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Genome-wide analysis of AR binding and comparison with transcript expression in primary human fetal prostate fibroblasts and cancer associated fibroblasts

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ABSTRACT

The androgen receptor (AR) is a transcription factor, and key regulator of prostate development and cancer, which has discrete functions in stromal versus epithelial cells. AR expressed in mesenchyme is necessary and sufficient for prostate development while loss of stromal AR is predictive of prostate cancer progression. Many studies have characterized genome-wide binding of AR in prostate tumour cells but none have used primary mesenchyme or stroma.

We applied ChIPseq to identify genomic AR binding sites in primary human fetal prostate fibroblasts and patient derived cancer associated fibroblasts, as well as the WPMY1 cell line overexpressing AR. We identified AR binding sites that were specific to fetal prostate fibroblasts (7534), cancer fibroblasts (629), WPMY1-AR (2561) as well as those common among all (783). Primary fibroblasts had a distinct AR binding profile versus prostate cancer cell lines and tissue, and showed a localisation to gene promoter binding sites 1 kb upstream of the transcriptional start site, as well as non-classical AR binding sequence motifs.

We used RNAseq to define transcribed genes associated with AR binding sites and derived cistromes for embryonic and cancer fibroblasts as well as a cistrome common to both. These were compared to several in vivo ChIPseq and transcript expression datasets; which identified subsets of AR targets that were expressed in vivo and regulated by androgens. This analysis enabled us to deconvolute stromal AR targets active in stroma within tumour samples.

Taken together, our data suggest that the AR shows significantly different genomic binding site locations in primary prostate fibroblasts compared to that observed in tumour cells. Validation of our AR binding site data with transcript expression in vitro and in vivo suggests that the AR target genes we have identified in primary fibroblasts may contribute to clinically significant and biologically important AR-regulated changes in prostate tissue.

Contents

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1. Introduction

The androgen receptor (AR) is known to be essential for masculinisation of sex-accessory reproductive organs such as the prostate, seminal vesicle and penis, as well as playing a central role in prostate cancer.

Paracrine signalling mechanisms active in the developing prostate are often dysregulated in prostate tumours (Hayward et al., 1996; Vanpoucke et al., 2007; Schaeffer et al., 2008). Stromal-specific secreted molecules identified in embryonic rat and human prostate tissues have been shown to be re-expressed in cancer-associated fibroblasts (CAFs) and can modulate tumour growth (Orr et al, 2012, 2013). In addition, stromal markers have been shown to correlate with disease progression and may be predictors of aggressive prostate tumour subtypes (Rochette et al., 2017).

During development, AR expressed in mesenchymal cells is both necessary and sufficient for androgen mediated reproductive development; AR expressed in epithelial cells is not required (Cunha and Chung, 1981; Murashima et al., 2015). In prostate cancer, AR functions in both the tumour cells and via CAFs. CAFs are known to play an important role in prostate tumour growth (Olumi et al., 1999) and AR action within CAFs is able to contribute to hormone sensitive tumour growth (Ricke et al., 2012). In these experiments, AR was absent from the tumour cells, demonstrating that hormonal effects upon growth are likely to be mediated by AR in CAFs (Ricke et al., 2012). The function of AR has not been extensively examined in mesenchymal or stromal cells, despite their physiological importance, and our current knowledge of AR activity is derived from studies in prostate cell lines and patient tumour samples. Thus, the current AR cistrome reflects AR activity in cells that do not reflect androgen function in vivo during development or androgen action in fibroblasts present in prostate cancer stroma.

Recent advances in ChIP sequencing (ChIPseq) and gene profiling technologies have enabled the characterization of genome-wide AR binding and the AR transcriptional network (reviewed in (Lamb et al., 2014)). The majority of these studies have been conducted on prostate cancer cell lines and considerable effort has been made in defining AR targets in tumour epithelium (Andreu-Vieyra et al., 2011; Decker et al., 2012; Jin et al., 2013; Tao et al., 2014; Tewari et al., 2012; Zhu et al., 2012). This has revealed a transcriptional role for AR in tumour cells regulating cell proliferation, metabolism, survival and DNA repair (Massie et al., 2011; Polkinghorn et al., 2013). Prostate cancer cell lines have also been used to determine the changes in AR transcriptional networks in both a hormone-naïve- and castrate-resistant setting and numerous studies have revealed distinct binding profiles in the androgen-independent setting (Decker et al., 2012; Zhu et al., 2012; Massie et al., 2011; Wilson et al., 2016; Wang et al., 2009). More recently, the genomic binding profile of AR variants have also been explored and have been shown to compensate for full length AR in an endocrine therapy-like setting (Chan et al., 2015). These studies have provided valuable insights in to the molecular biology and function of the AR and have identified several co-activators and co-repressors that modulate AR transcriptional activity (Chng et al., 2012; Jehle et al., 2014; Jia et al., 2008; Jin et al., 2014; Pihlajamaa et al., 2014; Yu et al., 2010).

It has been shown that the genome-wide AR binding profiles identified in prostate cancer cell line models are not reflective of in vivo tissues and that AR target gene signatures identified in prostate cancer tissues are not represented in prostate cancer cell line models (Sharma et al., 2013). This has highlighted the importance of studying AR genomic binding in primary cells or tissues. Therefore, more recently, the genomic behavior of AR has been investigated in prostate cancer tissues and has revealed that AR genomic binding and interactions are reprogrammed with different tumour stages and have prognostic value (Chen et al., 2015; Nevedomskaya et al., 2016; Pomerantz et al., 2015; Stelloo et al., 2015). However, tissue samples typically contain multiple distinct cellular components and the majority of studies to date have neglected the role of the AR in CAFs present in the tumour microenvironment. It has been shown that human prostate myofibroblast cell lines have a different AR binding profile to prostate cancer epithelium and are not dependent on the classic AR pioneer factors such as FOXA1 (Leach et al., 2017). The role of AR in stroma is likely to be important, since stromal expression of AR is prognostic (Leach et al., 2015; Huber et al., 2015) and the genes regulated by AR in stroma may themselves be prognostic and control cell proliferation and differentiation. However, no study has addressed the genomic behavior of AR in primary prostate developmental or cancer-associated fibroblasts. In addition, the majority of studies to date investigating the AR transcriptional network have been limited by genome coverage on microarrays and thus may not adequately capture the full landscape of AR binding and transcriptional regulation in cells and tissues.

We have applied ChIPseq and RNA sequencing of the transcriptome (RNAseq) to provide the first AR cistrome in primary prostate embryonic and cancer-associated fibroblasts. In fibroblasts, the AR genomic distribution was distinct from prostate cancer cell lines, with a strong enrichment at proximal promoter regions of genes and with preference for non-classical AR binding motifs. Genes associated with these binding sites correlated well with the fibroblast transcriptomes and allowed us to define cancer-fibroblast specific and embryonic prostate fibroblast specific AR cistromes. The AR cistromes showed a high degree of overlap with

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