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Abstract

The hormone testosterone plays crucial roles during male development and puberty and throughout life, as an anabolic regulator of muscle and bone structure and function. The actions of testosterone are mediated, primarily, through the androgen receptor, a member of the nuclear receptor superfamily. The androgen receptor gene is located on the X-chromosome and receptor levels are tightly controlled both at the level of transcription of the gene and post-translationally at the protein level. Sp1 has emerged as the major driver of expression of the androgen receptor gene, while auto-regulation by androgens is associated with both positive and negative regulation in a possible cell-selective manner. Research into the networks of positive and negative regulators of the androgen receptor gene are vital in order to understand the temporal and spatial control of receptor levels and the consequences for healthy aging and disease. A clear understanding of the multiple transcription factors participating in regulation of the androgen receptor gene will likely aid in the development and application of hormone therapies to boast or curb receptor activity.

Introduction

The androgens testosterone and dihydrotestosterone act through the androgen receptor (AR). Testosterone is produced by the Leydig cells of the testes and the theca cells of the ovaries and the weaker androgen, dehydroepiandrosterone, is synthesised in the adrenal gland of both men and women (Smith et al 2013). Testosterone acts as both a hormone and a pro-hormone, being converted to its more powerful derivative dihydrotestosterone (DHT) by 5-α-reductase in peripheral tissues (skin, hair follicle, bone, prostate, liver) or by aromatase to the potent oestrogen, 17β-oestradiol (ovaries, bone, brain, adipose tissue, prostate) (reviewed in Smith et al 2013; Li and Rahman 2008; Ellem and Risbridger 2010). DHT has two fold greater affinity for the AR and a five-fold lower rate of dissociation than testosterone (Grino et al 1990).

The levels of circulating androgens decline with age in both sexes, which can impact on bone and muscle integrity, sexual drive and general wellbeing. In addition, the expression and activity of the AR has been shown to play an important role in the development or progression of a number of cancers (prostate, breast, endometrial, bladder, kidney) (Ma et al 2008; Chang et al 2014; Godoy et al 2016) and a range of other conditions including acne, male-pattern baldness and polycystic ovarian syndrome (Smith et al 2013). The AR is expressed ubiquitously in human and mouse tissues, although the amount of the mRNA varies, with the highest levels reported for reproductive tissues (testes, prostate, ovaries, uterus), liver, breast, adipose and muscle (see Ruizeveld de Winter et al 1991; Kumar and Thakur 2004; Bookout et al 2006); Human Protein Atlas [www.proteinatlas.org]). Despite considerable efforts to identify regulatory sequences in the promoter region and within the coding sequence of the AR gene a comprehensive understanding of the mechanisms controlling expression of the receptor mRNA and protein in different target tissues is lacking. In this review we will consider recent developments and discuss these in the context of older literature to better understand the regulatory networks controlling tissue-selective expression of androgen receptor levels. Our main focus will be on breast and bone and include comparisons with the prostate gland where appropriate.

The AR Gene: a TATA-less Promoter

The human AR (hAR) gene lies on the X chromosome at the locus Xq11 – Xq12 and consists of eight exons spanning approximately 180 kbp. The transcript of 4.3 kb includes a long 5' untranslated region (5'UTR) of 1.1 kb (Figure 1) and a 3'UTR of 6.8 kb (Tilley et al 1990; Faber et al 1991). The 5'UTR is uncommonly long as the median and average lengths of human 5'UTRs are 160 and 220 bp respectively (Chen et al 2011). Regulatory genes often encode long 5'UTRs and the other human steroid hormone receptor genes also contain untranslated regions that are similarly longer than average.

A clearly discernible correlation exists between the exons and receptor domains, implying that exon shuffling has played a key role in the evolution of the AR (Figure 1) (Choong et al 1998). The introns become progressively smaller in a downstream direction and vary enormously in size from 96.5 kbp to 0.7 kbp. The sizes of exons encoding the highly conserved DNA binding (DBD) and ligand binding (LBD) domains show little divergence with no variation between those of human and mouse or rat, the most commonly used animal models in receptor research. In contrast, the introns and 5'UTR sequences of these species all show marked differences in length (Figure 1).

While introns are well known to exhibit marked evolutionary variation between species, long 5'UTRs are unusual and contain increased numbers of regulatory elements with potential implications for gene regulation. Comparison of the lengths of 5'UTRs is insufficient to reveal the species-specific regulatory complexity of this region. For example, the 5'UTRs in rat and mouse, which diverged from humans about 75 Mya, are 11 and 10 % shorter than human whilst those in dog and cow, which diverged from humans about 100 Mya, are 1 and 5 % longer. In comparison to the human AR sequence, the percentage identity of the 5'UTRs in rat, mouse, dog and cow are 71, 72, 76 and 70 % respectively. However, these values belie the fact that the 5'UTRs contain multiple deletions and insertions. Alignments of the AR 5'UTR region in different

4

species reveals poor conservation and only primates possess some of the major human 5'UTR regulatory elements with no equivalent sequences in avian or piscine species (Hay et al 2015). The AR 5'UTR is predicted to be enriched in GC-rich low-complexity transposon-derived and as transposable elements gain mutations rapidly (Ward et al 2013) and act as generators of new binding motifs with fewer point mutations than most promoters (Bourque et al 2008) the AR gene 5'UTR could play an important role in species-specific expression.

Due to its location on the X chromosome, the AR gene exists in a hemizygous state in males so that mutations in exons or regulatory elements have a direct phenotypic manifestation. The absence of AR expression or activity results in androgen insensitivity syndrome (AIS), where XY individuals develop partially or completely as phenotypic females (Hughes 2008). Women, on the other hand, have two copies of the gene and studies utilising differences in the hypervarible number of CAG repeats in exon 1 (polyQ repeats in the amino-terminal domain, Figure 1) show that 90% of women are heterozygous (Edwards et al 1992). The expression of the AR in women is further complicated by the process of Lyonisation in which one X chromosome is inactivated in the early embryo by DNA hypermethylation and histone hypoacetylation. Although Lyonisation is considered to occur in a random manner, studies have shown that X chromosome inactivation is frequently skewed, deviation from a ratio of one for Xp active: Xm active, with the imbalance increasing as women age (Busque et al 1996). The initial apparent non-random inactivation is thought to occur by chance or due to, as yet uncharacterised, genetic differences between the two X chromosomes. Inactivation is faithfully maintained by cells, however, with the passage of time skewing can increase to the extent that the allelic ratios can exceed 10 fold in women over 60 years old. Several mechanisms have been proposed including low initial stem cell numbers, somatic selection of cells and stem cell depletion; all of which can occur differently in dissimilar tissue. The ultimate effect on AR expression and activity, if any, will depend upon the occurrence of differences in regulatory element sequences and the epigenetic status of the two alleles and possible polymorphisms in the poly Q repeat.

Role of transcription factor Sp1

Of all the transcription factors that regulate AR expression (summarised in Table 1) Sp1 is the main driver of expression of the AR gene which lacks TATA and CAAT boxes. By convention, gene architecture is denoted with the transcription start site (TSS) being assigned as +1 and values increasing in a downstream (3') direction mirroring transcription. Conversely, DNA sequence upstream (5') of the TSS has negative values (Figure 2). The core promoter has been mapped to between -74 and +87 bp (Takane and McPhaul 1996) and contains the principal GC box (-45 to -40 bp), which is a distinct 6 bp sequence, 5' GGGCGG 3', to which Sp1 can bind (Figure 2). The principal GC box is also referred to as Sp1-1 (Faber et al 1993) and lies downstream of an approximately 90 bp stretch of homopurine/homopyrimidine (-150 to -60 bp) that provides an abundant supply of bound Sp1 (Chen et al 1997). These elements and the region surrounding the TSS are highly conserved in the rat receptor gene (Figure 2). Although the core promoter GC box is the main contributor to establishing AR transcription, it is not absolutely essential, as other GC boxes in the 5'UTR also participate (Hay et al 2015). Two such sites that have been confirmed to be transcriptionally active are at +328 to +332 bp (Hay et al 2015), and +429 and +442 bp (Wang and Ferrari 2006). The latter regulatory element contains two Sp1 binding sites, 2 bp apart, that are both active, but appear to operate singly, most probably due to steric restrictions. Mechanistically, Sp1 can interact directly with the transcriptional machinery (TFIID) to initiate transcription. However, more recently Sp1 was found to recruit the chromatin modifying enzymes Brg1 and protein arginine methyltransferase, PRMT 5, to the AR promoter (Deng et al 2017). This epigenetic regulation was correlated with increased AR expression and prostate cancer cell growth.

There is no evidence for androgens directly affecting transcription of the Sp1 gene. However, the activated AR can inhibit Sp1 transactivation by interacting with the protein and impeding its binding to GC boxes. Sp1 directed expression of c-Met in the prostate cancer cell line WR22Rv1 and LNCaP xenografts can be downregulated by this process (Verras et al 2007).

Importance of regulatory sequences in the promoter and 5′UTR

A CpG island spanning the proximal promoter and 5′UTR (ca -500 to + 700 bp) was found to have variable levels of DNA methylation in normal tissues (breast, prostate and ovary) and prostate cancer cells and tissues associated with silencing of the AR gene (Kinoshita et al 2000; Jarrard et al 1998). The 5′UTR also has a high GC-content (58 % overall) with the first 500 to 600 bp downstream of the transcription start site (TSS) being almost 70 % GC. Early studies on the mouse AR gene identified two regions, called androgen receptor suppressor (ARS) sequences, which mediated downregulation of receptor expression (Grossmann et al 1994; Kumar et al 1994). Subsequent work identified a role for the transcription factor Pur-α (Purine-rich element-binding protein α), which binds to purine-rich repeats of (GGN) in a sequence specific manner, although there is no definitive consensus sequence (Table 1). Transcription of hAR is inhibited by pur-α binding to two sites in the 5′UTR that lie at +324 to +344 bp (Wang et al 2004) and +434 to +443 bp (Hay et al 2015) (Figure 2). Unlike most transcription factors, pur-α binds to single stranded DNA and elicits its effect on the hAR gene by acting in concert with the ubiquitous heterogeneous nuclear ribonucleoprotein-K (hnRNP-K) that binds to the opposite pyrimidine-rich strand leading to strand separation.

Of significance, is the recent observation that the inhibitory pur-α binding sites overlap with the 5′UTR Sp1-binding sites with the effect on transcription being determined by the relative amounts of these antagonistic transcription factors (Figure 2; Hay et al 2015). Many cancers display elevated levels of Sp1 (Sankpal et al 2011) and studies have shown that this leads to increased AR transcription in the prostate cancer cell line LNCaP (Yuan et al 2005).

Androgens have also been shown to downregulate transcription of the pur-α gene in prostate cancer (DePrimo et al 2002). Greatly reduced levels of pur-α are a feature of castrate resistant prostate cancer (CRPC) (Inoue et al 2008). This suppression of the pur-α gene leads to attenuated pur-α downregulation of the hAR gene and contributes to increased AR expression (Wang et al 2008).

7

This effect will be further compounded by subsequent enhanced access of Sp1 to the 5'UTR regulatory elements, further stimulating AR production.

Clinical Mutations in the 5'UTR

The regulation of AR expression involving the 5'UTR can occur at the level of transcription, as detailed above, and/or translation. Given the importance of this region several groups have investigated the occurrence of mutations or polymorphisms that could impact on receptor expression. An early study found two changes at +2 (G->T) and +214 (C->A) in two men with prostate cancer (Crocitto et al 1997). Although no functional analysis was described, it is interesting that the C214A change could disrupt a potential, but functionally unconfirmed, Sp1 binding site (see Wang and Ferrari 2006). However, a later study examining tumour and normal prostate tissue, together with five prostate cancer cell lines and xenograft samples, concluded that mutations were only present in cultured cell lines (DU145 and LAPC4) and a small number of xenografts (Waltering et al 2006). There was no evidence of recurrent mutations in the promoter, 5' and 3 UTRs in patient samples.

Studies based on a cohort of breast cancer patients identified the mutation at +116 (T->A) in two AR negative tumours (Peters et al 2012). However, functional analysis found no evidence that this genetic change was responsible for loss of receptor expression. In contrast, a change of C to T at position +580, that creates an 'ATG' codon and an upstream open reading frame (uORF), was identified in two unrelated individuals with CAIS (Hornig et al 2016). This mutation results in a functional initiation codon and expression of a short polypeptide that impaired translation from the normal downstream ATG and as a consequence, reduced AR protein and activity (Hornig et al 2016). Collectively, these studies suggest that mutations in the promoter and 5'UTR are rare. However, such genetic changes can, or have the potential to, impact on AR expression and function.

AR Expression in Breast Tissue

Although androgenic signalling is chiefly associated with the development and maintenance of primary and secondary male characteristics, especially during puberty when the testes are by far the major source of testosterone, the AR also plays important functions in females where the adrenal glands and ovaries form the main supply of androgens (Smith et al 2013). Indeed, androgens are required for normal development and fertility of both sexes and studies using female AR knockout mice show that the receptor is vital for the development of ovarian follicles and ovulation (Walters et al 2010). Visualisation of AR function in transgenic female mice shows robust activity in the mammary glands, ovaries, omentum tissue and uterus, and a strong correlation with AR expression in humans (Dart et al 2013). Importantly, these studies confirm that androgens can act directly through AR in women rather than via androgen aromatisation to oestrogens which are the main drivers of female sexual development. The importance of androgens in women increases dramatically with age, especially after menopause when circulating levels of testosterone and oestradiol decrease by 1.5 and 10 fold respectively (Rothman et al 2011) with androgens becoming the predominant sex hormone (Nicolas Diaz-Chico et al 2007).

The AR is expressed in glandular epithelial and stromal/fibroblast cells in the breast (Hickey et al 2012). Down-regulation of receptor mRNA has been reported in response to DHT in a number of breast cancer cell-lines (T-47D, MFM-2333, MDA-453) (Hall et al 1992; Hackenberg et al 1993; Yeap et al 1999). Interestingly, in the latter cell model the response was at the level of stability of the receptor mRNA (Yeap et al 1999). In our studies we also observed down-regulation of the AR mRNA in MCF-7 cells, but not in BT-474 cells (Figure 3). These two cell lines represent different subtypes of breast cancer: luminal A and B respectively (Holliday and Speirs 2011). The significance of this differential response is a topic of ongoing research.

It has been understood for over a quarter of a century that the AR directly autoregulates its own gene (Table 1), with down-regulation observed in rat prostate, seminal vesicles, epididymis, kidney and brain, after castration (see Shan et al 1990; Quarmby et al 1990). Interestingly, this was not the case in

the rat testes, suggesting tissue-selective regulation plays a role in the hormonal control of AR levels (Blok et al 1992) . Auto-repression of the AR mRNA has also been observed in a number of human cell-lines (Shan et al 1990; Quarmby et al 1990; Krongrad et al 1991; Wolf et al 1993) ; Figure 3) and recent studies have focused on prostate epithelial cells (Cai et al 2011; Hay et al 2014). There are several confirmed AREs within the hAR gene that have been shown to alter expression (Figure 2). The ARE closest to the main promoter and transcription start site lies +611 bp downstream within the 5'UTR (Hay et al 2014). The sequence of this regulatory element, 5'-AGAACCctcTGTTTT-3', differs from the consensus ARE, 5'- GGA/TACAnnnTGTTCT-3', and is unique to primates. Binding of the AR to this element inhibits transcription of the gene and a reporter gene driven by the promoter and 5'UTR of the human AR gene was similarly down regulated by DHT in prostate (Hay et al 2014) and MCF-7 cells (Hunter and McEwan unpublished observations) supporting the role of the identified receptor response element in negative auto-regulation.

A second inhibitory ARE is situated in intron 2 over 100k bp downstream of the main promoter (Cai et al 2011) (Figure 2). The mechanism of action has been elucidated, with the chromatin remodelling enzyme LSD1 being recruited by ARE-bound activated AR resulting in histone H3K4me1,2 demethylation and subsequent downregulation (Cai et al 2014). A role for the pioneer transcription factor FOXA1, in this negative regulation, has also been identified (Jones et al 2015).

Intriguingly androgens may also have an indirect effect on AR gene expression through inhibition of the GATA2 transcription factor gene (He et al 2014) (Table 1). Using ChIP-seq, GATA2 was found to bind to a regulatory element 5.5 kb upstream of the TSS of the AR gene and enhanced transcription, while treatment of prostate cells with an AR agonist led to repression of both GATA2 and AR mRNA (He et al 2014). In addition, a GATA2 binding site downstream of the TSS at +762 has also been correlated with positive regulation of the AR gene (Wu et al 2014) (Figure 2).

Taken together, the findings from the above studies support multiple pathways for auto-repression of the AR gene, in prostate and breast cells. These mechanisms involve direct binding of the AR to DNA elements within the gene (5'UTR and Intron 2) and interference with transcription factor activity (Sp1) or expression (GATA2). It will be important to now determine the role of the identified negative AREs in different cell types and tissues and the mechanisms underlying tissue regulation of receptor levels.

AR Expression in Bone

The AR is expressed in both bone forming osteoblasts and bone remodelling osteoclasts (Vanderschueren et al 2004; Manolagas et al 2013). The actions of androgens in bone are complex involving both direct, AR-mediated, and indirect, oestrogen receptor α (ERα) action. DHT has been shown to increase expression of the receptor mRNA (Wiren et al 1997). The response was mapped to a 3 kb stretch of DNA encompassing the promoter and part of the 5'UTR (-2330 to +573) (Wiren et al 1997). In the same osteoblast cell-line, SaOS-2, derived from an 11 year old girl, we also observe an increase in receptor mRNA in response to DHT treatment (Figure 3). The use of DHT in both studies would support this regulation being a direct action of the AR. However, binding of the AR to sequences within the putative regulatory region has not be demonstrated. The outstanding question concerns the mechanism(s) involved in this upregulation and how this compares with the reported down–regulation observed in other cells, notably prostate and breast.

Importantly, not all AREs in the hAR gene are inhibitory and early studies have suggested that the AR can enhance expression of the receptor message through a composite binding site spanning exons 4 and 5 (Reviewed in Burnstein 2005). The AREs furthest from the promoter are somewhat unusual in that the half sites lie in two exons (4 and 5) separated by a 5,790 bp intron (Dai and Burnstein 1996) and together with Myc, upregulate AR transcription (Grad et al 1999) (Figure 2). Two other non-consensus AREs are also present and these bind AR much more weakly in EMSA studies, but are required for maximal androgen regulation. Myc has been shown to directly upregulate hAR expression by

binding to a consensus E box in exon 4 at +167,636 bp (Grad et al 1999) (Figure 2). Bound Myc interacts with its binding partner Max via a basic helix-loop-helix leucine zipper domain and both proteins are required for AR stimulation of the AREs in exons 4 and 5 described above. Interestingly, the expression of Myc is repressed by androgens in the prostate cancer cell line LNCaP (Bolton et al 2007) and upregulated by oestrogen (17β-estradiol) in breast cancer cells (Cicatiello et al 2004).

AR Expression and Ageing

Rats and mice maintained on calorie restricted diets have demonstrably longer life spans (Guarente 2013). It is therefore interesting that a calorie restricted diet can rescue the down-regulation of the AR mRNA in the ageing rat liver (Song et al 1991). The regulatory sequences mediating the age-dependent down-regulation of the rat AR were mapped to the promoter of the receptor gene, and termed 'age-dependent factor' (ADF: -301 to -330 bp) and 'associated factor' (AF: -340 to -372) (Supakar et al 1993). Loss of binding at these elements was associated with aging in the rat liver. Further work from the Chatterjee laboratory, identified the composition of the transcription factor complexes binding to the ADF element, which included the proto-oncoproteins B-Myb and/or c-Myb associated with either PARP-1 and hnRNPK (activation) or p53 and a co-repressor complex (repression) (Shi et al 2008) (Table 1). Furthermore, the transcriptional repression complex was correlated with down regulation of the AR gene in response to oxidative stress. However, the regulatory elements identified in the rat AR promoter do not appear to be conserved in the human gene.

Negative regulation of AR expression by NFκB was also correlated with the 'age-dependent desensitisation' of androgen action in the rat liver (Supakar et al 1995). In this case the binding site was mapped upstream of the gene to sequences -555 to -565 bp, that binds heterodimers of p65 and p50, as well as p50 homodimers (Figure 2). The ratio of p50 homodimers to heterodimers increases 10 fold with age leading to recruitment of histone deacetylase leading to downregulation of the AR gene (Supakar et al 1995).

12

Using protein extracts from rat Sertoli cells an additional binding site was described at -482 to -491 bp (Delfino et al 2003). In contrast to the liver, binding of NFκB was associated with stimulation of AR expression in Sertoli cells (Delfino et al 2003; Zhang et al 2004). NFκB has also been shown to strongly upregulate hAR promoter activity in LNCaP through the use of reporter gene assays (Zhang et al 2009). The consensus binding sequence for NFκB incorporates a high degree of variability resulting in seven potential sites within the hAR promoter between approximately -1600 and +1 (Figure 2): and an eighth site greater than 3.2 kb upstream. ChIP analysis using antibody against the p50 subunit confirmed binding of NFκB to the promoter. However, as the average lengths of DNA fragments used in the ChIP assays was 1 kb, it is impossible to determine which of the potential sites are functional.

However, a B-Myb binding site (+176 to +181) and associated NFκB element that lies three turns of the DNA helix (+145 to +154) have been delineated in the 5'UTR of the human AR gene (Ko et al 2008) (Figure 2). These sequences lie on the same face of the DNA double helix and form a composite regulatory element required for the negative regulation of AR mRNA by the proinflammatory cytokine, TNFα, in prostate calls (Figure 2). B-Myb is expressed in virtually all proliferating cells where it is involved in cell cycle progression and is implicated in carcinogenesis and cellular senescence. As a transcription factor it can either stimulate or repress target genes, however, in the context of hAR transcription, the main function appears to be to stabilise binding of NFκB to its adjacent site and to participate in forming a multi-protein complex that includes histone deacetylase 1 (Ko et al 2008). As a consequence, chromatin remodelling leads to downregulation of the hAR gene.

It is also noteworthy that the binding of p53 to sequences upstream of the TSS, -488 to-469 bp, has been described in several human cell-lines, including prostate (PrECs, LNCaP), bone (SaOS-2) and human colorectal cells (HCT116) (Alimirah et al 2007) (Figure 2). The binding of p53 was associated with repression of AR mRNA transcription. p53 is activated in response to various

13

forms of cell stress e.g. DNA damage, oxidative stress and hypoxia, and can either stimulate or repress target genes which are predominantly involved in cell cycle progression, apoptosis and DNA repair. Impaired functioning of the p53 pathway is a major factor in over half of all cancers, and mutations in the p53 gene (*TP53*) resulting in loss of function in prostate cancer are associated with tumour progression and poor prognosis (Burchardt et al 2001; Heidenberg et al 1995). These findings are consistent with loss of p53 suppression of hAR expression leading to high levels of AR. It will be important to determine if the action of p53 and B-Myb (and NFκB), cooperate in a manner analogous to their function on the rat promoter to regulate the human AR gene in an age-dependent manner.

Androgens and the Process of Epithelial Mesenchymal Transition

Epithelial mesenchymal transition (EMT) acts under different physiological conditions as a source of mesenchymal cells during development, tissue repair and fibrosis and cancer cell invasiveness and metastasis (Kalluri and Weinberg 2009). In the context of AR expression and signalling, EMT has primarily been studied in terms of cancer progression and/or development of CRPC. Interestingly, two bHLH transcription factors, Twist 1 (Soini et al 2011; Shiota et al 2015) and ZEB1 (Martin S.K. et al 2013), associated with the EMT response have been shown to regulate expression of the AR gene (Table 1).

Expression of Twist1 and the AR are increased by oxidative stress, but the change in the receptor (mRNA/protein) is lost after treatment with siRNAs that target Twist 1 (Shiota et al 2010). Twist 1 was found to bind to E-boxes, 5'-CANNTG-3', in the proximal promoter (-442 to +51 bp) and upstream regions (-539 to -974 bp and -1187 to -1589 bp) of the human AR gene. Functional and mutational mapping highlighted the importance of the E-boxes in the proximal promoter (Figure 2: -168 to -150 and -26 to -8 bp), for Twist 1 stimulation of AR mRNA expression (Shiota et al 2010). In a further twist, expression of Twist 1 can be indirectly repressed by androgens in prostate cancer cells by NKX3-1. Androgens strongly upregulate NKX3-1 production in prostate epithelial cells and in tumour cell lines (DePrimo et al 2002; Prescott et al 1998), where NKX3-1

14

binds to the promoter of the Twist 1 gene and robustly represses transcription (Eide et al 2013). However, it has also been reported that androgens upregulate Twist 1 expression, observed in prostate cancer microarrays (Ngan et al 2009), and in LNCaP cells after 72 hours of treatment with the powerful androgen agonist R1881 (Eide et al 2013). These apparently contradictory findings appear to be due to the differing timescales used, with an initial downregulation of Twist 1 expression by increased NKX3-1 eventually being overcome by androgen upregulation.

Zeb1 (Table 1) is a transcription factor that can act as a repressor or activator of target gene expression, and ChIP analysis has confirmed that it binds directly to an E box in the hAR 5'-UTR ,at position +1001 to +1006 bp (Figure 2) where it induces increased AR transcription in triple negative breast cancer cells and human foreskin cells (Graham et al 2010; Qiao et al 2012). Zeb1 is intimately associated with cancer by promoting EMT and metastasis (Zhang et al 2015) and overexpression correlates with a shorter time of progression to advanced prostate cancer after radiotherapy (Marin-Aguilera et al 2014). Invasive ductal breast carcinomas have higher stromal expression of Zeb1 than do *in situ* tumours (Soini et al 2011). In a feed forward mechanism, expression of the gene for Zeb1 is upregulated by androgens through two AREs (Anose and Sanders 2011) and use of the androgen antagonist bicalutamide in triple negative breast cancer reduces levels of Zeb1 (Graham et al 2010). Oestradiol upregulation of Zeb1 expression has also been reported in both human foreskin cells (Qiao et al 2012) and in the endometrial stroma and myometrium of the mouse and human uterus (Spoelstra et al 2006).

The above studies emphasise the functional links between AR levels and activity and EMT during tumour progression and metastasis. It will be important to investigate further the role of AR expression in other forms of EMT, namely wound repair and development and embryo implantation.

Conclusions and Future Perspectives

In this review we have focused on the direct transcriptional regulation of the human AR gene in different tissues. However, it is worth noting that RNA interference mechanisms, resulting in DNA methylation of the promoter (Cho et al 2014) or targeting positive transcriptional regulators of the receptor gene (Nadiminty et al 2012), have also been found to down-regulate AR expression. Similarly, RNA binding proteins, PCBP1 (polyC-binding protein) (Cloke et al 2010) and EBP-1 (Erb3-binding protein) (Zhou et al 2010) have been shown to target the 3'UTR of the AR mRNA regulating RNA stability in endometrial stromal cells and prostate cancer respectively. The diversity of mechanism that have been identified clearly emphasise the importance of tight regulation of AR mRNA and protein in different target tissues.

The two main themes that emerge from this research are (1) auto-regulation of the AR gene in different cell types in response to androgens and (2) the interplay between positive and negative regulatory elements, and transcription factors, in the control of receptor mRNA. A fuller appreciation of androgen action, throughout the life course, will depend upon a detailed understanding of these mechanisms controlling the temporal and spatial expression of the AR. It will therefore be paramount to determine the influence of the different signalling and transcription factor networks, which operate during embryo development and in adult tissues, to control the levels of receptor and crucially androgen responsiveness. For example, do the mechanisms observed in the ageing rodent liver, involving the transcription factors NFκB, c-Myb and p53, operate in human non-reproductive tissues such as bone and muscle?

The situation is further complicated in that the most commonly used rodent models may not fully capture the regulation of the human AR gene. The development of more physiologically representative culture approaches offers an exciting alternative approach (Ellem et al 2014). Notwithstanding the technical difficulties inherent in the use of spheroid cultures or tissue explants, such approaches over the ability to consider 3D cell interactions and tissue architecture. More tractable, co-cultures will allow for epithelial-stromal

16

interactions and functional contacts with the extra cellular matrix to be investigated in terms of regulation of AR expression and function.

Understanding the cell and tissue-selective regulation of the AR levels is fundamentally important to a fuller comprehension of receptor signalling throughout the life course. Such an appreciation is also likely to have a significant impact on the treatment of hormone-dependent diseases and the age-dependent decline in circulating testosterone in both men and women.

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Figure Legends

Figure 1. Androgen Receptor Gene and Protein B. Androgen receptor gene structure for the indicated species. Bent arrow indicates the transcriptional start site (TSS +1). Note, the AR mRNA represents only 2% of the genomic sequence necessitating the use of 50-fold different scales for exons and introns in order to accurately display the relative sizes of the different elements of the gene. Panel below, shows a schematic representation of the human AR protein with domains: LBD, ligand binding domain; DBD, DNA binding domain; NTD, amino-terminal domain; NLS, nuclear localisation signal; AF1, activation domain 1 and subdomains TAU1 and TAU5.

Figure 2. Regulation of the Androgen Receptor Gene. Schematic to-scale description of key regulatory elements and transcription factor binding to the androgen receptor gene promoter and enhancers. The DNA is represented as a straight line with ovals for transcription factor binding: TSS, transcription start site and 'ATG' represents the first codon of the receptor protein respectively. For clarity factors positively (top line) and negatively (bottom line) regulating transcription of the gene are shown separately: upregulation ⬆; downregulation ⬇. Key to transcription factors is shown below. The boxed shaded regions indicate sequence conservation between the human and rat AR gene promoter and 5'UTR. A more comprehensive description of transcription factors regulated the AR gene is provided in Table 1.

Figure 3. Regulation of the Androgen Receptor Gene in Different Human Cell Types. Semi-quantitative qPCR for the AR gene and the house keeping gene, GAPDH. The charts show the quantitated levels of receptor mRNA relative to a 0 hormone control for at least three independent experiments.

Table 1 Transcription Factors Regulating Expression of the AR gene

Factor	Comments	Effect of androgens	Effect of oestrogens	References
AR	Four active AREs can up- or downregulate.	Auto-downregulation/ upregulation.	Indirect upregulation through increased Zeb1 expression.	(Cai et al 2011,Hay et al 2014,Dai and Burnstein 1996,Grad et al 1999)
B-myb	Forms complex with NFκB that recruits histone deacetylase 1 resulting in downregulation.		Upregulation in BCa cells.	(Cicatiello et al 2004,Ko et al 2008)
c-myc	Upregulates through binding to Max.	Downregulation in LNCaP.	Upregulation in BCa cells.	(Grad et al 1999,Bolton et al 2007,Cicatiello et al 2004,Ko et al 2008)
CRE	Upregulation in response to cAMP but the transcription factors have not been determined.			(Mizokami et al 1994)
E2F1/Rb	Acting in concert, they downregulate.		Both E2F1 and Rb are either up- or downregulated by oestrogens depending upon the BCa cell line.	(Davis et al 2006,Stender et al 2007)
Foxo3a	Upregulation.			(Yang et al 2005)
GATA2	Upregulation.	Downregulation in prostate cells		(He et al 2014)(Wu et al 2014)
LEF1	Upregulation.			(Yang et al 2006)
NFκB	Downregulation through the binding site in the 5'UTR, however, upregulation is seen at an undetermined site between the TSS and -1,600.	Downregulation of both p50 and p65 in PCa cells.		(DePrimo et al 2002,Zhang et al 2009,Ko et al 2008)
p53	Downregulation.			(Alimirah et al 2007)
Pur α	Downregulation through two sites in 5'UTR that overlap with stimulatory Sp1 sites.	Downregulation in PCa cells		(Hay et al 2015,Wang et al 2004,DePrimo et al 2002)
Smad2, 3	Upregulation.	Downregulation of Smad3.		(Kang et al 2009)
Sp1	Sp1 is the main driver of AR expression and binds to the core promoter and additional sites in the 5'UTR.	Nontranscriptional inhibition through binding of activated AR.	Nontranscriptional inhibition through binding of activated ERα and ERβ.	(Faber et al 1993,Hay et al 2015,Wang and Ferrari 2006,Verras et al 2007,Bartella et al 2012)
SREBP1	Upregulation.	Indirect upregulation through increased expression of β2-microglobulin.		(Huang et al 2010,Yoon and Wong 2006)
Twist1	Upregulation.	Indirect downregulation through increased expression of NKX3-1.		(DePrimo et al 2002,Shiota et al 2010,Eide et al 2013)
Zeb1	Upregulation.	Upregulation.	Upregulation.	(Graham et al 2010,Qiao et al 2012,Anose and Sanders 2011)

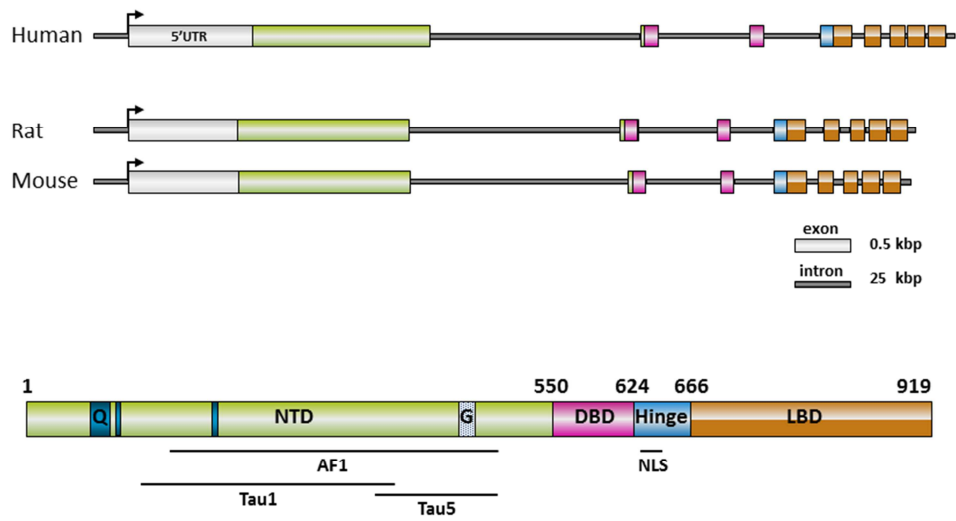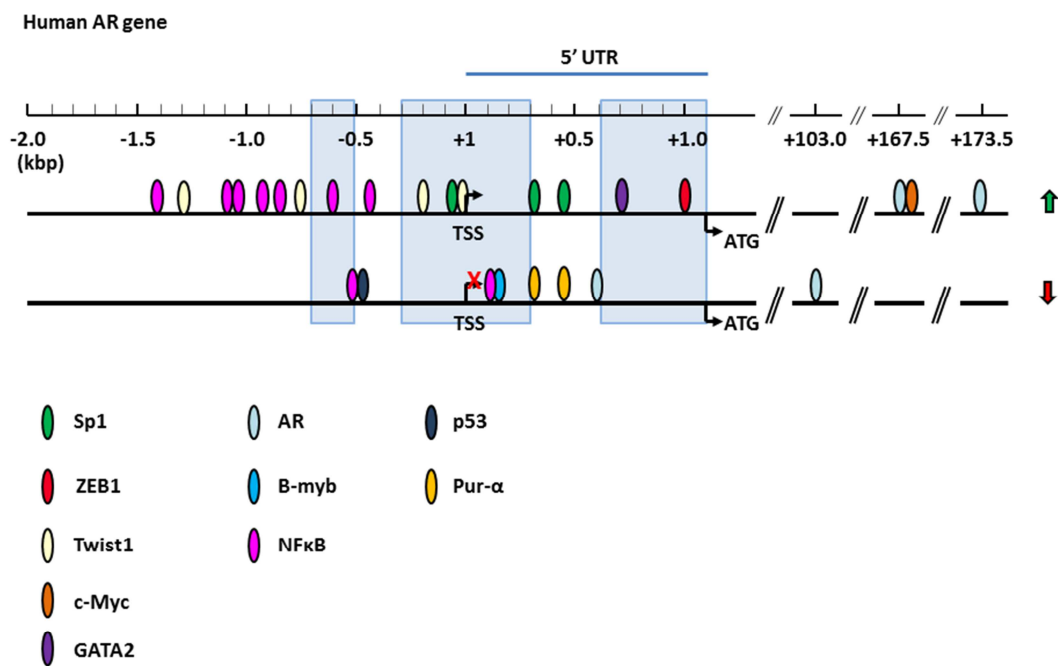BCa, breast cancer; PCa, prostate cancer.
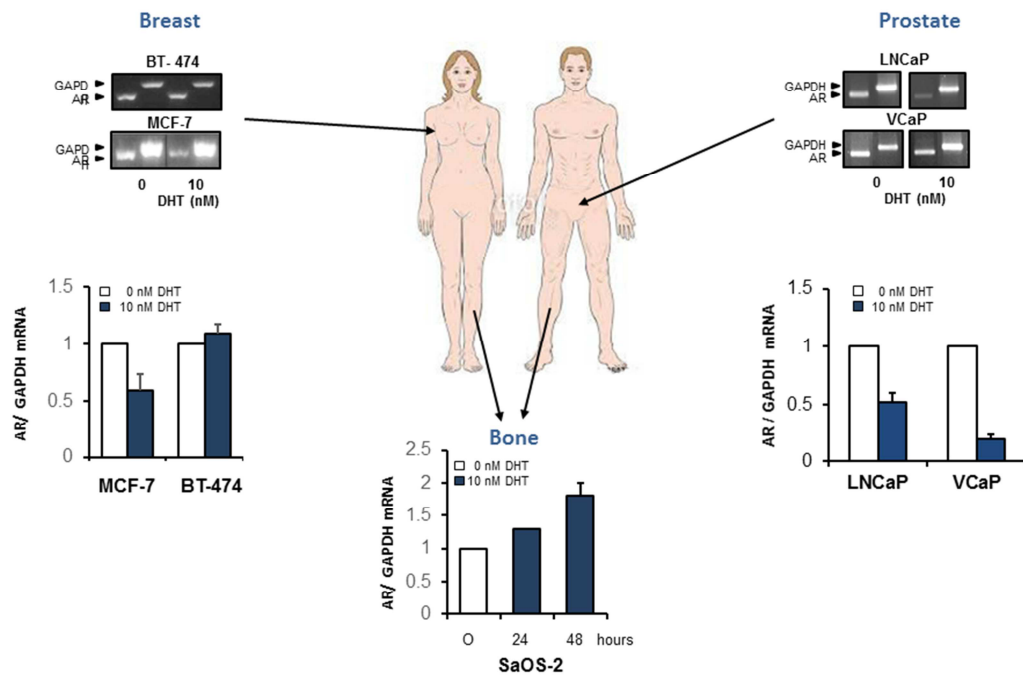
Figure 1

Human AR gene

Figure 2

Figure 3

Highlights

- The androgen receptor gene is X-linked and lacks a TATA-sequence in the promoter
- Sp1 is a major driver for receptor mRNA expression
- The 5'UTR contains several transcription factor binding sites mediating both positive and negative regulation
- Androgens downs regulate receptor mRNA in breast and prostate cells but upregulate expression in bone.