the presence of rs339331 T-variant increases the binding of HOXB13 proteins thus increasing expression of the RFX6 gene (Huang *et al* 2014).

RFX6 gene codes the protein that functions as a transcription factor that is known to be required, for example, for pancreatic islet cell development (Soyer *et al* 2014). But there is also evidence that RFX6 upregulation is associated with prostate cancer progression (Huang *et al* 2014). Therefore, it was suggested that rs339331 T-variant may function as a genetic predisposition to prostate cancer and also a possible target for diagnostic sequencing.

4.2. Breast cancer

4.2.1. HOXA5 and apoptosis

The activation of HOXA5 expression was observed to be directly associated with apoptosis in breast cancer cells. As for MCF7 cells with non-mutated p53 gene HOXA5 activated its transcription and subsequent apoptosis cascade (Gupta 2002). But interestingly, p53 was shown not to be the absolute requirement for HOXA5 to activate the apoptosis in Hs578T cells. The DNA of this cell line contains a mutated p53 gene, the mutation affects the binding domain of this gene, and therefore HOXA5 is not able to upregulate this gene (Chen *et al* 2004).

However, the mechanism, in this case, is not well-understood. It is almost definite that HOXA5 activation leads to increased caspase 2 and caspase 8 activity, but the exact way how it happens is not clear, although there are several worthy deductive hypotheses (Chen *et al* 2004). HOXA5 is not able to directly upregulate caspase 2 and caspase 8 proteins, also apoptotic pathway cannot be activated through p53 in Hs578T cells. The first hypothesis proposes an idea of some unknown molecule functioning as proteinase for procaspase 2 and 8 cleavage whose activation is regulated by HOXA5. The next hypothesis is connected to another pathway that starts with the tumor necrosis factor receptor (TNFR) family. These receptors relay a signal through different TNFR-associated proteins with death domains (for example, FADD). So, it is proposed that some adaptor molecules as FADD might exist and be activated directly or indirectly by HOXA5 (Chen *et al* 2004).

Another suggestion tells that HOXA5 may relate to the procaspase-2 release from the mitochondrion. It was found that mitochondria of different organs contain procaspase-2 and procaspase-9 that are released upon opening of the mitochondrial permeability transition pore (PTP) by different sources of activation (Susin *et al* 1999). In this case, it is assumed that HOXA5 may activate PTP opening, although bongkrekic acid (known PTP blocker) had no effect on apoptosis driven by HOXA5.

In summary, HOXA5 activation leads to apoptosis induction by caspase-2 and caspase-8 activation. Also, HOXA5 upregulation plays a significant role as a factor determining the balance of TNFR-pathway. Even in the presence of a small dose of TNF-α the process more likely ends in apoptosis if the HOXA5 gene is induced in the cell. As for numbers, quite a significant difference is observed when comparing the effect of concentration of TNF-α in different cells. Cells with inactive HOXA5 did not start the apoptosis cascade even at 100

ng/mL TNF-α concentration, whereas in HOXA5-induced cells 1 ng/mL of TNF-α was enough to activate apoptosis (Chen *et al* 2004). The balance mentioned above occurs since TNFR activation may stimulate different targets in cell metabolism. Besides caspase activation through FADD adapter molecules, it may also lead to activation of MAP-kinase (mitogen-activated pathway) and NFkB (nuclear factor kappa-light-chain-enhancer of activated B cells). MAP kinase activates proliferative pathways; NFkB is a protein complex known for being able to activate apoptosis inhibition in cells (Gupta 2002).45 So, in the presence of active HOXA5, the balance between the pathways is determined by supportive activation of caspases-2 and -8 and therefore apoptosis prevails (Chen *et al* 2004).

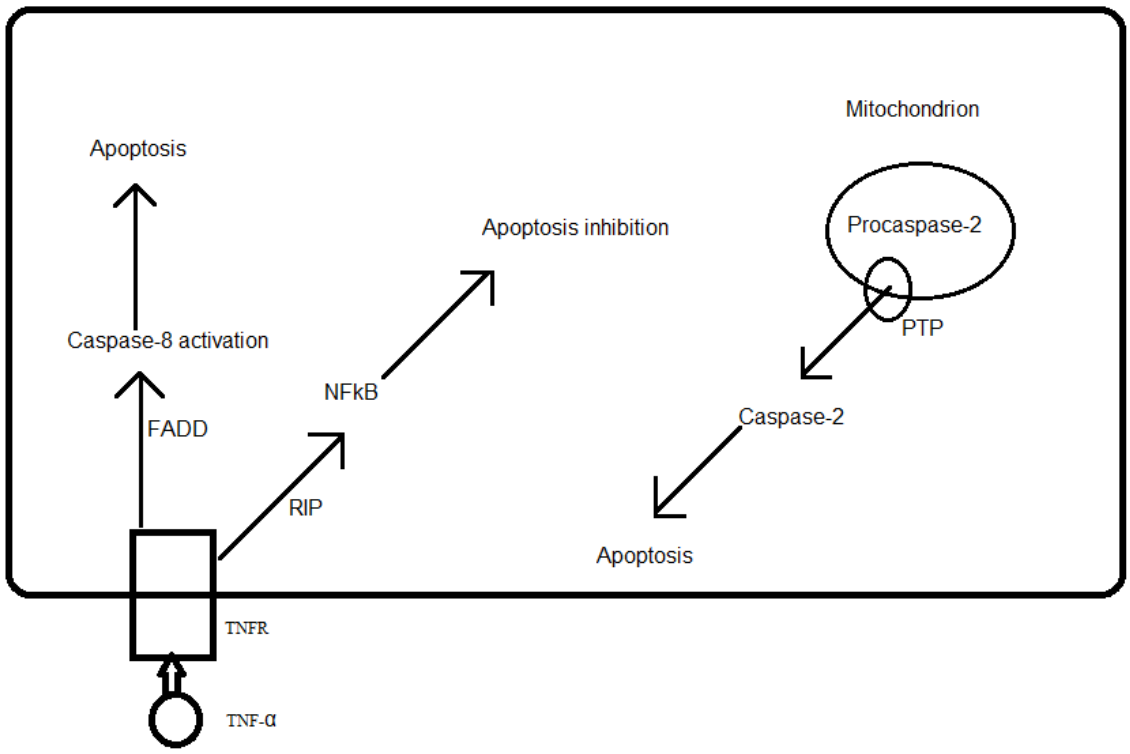

Figure 3. Schematic vies of pathways described in chapter 4.2.1. TNFR is able to mediate both proliferative and apoptotic pathways. It was noticed that apoptotic pathway prevails in presence of HOXA5. The right side of the figure shows an alternative apoptotic pathway that includes the mitochondrion. It is hypothesized that HOXA5 might activate the opening of the PTP. Material for the figure is taken from Chen *et al* 2004.

4.2.2. HOXA5 activation by retinoic acid.

The expression of HOXA5 was observed to be activated by retinoic acid through different retinoic acid receptors.

Retinoic acid is a vitamin A derivative that is able to activate HOX-proteins' expression during embryogenesis (Chen *et al* 2007). It was also tested whether retinoic acid is able to induce HOXA5 expression as a way to activate apoptosis (Chen *et al* 2007).

It was found that activated retinoic acid receptors (primarily RARα and RARβ) bind to specific retinoic acid responsive element (RARE) downstream from HOXA5 gene (in 3' direction). This RARE contains a common RAR motif needed for binding of RAR. The sequence of this motif is AGGTCA which is believed to be recognized not only by RARα and RARβ but also by other RARs.

It means that retinoic acid could potentially be used as chemopreventive agent; although it is important to mention that not all the breast cancer cell lines reacted to retinoic acid in the same way, so it can be used only in some specific cases. Firstly, only RARβ-positive breast

cancer cells may react to retinoic acid efficiently (RARβ-positive means that cell line contains active RARβs). Secondly, there was observed breast cancer cell line with high expression of RARβ but the upregulation of HOXA5 was not induced; this cell line was MDAMB435 (Chen-Zhang *et al* 2007).

4.2.3. Apoptosis induction by HXR9 activating HOXB1 – HOXB9 and connection with c-Fos protein.

As discussed previously (chapter **4.1.4.**), PBX is a transcriptional co-factor that may influence the mechanism by which HOX-proteins regulate transcription; in a form of HOX-PBX dimer, it is able to achieve stronger DNA binding and thus more efficient activation of transcription (Chang *et al* 1995). HXR9 is a synthetic peptide containing specific amino acid sequence similar to that of HOX-proteins with help of which they bind to PBX co-factors. So HXR9 acts as an antagonist and prevents the formation of HOX-PBX dimers.

The effect of HXR9 was tested on 78 different breast cancer cell lines and it was observed that its effect depends on the expression level of HOX-proteins. In the case of high expression of HOXB1-HOXB9 in particular cell lines, it was seen that HXR9 induces apoptosis, or in other words, the lack of HOX-PBX dimers induces apoptosis, possibly meaning that HOX proteins activate different genes or in a different way, if they act without interaction with PBX. In this case, the successful induction of apoptosis by HXR9 correlated with the c-Fos protein upregulation (Morgan *et al* 2012). c-Fos protein is a proto-oncogene and proteins coded by this gene have many different functions. It codes a protein localized in the nucleus that binds to Jun-family proteins to form activation protein-1 (AP-1) (Preston *et al* 1996). These dimers function as transcription factors inducing such processes as cell development and growth. Moreover, these dimers are also able to induce apoptotic pathways, although the exact pathway is not known yet. Certain tests determined that c-Fos apoptosis induction is connected with p53 activation, the blocking of apoptosis was observed in the colorectal carcinoma (RKO) cells with the non-functional p53 gene. Moreover, blocking by B-cell lymphoma 2 (Bcl-2) protein was observed (Preston *et al* 1996).

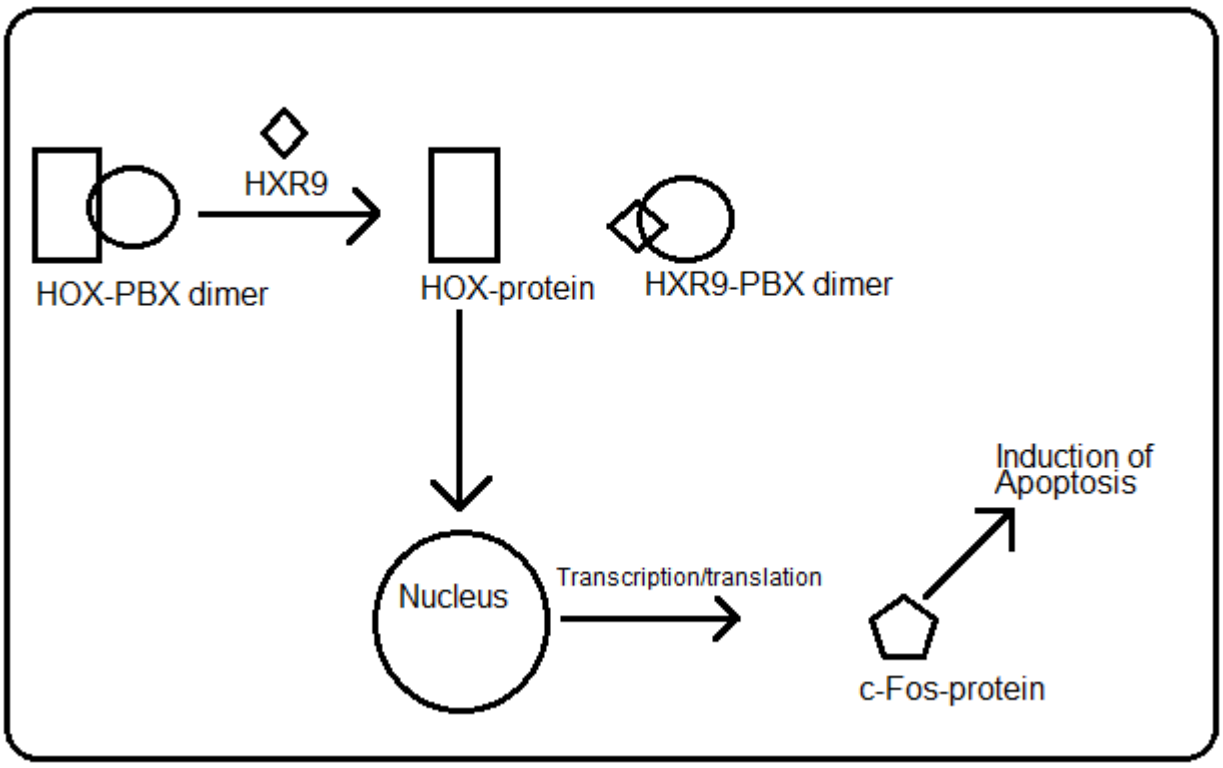

Figure 4. Schematic view of the theory described in chapter 4.2.3. HXR9 acts as an antagonists and prevents the formation of HOX-PBX-dimers. HOX-proteins induce the expression of c-Fos in breast cancer. c-Fos protein induces the apoptosis. Theory for the figure is taken from Morgan-Pirard 2003, Morgan *et al* 2012.

4.2.4. HOXB13 downregulates estrogen receptor expression in breast cancer cells.

Tamoxifen is known as a drug for estrogen receptor-positive breast cancer cells. Its metabolites act as estrogen antagonists, thus decreasing estrogen effect on its target genes, it helps to decrease proliferation rate.

But some patients with breast cancer still become resistant to tamoxifen treatment within the time due to decreased estrogen receptor expression. Correlation between tamoxifen resistance and HOXB13 upregulation has been observed. Therefore, this transcription factor was tested to have a connection with estrogen receptor expression levels. Experiments show that there is a HOX-binding motif in the promoter region of the estrogen receptor-α (ER-α) gene, so it is apparent that HOXB13 is able to affect estrogen receptor-α expression. So, due to decreased ER-α amount in the cell tamoxifen and its metabolites do not have a target to bind to (Shah *et al* 2013).

4.2.5. ER-α downregulated by HOXB13, but cancer cells find an alternative way to stimulate proliferation. HOXB13 and interleukin-6 (IL-6).

Although HOXB13 negatively affects ER- α expression in breast cancer cells, the latter still manage to continue growing. The increased amount of HOXB13 correlated with the increased size of the stroma in the breast cancer cell line. IL-6 expression showed the highest correlation with HOXB13 expression. Proliferation of stromal cells is usually more active during inflammation, for example, during wound healing, but interestingly it is also active in different carcinomas as well as in breast cancer. Therefore, it was concluded that cancer cells were able to activate inflammatory pathways by expressing such substances as cytokines (Dvorak 2015). In this case, IL-6 activates stromal growth stimulating protein kinase B pathway in fibroblast that surrounds breast cancer cells. Of course, this proliferative pathway is also activated in cancer cells and plays a role of an alternative metabolic path in case of inactive ER-α (Shah *et al* 2013).

HOXB13 is suggested to have binding sites in the promoter of the IL-6 gene according to ChIP-seq analysis (Shah *et al* 2013). So, all these facts together may give a good explanation of the HOXB13 role in breast cancer cells. HOXB13 downregulates the ER-α gene but upregulates IL-6 gene thus activating cells' proliferative pathway. Moreover, IL-6 plays a role in stromal growth stimulating fibroblast to act as in the inflammatory state.

Knowing this it was tested whether inhibition of protein kinase B pathway may influence cancer cell growth. mTOR was chosen as a target because it relays the signal form PKB. Rapamycin is known to be an inhibitor of mTOR, and it was shown to cause regression of HOXB13-expressing breast cancer (Shah *et al* 2013).

Considering the fact that HOXB13-expressing breast cancer cells still express a small amount of ER-α it was tested whether treatment with both tamoxifen and rapamycin would be more effective. This combination was significantly more effective than with tamoxifen alone and slightly more effective than with rapamycin alone (Shah *et al* 2013). Therefore, treatment with the rapamycin-tamoxifen combination could become a good candidate for ER+ breast cancer cases with high HOXB13 expression (Shah *et al* 2013).

4.3. Lung cancer

Lung cancer can be divided into two main groups: non-small-cell lung carcinoma (NSCLC) and small-cell lung carcinoma (SCLC). The most amount of lung cancer cases are caused

by NSCLC that can be divided into 3 main subclasses: adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma. These three subclasses differ in cell morphology and location of cancer origin.

4.3.1. HOX-proteins' expression in NSCLC.

Increased expression of HOXA5 and HOXA10 was observed in both adenocarcinoma and squamous-cell carcinoma. This fact seems to be self-contradictory in comparison to the role of these proteins in other cancer-types because HOXA5 and HOXA10 are believed to be antioncogenic factors according to other studies (Raman *et al* 2000, Bromleigh-Freedman 2000).

However, there are certain observations explaining this contradiction. Immunohistochemical staining shows that HOXA5 and HOXA10 are found mostly in the cytoplasm, but not in the nucleus. That is why there is a hypothesis about possible non-transcriptional action of HOXA5 and HOXA10, which might not have a chance to activate transcription in the nucleus. So, they might have interactions with other proteins and thus preventing apoptosis (Abe-Hamada *et al* 2006).

In addition to HOXA5 and HOXA10, increased expression of HOXA1 and HOXC6 was also observed. Taking into account that the amount of HOXA1 correlates with increased cell growth in breast cancer and that HOXC6 is a possible apoptosis inhibitor in prostate cancer it was suggested that they could play the same role in squamous-cell carcinoma (Zhang *et al* 2003, Ramachandran *et al* 2005, Abe *et al* 2006).

4.4. Ovarian cancer

There are two main groups ovarian cancer is divided to: ovarian carcinoma and sex cord-gonadal stromal tumors. The former is connected with the serous layer of cover tissues of ovaries, it includes, for example, high-and low-grade serous carcinoma. The second is associated with stromal components or germ cells themselves, for example, granulosa cell tumor, thecoma, and fibroma. Granulosa and theca are names of layers of ovarian follicles (Figure 5).

Sex cord-gonadal stromal tumors are much rarer than ovarian carcinoma, of which high-grade serous carcinoma is known to be the main cause of ovarian cancer (Shih *et al* 2004).

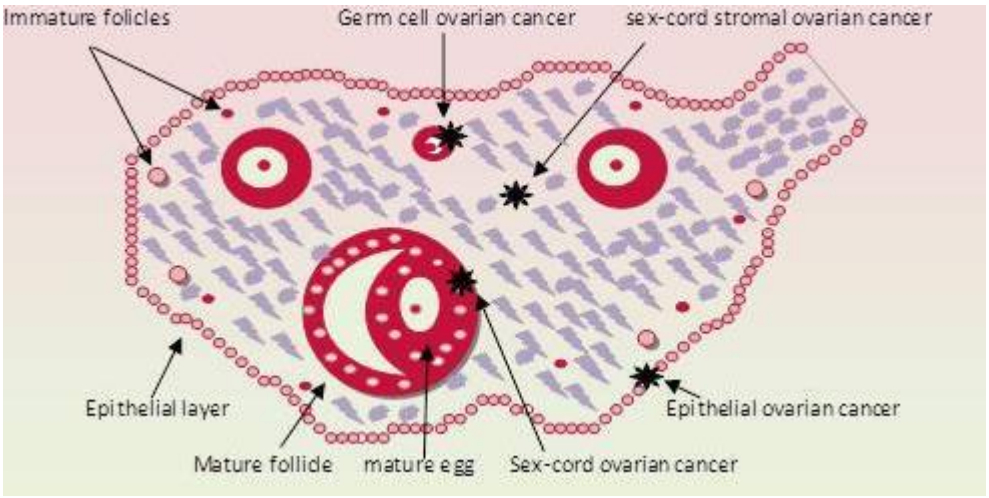

Figure 5. Origins of ovarian cancer. It may develop from the epithelial cells, germ cells and stromal cells. Fibromas develop from connective tissue. Granulosa cell tumor and thecoma develop from the layers of ovarian follicles. Figure from Janbaz *et al* 2014.

4.4.1. HOXA5 and effect on estrous cycle

Expression patterns of HOXA5 were tested in mice of different ages (four and ten months) at different phases of the estrous cycle. HOXA5 expression was observed mostly in the ovarian stroma in comparison to follicles, corpora lutea and ovarian stromal epithelium (OSE) where the expression of HOXA5 was not counted as significant. The same pattern was true for oviduct, where the expression of HOXA5 was more significant in the stroma.

Reproductive cycle of mice consists of the following stages: diestrus, proestrus, estrus and metestrus. Growth of the follicles occurs during diestrus and proestrus. Ovulation takes place in estrus that is followed by metestrus involving hormonal secretion by corpora lutea.

For 4-month old mice, there was no significant difference in HOXA5 expression between proestrus, estrus, and diestrus. But it was noticeably lower during the metestrus. Interestingly, for 10-month mice, HOXA5 expression was increased at diestrus (Gendronneau *et al* 2012). Also, expression patterns of HOXA5 were tested for the gestation period in mice. At the very beginning of the gestation, the expression of HOXA5 was almost the same as during the estrous cycle and located in the stroma. There was also observed a clear pattern of HOXA5 upregulation from day 6,5 to 12,5, although the difference between the beginning and day 12,5 was not statistically significant. However, all this data suggests that HOXA5 is connected with the cycle stage, age, and gestation (Gendronneau

The comparison between inactive and active HOXA5 gene was performed. There was observed a significant difference in the number of estruses per month in 4,5-6 and 8-10-month-old mice; in HOXA5$^{-/-}$ mice it was increased on around 1 and 2 times per month respectively. In age groups with a decreased number of estruses per month was also observed a prolonged time of diestrus and metestrus phases.

As for hormones, 2-fold higher 17ß-estradiol concentration at estrus was measured in 10 month-old mice, but there was no correlation between this increase and concentration of luteinizing hormone (Gendronneau *et al* 2012).

4.4.2. HOXA5 and ovarian cyst formation

In HOXA5$^{-/-}$ 10-month-old mice cyst formation was observed (Gendronneau *et al* 2012). The cyst is a rounded hollow lumen typically enclosed by epithelial cells. There are many different ways of cyst formation for different tissues, but here the one for ovaries will be described.

EMT is a process that is generally involved in embryogenesis during the formation of several different tissue layers from one primordial layer. EMT is transition of apico-basally polarized epithelial cells into more fibroblast-like mesenchymal and migratory cell type . For example, the formation of mesoderm and endoderm is done with EMT during gastrulation period (Hay 1995). Also, EMT is a way to repair tissues after different processes. In the case of ovaries, after ovulation, the mature ovum leaves the ovary and leaves there corporal luteum. After this, the formed hole in the ovarian surface epithelium (OSE) can be repaired by EMT. But sometimes this process may not proceed properly, and epithelium cells get entrapped in the stroma and are able to form cysts.

The molecular and genetic level of this process is complicated and is mostly correlational with some subsequent hypotheses. Firstly, it was observed that the dominant negative form of transcription factor Smad-2 correlated with cyst formation in ovarian granulosa cells (Bristol-Gould *et al* 2005). Secondly, the decreased activity (heterozygote) of the GATA6 transcription factor also correlates with the formation of cysts in ovaries (Cai *et al* 2009). It was checked whether the expression of GATA6 and Smad-2 is affected by the loss of HOXA5. No difference was found for GATA6 expression in HOXA5$^{+/+}$ and HOXA5$^{-/-}$ ovaries. An increased amount of phosphorylated Smad-2 (its activated form) was noted in HOXA5$^{-/-}$

mice. But it is not clear yet how exactly the loss of HOXA5 is connected with activation of Smad-2, because there was no difference in expression of Smad-2 ligands between control and HOXA5-knockout mice (Gendronneau *et al* 2012).

The third regulation of EMT is EGFR (epidermal growth factor receptor) signaling, which is believed to control the EMT and the process of ovulation as such (Ahmed- Maines *et al* 2006, Conti *et al* 2006). Ovulation is stimulated by the high pick of the luteinizing hormone that induces the transcription of amphiregulin (Areg), epiregulin (Ereg) and betacellulin (Btc) that are epidermal growth factors. Therefore, the expression of EGFR and its three ligands Areg, Ereg and Btc was analyzed. In HOXA5$^{-/-}$ 10-month-old mice there was observed a clearly decreased expression of these proteins suggesting that it may partially connect the loss of HOXA5 and cyst formation through diminished stimulation of EGFR signaling (Gendronneau *et al* 2012).

4.4.3. The connection between cyst formation and ovarian cancer.

All the above-listed examples are possible explanations of the cyst formation and its hypothetical dependence on the loss of HOXA5 transcription factor. But it is not enough to connect cyst formation and ovarian cancer. To this one more issue has to be described, and that is paired box gene 8 (PAX8) transcription factor.

PAX8 is known to be active during embryogenesis when it stimulates the growth of Müllerian organs (ones formed by developing from the Müllerian ducts of embryo), however, in the mature organism this transcription factor is usually active in epithelium cells of the uterus and oviduct, but it is not normally expressed in ovary of the mature organism. PAX8 is used as a tumor biomarker for different tissues. For example, it is expressed in ovarian cysts in the case of ovarian cancer (Ozcan *et al* 2011). One of the transcription targets of PAX8 is Wilms tumor protein (Wt1) (Dehbi-Pelletier 1996). Expression of PAX8 and Wt1 was studied in different parts of ovaries on mice; it was observed that these two transcription factors PAX8 and Wt1 are expressed in the epithelium of the cysts inside the ovarian stroma. Moreover, in 4-month-old HOXA5$^{-/-}$ mice there were some cells observed in the ovaries expressing PAX8 (Gendronneau *et al* 2012). It was suggested that the loss of HOXA5 may affect the expression of many other different transcription factors including those ones regulating EMT and other being a master regulator of the oviduct development (PAX8). Altered expression disturbs estrous cycle periods' length and also is a predisposition to ovarian cyst formation that may play a role of the first step of cells becoming cancerous.

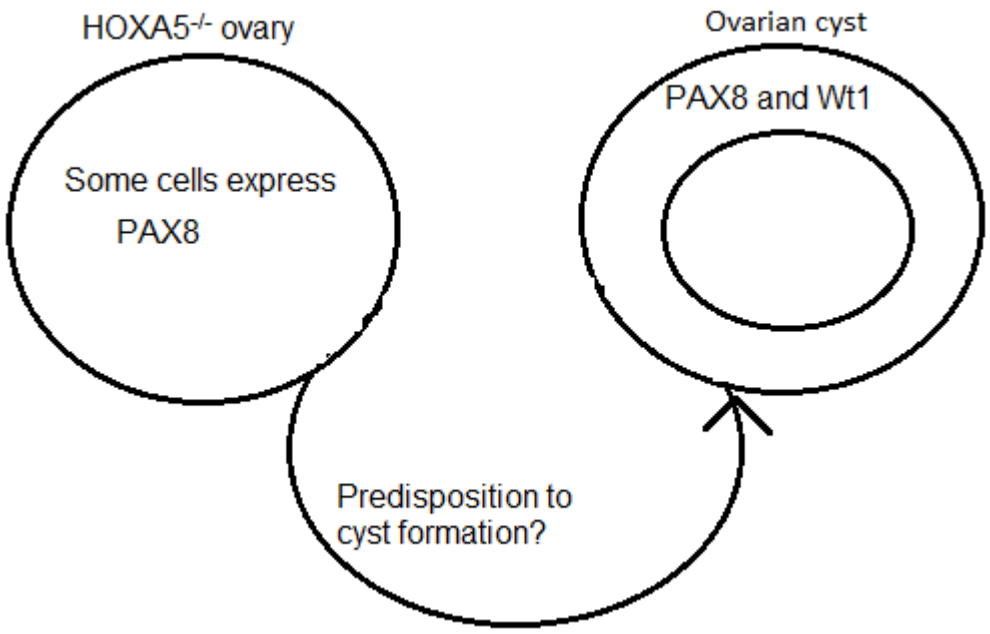

Figure 6. Schematic view of the hypothesis presented in chapter 4.4.3. Upregulation of PAX8 correlated with decreased amount of HOXA5 in ovaries of mice. Upregulation of PAX8 and Wt1 was observed in ovarian cysts. It was suggested that downregulation of HOXA5 might predispose ovaries to development of ovarian cysts through upregulation of PAX8. Figure is based on Gendronneau *et al* 2012.

4.4.4. HOXA9, HOXA10, HOXA11 versus serous, endometrioid and mucinous EOCs respectively.

Epithelial ovarian cancers (EOC) originates from ovarian epithelium cells. EOCs can be divided into three histologically different classes: serous, endometrioid and mucinous EOC. In the case of serous EOC, it resembles the cancer of fallopian tubes, whereas the endometrioid EOC is known for the formation of endometrial-like glands. And finally, mucinous EOC – resembles cervical and intestinal tissues.

Fallopian tubes, uterus, and cervix arise from the müllerian ducts during the embryogenesis. The development of the müllerian ducts into the above-listed parts of the reproductive system is known to be regulated by the certain cluster of HOX-proteins called "Abdominal-B gene" that involves three HOX-proteins: HOXA9, HOXA10, and HOXA11 (Hsieh *et al* 1995, Benson *et al* 1996). The tissue of expression for each HOX-member of the abdominal-B gene was analyzed in an adult rabbit to determine for which tissue type each member is responsible. It was found, that HOXA9 is expressed in fallopian tubes, endometrium, and cervix, whereas HOXA10 was mostly expressed in the mid-and posterior section of uterus and HOXA11 mostly in the posterior section of uterus (Figure 6) (Chen *et al* 2005). Also, it was checked whether the pattern is the same in three EOC types listed above (Figure 8). HOXA9 was expressed in all types of EOC, HOXA10 in endometrioid and mucinous EOC and HOXA11 expression was observed in mucinous EOC only (Chen *et al* 2005). So this pattern might serve an explanation of why EOCs have exceedingly similar histological features to the parts of the müllerian duct. Also, it demonstrates how a certain type of EOC might develop depending on the different expression of abdominal-B gene group.

Also, here is one more HOX-protein that is thought to have ab effect on the EOCs. According to the level of differentiation, the above-listed examples of EOCs are of high-grade and poorly differentiated. There are also low-grade EOCs meaning that their degree of differentiation is higher than in high-grade carcinomas. The potential HOX-protein that determines whether carcinoma is low-or high-grade is HOXA7 (Chen *et al* 2005). The presence of expressed HOXA7 along with members of Abdominal-B gene group members showed, that HOXA7 affects the degree of the differentiation, but does not influence the histological type of EOCs (Figure 8).

So, it is hypothesized that OSE cells that normally do not express HOXA9-HOXA11 proteins might start producing these transcription factors. It results in three different types of EOCs depending on what member of the Abdominal-B gene group is expressed, moreover, whether it will be high-or low-grade carcinoma is potentially determined by the level of HOXA7 expression, the presence of which increases the degree of the differentiation.

It is not known what makes OSE repress these genes after embryogenesis as well as to express them in the case of EOCs. But it is known that the expression of HOXA9-HOXA11 in the reproductive tract may be activated by progesterone or inhibited by estrogen (Benson *et al* 1998). Therefore, it is assumed that hormonal changes inside the peritoneal cavity (OSE faces this cavity) might change the expression of Abdominal-B gene group members in OSE and cause the müllerian duct-like differentiation (Chen *et al* 2005).

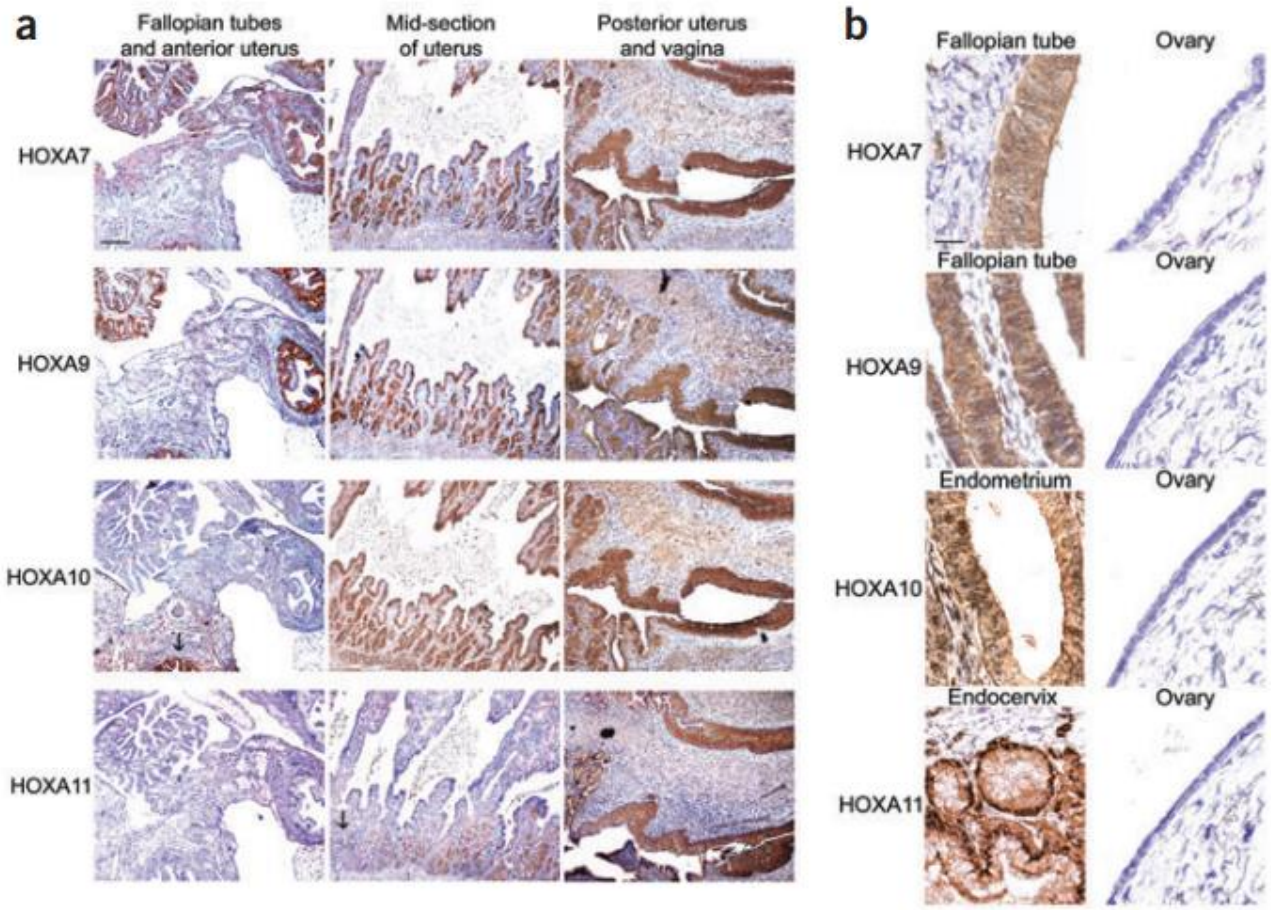

Figure 7. Spatially restricted HOX expression in the reproductive tract. (a) Immunohistochemical staining of HOXA7, HOXA9, HOXA10 and HOXA11 in normal tissues of fallopian tubes, uterus and vagina of the adult mouse. Scale bar, 200 μM. Anterior uterine epithelia that stained strongly for HOXA7, HOXA9 and HOXA10 but not for HOXA11 are indicated by arrows. (b) Staining of HOXA7 and HOXA9 in fallopian tube, of HOXA10 in endometrium, and of HOXA11 in endocervix of the normal adult human. Little or no staining of HOX proteins was detected in normal human OSE. Scale bar, 20 μM. Figure and text from Cheng *et al* 2005.

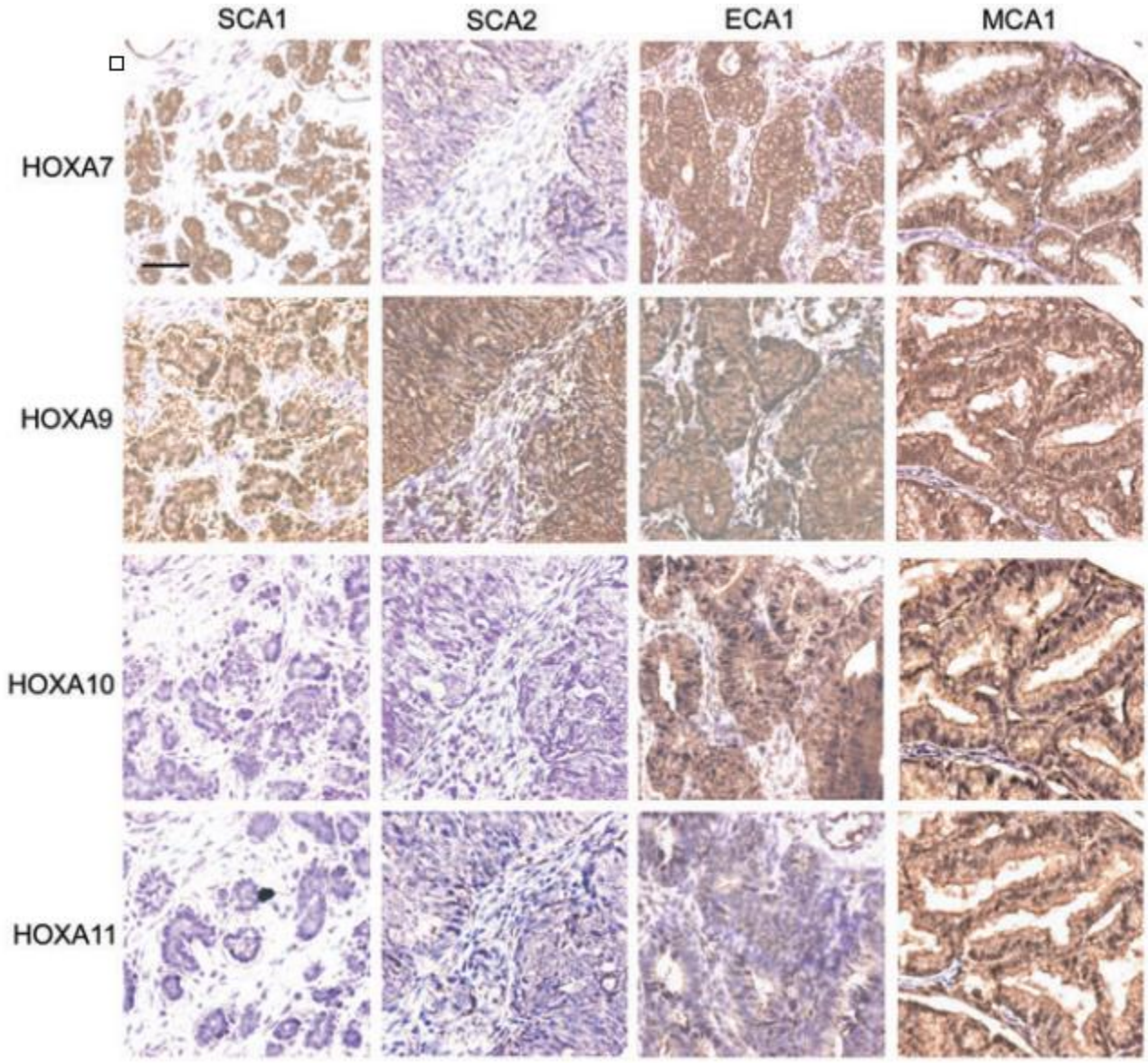

Figure 8. Immunohistochemical staining of HOXA7, HOXA9, HOXA10 and HOXA11 in specimens of low-grade (SCA1) and high-grade (SCA2) serous carcinoma, low-grade endometrioid carcinoma (ECA1) and mucinous carcinoma (MCA1). Scale bar, 50 μM. Figure and text from Cheng *et al* 2005.

4.4.5. HOXA4 – invasion promoter or suppressor?

The increased expression of HOXA4 was observed for invasive ovarian cancer cells. But studies show that bigger possibility is that overexpressed HOXA4, in fact, is a way of cells to respond to pathological transformations of cancer cells.

The term invasiveness is associated with such process as cell migration. This process may be activated by EGFR signaling through alterations in the expression of cells surface adhesion proteins (Katz *et al* 2007).

The effect of HOXA4 expression was tested in circumstances including EGFR signaling to check how it influences cell migration. It was observed that the amount of EGFR increased in cells with siRNA against HOXA4 mRNA. In other words, along with the downregulation of HOXA4, the amount of EGFR has increased, also in some cells phosphorylated form of EGFR has increased in proportional amount to EGFR. So it might mean that expression of HOXA4 inhibits EGFR signaling thus preventing cell migration and lowering the probability to become an invasive cell (Ota *et al* 2009).

Apart from EGFR signaling, there is also another possible connection between HOXA4 and cell motility. HOXA4 was reported to upregulate the expression of β1-integrin (Klausen *et al* 2009). This protein is a member of membrane receptors functioning in cell recognition and adhesion. Together with other members, it forms complexes on the cell membrane to allow adhesion. Therefore, the downregulation of any member would lead to fewer amount of complexes and could lead to worse adhesion. It was shown that knockdown of HOXA4 actually decreases cell-cell adhesion in high-grade ovarian carcinoma cells (Klausen *et al* 2009).

So, HOXA4 may be considered to be a metastatic suppressor, the expression of which has a regulatory effect in response to adhesion and motility changes of cells. Its expression inhibits EGFR signaling and upregulates β1-integrin thus supporting cell adhesion and suppressing the transformation to invasive state.

4.5. Bladder cancer

There are three main types of bladder cancer depending on the tissue that it is developed from. The most common type is transitional cell carcinoma arising from transitional epithelium as the name implies. Transitional epithelium is a subtype of stratified epithelium that forms a thick layer on the inner surface of the bladder tissue. Two other types of bladder cancer are squamous cell carcinoma and adenocarcinoma.

4.5.1. HOX-genes' expression in transitional cell carcinoma.

The discussion on associations between HOX-genes' expression and bladder cancer cases will start with the overall look at HOX-genes' up- and downregulation in all four HOX-clusters.

The expression of all four HOX-clusters was analyzed in normal bladder tissue and bladder cancer tissue. Some of HOX-genes are expressed in normal bladder tissue of adult humans, proposing that they might be essential for differentiation or tissue homeostasis. Obviously, some HOX-genes get silenced in the tissue after the development.

Although, there are many alterations in all the clusters in bladder cancer tissues, the ones of the C-cluster seem to be the most significant and noticeable. Part of the HOXC-cluster is silenced in the adult tissue of the urinary bladder, specifically HOXC4, HOXC5, HOXC6, HOXC9, HOXC11, and HOXC12. Four of these proteins get expressed in bladder cancer tissue, namely HOXC4, HOXC5, HOXC6, and HOXC11. Here it is important to mention that it is not a rule working for all the cases of the bladder cancer, but rather it is true for the majority of bladder cancer tissue samples analyzed. But the alterations in HOXC-cluster still appear to function as a good marker for bladder cancer cases.

As for HOXA-cluster, the correlations for the anterior part of the cluster are difficult to judge upon due to the differential expression even in the normal bladder tissue samples, specifically, it is true for HOXA1, HOXA2, HOXA4, and HOXA5. But the situation is different for the HOXA3 gene that is silent in all the samples of normal bladder tissue but gets upregulated in the majority of bladder cancer tissue samples. Speaking about the posterior region of HOXA-cluster, a relatively significant correlation is observed for the HOXA11 gene expression. It is active in 60 % of the normal bladder tissues and in only 6 % of the bladder cancer samples. But the significance of this correlation seems to be quite questionable due to the fact that for 2/15 pairs of samples the result of the comparison is opposite. HOXA11 is silenced in the normal bladder tissues and activated in bladder cancer tissues (Cantile *et al* 2003).

Correlations for the B-cluster are even less obvious than for the A-cluster. Most of the HOXB-genes are very heterogeneously expressed in both normal and bladder cancer tissues. Although no correlations have been reported for the middle and posterior parts of the B-

cluster, significant differences for the HOXB2-gene have been noted (Cantile *et al* 2003). It is inactive in 4/15 samples of normal bladder tissues, whereas it is upregulated in 11/15 samples of bladder cancer tissues. In other words its expression is more prevalent for cancer tissues than for normal bladder tissues. Also, the HOXB3 expression appears to behave in a pattern where it is silent for all the normal bladder tissue samples, while it gets activated in 4/15 samples of tumor tissue (Cantile *et al* 2003). Still, it does not seem to be enough to be used as a proper prediction marker.

And finally, the D-cluster shows the least amount of observable differences between normal and cancer tissue. HOXD1-D4, HOXD8-D9, and HOXD12-13 are almost always silenced in both normal and cancer bladder tissues with some relatively rare alterations. The clearest difference in expression is noticed for HOXD11 member of the D-cluster. This gene is observed to be inactive in all samples of normal bladder tissues, but it became active in 6/15 samples of bladder cancer tissues (Cantile *et al* 2003).

4.5.2. Cytokeratins in transitional cell carcinoma vs HOXC-cluster expression.

In this subchapter I will discuss the interpretation of the above-listed correlative expression of HOX genes in various cancers.

Cytokeratins are proteins that belong to a group of cytoskeletal components called intermediate filaments. They are needed to maintain the shape of the cell as well to enable mechanical cell-to-cell communication.

There are many cytokeratins that are normally expressed in normal transitional epithelium, for example, cytokeratins 8, 18 and 19. Depending on the subtype of transitional cell carcinoma there were observed different patterns of expression for these (and others) cytokeratins (Moll *et al* 1988).

Genes coding for cytokeratins 1, 3, 4, 5, 6A and 6B are located of the 12th chromosome and they are in physical contiguity to the 5' end of the HOXC-cluster, whereas genes coding for cytokeratin 7 and basic hair Keratin 1 and 6 are physically contiguous to the 3' end of the HOXC-cluster, moreover, the last two are transcriptionally regulated by HOXC13 (Jave-Suarez *et al* 2002). So this data suggests that alterations in HOXC-cluster expression and alterations in the expression of different cytokeratins might be connected in the case of transitional cell carcinoma.

4.5.3. HOXB13 in NMIBC and MIBC.

Transition cell carcinoma is also divided into two subtypes depending on its progression: non-muscle invasive bladder cancer (NMBIC) and muscle-invasive bladder cancer (MBIC). In the case of the MBIC cancer spreads to the muscle tissue, whereas in the case of the NMBIC it stays in the epithelial tissues.

Although no correlations were observed between posterior members of HOXB-cluster and bladder cancer (Cantile *et al* 2003), in another analysis it was noticed that HOXB13 may be a marker to distinguish between NMBIC and MBIC (Marra *et al* 2013). In this experiment, a very low expression of HOXB13 was shown for normal bladder tissue. When the tumors were analysed it was noticed that HOXB13 expression was significantly higher in MBIC when compared with NMBIC (Marra *et al* 2013).

Also, the observed expression was mostly cytoplasmic, showing that HOXB13 nuclear-cytoplasmic delocalization may correlate with the muscle invasiveness. This hypothesis is supported by the already observed cytoplasmic-nuclear delocalization of HOXB13 after skin development (Kömüves *et al* 2003).

4.5.4. Hypermethylated HOXA9 as a predictive marker of NMBIC

Methylation of DNA regions responsible for tumor suppressors is one of the common features for different types of cancers. Therefore, these methylated targets may play a role of good predictive markers as well as be aimed in therapy.

It was studied whether there are some DNA methylations associating with the NMBIC. Methylation of HOXA9, ISL1, and ALDHA3 was observed to correlate with tumor number, size, grade, and stage. It was suggested that HOXA9, ISL1, and ALDHA3 may play the role of independent markers for NMBIC cases (Marra *et al* 2013).

4.6. Kidney cancer

Kidney cancer is cancer affecting kidney cells and it is divided into two main types: renal cell carcinoma and transitional cell carcinoma of the renal pelvis. Renal cell carcinoma is a much more common type of kidney cancer; it arises from the primal tubule of the nephron.

4.6.1. HOX-genes' expression in renal cell carcinoma.

The expression of all four HOX-clusters was analyzed in renal cell carcinoma to compare with normal kidney tissue. The most significant difference was observed in HOXD-cluster, namely its posterior part. HOXD9 – being active in all samples of a normal kidney of an adult – was active in only 4/9 samples of renal cell carcinoma. HOXD11 was observed to have the same activity in normal kidneys but was inactive in all samples of renal cell carcinoma that appears to be more even more noticeable correlation than that in case of HOXD9. HOXD12 seems to be active more frequently in renal cell carcinoma than in normal kidney.

HOXA-cluster expression is almost the same for both normal kidney and renal cell carcinoma. But it appears that the expression of genes HOXA4-HOXA7 was slightly less frequent in renal cell carcinoma if one compares to a normal kidney.

There were no strong differences in HOXB-cluster, HOXB1-HOXB5 were heterogeneously expressed in both normal kidney and renal cell carcinoma, whereas HOXB6-HOXB8 were active in samples of both groups. HOXB9 and HOXB13 were inactive in both normal and cancer cells.

As for C-cluster, HOX-genes' expression did not have any noticeable differences between normal and cancer samples. So the only significant alteration was observed in the posterior part of the HOXD-cluster, therefore HOXD9 and HOXD11 might play the role of prognostic markers (Cantile *et al* 2011).

4.7. Melanoma.

Melanoma is a cancer type arising from the melanocytes, pigment-producing cells of the bottom layer of the skin's epidermis. Nevi (more commonly called moles) may be the starting point of the melanoma, therefore, the amount and shape of nevi, for example, are used in the diagnosis of melanoma.

4.7.1. HOXC13 and metastasis in melanoma.

The starting point of the cancer is called the primary tumor, in case of melanoma that may be an abnormal mole. Eventually, cancer cells may acquire the invasive ability, so they get separated from the primary tumor and cause the spread of cancer to other tissues.

The exact mechanism of this process is not understood completely. However, there was an association between metastasis and HOXC13 expression in melanoma. Its expression in nevi and primary tumors was compared to one in melanoma metastasis. The exact over-expression was observed for this member HOXC13 in melanoma metastasis suggesting that its overexpression correlates with the initiation of metastasis in melanoma (Cantile *et al* 2012).

Such integrins as very late antigens (VLA) - VLA-2, VLA-5 and VLA-6 and adhesion molecule ICAM-1 were observed to be highly expressed in cells with silent posterior HOXC-genes, whereas the opposite was true for cells with actively expressed posterior HOXC-genes (Cillo *et al* 1996). It suggests the above-mentioned overexpression of HOXC13 might cause the metastasis by affecting the expression of adhesion molecules (Cantile *et al* 2012).

HOXC13 can be used as a marker and also as a therapeutic target. For example, HXR9 (hexapeptide analog) could potentially be used to prevent interaction between HOX proteins and their assisting PBX proteins and thus to prevent the transcriptional function of the HOX-protein.

Recently, HXR9 was shown to cause apoptosis in melanoma cells. It was observed that HXR9 caused an increased amount of different proapoptotic factors such as Fos, for example. Fos could possibly be a part of activator protein (AP-1) together with JUN protein. AP-1 acts as transcription factor influencing cell cycle, but the exact mechanism of how it could cause apoptosis is not known. This data suggests that overexpressed HOX-proteins could act as inhibitors of proapoptotic factors, therefore, the inactivation of HOX/PBX interaction could cause elevated levels of proteins responsible for mediating of apoptosis (Morgan *et al* 2007).

5. Conclusions and future perspectives.

HOX-proteins are transcription factors that evolve from the dispersed structure and form a very unique organization in vertebrates where they are split into four tight clusters on four different chromosomes. HOX-proteins play a fundamental role in embryogenesis controlling the formation of the future body plan.

Expression of HOX-protein greatly varies among different tissues of different parts of the body and some of them remain active even in the adult organism, whereas some get silenced. The exact role of HOX-proteins in adult organisms is not clear, but there are many observations and correlations between their expression and different cancer types.

Depending on the cancer type, the exact role of HOX-protein involvement is understood to a different extent. However, even these correlations seem to propose potential diagnostic and therapeutic targets for different cancer types. HOX-proteins are able to affect different metabolic pathways, for example, some HOX-proteins may activate apoptosis, whereas other are able to inactivate it. In some cases upregulation of HOX-proteins may result in drug-resistance, while in other cases it is more difficult to find a certain association between altered expression of particular HOX-protein and a certain cancer type.

The majority of research data about HOX-proteins is made up of pure correlational analysis including some hypothetical interpretations. It is not possible to say conclusively whether HOX-proteins induce the development of certain cancer type or vice versa. The probability of the latter scenario appears to be slightly higher due to the observed effect of altered HOX-protein expression serving a predominantly supportive function

Because of the inherent complexity of cancers, the altered HOX-expression profiles cannot be the absolute target for cancer treatment, but further research and the unveiling of molecular mechanisms connected with HOX-proteins might make the picture of cancer more complete and reveal even more potential targets.

6. References

Abe, M., Hamada, J., Takahashi, O., Takahashi, Y., Tada, M., Miyamoto, M., Morikawa, T., Kondo, S. & Moriuchi, T. 2006, "Disordered expression of HOX genes in human non-small cell lung cancer", Oncology reports, vol. 15, no. 4, pp. 797-802.

Ahmed, N., Maines-Bandiera, S., Quinn, M.A., Unger, W.G., Dedhar, S. & Auersperg, N. 2006, "Molecular pathways regulating EGF-induced epithelio-mesenchymal transition in human ovarian surface epithelium", American Journal of Physiology-Cell Physiology, vol. 290, no. 6, pp. C1532-C1542.

Axlund, S.D., Lambert, J.R. & Nordeen, S.K. 2010, "HOXC8 inhibits androgen receptor signaling in human prostate cancer cells by inhibiting SRC-3 recruitment to direct androgen target genes", Molecular cancer research : MCR, vol. 8, no. 12, pp. 1643-1655

Benson, G.V., Lim, H., Paria, B., Satokata, I., Dey, S.K. & Maas, R.L. 1996, "Mechanisms of reduced fertility in Hoxa-10 mutant mice: uterine homeosis and loss of maternal Hoxa-10 expression", Development, vol. 122, no. 9, pp. 2687-2696.

Boube, M., Hudry, B., Immarigeon, C., Carrier, Y., Bernat-Fabre, S., Merabet, S., Graba, Y., Bourbon, H. & Cribbs, D.L. 2014, "Drosophila melanogaster Hox transcription factors access the RNA polymerase II machinery through direct homeodomain binding to a conserved motif of mediator subunit Med19", PLoS genetics, vol. 10, no. 5, pp. e1004303.

Bristol-Gould, S.K., Hutten, C.G., Sturgis, C., Kilen, S.M., Mayo, K.E. & Woodruff, T.K. 2005, "The development of a mouse model of ovarian endosalpingiosis", Endocrinology, vol. 146, no. 12, pp. 5228-5236.

Bromleigh, V.C. & Freedman, L.P. 2000, "p21 is a transcriptional target of HOXA10 in differentiating myelomonocytic cells", Genes & development, vol. 14, no. 20, pp. 2581-2586.

Bürglin, T.R. 1997, "Analysis of TALE superclass homeobox genes (MEIS, PBC, KNOX, Iroquois, TGIF) reveals a novel domain conserved between plants and animals", Nucleic acids research, vol. 25, no. 21, pp. 4173-4180.

Bürglin, T.R. 1998, "The PBC domain contains a MEINOX domain: coevolution of Hox and TALE homeobox genes?", Development genes and evolution, vol. 208, no. 2, pp. 113-116

Cabart, P., Ujvari, A., Pal, M. & Luse, D.S. 2011, "Transcription factor TFIIF is not required for initiation by RNA polymerase II, but it is essential to stabilize transcription factor TFIIB in early elongation complexes", Proceedings of the National Academy of Sciences of the United States of America, vol. 108, no. 38, pp. 15786-15791.

Cai, K.Q., Caslini, C., Capo-Chichi, C.D., Slater, C., Smith, E.R., Wu, H., Klein-Szanto, A.J., Godwin, A.K. & Xu, X. 2009, "Loss of GATA4 and GATA6 expression specifies ovarian cancer histological subtypes and precedes neoplastic transformation of ovarian surface epithelia", PloS one, vol. 4, no. 7, pp. e6454.

Cantile, M., Cindolo, L., Napodano, G., Altieri, V. & Cillo, C. 2003, "Hyperexpression of locus C genes in the HOX network is strongly associated in vivo with human bladder transitional cell carcinomas", Oncogene, vol. 22, no. 41, pp. 6462.

Cantile, M., Schiavo, G., Franco, R., Cindolo, L., Procino, A., D'Armiento, M., Facchini, G., Terracciano, L., Botti, G. & Cillo, C. 2011, "Expression of lumbosacral HOX genes, crucial in kidney organogenesis, is systematically deregulated in clear cell kidney cancers", Anti-Cancer Drugs, vol. 22, no. 5, pp. 392-401.

Cantile, M., Scognamiglio, G., Anniciello, A., Farina, M., Gentilcore, G., Santonastaso, C., Fulciniti, F., Cillo, C., Franco, R. & Ascierto, P.A. 2012, "Increased HOX C13 expression in metastatic melanoma progression", Journal of translational medicine, vol. 10, no. 1, pp. 91.

Chang, C.P., Shen, W.F., Rozenfeld, S., Lawrence, H.J., Largman, C. & Cleary, M.L. 1995, "Pbx proteins display hexapeptide-dependent cooperative DNA binding with a subset of Hox proteins", Genes & development, vol. 9, no. 6, pp. 663-674.

Chen, H., Chung, S. & Sukumar, S. 2004, "HOXA5-induced apoptosis in breast cancer cells is mediated by caspases 2 and 8", Molecular and cellular biology, vol. 24, no. 2, pp. 924-935

Chen, H., Zhang, H., Lee, J., Liang, X., Wu, X., Zhu, T., Lo, P.K., Zhang, X. & Sukumar, S. 2007, "HOXA5 acts directly downstream of retinoic acid receptor beta and contributes to retinoic acid-induced apoptosis and growth inhibition", Cancer research, vol. 67, no. 17, pp. 8007-8013.

Chen, J., Zhu, S., Jiang, N., Shang, Z., Quan, C. & Niu, Y. 2013, HoxB3 promotes prostate cancer cell progression by transactivating CDCA3.

Chen, J.L., Li, J., Kiriluk, K.J., Rosen, A.M., Paner, G.P., Antic, T., Lussier, Y.A. & Vander Griend, D.J. 2012, "Deregulation of a Hox protein regulatory network spanning prostate cancer initiation and progression", Clinical cancer research : an official journal of the American Association for Cancer Research, vol. 18, no. 16, pp. 4291-4302.

Cheng, W., Liu, J., Yoshida, H., Rosen, D. & Naora, H. 2005, "Lineage infidelity of epithelial ovarian cancers is controlled by HOX genes that specify regional identity in the reproductive tract", Nature medicine, vol. 11, no. 5, pp. 531.

Cillo, C., Cantile, M., Mortarini, R., Barba, P., Parmiani, G. & Anichini, A. 1996, "Differential patterns of HOX gene expression are associated with specific integrin and ICAM profiles in clonal populations isolated from a single human melanoma metastasis", International journal of cancer, vol. 66, no. 5, pp. 692-697.

Conti, M., Hsieh, M., Park, J. & Su, Y. 2006, "Role of the epidermal growth factor network in ovarian follicles", Molecular Endocrinology, vol. 20, no. 4, pp. 715-723.

Dehbi, M. & Pelletier, J. 1996, "PAX8-mediated activation of the wt1 tumor suppressor gene.", The EMBO journal, vol. 15, no. 16, pp. 4297-4306.

Duan, H. & Dixit, V.M. 1997, "RAIDD is a new'death'adaptor molecule", Nature, vol. 385, no. 6611, pp. 86.

Dvorak, H.F. 2015, "Tumors: wounds that do not heal-redux", Cancer immunology research, vol. 3, no. 1, pp. 1-11.

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. 2012, "Germline mutations in HOXB13 and prostate-cancer risk", New England Journal of Medicine, vol. 366, no. 2, pp. 141-149.

Fanayan, S., Firth, S.M., Butt, A.J. & Baxter, R.C. 2000, "Growth inhibition by insulin-like growth factor-binding protein3 in T47D breast cancer cells requires transforming growth factor-beta (TGF-beta) and the type II TGF-beta receptor", The Journal of biological chemistry, vol. 275, no. 50, pp. 39146-39151.

Gary J. Miller, Heidi L. Miller, Adrie van Bokhoven, James R. Lambert, Priya N. Werahera, Osvaldo Schirripa, M. Scott Lucia and Steven K. Nordeen 2003, "Aberrant HOXC Expression Accompanies the Malignant Phenotype in Human Prostate", vol. 63, no. 18.

Gaunt, S.J. & Strachan, L. 1996, "Temporal colinearity in expression of anterior Hox genes in developing chick embryos", Developmental dynamics, vol. 207, no. 3, pp. 270-280.

Gendronneau, G., Boucherat, O., Aubin, J., Lemieux, M. & Jeannotte, L. 2012, "The loss of Hoxa5 function causes estrous acyclicity and ovarian epithelial inclusion cysts", Endocrinology, vol. 153, no. 3, pp. 1484-1497.

Gupta, S. 2002, "A decision between life and death during TNF-α-induced signaling", Journal of clinical immunology, vol. 22, no. 4, pp. 185-194.

Hay, E.D. 1995, "An overview of epithelio-mesenchymal transformation", Cells Tissues Organs, vol. 154, no. 1, pp. 8- 20.

Hsieh-Li, H.M., Witte, D.P., Weinstein, M., Branford, W., Li, H., Small, K. & Potter, S.S. 1995, "Hoxa 11 structure, extensive antisense transcription, and function in male and female fertility", Development (Cambridge, England), vol. 121, no. 5, pp. 1373-1385.

Huang, Q., Whitington, T., Gao, P., Lindberg, J.F., Yang, Y., Sun, J., Väisänen, M., Szulkin, R., Annala, M. & Yan, J. 2014, "A prostate cancer susceptibility allele at 6q22 increases RFX6 expression by modulating HOXB13 chromatin binding", Nature genetics, vol. 46, no. 2, pp. 126.

Jave-Suarez, L.F., Winter, H., Langbein, L., Rogers, M.A. & Schweizer, J. 2002, "HOXC13 is involved in the regulation of human hair keratin gene expression", The Journal of biological chemistry, vol. 277, no. 5, pp. 3718-3726.

Katz, M., Amit, I., Citri, A., Shay, T., Carvalho, S., Lavi, S., Milanezi, F., Lyass, L., Amariglio, N. & Jacob-Hirsch, J. 2007, "A reciprocal tensin-3–cten switch mediates EGF-driven mammary cell migration", Nature cell biology, vol. 9, no. 8, pp. 961.

Kim, S.D., Park, R., Kim, Y., Kim, I., Kang, T.W., Nam, K.I., Ahn, K.Y., Bae, C.S., Kim, B.Y., Park, S.S. & Jung, C. 2010, "HOXB13 is co-localized with androgen receptor to suppress androgen-stimulated prostate-specific antigen expression", Anat Cell Biol, vol. 43, no. 4, pp. 284-293.

Kim, Y., Yoon, H., Kim, J.S., Kang, H.W., Min, B., Kim, S., Ha, Y., Kim, I.Y., Ryu, K.H. & Lee, S. 2013, "HOXA9, ISL1 and ALDH1A3 methylation patterns as prognostic markers for nonmuscle invasive bladder cancer: Array-based DNA methylation and expression profiling", International journal of cancer, vol. 133, no. 5, pp. 1135-1142.

Klausen, C., Leung, P.C. & Auersperg, N. 2009, "Cell motility and spreading are suppressed by HOXA4 in ovarian cancer cells: possible involvement of beta1 integrin", Molecular cancer research : MCR, vol. 7, no. 9, pp. 1425- 1437.

Kömüves, L.G., Ma, X., Stelnicki, E., Rozenfeld, S., Oda, Y. & Largman, C. 2003, "HOXB13 homeodomain protein is cytoplasmic throughout fetal skin development", Developmental dynamics, vol. 227, no. 2, pp. 192-202.

LaRonde-LeBlanc, N.A. & Wolberger, C. 2003, "Structure of HoxA9 and Pbx1 bound to DNA: Hox hexapeptide and DNA recognition anterior to posterior", Genes & development, vol. 17, no. 16, pp. 2060-2072.

Liu, X., Wang, L., Zhao, K., Thompson, P.R., Hwang, Y., Marmorstein, R. & Cole, P.A. 2008, "The structural basis of protein acetylation by the p300/CBP transcriptional coactivator", Nature, vol. 451, pp. 846.

Ma, L., Benson, G.V., Lim, H., Dey, S.K. & Maas, R.L. 1998, "Abdominal B (AbdB) HoxaGenes: regulation in adult Uterus by estrogen and progesterone and repression in

müllerian duct by the synthetic estrogen diethylstilbestrol (DES)", Developmental biology, vol. 197, no. 2, pp. 141-154.

Marra, L., Cantile, M., Scognamiglio, G., Perdonà, S., La Mantia, E., Cerrone, M., Gigantino, V., Cillo, C., Caraglia, M. & Pignata, S. 2013, "Deregulation of HOX B13 expression in urinary bladder cancer progression", Current medicinal chemistry, vol. 20, no. 6, pp. 833-839.

Moll, R., Achtstatter, T., Becht, E., Balcarova-Stander, J., Ittensohn, M. & Franke, W.W. 1988, "Cytokeratins in normal and malignant transitional epithelium. Maintenance of expression of urothelial differentiation features in transitional cell carcinomas and bladder carcinoma cell culture lines", The American journal of pathology, vol. 132, no. 1, pp. 123-144.

Morgan, R., Boxall, A., Harrington, K.J., Simpson, G.R., Gillett, C., Michael, A. & Pandha, H.S. 2012, "Targeting the HOX/PBX dimer in breast cancer", Breast cancer research and treatment, vol. 136, no. 2, pp. 389-398.

Morgan, R., Pirard, P.M., Shears, L., Sohal, J., Pettengell, R. & Pandha, H.S. 2007, "Antagonism of HOX/PBX dimer formation blocks the in vivo proliferation of melanoma", Cancer research, vol. 67, no. 12, pp. 5806-5813.

Nakayama, K.I. & Nakayama, K. 2005, "Regulation of the cell cycle by SCF-type ubiquitin ligases", Seminars in cell & developmental biologyElsevier, , pp. 323.

Neijts, R. & Deschamps, J. 2017, "At the base of colinear Hox gene expression: cis-features and trans-factors orchestrating the initial phase of Hox cluster activation", Developmental biology, vol. 428, no. 2, pp. 293-299.

Neijts, R., Amin, S., Van Rooijen, C. & Deschamps, J. 2017, "Cdx is crucial for the timing mechanism driving colinear Hox activation and defines a trunk segment in the Hox cluster topology", Developmental biology, vol. 422, no. 2, pp. 146-154.

Neijts, R., Amin, S., van Rooijen, C., Tan, S., Creyghton, M.P., de Laat, W. & Deschamps, J. 2016, "Polarized regulatory landscape and Wnt responsiveness underlie Hox activation in embryos", Genes & development, vol. 30, no. 17, pp. 1937-1942.

Okamoto, T., Yamamoto, S., Watanabe, Y., Ohta, T., Hanaoka, F., Roeder, R.G. & Ohkuma, Y. 1998, "Analysis of the role of TFIIE in transcriptional regulation through structure-function studies of the TFIIEbeta subunit", The Journal of biological chemistry, vol. 273, no. 31, pp. 19866-19876.

Ota, T., Klausen, C., Salamanca, M.C., Woo, H.L., Leung, P.C. & Auersperg, N. 2009, "Expression and function of HOXA genes in normal and neoplastic ovarian epithelial cells", Differentiation, vol. 77, no. 2, pp. 162-171.

Ozcan, A., Shen, S.S., Hamilton, C., Anjana, K., Coffey, D., Krishnan, B. & Truong, L.D. 2011, "PAX 8 expression in non-neoplastic tissues, primary tumors, and metastatic tumors: a comprehensive immunohistochemical study", Modern Pathology, vol. 24, no. 6, pp. 751.

Piper, D.E., Batchelor, A.H., Chang, C., Cleary, M.L. & Wolberger, C. 1999, "Structure of a HoxB1–Pbx1 heterodimer bound to DNA: role of the hexapeptide and a fourth homeodomain helix in complex formation", Cell, vol. 96, no. 4, pp. 587-597.

Piper, D.E., Batchelor, A.H., Chang, C., Cleary, M.L. & Wolberger, C. 1999, "Structure of a HoxB1–Pbx1 heterodimer bound to DNA: role of the hexapeptide and a fourth homeodomain helix in complex formation", Cell, vol. 96, no. 4, pp. 587-597.

Preston, G.A., Lyon, T.T., Yin, Y., Lang, J.E., Solomon, G., Annab, L., Srinivasan, D.G., Alcorta, D.A. & Barrett, J.C. 1996, "Induction of apoptosis by c-Fos protein", Molecular and cellular biology, vol. 16, no. 1, pp. 211-218.

Ramachandran, S., Liu, P., Young, A.N., Yin-Goen, Q., Lim, S.D., Laycock, N., Amin, M.B., Carney, J.K., Marshall, F.F. & Petros, J.A. 2005, "Loss of HOXC6 expression induces apoptosis in prostate cancer cells", Oncogene, vol. 24, no. 1, pp. 188.

Raman, V., Martensen, S.A., Reisman, D., Evron, E., Odenwald, W.F., Jaffee, E., Marks, J. & Sukumar, S. 2000, "Compromised HOXA5 function can limit p53 expression in human breast tumours", Nature, vol. 405, no. 6789, pp. 974.

Reese, J.C. 2003, Basal transcription factors.

Rivera-Pérez, J.A. & Magnuson, T. 2005, "Primitive streak formation in mice is preceded by localized activation of Brachyury and Wnt3", Developmental biology, vol. 288, no. 2, pp. 363-371.

Ryoo, H.D., Marty, T., Casares, F., Affolter, M. & Mann, R.S. 1999, "Regulation of Hox target genes by a DNA bound Homothorax/Hox/Extradenticle complex", Development (Cambridge, England), vol. 126, no. 22, pp. 5137-5148.

Shah, N., Jin, K., Cruz, L.A., Park, S., Sadik, H., Cho, S., Goswami, C.P., Nakshatri, H., Gupta, R., Chang, H.Y., Zhang, Z., Cimino-Mathews, A., Cope, L., Umbricht, C. & Sukumar, S. 2013, "HOXB13 mediates tamoxifen resistance and invasiveness in human breast cancer by suppressing ERalpha and inducing IL-6 expression", Cancer research, vol. 73, no. 17, pp. 5449-5458.

Shen, R., Sumitomo, M., Dai, J., Hardy, D.O., Navarro, D., Usmani, B., Papandreou, C.N., Hersh, L.B., Shipp, M.A. & Freedman, L.P. 2000, "Identification and characterization of two androgen response regions in the human neutral endopeptidase gene", Molecular and cellular endocrinology, vol. 170, no. 1-2, pp. 131-142.

Shen, W.F., Krishnan, K., Lawrence, H.J. & Largman, C. 2001, "The HOX homeodomain proteins block CBP histone acetyltransferase activity", Molecular and cellular biology, vol. 21, no. 21, pp. 7509-7522.

Shih, I. & Kurman, R.J. 2004, "Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis", The American journal of pathology, vol. 164, no. 5, pp. 1511-1518.

Soyer, J., Flasse, L., Raffelsberger, W., Beucher, A., Orvain, C., Peers, B., Ravassard, P., Vermot, J., Voz, M.L., Mellitzer, G. & Gradwohl, G. 2010, "Rfx6 is an Ngn3-dependent winged helix transcription factor required for pancreatic islet cell development", Development (Cambridge, England), vol. 137, no. 2, pp. 203-212.

Sumitomo, M., Milowsky, M.I., Shen, R., Navarro, D., Dai, J., Asano, T., Hayakawa, M. & Nanus, D.M. 2001, "Neutral endopeptidase inhibits neuropeptide-mediated transactivation of the insulin-like growth factor receptor-Akt cell survival pathway", Cancer research, vol. 61, no. 8, pp. 3294-3298.

Sumitomo, M., Shen, R., Goldberg, J.S., Dai, J., Navarro, D. & Nanus, D.M. 2000, "Neutral endopeptidase promotes phorbol ester-induced apoptosis in prostate cancer cells by inhibiting neuropeptide-induced protein kinase C delta degradation", Cancer research, vol. 60, no. 23, pp. 6590-6596.

Susin, S.A., Lorenzo, H.K., Zamzami, N., Marzo, I., Brenner, C., Larochette, N., Prevost, M.C., Alzari, P.M. & Kroemer, G. 1999, "Mitochondrial release of caspase-2 and -9 during the apoptotic process", The Journal of experimental medicine, vol. 189, no. 2, pp. 381-394.

Thelen, P., Burfeind, P., Grzmil, M., Voigt, S., Ringert, R. & Hemmerlein, B. 2004, "cDNA microarray analysis with amplified RNA after isolation of intact cellular RNA from neoplastic and non-neoplastic prostate tissue separated by laser microdissections", International journal of oncology, vol. 24, no. 5, pp. 1085-1092.

Waltregny, D., Alami, Y., Clausse, N., Leval, J.d. & Castronovo, V. 2002, "Overexpression of the homeobox gene HOXC8 in human prostate cancer correlates with loss of tumor differentiation*", The Prostate, vol. 50, no. 3, pp. 162- 169.

Zhang, X., Zhu, T., Chen, Y., Mertani, H.C., Lee, K.O. & Lobie, P.E. 2003, "Human growth hormone-regulated HOXA1 is a human mammary epithelial oncogene", The Journal of biological chemistry, vol. 278, no. 9, pp. 7580-7590.

Zhao, J., Stockwell, T., Roemer, A. & Chikritzhs, T. 2016, "Is alcohol consumption a risk factor for prostate cancer? A systematic review and meta–analysis", BMC cancer, vol. 16, no. 1, pp. 845.