

Chapter 3

Aptamers: Promising Molecules for Diagnosis and Treatment of Prostate Cancer

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Prostate Cancer

In developed countries, the Prostate Cancer is the most common in men and the mortality rate is in third place, after lung and colorectal cancer [1]. Prostate cancer is a clinically heterogeneous disease, which presents great variability in the risk stratification (low, intermediate and high) and within the Gleason scores [2].

The genetic origin of prostate cancer is not clear, studies have identified recurrent gene mutations and mutations hotspots in several types of cancer [3]. It is believed that epigenetic changes how DNA methylation changes can occur as previous events point mutations in prostate cancer [4]. The recent publication from John R. Packer and Norman J. Maitland (2016) [5] comment that the origin of human prostate cancer is divided into three parts:

1. Cellular origins of prostate;
2. Prostate cancer's inflammatory aetiology;
3. Pré-malignancies of the disease alongside the specific molecular defects of the cancer.

Human prostate is a gland located in the pelvis and is surrounded posteriorly by rectum and superiorly by the bladder, being responsible for the production and maintenance of sperm viability in semen production [6]. Prostate has a parenchyma epithelial and epithelial cells (basal and secretory), in addition to the neuroendocrine cells in adult prostate, which expresses neuropeptides (chromogranin and serotonin) [7]. The relative content of different epithelial cells in the normal prostate are luminal (60%),

basal (40%) with the stem cells constituting ~1% of total epithelia and cellular composition of a cancerous acinus are luminal cells make up 99% of tumours, basal cancer stem cells (CSCs) are estimated to constitute 0.1% of tumour epithelial cells[5]. Cancer stem cell (CSC) is within a malicious clonal population [8] and possesses similar characteristics to normal tissue stem cells, which poses the hypothesis that a cancer stem cell can reconstitute a tumour following treatment and thus promote resistance and recurrence [9].

The model of progression from a normal prostate differentiation hierarchy to the deregulated growth of a prostate tumour does not exclude the possibility that a progenitor cell could become the cancer stem cell following a series of mutations. This cancer stem cell is then able to maintain tumour growth and differentiate into the different cell types that compose the tumour. Furthermore, it is possible for other mutations to occur within bulk tumour cells altering their behaviour and contributing to a more aggressive cancer phenotype [10].

Em addition, evidences of an inflammatory aetiology for prostate cancer has been proposed for many years. Development of inflammation due to infection (prostatitis itself is the most common prostatic disorder) [11] stimulates the infiltration of immune cells and elevated intra-tumoural presence of immune cells can, counter intuitively, aid progression of the cancer [12].

Finally, the prostate Cancer pré-malignancies can be the origin of CaP. They are pré-neoplastic disorders, which the proliferative inflammatory atrophy (PIA) and PIN [13] (Figure 1) [5].

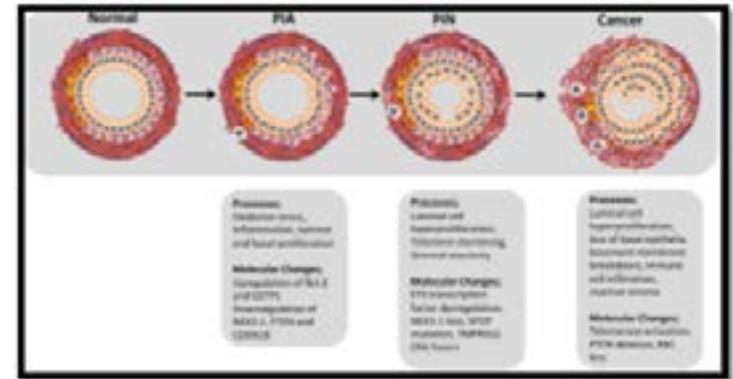


Figure 1: Phenotypic, micro-environmental and molecular changes incurred through the pre-malignant states of prostate cancer.

Proliferative inflammatory atrophy (PIA) is a focal regenerative hyper-proliferative response from epithelia and is considered a disorder to progression of carcinogenesis [14] and PIN is characterized by enhanced luminal cell hyperplasia and is similar to prostate cancer yet lacks any disruption of basement membrane [15]. Despite several studies in the field, there is not evidences of the how is the PIN advancement to prostate cancer (CAP).

Prostate cancer origin is important to development of efficient diagnosis and treatments. In the years 80, it

was introduced of diagnosis using prostate-specific antigen (PSA), which associates stage migration of cancer has helped to a lower disease risk [2]. Studies have developed new diagnostic tests based on gene analyses do to increase the accuracy in the various stages of prostate cancer (PCa) during the course of disease. Urine analysis of the expression of PCA3 levels and TMPRSS2: ERG has as aim to refine the selection of initial and repeat biopsy and gene expression tests in tissues has been developed to predict the occurrence of subsequent events of PCA, including adverse characteristics, biochemical recurrence, metastatic progression and mortality of the PCa [16]. Furthermore, biomarkers biosensor functionalization are used to prostate cancer detection [17] and aptamers show high selectivity, enabling it to be used as a biomarker to improve PCa diagnosis [18].

The most recent works have proposed different prostate cancer therapy. The inactivation of cancer stem cell providing a potential therapeutic target for combined treatment approaches with irradiation [19], B-cell-based antitumor strategies [20], microRNAs as therapeutic targets or tools [21], radioimmunotherapy [22], testosterone therapy [23]. Furthermore Khedri et al, (2015) [24] addressed progress in cancer immunotherapy with nucleic acid aptamers and highlighted recent developments either in immune system targeting or in immunotherapy methods involved aptamers.

Further studies are needed to investigate the clinical benefit of these new technologies and how they should be utilized in clinics.

Selex

Systematic Evolution of Ligands by Exponential was development in 1990, by three distinct researches teams. Ellington e Szostak (1990) used this technique to explain the existence of active sites, evaluating the ability of RNA molecules to form specific interactions with small molecules, in a way similar to proteins [25,26]. Meanwhile, Tuerk e Gold (1990) identified an eight nucleotides region in RNA capable of interacting with T4 DNA polymerase. In this work, two distinct sequences were selected by SELEX from a pool of approximately 65.536 sequences [27]. In the same year, Robertson & Joyce (1990), described RNAs with catalytic activity, selected in vitro [28].

Molecules selected by SELEX are called aptamers (from the Latin word “aptus”, meaning to fit, and from the Greek word “mers”, particle. They are structures composed by oligonucleotides, capable of specific binding to target molecules such as DNA, RNA, proteins and even bacteria or other cells [25,29]. When compared to monoclonal antibodies, aptamers present some advantages, such as: fast synthesis, low cost, low molecular weight, low immunogenicity, stability, easy purification and structural modification, high specificity, capability of active confor-

mational recovery under the right conditions, and a vast diversity of target molecules (toxins, enzymes, proteins, virus, bacteria, and even cells). These characteristics render aptamers ideal for utilization in therapeutics, biosensing and diagnosis [30]. The aptamer selection process is shown in Figure 2.

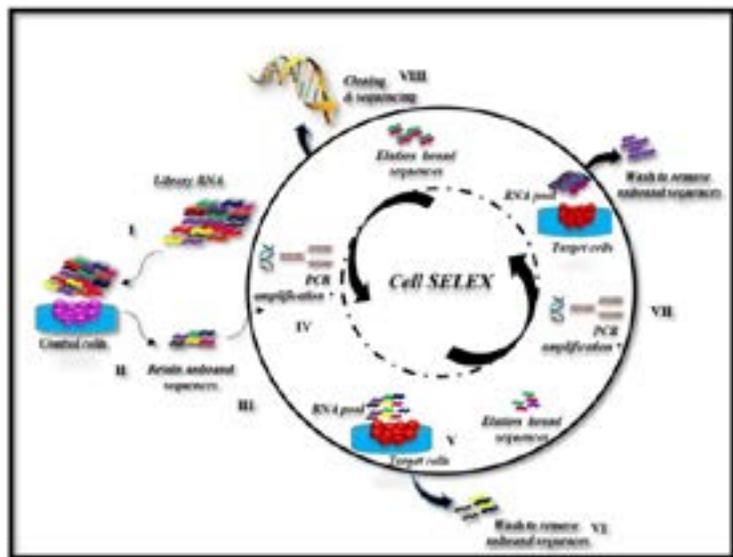


Figure 2: Schematic representation of the Cell SELEX aptamer selection. Adapted Souza et al.,(2015) [31].

SELEX stands out for high selectivity and specificity of aptamers obtained after selection. Thus, research related to diseases like cancer, explore this technique increasingly in the search to use the selected aptamers for earlier

and more accurate diagnosis, and direct strategy for the therapy of this disease. Since the discovery the number of papers published using the SELEX technique significantly increased Figure 3.

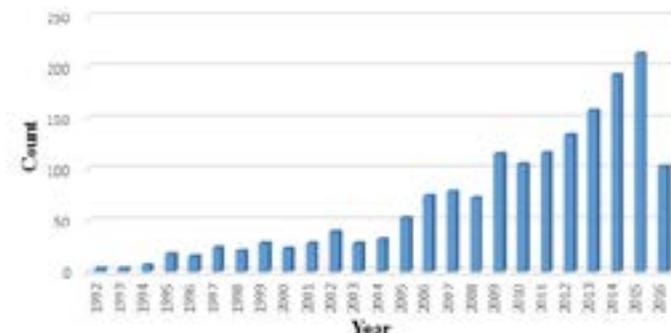


Figure 3: Number of papers published between 1992 and 2016.

After 25 years of the first selection process for SELEX, several modifications have been proposed in order to get more selective molecules and specific to a particular target. Table 1 shows the main SELEX and variants described in the literature.

Table 1: Principal changes of SELEX.

SELEX modification	References
CE-SELEX	[32]
SELEX-SAGE	[33]
Multi-stage-SELEX	[34]
Deconvolution-SELEX	[35]
Next Generation SELEX	[36]
In vivo SELEX	[37]
CE- SELEX	[38]
Non-SELEX	[39]
Cell SELEX	[40]
3D Cell SELEX	[18]
FluMAG	[41]

Aptamers and Prostate Cancer

Given the selectivity and specificity that aptamers offer, there are many publications for the application of these prostate cancer. Marangoni et al. (2015) described the selection of a novel nucleic acid antibody-like prostate cancer (PCa) that specifically binds to the single-stranded DNA molecule from a 277-nt fragment that may have been partially paired and bound to the PCA3 RNA conformational structure [42]. Min et al. (2011) designed a dual-aptamer complex specific to both prostate-specific membrane antigens (PSMA) (+) and (-) prostate cancer cells [43]. Wang et al. (2014) developed a aptamer probe

against PCa cell line PC-3 by cell-SELEX technique. Wy-5a shows high specificity to the target cells with dissociation constants in the nanomolar range and does not recognize other tested PCa cell lines and other tested tumor cell lines [44].

Jeong et al. (2010) developed two different kinds of counter-SELEX methods; one includes pre- clearance step with inactive proPSA protein, and the other with tagged GST protein. After 9 iterative selection cycles, several identical RNA aptamers can be identified from both counter-SELEX methods. Duan et al. (2016) adopted the cell-SELEX strategy to obtain a DNA aptamer, termed DML-7. DML-7 binds to the classical DU145 metastatic prostate cancer cell line with high affinity [45].

Also, Souza, et al. (2015) performed 3D Cell-SELEX against PC-3 prostate cancer cell line, a novel strategy to select specific nucleic acid ligands against spheroid cells in 3D cell culture. This original system combines Cell-SELEX, a process that exploits the cellular structure to generate specific ligands and 3D cell culture, an approach that mimics the tissue microenvironment in vitro. These results showed the aptamer A4 as a specific ligand to prostate tumor cells, with dissociation constant in the nanomolar scale.

All molecules have selected could be of potential use as specific diagnostic, imaging and/or therapeutic agents against prostate cancer. These studies highlight, after 60

years of the discovery of the nucleic acid structure by Watson and Crick, the important role and the functionality that this molecule may have, in addition to the transmission and translation of genetic information [46].

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