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Bromodomain-containing proteins in prostate cancer

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ABSTRACT

Several oncogenic factors have been involved in prostate cancer progression. However, therapeutic approaches still focus on suppression of androgen receptor (AR) signaling. In fact, whereas the full-length AR incorporates a ligand-binding domain, which has become a drug target for competitive inhibitors, other transcription factors often do not have tractable binding pockets that aid drug development. Consequently drug development efforts have turned to transcription co-regulators, often chromatin-modifying enzymes or factors that bind to epigenetic modifications to chromatin. Bromodomain (BRD)-containing proteins fall into the latter category and significant progress has been made in developing small molecule inhibitors that target a particular subgroup of BRD-containing proteins known as the Bromodomain and extra-terminal (BET) family proteins. These inhibitors have proven particularly effective in inactivating c-Myc in lymphoma but more recently members of the BET family have also been identified as AR-interacting proteins raising the prospect of using these inhibitors as an alternative strategy for targeting AR-driven cancers. In this review we will provide an overview of BRD-containing proteins and the potential for exploiting them as biomarkers and drug targets in prostate cancer.

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1. Introduction

Epigenetics can be defined as a heritable pattern of post-translational modifications leading to the emergence of new phenotypes that are not encoded within genomic DNA sequence (Graca et al., 2016). The epigenetic code is made of several chromatin modifications that can be found on histones in different combinations. Histone acetylation is one example of these modifications,

Abbreviations: TF, transcription factor; HAT, histone acetyltransferase; HDAC, histone deacetylase; BRD, bromodomain; BET, bromodomain and extra-terminal; NEPC, neuroendocrine prostate cancer; DDR, DNA damage Response.

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which is most significantly linked to regions of open chromatin accessible to DNA and RNA polymerases. Acetylation levels on histones are dysregulated in cancers and this reflects changes in the expression and/or activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs). Deregulation of HDACs changes the relative expression of oncogenes, tumour suppressors and subsets of microRNAs in many cancer types. This has motivated the development of HDAC inhibitors as oncological agents (Marrocco-Tallarigo et al., 2009; Anne et al., 2013). Epigenetic readers such as bromodomain (BRD)-containing proteins offer an alternative target class to disrupt pro-tumorigenic transcription arising from epigenetic modifications (Filippakopoulos and Knapp, 2014). BRD inhibitors that specifically target a subset of BRD-containing proteins, BET (bromodomain and extra-terminal) proteins have been developed. These inhibitors impact on gene expression programmes by altering how protein complexes utilize histone acetyl marks (Filippakopoulos and Knapp, 2014). In particular, the effect of BRD inhibitors is focused on cell growth and resistance to apoptosis in cancer cells and on inflammatory signaling in immune cells (Muller et al., 2011; Bachu et al., 2016). The use of such inhibitors has helped to understand the cell/tissue-specific functions that these proteins perform. The main functional role of BET proteins is to promote transcriptional elongation/efficiency. Consequently the somewhat cell type-specific effects on transcription observed speak of the interplay between these proteins and transcription factors (TFs) driving cellular specialization and tumorigenesis (Di Micco et al., 2014; Liu et al., 2014).

2. Prostate cancer, the AR signaling axis, chromatin, and bromodomain-containing proteins

Prostate cancer (PC) is a heterogeneous disease and the etiology is still not completely known. From the molecular point of view, the main focus of research has always been the androgen receptor (AR), which is thought to be an important driver of the disease (Mills, 2014). In recent years a number of drugs such as Enzalutamide and Abiraterone acetate have received clinical approval due to their impact on AR signaling, acting as a potent antagonist to androgen binding to the ligand-binding domain and as a potent inhibitor of androgen synthesis respectively (Teply and Antonarakis, 2016). Each drug has enhanced the median survival of patients with lethal castrate-resistant metastatic disease by months and in the case of some individuals by years. In other cases however responses have been significantly less impressive due to a range of resistance mechanisms that include the expression of AR splice variants that lack a ligand-binding domain (for example AR-V7) (Ciccarese et al., 2016), AR copy number amplification (Visakorpi et al., 1995) and over-expression and the evolution of a dependency on other oncogenic signaling pathways driven by phosphatidyl inositol 3-kinase/Akt (PI3-kinase/Akt) signaling, receptor tyrosine kinases or indeed other oncogenic TFs such as c-Myc or N-Myc (Beltran et al., 2016; Robinson et al., 2015; Dardenne et al., 2016; Barfeld et al., 2017). Regardless of the oncogenic driver however transcriptional dysregulation is fundamental to cellular transformation and cancer progression. Changes in transcriptional profiles are used as cancer biomarkers and are in effect readouts of oncogenic signaling and changes that have occurred within the nucleus to both the accessibility of DNA sequences and the activity of TFs and co-regulators (Luo and Dean, 1999). DNA packed into nucleosomes forms the simplest and most immediate chromatin structure. Such chromatin structure is the ultimate determinant of the transcriptional output, which therefore exerts regulatory functions. In fact the recurring chromatin modifications at the nucleosomes associated with transcriptional changes have been defined as the “histone code”. Through these mechanisms, chromatin modifications and their alterations, have an indirect effect on cell identity and cancer development (Audia and Campbell, 2016). However, several other chromatin-associated proteins mediate transcriptional regulation. TFs and the basal transcription machinery are direct effectors in transcriptions, while chromatin writers and erasers modify chromatin landscapes (Speranzini et al., 2016; Zhang et al., 2015). Another class of chromatin-associated proteins is made of chromatin readers such as BRD-containing proteins. This class of proteins is overlapping with the above-mentioned other classes of chromatin-associated proteins as they recognize acetylated histones and perform a wide range of downstream functions. BRD-containing proteins can also present other domains and be TFs or transcriptional co-regulators and can act as primary recruiting factors for the assembly of larger protein complexes. They may also perform catalytic activities such as methyltransferase, ATP-dependent chromatin-remodeling, HAT and helicase (Fujisawa and Filippakopoulos, 2017) (Table 1). Therefore, BRD-containing proteins are transcriptional regulators that initiate chromatin reshaping in order to prepare for transcription.

3. Targeting bromodomain-containing proteins −BRD4 and associated therapeutics

Current therapies aim to shut down the signaling associated with the androgen/AR axes (Teply and Antonarakis, 2016). One of the drawbacks of this approach is that complete shutdown of the pathway has proven difficult to achieve, with little benefit for the patients, in terms of overall survival to the so called castration resistant prostate cancer (CRPC) that still relies on androgens, and therefore on the AR to progress. Forms of neuronendocrine prostate cancers (NEPCs) have been described, which may also represent clonal selection following treatment with next generation anti-androgens such as Enzalutamide (Vlachostergios et al., 2017). Therefore the current therapeutic strategy to reduce patients' side effects and limit the selection of resistant clones is to identify druggable downstream targets of AR that mediate its oncogenic effect. However, only a few have been found that associate with disease outcome (Urbanucci et al., 2013). Moreover it is not known how the AR switches from being the main driver of physiological healthy prostate cells differentiation, to being the main driver of PC cells growth and resistance to many therapeutic approaches. These clinical challenges have motivated a renewed focus on targeting co-activators supporting the transcriptional activity of not merely the AR but other important oncogenic TFs (Culig and Santer, 2014; Rohira and Lonard, 2017). Thanks to the pioneering work of Arul Chinnayian's laboratory, two BRD-containing proteins were shown to be involved in PC progression to CRPC (Asangani et al., 2014). In particular, both BRD4 (Asangani et al., 2014) and lysine methyltransferase 2A (KMT2A, alias MLL) (Grasso et al., 2012; Malik et al., 2015) were found to directly interact with the AR and act as transcriptional activator. BRD4 can be viewed as a multi-cancer target. It was firstly identified because of its role in NUT midline carcinoma (NMC) (French et al., 2008), a rare subtype of squamous cell carcinoma characterized by a translocation most often involving the *NUT* gene and BRD4 (Bauer et al., 2012). NMC typically arises from midline structures of the head, neck and respiratory tract principally the thorax and can be diagnosed in both adults and children. This extremely aggressive disease has a median overall survival of around 6 months and the BRD4 fusion blocks differentiation of NMC cells in part by promoting expression of c-MYC (Grayson et al., 2014). The use of a BET inhibitor, OTX015/MK-8628, was able to extend overall survival to 18 months in two patients (Stathis et al., 2016) and more recently has achieved significant impacts on a second lymphoma, also acting through c-MYC (Gaudio et al., 2016).

BET inhibitors have shown pre-clinical efficacy in CRPC but as