ΕΛΛΗΝΙΚΗ ΔΗΜΟΚΡΑΤΙΑ

Εθνικόν και Καποδιστριακόν Πανεπιστήμιον Αθηνών

ΙΔΡΥΘΕΝ ΤΟ 1837

ΙΑΤΡΙΚΗ ΣΧΟΛΗ

Α΄ ΕΡΓΑΣΤΗΡΙΟ ΠΑΘΟΛΟΓΙΚΗΣ ΑΝΑΤΟΜΙΚΗΣ

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ΜΕΤΑΠΤΥΧΙΑΚΟ ΠΡΟΓΡΑΜΜΑ ΣΠΟΥΔΩΝ:

«Νεοπλασματική Νόσος στον Άνθρωπο: Έρευνα και Κλινικοπαθολογοανατομική Προσέγγιση στα Πλαίσια της Εξατομικευμένης Ιατρικής (Διάγνωση και Στοχευμένη Θεραπεία)»

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ΔΙΠΛΩΜΑΤΙΚΗ ΕΡΓΑΣΙΑ

ΘΕΜΑ: Υποδοχείς Farnesoid X Reseptors (FXRs) και καρκίνος προστάτη

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Η παρούσα διπλωματική εργασία εκπονήθηκε στο πλαίσιο των σπουδών για την απόκτηση του Μεταπτυχιακού Διπλώματος Ειδίκευσης στη

«Νεοπλασματική Νόσος στον Άνθρωπο: Έρευνα και Κλινικοπαθολογοανατομική Προσέγγιση στα Πλαίσια της Εξατομικευμένης Ιατρικής (Διάγνωση και Στοχευμένη Θεραπεία)»

που απονέμει η Ιατρική Σχολή του Εθνικού & Καποδιστριακού Πανεπιστημίου Αθηνών.

Η ΤΡΙΜΕΛΗΣ ΕΠΙΤΡΟΠΗ

ΟΝΟΜΑΤΕΠΩΝΥΜΟ	***ΒΑΘΜΙΔΑ***	***ΥΠΟΓΡΑΦΗ***
Σ. ΘΕΟΧΑΡΗΣ (Επιβλέπων)	**ΚΑΘΗΓΗΤΗΣ**	**……**
Π. ΚΟΡΚΟΛΟΠΟΥΛΟΥ	**ΚΑΘΗΓΗΤΡΙΑ**	**…….**
Ι. ΑΝΑΣΤΑΣΙΟΥ	**ΑΝ. ΚΑΘΗΓΗΤΗΣ**	**……..**

ΠΕΡΙΕΧΟΜΕΝΑ

1. ΓΕΝΙΚΟ ΜΕΡΟΣ

1.1 ANATOMY-PHYSIOLOGY OF PROSTATE

Prostate is an exocrine gland of the male reproductive system, a musculoglandular, encapsulated organ of elastic composition that surrounds the first section of the urethra [1,2]. The word prostate originates from Ancient Greek προστάτης, literally meaning "one who stands before", "protector", because of its close anatomic relation to the bladder [1]. It constitutes the largest accessory gland in the male reproductive system. Prostate's typical dimensions are 5x3x3 cm, weight is around 20 grams and mean volume is 25 ml in young adults [3,4]. It is often described as having the size of big chestnut [1].

It secretes the prostatic fluid, a milk-coloured, watery, mildly acidic, of distinct odour liquid, which contains fibrinolysin, citric acid, prostatic acid phosphatase (PAP), proteolytic enzymes, one of which is a kallikrein called prostate-specific antigen (PSA), polyamines, like spermine, glucose and zinc with a concentration 500–1,000 times the concentration in blood [5,6]. Kallikreins (KLKs), a subfamily of 15 serine proteases and zinc (Zn^{2+}), a trace element stored within the cytoplasm of the prostatic epithelial cells with the highest concentration of all soft tissue in the human body (about 4% of the overall Zn^{2+} content) achieved by an androgen-dependent Zn^{2+} cellular uptake, when mixed with the other glandular excretions of semen lead to liquefaction, clotting cycle initiation and sperm motility, together termed sperm maturation [5]. The importance of zinc inside the prostate is dual. Firstly, Zn^{2+} stops the initial step of the Krebs cycle, the oxidation of citric acid in isocitric acid, resulting to the accumulation of high levels of citric acid, which is needed inside the prostatic fluid acting mainly as an energy substrate for sperm cells, increasing ATP production. Because of this special condition, prostatic epithelial cells are the only healthy human cells that produce energy by glycolysis, which is the typical energy source pathway of cancer cells, rather than Krebs cycle. Secondly, Zn^{2+} causes the temporary deactivation of prostatic tissue KLKs, which are stored inside the cells in the form of zymogens of pre-pro-KLKs, not allowing the proteolytic cascade pathway to begin [5]. During ejaculation, sperm is mixed with both prostatic (20-30% of it's volume) and seminal vesicle fluid, which is rich in semenogelins, inside the urethra. Zn^{2+} is transported from KLKs where it was connected to semenogelins

because they have a higher affinity for it, triggering the activation of both and resulting in the formation of a gelatinous mass from semenogelins and fibronectin at first. Then, within a few minutes, KLKs proteolytic actions act to liquefy semen and break down clotting factors in the ejaculate. This allows semen to remain in a fluid state, moving throughout the female reproductive tract for potential fertilization [5]. Prostatic acidic phosphatase is involved in the nutrition of the spermatozoa by hydrolysing phosphoryl choline into choline [6].

Prostate sits over the pelvic floor, between the lower limit of pubic bone and rectal ampulla, bellow the posterior wall of urinary bladder, seminal vesicle and ampulla of ductus deferens. Through the prostate passes a part of the urethra called the prostatic urethra coming from the bladder and inside the prostate it merges with the two ejaculatory ducts. For descriptive purposes the terms apex, at the exit of the urethra, base, under the bladder surface, and two anterolateral surfaces are used [7], picturing prostate like an inverted cone.

Confusion exists in current sources over the nature of prostatic capsule. Most authors agree that the organ is surrounded by a capsule, but with a more conventional sense of the word, being a dense fibromuscular band, which is an integral part of the prostate and is absent at prostate base and not clearly defined at the apex [8,9,10,11]. Outside this capsule there are fascial structures that wreathe the organ, prostatic, Denonvilliers' and endopelvic [11]. Others prefer the usage of the terms true capsule for the first and false capsule for the fasciae [9,10].

Prostate is divided anatomically into five lobes: anterior, posterior, two lateral and middle. The anterior lobe (or isthmus) contains the fibromuscular tissue positioned in front of the urethra, posterior lobe is the area bellow the entrance of ejaculatory ducts and behind the urethra, it is rather a clinical than an anatomical term, while it is the part of prostate palpated during digital rectal examination. Right and left lateral lobes form the main mass of the organ, located laterally and posteriorly of the urethra. Middle lobe, placed between urethra and ejaculatory ducts has a high density of prostatic parenchyma [7].

John E. McNeal [12] found that the relatively homogeneous cut surface of an adult prostate in no way resembled lobes and thus proposed the idea of three zones, that are more histologically oriented, peripheral, central and transitional. Peripheral zone (PZ) is the sub-capsular portion of the posterior aspect of the prostate gland that surrounds the distal urethra and is comprised of thick, multi-branched glands with elongated (up to 3 cm) secretory ducts that make up around 65% of the parenchymal tissue, compose posterior and side parts of the organ and end up mostly in the prostatic sinuses but also in side walls of urethra. The fact that these glands end up vertically in the prostatic urethra, may explain their tendency to permit urine reflux. Central zone (CZ) surrounds the ejaculatory ducts and it contains submucosal glands that end up in the prostatic sinuses and prostatic utricle, next to the main ducts,

forming almost 30% of the parenchymal tissue. CZ glands empty obliquely in the prostatic urethra, thus not allowing urine reflux. Transitional zone (TZ) surrounds proximal urethra and is made of simple, small glands inside the mucous membrane that end up mostly at the upper part of the prostatic urethra and constitute about 5% of the parenchyma. The rest non parenchymal tissue is called anterior fibro-muscular zone (or stroma), it is located mostly near the apex anteriorly and is composed only, as its name suggests, of muscle and fibrous tissue. It is considered by some writers as a fourth zone [7, 13, 14].

Part of TZ roughly corresponds to anterior lobe, PZ to posterior lobe, CZ to middle lobe and part of all zones contribute to lateral lobes [15].

Prostate is oxygenated mostly from branches of inferior vesical artery (internal iliac), also from branches of middle rectal artery and internal pudental artery. The veins that drain prostate start from prostatic venous plexus between capsule and fibromuscular band at the anterior surface and then blood is carried to internal iliac vein. Plexus is also connected with vesical venous plexus and internal vertebral venous plexus. As for lymph, it is drained through the prostatic capsule and forms the periprostatic lymphatic plexus from where lymph vessels begin and transport it to internal iliac lymph nodes mostly, but it can also go to sacral and obturator nodes and to some extent to external iliac nodes. Prostate's innervation is achieved through branches of the prostatic nerve plexus, which is an extension of inferior hypogastric plexus with sympathetic and parasympathetic fibers. The first are responsible for innervation of smooth muscle fibers of fibromuscular stroma and ejaculation and the second for innervation of glands, production and excretion of prostatic fluid and erection [7].

1.2 EMBRYOLOGY OF PROSTATE [2,13]

The prostatic part of the urethra develops from the middle pelvic part of the urogenital sinus, of endodermal origin. At 9 weeks of embryonic life, mesenchyme mostly to posterior and apical sides of urethra starts to thicken, at 10 weeks, epithelial buds from distal walls of urethra start to protrude into the condensed mesenchyme and around the end of the third month (12th week) they evolve into outgrowths and spread more into the surrounding tissue. The cells lining this part of the urethra differentiate into the glandular epithelium of the prostate. The associated mesenchyme differentiates into the dense stroma and the smooth muscle of the prostate. At the time of birth, prostate is a system of simple diverticula of urethral mucous membrane that go through the fibromuscular stroma. Between the 6th and 7th week after birth formulation of acinar glands and excretory ducts is initiated possibly from mother's estrogens. During puberty, the complete organ is formed and it's size doubles

with the development of preexisting acinar glands and the creation of new under the influence of testicular and in a lesser degree from adrenal and pituitary gland hormones. At the 3rd decade hyperplasia of glandular epithelium forms folds that protrude at acinar lumen, between the 4th and the 5th decade, prostate size stabilizes, folds disappear and amyloid bodies are increased. At older age, prostate undergoes either hyperplasia (75-80% of males after 60) or atrophy. As a result from the simultaneous embryological development from many diverticula of urethral membrane, prostatic glands, unlike other parenchymal organs, don't form lobes and lobules, but three concentric zones around urethra: outer, middle and inner, which after some time can't even be discerned. Wolffian ducts are also believed to contribute to the development of the central zone of the prostate, rendering the organ's embryonic derivation dual, mostly endodermic and partially mesodermic. To function properly, prostate needs male hormones (androgens), which are responsible for male sex characteristics. The main male hormone is testosterone, which is produced mainly by the testicles, but dihydrotestosterone (DHT), a metabolite of testosterone after a chemical reaction catalyzed by 5a-reductase, present also in prostatic tissue, is the hormone that predominantly regulates the prostate.

1.3 HISTOLOGY OF PROSTATE

Prostate comprises glandular elements (70-75%) and stroma-matrix (25-30%).

The first component consists of 30-50 independent compound tubuloalveolar glands whose secretory parts unite and form 12-20 prostatic secretory ducts, which end up in the prostatic sinus of urethra [7]. Ducts are lined by two layers of epithelium, the inner layer consisting of columnar and the outer of small cubical cells with the exception of the proximal 2 mm that are covered by urothelium [16]. Glandular epithelium, partially single-layered columnar and partially pseudostratified, is commonly described having three distinct categories of cells:

• secretory or luminal cells: columnar cells that protrude into the alveolar lumen, contain an abundance of small clear secretory vacuoles and stain positively for prostatic acid phosphatase (PAP), prostate specific antigen (PSA), vimentin, luminal keratins (CK8, CK18) [17], Leu7/CD57, high levels of androgen receptors (ARs) [18] and EMA. Novel immunohistochemical markers are NKX3.1 protein (tumour suppressor) and PSMA (prostate specific membrane antigen) [16, 19].

• basal cells: cuboidal cells attached to basement membrane, that may have prominent nucleoli, are considered reserve stem cells, can undergo myoepithelial metaplasia and stain positively for high molecular weight cytokeratins, p63, basal cytokeratins (CK5, CK14) [17]

and low levels of ARs. Reactive hyperplasia of basal cells during inflammation can lead to multilayered epithelium [16,19].

• neuroendocrine cells: scattered irregularly throughout all anatomic zones, they constitute less than 1% of prostatic epithelium [20] and can't be distinguished without special stains like chromogranin, synaptophysin, neuron specific enolase (NSE) and CD56. Two different cell types were found under the electron microscope, the open-type cells with apical processes reaching the lumen and with surface microvilli and the closed-type cells with dendritic-like processes spreading among neighbouring epithelial cells [21], without reaching the lumen. Apart from the abovementioned typical markers their granules have been found to contain a plethora of other neurotransmitters such as chromogranin B,C, serotonin, somatostatin and calsitonin [16,19, 22].

A putative intermediate cell state between basal and luminal lineages has been also identified on the basis of shared expression of basal and luminal cytokeratins [19].

Recently, two previously unknown epithelial cell types were identified mainly in the periurethral prostatic ducts, one positive for SCGB1A1 and similar to Club cells of lungs and the other positive for KRT13 and similar to Hillock Club cells of lungs. Their exact function remains to be specified [19].

Peripheral and transitional zone secretory cells have a paler cytoplasm (tightly packed vacuoles) and a more peripheral nucleus than central zone cells (darker cytoplasm, thickened epithelium, cell crowding, larger and more central nuclei, seem more stratified) [19].

Merocrine secretion is dominant amongst prostatic epithelial cells, but apocrine secretion has also been discovered to take place with the exact role of the involved vacuoles (prostasomes) remaining to be specified [6].

Over time, thickened amyloid forming secretions of glycoproteins called corpora amylacea or prostatic concretions can accumulate in the alveoli and ducts of the prostate. If grown in size and calcified they are called prostatic stones [13].

The stromal element is made up from randomly oriented bundles of smooth muscle cells and connective tissue with fibroblasts and matrix rich in elastic and collagenous fibres, which spread between the prostatic glands as cores coming from radial laminae from the prostatic fibromuscular band. Some stromal cells express ARs and ERs [23]. The intervening matrix is formed of laminin (LM5, LM10) and collagen (COLIV, COLVII) [17].

The term benign prostatic hyperplasia (BPH) refers to a non-malignant enlargement of the prostate that is very common in older men. It can result in a prostate twice the normal weight or more (33+-16g in autopsies, 100 g in surgical specimens, rarely over 800 g). Both glandular and fibromuscular elements are affected and hyperplasia initiates almost exclusively from the transitional zone [13]. It is often identified when prostate has already been enlarged enough to cause symptoms. The most common are grouped as lower urinary tract symptoms (LUTS) and are further divided into irritative (higher frequency, urge incontinence, nocturia, dysuria) and obstructive (hesitancy, straining, weaker flow, prolonged voiding, urinary retention and at advanced stages overflow incontinence) symptoms [24]. Also, a compromised pressure gradient at the bladder neck is measured, called bladder outlet obstruction, which can ultimately lead to urinary tract infections, formation of bladder stones and even renal failure [24]. BPH is present in about 50% of men in their 5th decade and 75% of men in their 8th decade, with only 5-10% having symptoms [13].

1.4 PROSTATE CANCER

1.4.1 Epidemiology

World (2018): incidence 1.276.106 (4th overall, 2nd in males*), mortality: 358.989 (8th overall, 3.8% of all deaths caused by cancer in men), 5-year prevalence: 3.724.658 [25].

Greece (2018): incidence: 6.457 (4th overall, 2nd in males*), mortality: 1.853 (5th overall), 5-year prevalence: 22.993 [25].

*excluding non-melanoma skin cancer

Prostate cancer is estimated to have a higher prevalence in developed countries, probably due to more frequent use of screening tests. Incidence is age dependent, rising in elderly men, with the median age of diagnosis being 66 years of age. Over 50% of men over 80 years old are believed to have a form of prostatic malignancy, including asymptomatic cases and autopsy data. An increase in incidence of 79.8% and in mortality of 105.6 % is expected until 2040 [25,26].

1.4.2 Pathogenesis

The exact mechanism of tumourigenesis remains unspecified and, like the preponderance of cancers, many factors are thought to lead to malignant transformation in prostate.

Hormonal: binding of testosterone and mostly dihydrotestosterone (DHT) to the androgen receptors of the prostate is certain to play a pivotal role in the development of prostate cancer [27,28]. If castration has been performed before puberty, no functional prostate, BPH or cancer are practically expected with minimal exceptions observed, which use a primary backdoor pathway to synthesise DHT [27].

Age: time progression is a definite risk factor [26].

Genetic/epigenetic: prostate cancer has a large genetic component, as up to 42% of the risk could be explained by inheritance from studies about twins [29]. It has been proposed that first degree relatives of patients with prostate cancer have a two to three times increased danger [30]. Notably, HOXB13 and BRCA2 germline mutations have been consistently shown to increase prostate cancer risk, and are more commonly observed among patients diagnosed with early-onset disease (3,1 and 2% respectively) [30,31]. Especially BRCA2 germline mutations have been found to raise the risk of developing prostate cancer (8.6-fold in men ≤65 years, lifetime risk in mutation carriers has been estimated to be 20%) and also to affect the prognosis and management of the disease, which is usually more aggressive [29]. Genome-wide association studies (GWAS) performed mostly in European populations, have identified around 170 genetic loci associated with increased risk [31]. Genetic translocations leading to overexpression of transcription factors of the ETS family: ERG (21q22.2, in over 90% of cases), ETV1 (7p21.2), ETV4 (17q21) and ETV5 (3q27) due to fusion with androgen-responsive serine protease gene TMPRSS2 (21q22.2) are calculated to be involved in around 50% of prostate adenocarcinomas. TMPRSS2-ERG fusion usually results from interstitial deletion at 21q22.2-22.3 causing juxtaposition of the two genes that are normally 3 Mb distant. The expression of this path acts as an early event in cancer being downregulated as disease progresses to androgen resistance. According to studies, ETS-fusion patients tend to have an invasive phenotype of cells and a more advanced disease [32]. Point mutations in SPOP gene have been discovered in 10% of cancers, commonly associated with CHD1 deletions. Loss of PTEN (Deletion or mutation has been reported to occur in 20-40% of localized prostate cancer [33]), TP53 mutations, alterations of RB tumour suppressor gene, HER-2 amplification in 1/3 of cases and mutations in mismatch repair gene (Lynch) are other molecular data that have been discovered. At sporadic forms, hypermethylation and loss of expression of GSTP-1 [also noticed in precursor lesion of high-grade prostate intraepithelial neoplasia (PIN)] [34] and of the newly investigated tumour suppressor gene SERPINB1 [35],

shortening of telomers (also in PIN) and activation of telomerase (role of MYC) [36] are common.

Ethnic and geographic factors: it is estimated that African-American populations tend to have a higher incidence rate of symptomatic cancer, younger age of diagnosis and a more aggressive type of disease than other racial and ethnic groups (1.7 times higher incidence and 2.4 times higher mortality rate [37]), due to social, environmental and genetic factors (more common risk variants of 8q24 locus [38]). Geographically, a significantly raised percentage of men with prostate cancer is noticed in Australia/New Zealand and Northern Europe (Scandinavian countries) [25], while Japan and other Asian countries have the lowest numbers [31,39].

Environmental: increase with exposure to pesticides, herbicides, chromium, cadmium, tobacco smoke and chronic infectious-inflammatory prostatic diseases [40,41].

Dietary: relation of abdominal obesity, saturated fat and alpha-linolenic acid (ALA) with advanced prostate cancer and cancer lethality and a suggested interaction with MYC protein [42, 43, 44]. Under investigation is the possibility of delaying the progress of disease by lowering saturated fat consumption in men with early stage cancer [42].

1.4.3 Zonal distribution of prostate cancer

PZ: the dominant site of prostate cancer with about 70% of tumours [45]. Possible explanation is the high incidence of acute and chronic inflammation found in this compartment because of urine reflux [14].

TZ: around 20% of cancer cases arise from this zone [45]. Tumours often have a particular histology, with small to medium glands of mostly tall columnar cells with clear cytoplasm [46] and tend to have an overall favourable prognosis [47].

CZ: the central zone accounts for roughly 3% of prostate cancers; these cancers tend to be highly aggressive and of higher grade and stage [48,49].

1.4.4 Diagnosis

Prostate cancer is possible to cause urine obstructive and irritative symptoms, which can be easily confused with signs of BPH, hematospermia, erectile dysfunction or decrease in ejaculatory volume, but usually in early stages of the disease there are little or no symptoms,

because of it's tendency to start peripherally. It is also not rare for the first symptoms to originate from metastatic sites. As a result, the preclinical diagnosis is of great importance and the three methods used are digital rectal exam, transrectal ultrasound and blood biomarkers [50].

About 200 genes are more specifically expressed in the prostate with about 20 genes being highly prostate specific [51] and potentially biomarkers. Some of the prostate specific proteins are enzymes, such as the prostate specific antigen (PSA), which is a 33kDa serine-protease of the kallikrein family secreted by prostatic ductal and acinar cells, as well as male periurethral glandular cells, that aids the liquefaction of the seminal coagulum in the ejaculate. In case of inflammation, hyperplasia or neoplasia, basement membrane's barrier is weakened and increased PSA amounts enter blood circulation. With a cutoff value of 4ng/ml, serum PSA is the most widespread marker for screening and following up prostate cancer, where it is increased ten times on average, but it is controversial and not very specific [50]. Other conditions that may raise PSA levels are severe constipation, extended sexual intercourse, catheterization, infarct or major trauma. Another protein of the same family as PSA is human kallikrein 2 (hK2), which was found according to a study that it may improve the diagnosis of cancer alone or together with PSA [50]. Immunohistochemical stain for a protein called Early Prostate Cancer Antigen (EPCA) in prostatic tissue was shown from experimental data to have 84% sensitivity and 85% specificity in detecting prostate cancer even 5 years before the current methods [50]. Continuing efforts are being made in order to find novel methods for the earliest possible diagnosis, as well as combinations of existing markers or urine levels of proteins. Prostate Cancer Antigen 3 (PCA3) is a newer promising non-coding RNA measured in urine after prostate massage that is used as a marker with increased specificity that can be used along with PSA [50]. Another prostate specific non-coding RNA found during genomic testing, called SChLAP1, has been clinically supported to have positive results in the prognosis of aggressive prostate cancer [50]. Under investigation as biomarkers in prostate cancer are also TMPRSS2:ERG rearrangement and the androgen receptor splice variant-7 (ARv7) [35]. A C-terminal fragment of PSA consisting of 19 amino acid residues is being studied as a possible urine biomarker for diagnosis of prostate cancer [52].

The role of pre-biopsy magnetic resonance imaging (MRI) for prostate cancer screening is also being investigated in ongoing trials [53].

As for the starting age for PSA screening, there is not yet a clear consensus throughout the scientific community, because apart from it's universally accepted importance, overdiagnosis and overtreatment should also be considered. Most guidelines suggest to start no later than the

age 55 (45, 50 or 55) to healthy men with a life expectancy of at least 10–15 years. On the other hand, it is agreed that groups that are considered high risk (positive family history and African-American men) should start earlier than the others. Re-screening intervals can be adjusted according the calculated risk [53].

1.4.5 Pathology [13]

Grossly, prostate cancer is difficult to identify, maybe as a gray or yellowish area with unclear border and solid composition that can spread to prostatic ducts, acini, fibromuscular stroma, perineural spaces and blood vessels. Extraprostatic invasion is common and in advanced disease cancerous cells may extend into surrounding structures, like seminal vesicles, apex, prostatic urethra and bladder.

Two precursors of cancer are known, atypical adenomatous hyperplasia (adenosis) and prostatic intraepithelial neoplasia (PIN). The latter is the most significant and involves ducts and acini. It can be seen at multiple sites in all anatomic regions of the organ. Only high-grade PIN (HG-PIN) is diagnosed and reported, noticed typically as a more basophilic area of cells comparing to the surrounding parenchyma with high n/c ratio, nuclear crowding, large nuclei with prominent nucleoli. Four major patterns have been described: flat/atrophic, tufting, micropapillary and cribriform [54]. These cells share similar molecular features with cancerous cells in prostates with both, rendering PIN a highly probable intermediate stage in the evolution of cancer. It is considered a high predictive value marker for cancer so patients with PIN are followed-up [54].

Microscopically, most are adenocarcinomas with different degrees of differentiation. Well-differentiated are composed of small glands that extend to stroma in an unorganised manner. Unlike hyperplasia, malignant glands grow close to each other (back to back) without stroma in between. As the level of differentiation decreases, more acanonical, disintegrated glandular forms are found with papillary and cribriform architecture and in extreme cases solid cell groups with low differentiation can be seen. Areas of prostatic intraepithelial neoplasia (PIN, high or low-grade) often surround the cancerous loci.

Cancerous cells in adenocarcinomas have been often found to express both basal and luminal keratins, such as CK5 and CK8, which may denote that the disruption of differentiation pathways may play an important role in tumourigenesis [17]. The exact cells that prostate cancer originates from are unknown so far. Initially, they were deemed to be the glandular luminal cells, but recent evidence show that basal cells may also be involved [55].

Prostatic adenocarcinomas can be divided into two major categories: acinar, which represent the majority, and ductal. At first, it was deemed that they were originated from separate areas, but it has been observed that both patterns can be seen in the same tumour and both types can coexist as anatomically separate lesions. So, an alternative proposal states that the site of growth is more important.

There is a wide spectrum of neoplastic cells from highly differentiated and difficultly distinguished from normal parenchyma to anaplastic. Four major architectural patterns have been described: medium sized glands, small glands, diffuse individual cell infiltration and cribriform. Small tumour nests (poorly formed glands) and glomeruloid (intraluminal ball-like clusters of tumour cells, pathognomonic of malignancy) should be added.

Medium sized glands: close arrangement, cells with luminal cytoplasm and nuclei oriented to the basement membrane, smooth inner luminal surface of glands and scanty intervening stroma. Small glands: expansive nodules or infiltrative population of individual glands with regular round shape, between benign parenchyma. Both patterns and particularly the second also have cytologic abnormalities (nuclear enlargement, hyperchromasia, prominent nucleoli, rare mitoses).

Diffuse cell infiltration: resembles lobular carcinoma of the breast, rare.

Cribriform: variety of size of epithelial aggregates from large expanses of anastomosing epithelium to small-medium glands with complex intraluminal proliferations.

It should be mentioned that the above patterns often are found in combination, synchronously or metachronously.

Usually glands are single-layered and only occasionally stratified epithelium like PIN is noticed in cancer. Squamous metaplasia is uncommon, mostly visible in high-grade types and after hormonal or radiation therapy accompanied by poor prognosis. Perineural infiltration is common, being a strong indicator of malignancy, but it is pathognomonic only when circumferential. The surrounding stroma is characterised by a combination of hypercellularity and the deposition of basophilic ground substance (‘’mucinous fibroplasia’’ or ‘’collagenous micronodules’’), suggested as pathognomonic, as are glands encountered in fat tissue. In 10-23% of carcinomas, deeply eosinophilic crystalloid structures of proteins have been observed inside the lumen of particularly medium-sized glands, but they can also exist in benign glands. Intraluminal secretion of malignant glands often has a blue tone, which indicative of mucinous composition.

Special types of acinar carcinoma

Foamy gland carcinoma: prominent glands with cuboidal to columnar cells that have clear or foamy cytoplasm, because of the accumulation of lipids and small, pyknotic nuclei with mostly not visible nucleoli. Most are low grade but a high grade form exists as well.

With atrophic features: similar to benign hyperplastic change, cells have little amount of cytoplasm, their enlarged nuclei occupies most of the cell space and have visible macronucleoli. They have infiltrative growth pattern and are found next to areas of ordinary carcinoma.

Pseudohyperplastic: like hyperplastic glands, with papillary infoldings and branching. Cells have a microcystic appearance on low power view, nuclei are enlarged and positioned over the basement membrane. Macronucleoli, mitoses and intraluminal crystalloids can be noticed.

PIN-like: presence of medium to large-sized glands, stratified or pseudostratified epithelial lining like PIN, but they have a denser arrangement.

P63 expressing: with p63 positive stain found on the secretory, cancerous cells, while basal cells are absent. Other characteristics are little amount of cellular cytoplasm, often atrophic appearance of glands, slit-like luminal spaces and eosinophilic secretions.

Microcystic carcinoma: glands are on average 10 times more dilated than usual small adenocarcinoma glands, flattening the luminal cell lining layer, which may show atrophic changes. Intraluminal crystalloids and blue mucin are commonly present. Most of the times, usual acinar adenocarcinoma coexists, comprising the majority of the tumour [56].

Ductal adenocarcinoma: more common in periurethral areas, appears as polypoid villous or infiltrative urethral component in cystoscopy, histologically it has papillary and cribriform architecture with columnar pseudostratified malignant epithelium. Many carcinomas with grossly visible cysts share ductal features. Rarely, pagetoid spread to urethra can occur. Because of the tendency of these cancers to grow intraductally, basal cells can still exist to some points.

Distinct types of prostatic cancer, of uncertain categorisation:

Neuroendocrine cancers: lack of consensus exists between experts for their classification. One grouping commonly accepted is: usual adenocarcinoma with neuroendocrine differentiation,

with Paneth cell-like neuroendocrine differentiation (cells with eosinophilic cytoplasmic granules, in nests, cords or as single cells, not graded because they are clinically indolent), well-differentiated neuroendocrine tumour (carcinoid) (rare, like in other locations, more common in syndromes) small cell (similar to it's equivalent in the lung, alone or with ordinary carcinoma, some resulted in Cushing syndrome or ADH secretion, markers are not always expressed, many apoptotic cells, very aggressive), large cell neuroendocrine carcinoma (some develop after long-term hormonal therapy) and mixed neuroendocrine carcinoma-acinar adenocarcinoma.

Mucinous adenocarcinoma: with large amounts of intra- and extracellular mucin covering 25% or more of the tumour area, similar to mucinous adenocarcinoma of the breast, with microglandular, comedo, cribriform, solid and hypernephroid patterns, PSAP and PSA are mostly positive, bone metastases are not common, hormonal receptors are not usually expressed and it is less responsive to radiation comparing to acinar type.

Signet ring carcinoma: highly malignant, solid, acinar or single file architecture, with intracellular accumulation of mucin.

Adenosquamous carcinoma: others grow de novo, others after radiation or hormonal therapy of acinar carcinoma.

Squamous cell carcinoma: extremely rare, de novo or after hormonal therapy, probably represents extreme form of metaplasia happening in adenosquamous.

Adenoid basal cell tumour: like adenoid cystic or basal cell carcinoma of salivary glands, with expansive growth, multiple nodular or cribriform architecture, fibromyxoid stroma, squamous differentiation is common, close to areas of basal cell hyperplasia, PSA and PSAP are negative or focally positive.

Basal cell carcinoma and adenoid cystic carcinoma: extremely rare, highly aggressive, like basaloid carcinoma of anal canal and basaloid squamous carcinoma of upper aerodigestive tract, MYB rearrangements sometimes.

Lymphoepithelioma-like carcinoma: like nasopharyngeal.

Sarcomatoid carcinoma: combination of recognisable carcinoma with sarcomatoid elements: nonspecific spindle or giant cells, differentiation towards cartilage, bone or skeletal muscle. One subtype is pleomorphic giant cell adenocarcinoma. The epithelial component may also have squamous features.

Clear cell adenocarcinoma: extremely rare, only a few cases mentioned in literature [57].

Renal-type clear cell carcinoma: only few cases described until now: similar to Müllerian tumours and metastatic renal cell carcinomas [58].

Primary transitional cell carcinoma of the prostate: highly aggressive and rare entity affecting younger men than acinar carcinoma [59].

‘’Minimal adenocarcinoma’’ and ‘’atypical small acinar proliferation’’ (ASAP) are terms proposed to be used in 4-6% of biopsies that cannot be categorised as benign or malignant with certainty, there is suspicion of malignancy and a second biopsy is suggested.

Immunohistochemistry

PSAP and PSA are only used to support the diagnosis of metastatic prostate cancer to other organs, as they are expressed in both benign and malignant cells. ProPSA (precursor form of PSA) and especially native type (-5/-7) has been proposed to be more efficient than PSAP and PSA for detecting metastatic adenocarcinomas [60]. A more recent nuclear stain is NKX3.1 (androgen-related tumour suppressor gene) that has improved sensitivity and specificity over PSA and PSAP, becoming the first line marker in many laboratories. Prostate-specific membrane antigen (PSMA) is a membrane glycoprotein staining positive in all types of adenocarcinoma with expression increasing from benign epithelium to carcinoma. P501S (prostein) has a perinuclear staining pattern in prostate cancer also expressed in poorly differentiated and metastatic cases and when utilised together with PSA could increase sensitivity in detecting metastases. Nevertheless, PSMA and P501S are also positive for some in situ and invasive bladder carcinomas [61]. A very promising novel marker is cytoplasmic protein P504S, an a-methylacyl-CoA racemase (AMACR) important in beta-oxidation of branched-chain fatty acids and their derivatives. It is highly sensitive for prostatic adenocarcinoma, but it can also stain cells in benign prostatic lesions, non-prostatic tumours, atypical adenomatous hyperplasia, atrophy, some benign glands and PIN. It can be used for detecting small loci of cancer in needle biopsies and for validating malignancy in difficult cases. Cancer cells are often positive for androgen and progesterone receptors, but much less for estrogen receptors. HER2/NEU is overexpressed in androgen-independent cancer. Low molecular weight cytokeratins are mostly positive in cancer cells, cytokeratins 7 and 20 are usually negative and CDX-2 can sometimes stain positively. HMW keratins (34βE12 antibody), p63 and keratin 5/6 that are expressed in basal cells which are absent in acinar carcinoma are helpful in the distinction from benign cases. It should be noted that positivity can be found in ductal type carcinoma and some cancer cells and negativity can be encountered in atypical glands, so careful evaluation is in order. A helpful method aiding

diagnosis in complicated situations is a multiplex composed of racemase, HMW keratins (34βE12) and p63.

Intraductal carcinoma is a distinct entity described, associated with high-grade and high-stage disease. It is almost exclusively accompanied by an aggressive carcinoma and results from it's intraductal spread at late stages. Microscopically, malignant cuboidal secretory cells with round nuclei are seen filling large prostatic ducts or acini with at least partial preservation of basal cells forming either: a) solid or dense cribriform patterns or b) loose cribriform or micropapillary patterns with either marked nuclear atypia (size at least 6x from normal) or nonfocal comedo necrosis. PTEN loss has been found to strongly correlate with intraductal carcinoma [33].

Commonest sites of metastasis are the skeletal system (multiple and osteoblastic mostly, sometimes precede urological manifestations, in lumbar spine, sacrum and pelvis via Batson's venous plexus) and lymph nodes (may metastasise early to pelvic and via pelvic chains to retroperitoneal nodes). Denonvillier's fascia renders the expansion to rectum particularly rare [7].

1.4.6 Staging [62]

TNM (2017):

Primary tumour (T)

Clinical T (cT)

Tx Primary tumour cannot be assessed

T0 No evidence of primary tumour

T1 Clinically inapparent tumour that is not palpable

T1a Tumour incidental histological finding in 5% or less of tissue resected

T1b Tumour incidental histological finding in more than 5% of tissue resected

T1c Tumour identified by needle biopsy found in one or both sides, but not palpable

T2 Tumour is palpable and confined within the prostate

T2a Tumour involves one half of one side or less

T2b Tumour involves more than half of one side, but not both sides

T2c Tumour involves both sides

T3 Extraprostatic tumour that is not fixed or does not invade adjacent structures

T3a Extraprostatic extension (unilateral or bilateral)

T3b Tumour invades seminal vesicle(s)

T4 Tumour is fixed or invades adjacent structures other than seminal vesicles such as external sphincter, rectum, bladder, levator muscles and/or pelvic wall

Pathological T (pT):

T2 Organ confined

T3 Extraprostatic extension

T3a extraprostatic extension (unilateral or bilateral) or microscopic invasion of bladder neck

T3b tumour invades seminal vesicle

T4 Tumour is fixed or invades adjacent structures other than seminal vesicles such as external sphincter, rectum, bladder, levator muscles and/or pelvic wall

Note: there is no pathological T1 classification.

Note: positive surgical margin should be indicated by an R1 descriptor, indicating residual microscopic disease.

Regional Lymph Node (N):

NX Regional lymph nodes cannot be assessed

N0 No positive regional nodes

N1 Metastasis in regional node(s)

Distant Metastasis (M)

M0 No distant metastasis

M1 Distant metastasis

M1a Nonregional lymph node(s)

M1b Bone(s)

M1c Othes site(s) with or without bone disease

Note: when more than one site of metastasis is present, the most advanced category is needed. M1c is most advanced.

1.4.7 Grading

Many histological grading systems have been proposed depending on the grade of glandular differentiation, architecture of glands, nuclear atypia and mitotic activity. Gleason scoring system predominantly preferred (regardless difficulties as insufficient biopsy material and subjectivity it is well associated with clinical stage and prognosis) utilising the degree of glandular architectural differentiation and tumour growth pattern relating to stroma on low-power examination. Predominant tumour pattern (primary) is graded from 1 to 5 and secondary pattern (if existing) similarly and then the two numbers are added to obtain Gleason score. If the tumour has no secondary pattern, the primary is multiplied by two. Some tumours have tertiary or minor pattern which is mentioned only if it is of a higher grade. Gleason patterns graded 1 and 2 are not clinically important so in practice Gleason score starts at 3+3=6. According to 2014 criteria:

pattern 3: when there are only well-formed glands with central lumina.

pattern 4: is heterogeneous including cribriform glands, glomerulations, fused and poorly formed glands.

pattern 5: no gland formation, sheets, cribriform glands with central comedo necrosis and single cells like lobular breast cancer invasion pattern.

Variants as pseudohyperplastic, atrophic and PIN-like carcinoma are graded as 3+3=6 score.

Grade Group system has been adopted to better report Gleason score categories using numbers from 1 to 5. Lower-risk tumours are graded as Grade Group 1 and 3+4=7 is

separated from 4+3=7 Gleason scores. Existing grading system correlates well with PSA and PSAP levels, clinical, pathologic staging, lymph node and bone metastases, survival rates and response to therapy.

2014 modified Gleason grading in correlation with Grade Group system:

1: <=3+3=6: only individual discrete well-formed glands.

2: 3+4=7: predominantly well-formed glands with lesser component of poorly formed glands, fused glands, glomerulations or cribriform glands.

3: 4+3=7: predominantly poorly formed glands, fused glands, glomerulations or cribriform glands with lesser component of well-formed glands (if>5%).

4: 4+4=8: only poorly formed glands, fused glands, glomerulations or cribriform glands. 3+5=8: Predominantly well-formed glands with lesser component of sheets, cribriform glands with comedonecrosis or single cells. 5+3=8: Predominantly sheets, cribriform glands with comedonecrosis or single cells with lesser component of well-formed glands (if>5%).

5: >=4+5=9: only sheets, cribriform glands with comedonecrosis or single cells.

Three of the acinar variants (atrophic, pseudohyperplastic, and microcystic) are rated low-grade Gleason pattern 3, whereas foamy gland adenocarcinoma can be either Gleason pattern 3 or high-grade pattern 4 or 5 [56].

Intraductal carcinoma is not graded with Gleason, it is mentioned separately with a prognostic comment. In almost half of the cases of metastatic disease Gleason score appears higher than the primary tumour.

1.4.8 Therapy

Prostate cancer development has been supported by multiple studies to be closely related to increased function of androgen receptors (ARs) in the prostatic cells. ARs are ligand-activated transcription factors belonging to the nuclear receptor family. Inactive, they are located in the cytoplasm bound to chaperone proteins, but when connected with dihydrotestosterone (DHT), testosterone, or other androgenic steroids, they enter the cell nucleus and promote molecular changes. At the time of diagnosis, in about 90% of cases, cancer is still limited inside the organ or it is only locally expanded. Taking under consideration multiple factors, like staging

and PSA levels, clinical decisions are being made for the strategy of treatment, such as active surveillance, local external radiotherapy, brachytherapy, recent focal therapies [cryotherapy and high-intensity focused ultrasound (HIFU)] or radical prostatectomy, as long as the disease is still confined inside the prostate. If it has spread outside of the organ, either locally or as metastatic hormone-sensitive prostate cancer (mHSPC), common practice is the use of androgen deprivation therapy (ADT), which is lowering the levels of circulating androgens by performing surgical (orchiectomy) or chemical (estrogens, gonadotropin-releasing hormone (GnRH) agonists and antiandrogens) castration. Unfortunately, at some point after 18 to 36 months, most patients will develop resistance to ADT, a condition termed castration-resistant prostate cancer (CRPC). Therapeutic options in this situation are limited with chemotherapeutic agents docetaxel and cabazitaxel often being used. The AR axis remains vital so the recently approved agent abiraterone acetate, an androgen biosynthesis inhibitor targeting CYP17A1 is used with highly beneficial results. In mHSPC, ADT together with abiraterone or docetaxel have shown significant improvement comparing to monotherapy. Also, novel second-generation AR antagonists (enzalutamide, apalutamide and darolutamide) have been developed [63]. Poly ADP-ribose polymerase inhibitors (PARPis) are a relatively newly discovered group of agents that cause cell death to neoplastic cells through not repairable summed up DNA damage. For their action they are usually correlated with homologous recombination deficiency (HRD) gene alterations, most commonly with BRCA1/2 mutations. For prostate cancer until now two PARPis have been approved, rucaparib and olaparib for treatment of mCRPC in tumours with BRCA1/2 mutations for both as well as twelve other HRD alterations for olaparib. Pending research investigates the efficacy of combined PARPis with other drugs in tumours with or without HRD alterations [64]. Radium-223 dichloride is a radiotherapy drug utilized as a last resort in resistant cancer with bone metastases [63].

Ultimately, the disease in most of the cases will still relapse with defense mechanisms developed by cancer cells, such as AR gene amplification, ligand binding domain mutations, splice variants and altered levels of AR cofactors, rendering it necessary for more effective therapies to be developed based on personalized medicine and tumour molecular profile [63]. This is pronounced by the poor prognosis to date of patients with metastatic CRPC (mCRPC) that have an estimated median overall survival of 13-32 months and a 5-year survival rate of 15% [65].

During the last years important progress has been made in the field of immunotherapy with FDA approving in 2010 the use of dendritic cell based vaccine Sipuleucel-T (Sip-T) for cases of asymptomatic metastatic prostate cancer. Autologous dendritic cells are modified ex vivo with the addition of fusion protein PA2024 consisting of PAP and granulocyte-macrophage

colony stimulating factor (GM-CSF) and then administered back to the patient. Although this method is efficient and safe, it is not widely used in clinical practice because of disproportioned cost [65].

Many other agents are being tested in ongoing clinical trials alone or combined with already approved therapeutic methods. A DNA vaccine encoding PAP and GM-CSF called pTVG-HP has been developed in order to boost immune response against tumour cells. Another vaccine named GVAX uses whole prostatic cancer cells LNCaP and PC3 that are manufactured to overproduce GM-CSF inducing a robust response of dendritic cells and T-cells. Vaccination with genetically engineered viral vectors like an adenovirus 5 (Ad5) with PSA gene is also under research. Certain monoclonal antibodies against proteins that suppress antitumoural T cells are being trialed in conjunction with prostatate cancer, like ipilimumab targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4), pembrolizumab and atezolizumab for programmed cell death-1 (PD-1) and programmed cell death ligand-1 (PD-L1) respectively and antibodies targeting B7-H3 on cancer cells. An oncolytic virus, that is a virus built to invade and eliminate only cancer cells, termed Ad5-yCD/mutTKSR39rep-hIL12, is currently researched for prostate cancer. It carries two suicide genes, cytosine deaminase (CD) and herpes simplex type 1 thymidine kinase (HSV-1 TK), and incorporates them inside the malignant cells [65].

On a preclinical level, promising fields of drug development could be antibodies against soluble major histocompatibility I chain-related molecule (sMIC), which is a protease-cleaved protein of tumour cells. Uncleaved, it is present on the cell surface and stimulates NK and T cells after interaction with NKG2D protein, but it's soluble form aids immunosuppression. A recently described immune cell subset named myeloid derived suppressor cells (MDSCs) with an immunosuppressive and oncogenic role through the production of arginase I, IL-10 and PD-L1 among others has been discovered to also produce IL-23, which according to research data promotes castration resistance in mice models by AR activation. The fact that this effect was reversed after blocking IL-23 provides a research goad for a possible therapeutic solution. Lastly, oncolytic viruses, that were mentioned previously, are being manufactured to also produce molecules of the immune system. For example, an oncolytic herpes simplex virus-1 (oHSV) secreting human IL-12 and vinblastine or an oncolytic group B adenovirus, enadenotucirev (EnAd) expressing a protein that activates fibroblasts and CD3ε with positive prospects until now [65].

1.4.9 Prognosis

Definite factors

- Clinical stage.
- Pathologic stage: most accurate prognostic factor so far.
- Microscopic grading: Gleason score system and related Grade Group system.
- Surgical margins.
- Tumour volume: many centres use length of cancer in mm per biopsy core.
- Race: men of African descent have almost twice mortality from prostate cancer than that of white men.
- PSA serum levels.
- Lymphovascular invasion.
- Neuroendocrine features: poor differentiation, poor prognosis (serum levels of Chromogranin A have been found increased in metastatic comparing to non-metastatic prostate cancer, and poorly associated with survival in patients with CRPC and Gleason score ≥8, [66]).
- Prominent reactive stroma.
- Androgen-receptor status: high levels of ARs correlation with more aggressive disease, mutations of AR gene detected in metastatic tumours and considered to be the reason for androgen independence.
- DNA ploidy.
- Proliferation index (Ki-67).
- Chromosomal abnormalities.
- P53 expression: mutated in some advanced stage cancers.
- PTEN loss: in advanced stage disease.

Potential factors

- Higher serum bone sialoprotein (BSP) levels are related to faster development of bone metastases in patients with prostate cancer [66].
- Serum levels of sarcosine, glutamate and methionine metabolites have been associated with disease progress [66].
- High levels of obesity and metabolic syndrome are linked to worse oncological outcomes [67].
- Organic anion transporting polypeptide (OATP)1B3 is a membrane transporter mainly present in liver cells but it has been also found in prostate epithelial cells mediating the uptake of testosterone. There is evidence that this protein is upregulated

in prostate cancer in comparison to normal prostate and benign prostate hyperplasia and also that a common mutation that affects the corresponding gene could impair this function resulting in less testosterone input and better prognosis of androgen-independent prostate cancer [68].

- Emerging experimental data link tumoural B7-H3 protein positivity with worse prognosis with higher Gleason score, mCRPC phenotype and advanced stage [65].
- High serum concentrations of sMICs have been affiliated with metastatic potential and poorer prognosis [65].
- PSA reduction rate after treatment [52].
- Lactate dehydrogenase (LDH) elevation [52].
- Alkaline phosphatase increase may indicate bone or liver metastasis [52].
- Albumin decrease [52].
- Low levels of hemoglobin [52].
- Neutrophil-lymphocyte ratio (NLR) in blood when high is shown to worsen overall survival [52].
- Testosterone levels in blood [52].
- Increased number of circulating tumour cells (CTCs) in blood is suggestive of poor prognosis [52].
- High concentration of cell-free DNA (cfDNA) in blood correlates with worse prognosis [52].
- Number of bone metastases and visceral metastases worsen prognosis [52].
- Eastern Cooperative Oncology Group (ECOG) performance status [52].
- Pain severity- opioid analgesic use [52].
- Metastatic site [52].
- Under examination as promising future prognostic markers are exosomal and serum mi-RNAs and AR splice variants (like ARv7) mRNA levels in circulating tumour cells [66].
- Cysteine-rich angiogenic inducer (Cyr)61 has been found to be overexpressed in prostate cancer and evidence show that it's levels relate to high aggressiveness and increased post-surgery recurrence rates [52].

Recently, efforts have been made to identify molecular subtypes of prostate cancer that would personalize each tumour and have prognostic and therapeutic significance. Based on molecular expression, prostate cancer has been divided in luminal A, luminal B and basal

type. Experimental data have shown so far that luminal B subtype has the worst overall prognosis, but also responds better than the others in post-surgery ADT [55].

Efforts for risk stratification of patients have been developed with genomic, proteomic and epigenetic tests as OncotypeDx, ProMark, and ConfirmMDx that analyse a panel of biomarkers. But until now, most useful elements for prognosis and therapeutic decisions are preoperative serum PSA level, TNM stage, grade and surgical margin [35].

Finally, combining the TNM stages with PSA values and Grade Group grading, four prognostic stage groups have been proposed with many subcategories [62].

1.4.10 Predictive factors

- Time period for progression to CRPC from the initiation of ADT [52].
- AR splice variants and especially ARv7 in CTCs in blood have been associated with meagre effect to enzalutamide and abiraterone treatment [52].
- Copy number and certain mutations in the ligand-binding domain of ARs in blood cfDNA have also been affiliated with worse response to enzalutamide and abiraterone [52].
- Certain somatic mutations in DNA repair genes, like BRCA, have been connected with a poor effect of ADT, but a favourable one of PARP inhibitors [52].
- In different studies, enzalutamide was found to be beneficial when patients presented with low Gleason score, good performance status, absence of bone or visceral metastases, no prior steroid or docetaxel treatment, low LDH and high hemoglobin levels [52].
- In metastatic CSPC, positive outcome of chemohormonal therapy was more apparent in patients with a higher burden of disease [52].

2. FXR

2.1 INRODUCTION

Cholic acid (CA) and chenodeoxycholic acid (CDCA), the two main bile acids produced in human liver, are the final derivatives of cholesterol catabolism. It has been recently discovered that bile acids can act as endogenous ligands connecting with specific receptors and allowing the control of their own levels with a negative feedback mechanism. The main bile acid receptor is Farnesoid x receptor (FXR) or Bile acid receptor (BAR) or NR1H4 (nuclear receptor subfamily 1, group H, member 4), a nuclear receptor expressed in humans by NR1H4 gene, which is a member of the nuclear receptor superfamily [69]. Initially the receptor was cloned from a rat liver cDNA library in 1995, when it was also named as such because it was found to be weakly activated by farnesoid, a mevalonate metabolism intermediate [70].

2.2 BILE ACID PHYSIOLOGY

Human liver daily creates 200 to 600 mg of bile acids. After conjugation with amino acids taurine or glycine and the formation of salts in order to increase their solubility, they are excreted with phospholipids, cholesterol and other components and with water constitute bile, which is then stored inside the gallbladder and under the correct chemical signals, it is released inside the intestinal lumen. Inside bile, all the above mentioned molecules form mixed micelles acting as surfactants and helping the digestion and absorption of dietary cholesterol, triglycerides, and fat-soluble vitamins.

In the conversion of cholesterol to bile acids there are two major biochemical pathways, the classic and the alternative (responsible for about 9% of bile acid synthesis according to some reports, maybe more important in neonates), involving overall 17 different enzymes, found in the cytosol, endoplasmic reticulum, mitochondria, and peroxisomes, catalysing steroid ring modifications and oxidative side chain carbon cleavage [71]. In the classic pathway, modification stage is followed by the cleavage, while in the alternative pathway the opposite. The classic pathway begins with enzyme cholesterol 7α-hydroxylase (CYP7A1), inside the endoplasmic reticulum of liver cells, whereas the alternative with a mitochondrial cytochrome

P450 enzyme, sterol 27-hydroxylase (CYP27A1), located in many tissues as well as in macrophages [71].

In the liver, cholesterol is catalysed to 7α-hydroxycholesterol with CYP7A1, which then becomes 7α-hydroxy-4-cholesten-3-one, that is converted to 7α, 12α-dihydroxy-4-cholesten-3-one by the enzyme sterol 12α-hydroxylase (CYP8B1) ultimately leading to synthesis of CA or to 5β-cholestan-3α,7α-diol leading to CDCA [71]. Enzymes like Aldo-keto reductases D1 and C4 (AKR1D1, AKR1C4), CYP27A1, bile acid-CoA synthase (BACS), α-methylacyl-CoA racemase (AMACR) and β-oxidation enzymes are necessary for CA and CDCA formation. Finally, amino-acid transferase (BAAT) attaches glycine or taurine to form glycol- or tauro- CA or CDCA, with a frequency ratio of 3:1, rendering them more soluble and resistant to Ca2+ precipitation, cleavage by pancreatic enzymes and passive absorption. Most of the conjugated bile acids are reabsorbed from the small intestine, while a smaller proportion undergoes deconjugation and transformation from enzymes of the gut flora inside the ileum and mostly the colon, where about 15% of the conjugated bile acids enter, forming the secondary bile acids deoxycholic (DCA) and lithocholic (LCA) from CA and CDCA respectively and tertiary bile acids, such as ursodeoxycholic acid (UDCA) with further chemical reactions [71, 72]. Throughout their route, about 95% of excreted bile acids are reabsorbed at the terminal ileum or colon, via active transport from apical sodium-dependent bile acid transporter (ASBT) or passive transport and are redirected with portal vein back to the liver, constituting the circuit called enterohepatic circulation. Only around 5% of bile acids, equal to their daily production, are lost in feces, consisting mostly of DCA and LCA. About half of the quantity of DCA and a smaller percentage of LCA and UDCA are also reabsorbed and circulated back to the liver with enterohepatic circulation and with the assistance of active hepatic transporters sodium taurocholate cotransporting polypeptide (NTCP) and organic anion transporting polypeptide (OATP). Just a small amount of bile acids exits the enterohepatic circulation and enters the systemic blood circulation, reabsorbed from renal tubules or excreted through urine [71, 72]. In cholestatic conditions, bile acids undergo sulfation in the liver mostly by the enzyme sulfotransferase 2A1 (SUL2A1). Sulfated bile acids are more water soluble and thus difficultly absorbed and easily excreted from the intestine, while their elimination through urine rises about 100 times, leading to a total decrease in bile acid burden [72].

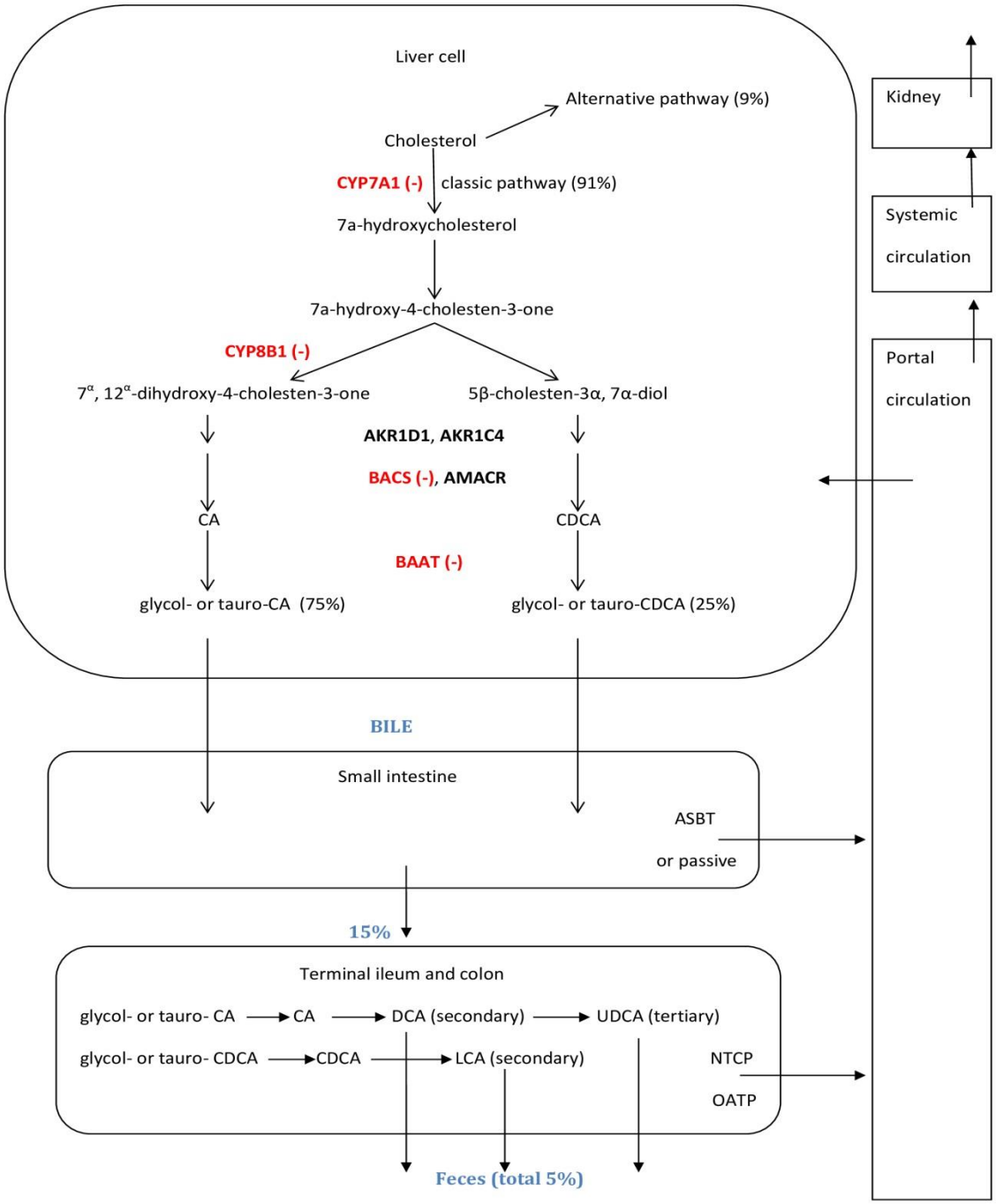

Figure 1: Bile acid metabolism

2.3 FXR MOLECULE

There are two FXR genes: FXRα and FXRβ. FXRβ is a pseudogene in humans encoding a functional protein in rodents, rabbits and dogs. FXRα (chromosome 12q23.1) has four isoforms (FXRα1, FXRα2, FXRα3, FXRα4) that result from two promoters each one with an internal cryptic splicing site. [70,73].

As all the members of the superfamily, it contains an N-terminal ligand-independent activation function 1 (AF1) domain, a highly conserved DNA binding domain (DBD) with two cysteine-rich Zn2+ finger motifs that is linked to a moderately conserved ligand binding domain (LBD) and a C-terminal ligand-dependent activation function 2 (AF2) domain [70, 74].

It is expressed mainly at liver, intestinal, kidney and adrenal gland cells with lower levels seen also in adipose tissue, heart and lung [75].

Natural ligands of FXR are bile acids, which is why it is considered a primary sensor of endogenous bile acids. The extent of activation of the receptor varies depending on bile acid affinity with CDCA having the strongest, followed by LCA. CA and DCA have a lower effect, while the more hydrophilic molecules, like UDCA are poor partial agonists, showing the importance of the hydroxyl group in position 7 for the activation of FXR [74, 76]. Interestingly, androgen metabolites, such as androsterone and etiocholanolone, have been shown to bind to the receptors too [67, 77]. In vitro, polyunsaturated fatty acids (PUFA) like arachidonic, linolenic and docosahexaenoic acid as well as intermediates of bile acid synthetic pathways were discovered to be also weak FXR ligands [73]. Moreover, β-catenin was recently suggested as a possible antagonist of FXR [78].

Due to the high toxicity of bile acids, they are not suitable to be used as pharmacological agents and thus in recent years many synthetic FXR ligands have been developed. These are divided into steroidal agonists, with similar formula with bile acids, and non-steroidal agonists, with a more selective to FXR approach in order to limit side effects from binding to other bile acid receptors, transporters and proteins. One crucial challenge in developing synthetic ligands remains the fact that FXR is designed to react with lipophilic molecules which have a poorer distribution to the body comparing to hydrophilic drugs. The first group is consisted of molecules like 6-ethylchenodeoxycholic acid (6-ECDCA), also known as obeticholic acid (OCA) or INT-747, with an additional hydrophobic cage inside the LBD of FXR, INT-767, a sulfate homologue with a slightly higher binding potency, 23-N-(carbocinnamyloxy)-3a-7adihydroxy-6a-ethyl-24-nor-5b-cholane-23-amine, a cinnamylcarbamate derivative of CDCA with similar effects with OCA and MFA-1, which is

a potential agonist containing the same methylation pattern as CDCA but without hydroxyl groups at positions 3 and 7, but has not been put to clinical trials yet [74]. The other group includes ligands like GW4064, being one of the most important means to study FXR in vitro and in vivo with no activity on other nuclear receptors at concentrations up to 1μM, albeit due to poor bioavailability and toxicological effects it is not incorporated in clinical trials, feraxamine, AGN29 and AGN31, activating in a medium degree the FXR–RXR heterodimer, pyrazolidine-3,5-diones, FXR-450, a compound that after some modifications surpasses the effectiveness of GW4064 in activating FXR having around 200% efficacy compared with it, good solubility in aqueous vehicles and high microsomal stability, some benzimidazole derivatives that have proven to be potent agonists with selective FXR activation and superior physicochemical properties providing a promising solution and Xanthohumol, a natural prenylated flavonoid from beer hops [74]. Apart from the abovementioned categories, some known drugs have been found to activate FXR to some degree, such as troglitazone with a partial agonistic activity, but also an antagonistic one in the presence of bile acids, some statins with significant in vitro agonistic properties and some sulfonylureas that modulate FXR.

On the other hand, some molecules have been described that act as antagonists to FXR. The first described is guggulsterone, the active compound in guggulipid, an extract from Commiphora mukul, that has two isomers, E and Z, it is active at many other nuclear receptors as well, like estrogen receptor, glucocorticoid receptor, mineralocorticoid receptor and pregnane X receptor and also has agonistic influence on BSEP. Another antagonist recently discovered with an important potency is a sulfated sterol from the marine organism Ophialepis superba [74].

Because activation or inhibition of every FXR target gene could result in undesired results and the disruption of bile acid homeostasis, efforts have been made to develop modulators, molecules that have selective agonistic or inhibiting properties on some targets and don't influence other. The only molecule so far exhibiting such behaviour is the synthetic retinoid derivative, AGN34, which augments CYP7A1 expression and reduces IBABP expression leaving other FXR target genes, such as SHP unaffected, but it binds only to the FXR– RXR heterodimer, so it must be considered as an allosteric FXR modulator [74].

When the substrate is bound, FXR moves to the nucleus and connects to specific FXR response elements on gene promoters, either as a monomer or after forming a heterodimer with retinoid X receptor (RXR), controlling their expression [73].

2.4 FXR AND LIVER-METABOLISM

One of the basic roles of FXR activation is lowering bile acid synthesis from cholesterol through upregulation of small heterodimer partner (SHP), an atypical receptor not possessing a DNA-binding domain, which inhibits nuclear receptors liver related homologue-1 (LRH-1) and hepatocyte nuclear factor 4 (HNF4) lowering the transcription of CYP7A1 and CYP8B1 genes in hepatocytes [71]. This way bile acid levels are adjusted from this negative feedback pathway in order not to reach toxic levels, because it is established that intrahepatic accumulation of bile acids can promote liver damage, possible outcomes of which are biliary fibrosis and cirrhosis [78, 79]. This is also achieved by upregulation of the major bile acid transporter, bile salt export pump (BSEP), expression in hepatic cells by FXR [80]. BSEP has a dominant role in cholestasis and liver injury [78]. Except BSEP, other membrane transporters have also been proposed to be upregulated inside hepatocytes by FXR in order to control bile acid levels, such as multidrug resistance protein 2/3 (MDR2/3), which transfers phosphatidylcholine to bile, multidrug resistance-associated protein 2 (MRP2), which transports organic anions including conjugated and sulfated bile acids into bile, ATP binding casette G5 (ABCG5), that facilitates the emission of cholesterol into bile canalicula, MRP3/4 to efflux bile acids and organic solute transporter (OST) α/β to move bile acids into sinusoidal blood. FXR also downregulates the Na+-dependent taurocholate cotransport peptide (NTCP), which is responsible for reabsorbing bile acids from sinusoidal circulation into hepatocytes, reducing bile acid uptake. FXR also controls bile acid conjugation by inducing BACS and BAAT genes [71] (fig.1). Experimental data show a reduction of FXR levels in human and mice fibrotic liver tissue, which when raised corrected mitochondrial dysfunction and hepatocyte death by blocking miR-199a-3p targeting liver kinase B1, a forerunner molecule of AMP-activated protein kinase (AMPK) [78]. FXR-SHP cascade has been discovered to limit activated hepatic stellate cells (HSCs), the cells that produce fibrogenic elements that initiate and preserve liver fibrosis [78]. In contrast, some data suggest that in obstructive cholestasis, FXR is found to have an assisting role in liver injury suppressing a protective mechanism mediated by constitutive androstane receptor (CAR) [78].

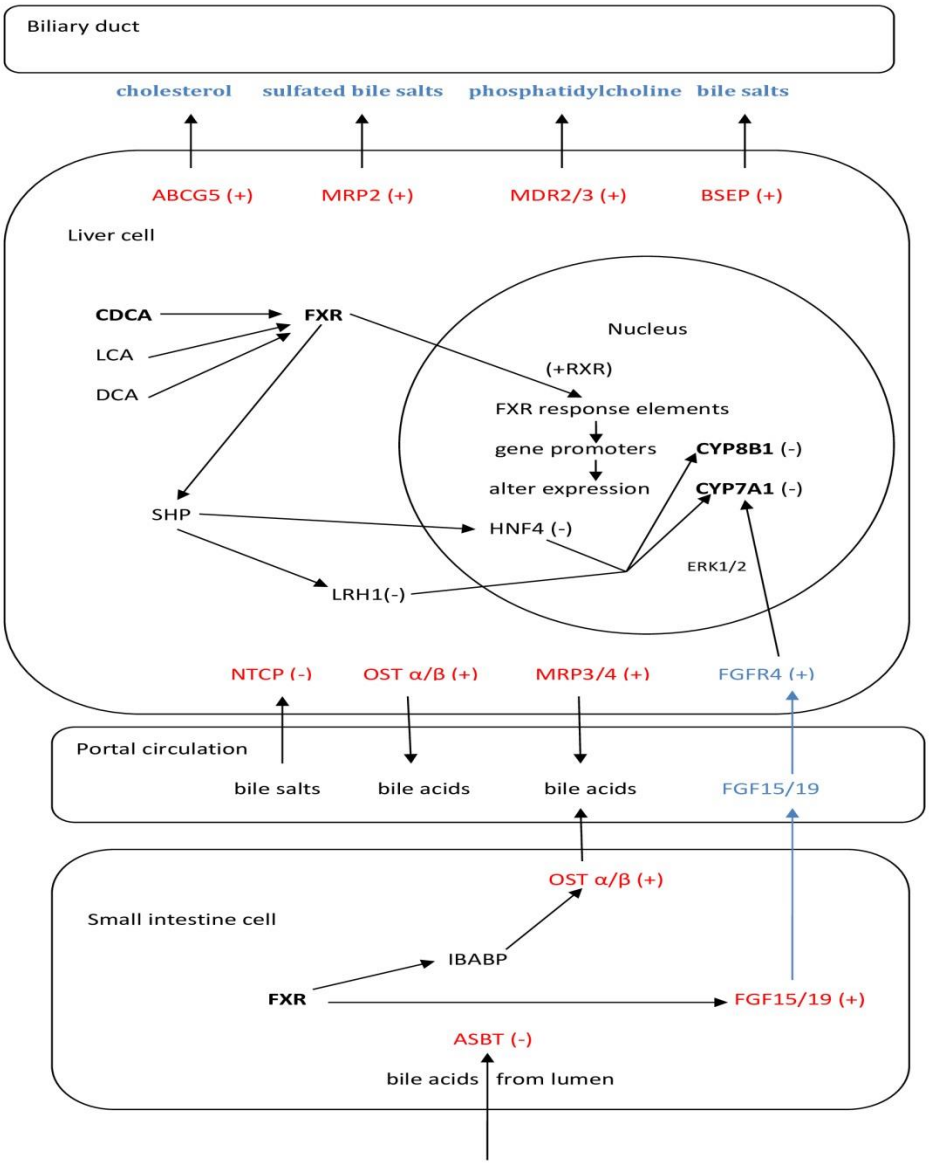

Figure 2: FXR's role on bile acid level regulation and enterohepatic communication

FXR also seems to have an important role in metabolism and body energy management. When activated, it reduces liver steatosis and hyperlipidemia throughout suppression of de novo lipogenesis and promotion of triglyceride oxidation and clearance [70]. This is mediated by a signaling cascade involving again SHP which results in the inhibition of sterol regulatory

element-binding transcription factor 1c (SREBP-1c), a critical transcription factor that regulates hepatic triglyceride synthesis by inducing key enzymes involved in lipogenesis, such as fatty acid synthase (FAS). Moreover, there is evidence that the triggering of FXR induces hepatic carboxylesterase 1, which is also essential for lipid homeostasis [70]. The FXR/SHP pathway also inhibits SREBP-2, a factor responsible for the activation of de novo cholesterol synthesis genes [71] (fig.2).

Apart from that, FXR is responsible for the increase of free fatty acid oxidation by the expression of peroxisome proliferator-activated receptor alpha (PPARα) and its target genes. Another role is the diminution of the expression of microsomal triglyceride transfer protein (MTTP) via SHP, which in turn leads to the decreasing of hepatic VLDL production. Furthermore, FXR has been found to increase the activity of lipoprotein lipase (LPL), phospholipid transfer protein (PLTP), induce VLDL receptor, hepatic scavenger receptor B1 (SR-B1), promote triglyceride hydrolysis by inducing apolipoprotein CII (APO CII) and inhibiting apolipoprotein CIII (APO CIII) expression and downregulate apolipoprotein AI (APO AI) leading to attenuated HDL [70, 81]. On the other hand, OCA has been discovered to result to an increase in macrophage reverse cholesterol transport (RCT) via an FXR dependent way contributing to HDL production [81]. It has been shown that the activation of FXR reduces hepatic gluconeogenesis, by deactivating enzymes phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase), suppresses glycolysis, induces glycogen synthesis, via phosphorylation and deactivation of glycogenesis inhibitor glycogen synthase kinase 3β (GSK3β) and controls glucose production in fasting conditions [70, 71,78] (fig.2).

The activation of FXR by natural and synthetic FXR agonists (GW4064, INT747) or the overexpression of FXR reduces plasma glucose levels and improves insulin sensitivity in diabetic mice [70]. According to experimental data this could result from interaction of FXR with peroxisome proliferator- activated receptor gamma coactivator 1-alpha (PPARGC1A or PGC-1α) protein, which in turn activates peroxisome proliferator-activated receptor gamma (PPARγ) nuclear receptor [82,83] (fig.2).

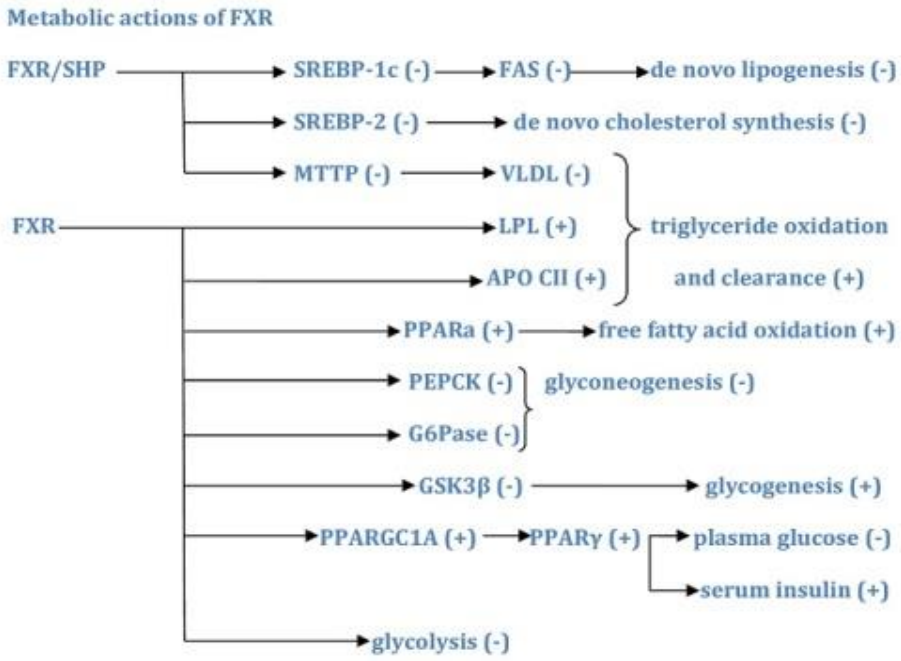

Figure 3

Other research results propose that FXR also augments the actions of Takeda G-protein receptor 5 (TGR5), another bile acid receptor in small intestinal enteroendocrine L cells to provoke GLP-1 secretion and thus enhance hepatic glucose and lipid metabolism [84].

FXR gene activity has been found to diminish during obesity and aging [70] which is confirmed by the promising results of the administration of FXR agonists against obesity-associated metabolic disorders [85].

In white adipose tissue, FXR increases insulin sensitivity and displays adipogenic and anti-inflammatory actions in mice and rat models mediating in the reduction of many pro-inflammatory adipokines, such as tumour necrosis factor-α (TNF-a) and interleukin-6 (IL-6), as well as the production of anti-inflammatory and insulin sensitising molecules, like leptin and adiponectin [78].

Other data demonstrate that binding of bile acids to FXRs reduces the production of the enzymes UDP-glucuronosyl transferases (UGTs) 2B15 and 2B17 whose role is to degrade androgens, thus leading to a decrease in their metabolism and excretion [86].

FXR could be also important in hepatocellular drug uptake and consequent drug-induced liver injury (DILI) and cholestasis via control of anion transporting polypeptide 1 B1 (OATP1B1), a molecule regulating the transport of many endogenous factors [78]. It has been suggested that nonsteroidal anti-inflammatory drugs act as antagonists to FXR and could cause DILI [78].

In research outcomes, FXR levels in hepatic cells were measured lower than normal in patients with Nonalcoholic Fatty Liver Disease (NAFLD) along with liver steatosis [78].

Recent data in mice models refer to the importance of FXR expression in ureagenesis and glutamine synthesis inside the liver, protecting from the toxicity of excessive amounts of ammonium in the blood [78].

FXR is suggested to have liver reparatory and regenerative properties possibly through the bile acid signaling cascade and by reducing apoptosis, as demonstrated in autoimmune hepatitis-induced mice models [78]. This receptor's activation resulted in increased action of the suppressor of cytokine signaling 3 (SOCS3), a down-regulator of cytokine signal transducer and activator of transcription 3 (STAT3), with anti-inflammatory effects on the liver [78]. Furthermore, FXR has been found to downregulate the inflammatory actions of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) protein complex in the transcription of DNA of hepatocytes, while NF-kB's subunits p65 and p50 according to other data could inhibit FXR activity, controlling this molecular circuit [71].

2.5 FXR AND OTHER SYSTEMS

In intestinal cells, the expression of FXR has been indicated to be anti-inflammatory and enteroprotective against bacterial overgrowth and damage [78]. FXR is found to inhibit the apical sodium bile salt transporter (ASBT) from the brush border membrane of ileal enterocytes reducing the amount of bile acids reabsorbed. FXR also activates ileum bile acid binding protein (IBABP) inside enterocytes' cytoplasm to bind bile salts and expel them into portal circulation with the action of transporter OSTα/β located at the basolateral membrane [71]. Experimental data also suggest that FXR plays a vital role in intestinal-liver coordinated actions in bile acid homeostasis. This is possible through the activation of fibroblast growth factor (FGF) 15 (in mice) or 19 (ortholog in humans) from FXR in small intestinal cells. FGF15/19 in turn is transported via blood circulation and connects with the FGF receptor 4 (FGFR4) on liver cells and with the aid of a membrane bound glycosidase β-Klotho, eventually leads to the suppression of CYP7A1 via activation of the extracellular stress-

activated receptor kinase 1/2 (ERK1/2) pathway [2, 71] (fig.2). In mice models with high expression of FXR in intestinal cells, the liver displayed increased cholestasis resistance and regenerative properties against injury, collaborating with liver cell FXRs [78]. Inhibition of CYP8B1 seems to be more dependent on liver FXR and FGF15 appears to downregulate CYP7A1 and not CYP8B1 in mice [71]. FGF19 levels have been measured to have a daylight rhythm with two major peaks at 15:00 and 21:00 lasting about 90 to 120 minutes and following the corresponding bile acid peaks. In human hepatocytes, bile acids are capable to induce FGF19 synthesis and secretion so that it could activate FGFR4 via an autocrine or paracrine manner [71]. Expression control of angiogenin (ANG), inducible nitric oxide synthase (iNOS) and IL-18 genes in intestinal cells from FXR contributes crucially in enteroprotection [87].

In the kidney, FXR displays a protective role against nephrotoxic factors, diabetic nephropathy, renal fibrosis and ischemia-reperfusion (I/R) damage through control of the expression of lipid metabolism proteins, inflammatory cytokines, fibrotic growth factors and suppression of Mothers against decapentaplegic homolog 3 (SMAD3) in mice models with unilateral ureteral obstruction and fibrosis [78]. Moreover, administration of obeticholic acid (OCA), a semi-synthetic analogue of CDCA hampered kidney injury effectuated by chemotherapeutic agent cisplatin via interaction with SHP [78].

In pancreatic β-cells, FXR has been found to contribute to insulin expression and glucose control via genomic and non-genomic actions, through regulation of the production of Krueppel-like factor 11 (KLF1) transcription factor and movement of glucose transporter 2 (GLUT2) at the cellular membrane respectively [88]. Also, it defends islet cells from lipotoxicity [78].

Given the aforementioned involvement of FXR with triglycerides, HDL, LDL and lipid metabolism in general, it's role in the cardiovascular system has also been investigated. Agonists have been shown to have preventive and protective roles against atherosclerosis and deactivating effects in platelets and consequent thrombus formation through possible nongenomic actions [78]. This receptor's anti-inflammatory actions pertaining to nitric oxide synthase (NOS) and Cyclooxygenase-2 (COX-2) could intervene positively in the atherosclerotic procedures [81]. For example, treatment with synthetic FXR agonist INT-767 resulted in less measured atherosclerosis and vascular wall inflammation in the aorta of mice [81]. Also, cardiac dysfunction after infarction in mice was found to be less after treatment with GW4064 via stimulation of adiponectin secretion [78].

Interestingly, certain alterations in the FXR gene and especially single nucleotide polymorphisms (SNPs) have been associated with certain conditions and outcomes such as

glucose homeostasis deregulation, gallstone creation, intrahepatic cholestasis of pregnancy, inflammatory bowel disease and the effectiveness of hypolipidemic therapy [87].

2.6 FXR AND CANCER IN OTHER ORGANS

In the liver, as a result of the abovementioned metabolic actions of FXR, it is sensible that it has a protective role against liver cancer according to studies. It has injury restoring, anti-inflammatory and cell survival properties deriving from gene expression control [76]. CDCA and agonist GW4064 have been proven to raise the expression of tumour suppressor gene NDRG2 (N-Myc downstream regulated gene 2) [76] and also FXR knockout mice had lower quantity of NDRG2 and spontaneously developed liver cancer [89, 90]. Added to that, FXR was downregulated in human liver cancer cell samples and FXR agonists inhibited tumour growth in orthotopic xenograft mice models [89, 90]. Also, FXR has been measured to be downregulated in human cholangiocarcinoma tissues in comparison to the surrounding normal liver tissue and was correlated with tumour differentiation, while OCA inhibited cancer growth and migration in in vitro studies in relation with reduced mitochondrial energy metabolism [91].

In the intestine, high FXR expression causes alteration of genes involved in cell proliferation and inflammation resulting in protection against cancer, as well as lower stage and better prognosis of disease [76, 78]. Interestingly, FXR mRNA expression in tissue samples of adenomas has been found to be reduced significantly and even more in colon cancer. Moreover, in metastatic colon adenocarcinoma cell lines SW-480 and SW-620 the detection of FXR expression was not possible, whereas in more differentiated cancer cell lines Caco-2 and HT-29 it was, providing evidence that FXR expression could be downregulated in concordance with dedifferentiation and loss in apoptotic capacity [92]. Other research data add that FXR expression stops tumour development and promotes a series of proapoptotic genes [92].

In oesophageal cancer, it has been found that FXR possibly modifies the function of genes responsible for cell growth [retinoid acid receptor-β2 (RAR-β2) and cyclooxygenase-2 (COX-2), with it's overexpression linked with higher tumour grade, larger tumour size and lymph node metastasis [76]. Trial results show that FXR levels are increased in esophagitis, Barrett's oesophagus and oesophageal adenocarcinoma in comparison to normal epithelium [92].

A role in the development of gastric cancer has also been investigated with FXR agonist GW4064, according to a study, inducing the expression of intestinal metaplasia promoters

caudal type homeobox protein 2 (cdx2) and mucin 2 (MUC2) in rat gastric epithelial cells RGM-1. Besides, bile acid reflux is a suspected mechanism of gastric carcinogenesis [92].

In pancreatic cancer, high function of FXR is correlated with higher expression of oncogenic MUC4 and poor prognosis [76]. This is further supported by the fact that FXR expression was measured in about 70% of pancreatic cancer cells that had metastasised in lymph nodes in comparison with only about 17% of cancer cells without metastasis suggesting the possible importance of FXR in a worse outcome disease [92]. Further in vitro evidence from the same study support this deduction as administration of agonist GW4064 in pancreatic cancer cell lines MIA-PaCa2 and PANC-1 induced migration and invasiveness, while antagonist guggulsterone had the opposite effects [92]. Despite all previously mentioned data, another published study, utilising immunohistochemical and statistical analysis of 55 tissue samples of pancreatic adenocarcinoma concludes that increased FXR expression was related with longer survival time and an overall better prognosis of disease [93].

In invasive breast cancer FXR's role seems more complicated. High levels of FXR in cancer cells have been correlated with smaller tumour size, higher proliferative rate and better overall and disease-free survival, acting as an independent factor of positive prognosis [94]. Another study performed, showed the inhibition of the oncogenic dynamic of cancer associated fibroblasts (CAFs) of the nearby stroma after activation with the agonist GW4064. This is rendered through changes in both mechanical attributes and paracrine signals of the neoplastic cells [95]. Other recent data relate FXR activation with osteomimetism and bone metastasis potential. Cancerous cells overexpressed Runt-related transcription factor 2 (RUNX2) and produced bone proteins osteopontin (OPN), osteocalcin (OC) and bone sialoprotein (BSP) providing a possible mechanism for the osteotropism of breast cancer metastases and making FXR a plausible predictive biomarker [96]. However, there is also evidence that FXR and ER expressions are interrelated and in ER positive cases in postmenopausal women tumour propagation and Ki67 values are higher when FXR is overproduced. This is further supported by the association of FXR with proliferating factors cyclinD1, p27 and c-myc with the latter relation correlated with a poorer outcome. This way, FXR could be used as a proliferation biomarker in ER-positive breast cancer in estrogen deprived patients [97].

In non-small cell lung cancer (NSCLC), FXR levels have been measured higher than normal, activating cyclin D1 gene which in turn displayed tumourigenic effects and worsened prognosis [76, 78].

In testicular cancer and more specifically in rat Leydig tumour cell line R2C, an in vitro investigation pointed out that CDCA reduced aromatase expression at all levels leading to

inhibition of cancer cell proliferation, which was inverted after knocking down FXR gene with a specific siRNA, providing a probable role of FXR as an antagonist of estrogen signaling [92].

In a recent study FXR levels were revealed as augmented in paraffin blocks of papillary thyroid carcinoma in relation to hyperplastic nodules and were associated with larger tumour size, more frequent lymph node metastases, extraglandular expansion, vascular invasion and generally a more aggressive disease [98].

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2.7 FXR AND PROSTATE CANCER

The association between FXR and prostate cancer has been the objective of many studies and research publications. The expression of FXR along with other molecules was investigated in conjunction with prostate cancer using immunohistochemical stains in formalin-fixed and paraffin-embedded tissue material retrieved from three groups, the first comprising 84 patients with clinically localised, low-risk prostate cancer that have undergone radical prostatectomy, the second 65 patients with advanced metastatic castration-resistant prostate cancer following transurethral resection and the last one 84 cases with BPH after transurethral resection for control. Unfortunately, FXR was not evaluated in this study because of stain diffusion [99].

Another more recent study, utilised prostatic cancer cell lines LNCaP (androgen dependent), and DU-145 (androgen independent) as well as normal prostate epithelial cell line RWPE-1 to prove in vitro that in prostatic cancer cells, lipid accumulation is higher, it is reduced after the administration of FXR's most potent natural agonist CDCA, it is correlated with transcription factor SREBP1, which is shown to be downregulated by FXR and most importantly that cancer cell proliferation is directly contingent on the FXR controlled SREBP1 lipid regulation pathway, providing to FXR in vitro anti-tumoural properties on prostate cancer [100].

In one more research article using prostatic cancer cell line LNCaP along with malignant and normal prostatic tissue samples, investigators came across some interesting deductions. FXR RNA and protein levels were found lower in prostate cancer tissues, in vitro administration of FXR agonists CDCA and GW4064 in LNCaP cells, reduced their proliferation rate, effect that was undone after FXR silencing by siRNA and augmented after transfection with plasmid encoding FXR's cDNA. Importantly, this action of FXR was correlated in vitro with the upregulation of well-known tumour suppressor gene PTEN and the subsequent diminution in

Akt phosphorylation providing to FXR the possible role of a tumour suppressor in prostate cancer [101].

A different research article performed, examines the role of FXR in androgen homeostasis using malignant (LNCaP) and normal prostatic epithelial (PrEC) cell lines in addition to prostatic tissue samples. The results suggest that FXR is expressed in vitro in LNCaP and immunohistochemically in normal prostatic cells and FXR agonists CDCA and GW4064 as well as androgen derivative androsterone cause in vitro downregulation of glucuronidating enzymes glucuronosyltransferase (UGT) 2B15 and 17 in LNCaP cells in both mRNA and protein level in an FXR-dependent manner and without affecting cell survival leading to reduced potent androgen deactivation in prostatic cells and a possible relation with increased AR activity and thus proliferation and cancer. The authors conclude stating that these results could partially explain the observation that many prostate cancer patients also have cholestasis and that patients receiving GW4064 as treatment for cholestasis should also be screened for prostate cancer markers [77].

A review article published some years later, emphasises that despite evidence like the increase of bile acid concentration in prostate cancer patients receiving ADT, the inhibition of proliferation in LNCaP cell lines after administration of FXR agonists CDCA and GW4065 via inducing PTEN, which inhibits the AKT pathway and the apoptotic effects of novel derivatives of UDCA and CDCA on PC-3 androgen-insensitive prostate carcinoma cell lines there are not until now many conclusive evidence on the direct connection of FXR activity with prostate cancer regression besides the documented evidence that FXR reduces cell proliferation by lowering lipid metabolism through blocking of SREBP1c and the finding that FXR mRNA and protein have been measured lower in human tissues with prostate cancer in comparison to normal cells (both papers described previously). This review continues presenting data suggesting a conflicting role of FXR in prostate cancer development, stating that androsterone, a potential FXR agonist has been found from the study forementioned to reduce androgen glucuronidation in LNCaP cells activating AR from the increase of androgens and mainly DHT, but at the same time possibly increasing the activation of FXR from the increase of androsterone, neutralising cancer proliferation induced by AR signaling. It concludes that more research data on in vivo models, SHP mRNA and protein studies in prostate cancer tissues and focus on the molecular outcomes and association with tumour grading and staging are vital in order to enlighten the role of FXR in prostate cancer and provide possible therapeutic targets [67].

A second review article examining the connection of bile acids with cancer in many organs again cites two of the previously mentioned experimental studies when referring to prostate cancer, the one with SREBP1 and the one with PTEN [76].

Lastly, a brief reference of FXR and it's relation to prostate cancer is made in one more review article mainly written about the role of enzyme AMACR. Namely, it is mentioned that high concentrations of AMACR have been found in prostate cancer possibly because of the enzyme's collaboration with FXR in bile acid synthesis. Furthermore, the article described before about FXR and the upregulation of (UGT) 2B15 and 17 in prostate is mentioned once more [86].

In conclusion, it is unambiguous that FXR is an important molecule for many different organs and systems and for many metabolic functions having complex interactions with many other molecules that seem to benefit or sometimes impede cancer formation. One of these actions is lipid control, which obviously plays an important role in prostate cancer that is driven by androgens. On the other hand only four research papers have investigated this connection, one of which didn't provide any results leaving only three, one of which has somewhat conflicting results, as well as three more review papers, all leading to very important findings and observations. FXR could indeed provide medicine with a novel therapeutic weapon against prostate cancer, which is a very common cause of morbidity and fatality, and especially against CRPC, which is so difficultly managed. Modern therapeutic agents could be designed that would target this gene's expression and augment it changing the course of the disease or a more potent agonist could be engineered that would act directly on FXR's target molecules and replace it. Yet, another very intriguing use of FXR would be as a powerful prognostic and predictive factor. Prostate needle biopsies and the subsequent Gleason score are vital for the first approach of prostate cancer and the determination of the initial therapy. Nevertheless, biopsy and regrading is not performed when the disease evolves, develops resistance and first-line therapy needs to be changed. To that end, it is of great importance that new minimally invasive markers be found that would allow tailoring of therapy at the procession of prostate cancer and FXR offers promise to that extent. For all the above reasons, the implementation of more research concerning the role of FXR molecule in prostate cancer is deemed crucial.

ABSTRACT

The purpose of this literature review was to investigate current published scientific data on the relation of bile acid nuclear receptor FXR and prostate cancer development and evolution. Constantly announced evidence on the importance of FXR on metabolism and especially on lipid control combined with the certain role of androgens and their receptors on prostate tumourigenesis, as well as already known involution of FXR and other cancers like breast, provide an appealing research field and a promising pharmacological target and therapeutic option. This review concluded that, unfortunately, there is not enough evidence yet on this matter, although the first results are very encouraging, rendering further research publications, including animal models, of paramount significance.

ΠΕΡΙΛΗΨΗ

Σκοπός της παρούσας βιβλιογραφικής ανασκόπησης ήταν η μελέτη του παρόντος δημοσιευμένου υλικού επάνω στη συσχέτιση του πυρηνικού υποδοχέα χολικών οξέων FXR με τη δημιουργία και εξέλιξη του καρκίνου του προστάτη. Ο διαρκώς αυξανόμενος όγκος ερευνών τονίζει τη σημασία του εν λόγω υποδοχέα στο μεταβολισμό και συγκεκριμένα στη διαχείριση των λιπιδίων σε συνδυασμό με τον παγιωμένο ρόλο των ανδρογόνων και των υποδοχέων τους στην ογκογένεση του καρκίνου του προστάτη, αλλά και η ήδη εξακριβωμένη εμπλοκή του με κακοήθεις νεοπλασίες άλλων οργάνων, όπως του μαστού, τον καθιστούν ως ελκυστικό πεδίο έρευνας και στόχο ανάπτυξης νέων φαρμάκων και θεραπευτικών επιλογών. Η ανασκόπηση έφτασε στο συμπέρασμα ότι, παρά τα πολύ υποσχόμενα υπάρχοντα δεδομένα, δυστυχώς, δεν υπάρχουν επαρκή στοιχεία ακόμα για ασφαλή συμπεράσματα. Επομένως, καθίσταται υψίστης σημασίας ο σχεδιασμός περισσότερων ερευνών οι οποίες να περιλαμβάνουν και ζωικά μοντέλα για τη διεξαγωγή ασφαλέστερων συμπερασμάτων σχετικά με αυτό το πολλά υποσχόμενο αντικείμενο.

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