Epigenetic and Epitranscriptomic Control in Prostate Cancer

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The initiation of prostate cancer has been long associated with DNA copy-number alterations, the loss of specific chromosomal regions and gene fusions, and driver mutations, especially those of the Androgen Receptor. Non-mutational events, particularly DNA and RNA epigenetic dysregulation, are emerging as key players in tumorigenesis. Researchers summarize the molecular changes linked to epigenetic and epitranscriptomic dysregulation in prostate cancer and the role that alterations to DNA and RNA modifications play in the initiation and progression of prostate cancer.

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1. Prostate Cancer

Prostate cancer (PCa) is the second-most diagnosed cancer in men worldwide. In 2019 it accounted for nearly one in five new diagnoses. It is the first cancer in terms of prevalence and is also a leading cause of male cancer-associated deaths [1][2]. Early detection through testing for the prostate specific antigen (PSA) and the improvement of procedures for surgical intervention radiation therapy and androgen deprivation therapy (ADT) have significantly reduced the number of deaths [3]. However, in more advanced or aggressive forms of the pathology, PCa can evolve to stages characterised by invasion of the seminal vesicles followed by metastasis especially in the bone, usually resulting in the death of the patient. The progression to metastatic disease is commonly linked to the fact that the cancer becomes androgen-independent, a frequent feature in advanced prostate cancer [1]. In fact, while ADT is initially effective in the majority of men with PCa, in around 20% of cases, patients progress to castration-resistant prostate cancer (CRPC) for which treatment options are very limited, revealing that other genetic or non-mutational factors may account for the initiation and progression of the disease [4]. Until recently, the first-line treatment options for metastatic CRPC were taxane chemotherapeutic agents [5]; unfortunately, one-third of patients fail to respond to initial treatment and, within 24 months, those who initially respond will develop resistance [6], emphasizing the need to find new therapeutic targets.

2. Epigenetic Alterations in Prostate Cancer

Until now, profiling studies of primary PCa have been focused on the most studied alterations of this tumour type, such as *AR* alterations, DNA copy number and single point mutations or mRNA expression [7][8][9]. However, with

the increase in large-scale genome sequencing and integrated multi-dimensional analyses projects such as The Cancer Genome Atlas (TCGA), the “Encyclopedia of DNA Elements” (ENCODE) or the International Cancer Genome Consortium (ICGC), a different picture started to emerge, where epigenetic changes can lead to chromatin remodelling and aberrant gene expression, which can have severe pathological consequences [10]. In cancer research, recent studies have developed a comprehensive profile of hundreds of primary prostate carcinomas by combining epigenetics, RNA-seq and ChIP-seq [11]. Through multiparametric genomic data integration, it was possible to uncover three subtypes of PCa with differential biological and clinical features, for a tumour type known to be difficult to classify [11]. Other studies have also established PCa subtypes based on distinct epigenetic changes. For instance, in the study by Armenia et al., the authors identified a new class of ETS-fusion-negative PCa defined by epigenetic alterations [12]. Using TCGA methylation and RNA-seq data, Xu et al. performed an epigenetic integrative analysis between normal and PCa tissue, in order to detect the pathways in which DNA methylation-driven genes were significantly enriched [13]. More recently, in the study by Lin et al., using single-cell RNA-seq profiles, the authors identified new signature genes and cell subtypes among CRPC cells [14]. All this evidence brings out a clear role for epigenetic regulation in PCa control.

Mechanistically many studies have shed light on the molecular effects underlying epigenetic dysregulation in PCa. One of the most frequent DNA methylation changes occurs at the *GSTP1* promoter, a fact which was already described 20 years ago. GSTP1 modulates several signalling pathways involved in proliferation, differentiation and apoptosis [15]. After this finding, many other recurrent epigenetic alterations have been described, and may be used in the future as a biomarker for the evaluation of PCa diagnosis and prognosis. Others include the promoter CpG island hypermethylation of *PTEN*, which causes its silencing [16], or the hypermethylation of the tumour suppressor gene *CDKN2A* (which encodes p16) that leads to increased proliferation, thus contributing to carcinogenesis [17]. Even the loss of *AR* expression is regulated in 30% of CRPC by hypermethylation of its promoter [18]. More interestingly, recent studies have described that, in metastatic CRPC and tumours that progress to AR-independency, epigenetic principal regulators are clearly altered, as well as key factor players in chromatin biology [18].

Besides DNA methylation, other epigenetic marks regulate chromatin structure and gene expression.

3. Epitranscriptomics Alterations in Prostate Cancer

Similarly to DNA, RNA can also be modified. Despite being known for over 50 years, the study of RNA modifications has suffered a delay regarding epigenetics, probably due to the lack of suitable tools for their study [19]. Thus, the emergence of this field, known as epitranscriptomics, is closely linked to the recent refinement of tools such as mass spectrometry, next-generation sequencing [19] or cryo-electron microscopy [20], which have enabled the discovery of over 170 RNA modifications [21][22].

These modifications are found in all types of RNA, from messenger RNA (mRNA) to non-coding RNAs such as ribosomal RNA (rRNA), transfer RNA (tRNA), microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) among many others [21]. tRNAs are the more extensively modified, with an average of 15% modified nucleotides per

molecule and involving a large number of enzymes and a high diversity of modifications (reviewed in [23][24]). In rRNA, around 130 individual modifications can be found, with 2'-O-methylation of the ribose and pseudouridine (Ψ) being the most frequent modification (reviewed in [25]). In the case of mRNA, the most abundant internal modification is N^6-Methyladenosine (m^6A), with around 0.1-0.4% adenines of all mRNAs being modified [26].

In contrast with DNA modifications, which are known to mainly regulate gene expression [27], RNA modifications control many functions apart from transcription such as RNA stability, location, splicing, degradation or translation efficiency [25][28][29]. For instance, 5-methylcytosine (m^5C) methylation of tRNAs stabilises their structure and protects them from nuclease-mediated cleavage [28]. However, the role or importance of most of these modifications are still unknown and, for others, it is only starting to emerge.

Despite the great diversity of modified nucleotides in RNA and the huge expansion of the field in the past years, little is known about the role of RNA modifications in PCa.

4. Conclusion

Despite the initial response to hormone-deprivation treatment, one of the main problems in PCa management is the relapse and progression rate to metastatic tumour, which has limited therapeutic options, none of them completely curative [18][30]. This intensifies the urgent need for the investigation of new therapeutic approaches.

Evidence has highlighted that epigenetic alterations are emerging as potential biomarkers to stratify PCa patients and predict clinical outcomes [18]. Epigenetic alterations are most common in advanced PCa, being especially dysregulated in metastatic CRPC [31]. These findings suggest an important role of epigenetic regulation in advanced phases of the disease and indicate that epigenetic mechanisms may regulate tumour selective pressures. The use of epigenetic modulators has been growing in recent years, and currently, six epigenetic drugs are approved by FDA for cancer treatment, mainly for haematological malignancies [32]. Regarding PCa, despite the huge number of studies pointing to epigenetic modulators as prognostic markers, none of them are used nowadays in clinical practice. However, clinical trials have shown only mild results in PCa patients, probably because most of them have been undertaken in late-stage, heavily pre-treated patients and without considering tumour subtypes [32][33][34]. A deep understanding of the molecular mechanism underlying the epigenetic mechanism and tumour biology will allow the development of successful clinical trials and the eventual approval of epigenetics-based therapies for PCa.

As in DNA, RNA modifications are also known to regulate responses to environmental signals [28][35] suggesting that they too may regulate cancer cells' survival of challenges occurred during tumour expansion or therapies, making them attractive therapeutic targets. However, unlike epigenetics, epitranscriptomics has not reached the clinic yet. Changes in several RNA modifications have been linked to different tumours including PCa [36][37][38][39][40][41][42][43][34], revealing their potential role as tumour biomarkers. However, their use is still limited by the lack of easy, sensitive, cost-effective and reliable high-throughput detection methods. In addition, the aberrant expression

of RNA-modifying enzymes has also been reported in PCa [36][37][38][40][44], but their specific roles in regulating tumorigenesis remain to be further characterised.

Similarly to epigenetics, RNA modifications are emerging as promising therapeutic targets, and great efforts are now being made to develop small molecule inhibitors to rewire the aberrant cancer epitranscriptomes. However, targeting RNA modifications could be fairly complicated since they are linked to most aspects of RNA biology, and their alteration could involve undesirable toxic effects. Moreover, the role of RNA modifications is context-dependent and could differ between cancers or even between different cell populations [35][45]. Thus, there is still a long road ahead that will require great research efforts in order to fully understand the biology of RNA modifications and the means to effectively target them, so that ground-breaking epitranscriptomics can finally reach the clinic.

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