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Histone H2A.Z prepares the prostate specific antigen (PSA) gene for androgen receptor-mediated transcription and is upregulated in a model of prostate cancer progression

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ABSTRACT

The histone variant H2A.Z is present at many eukaryotic gene regulatory regions and can affect rates of transcription. Here we show that total H2A.Z and an acetylated form of H2A.Z is mainly present at the prostate specific antigen (PSA) enhancer and promoter in prostate cancer cell lines where the gene is expressed, but the levels decrease during rapid cycles of transcription. Treatment of prostate cancer cells with androgen results in increased H2A.Z levels due to upregulation of the H2A.Z-1, but not the H2A.Z-2 gene. This upregulation is likely the result of increased MYC transcription factor binding that occurs in response to androgen at the H2A.Z-1 promoter. Furthermore, we show that in a LNCaP xenograft model of prostate cancer progression, there is a significant increase of H2A.Z protein in castration resistant LNCaP tumors resulting from increased expression of the H2A.Z-1 gene. While a similar trend was observed in samples from prostate cancer patients, the results were not statistically significant. Nevertheless, there may be a subset of prostate cancers where elevated expression of H2A.Z-1 is indicative of prostate cancer progression to androgen independence.

1. Introduction

The structure of chromatin in general has a repressive effect on transcription because it limits access to the underlying DNA sequence. In order to overcome this barrier, the cell employs three interconnected processes to modify the chromatin template which are: (1) post-translational modification of histones, (2) chromatin remodeling, and (3) incorporation of histone variants [1]. The synergistic action of these three processes in transcriptional modulation is exemplified by incorporation of the histone variant H2A.Z into chromatin which occurs at many human promoters genome-wide via the action of complexes that contain at least one ATP-dependent chromatin remodeling subunit (SRCAP, p400, TIP48/49) as well as histone acetyltransferase enzymes such as Tip60, among others [1]. H2A.Z is found associated with gene regulatory regions including promoters and enhancers [2,3] and while the exact mechanistic details of how it participates in facilitating transcription are not entirely clear, recent evidence indicates that H2A.Z may help in directly recruiting RNA Pol II at promoters [4]. Several studies show that H2A.Z helps to poise promoter chromatin for transcriptional activation and suggest that it may not be required after the initiation stages of transcription [5,6]. H2A.Z has been shown

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to play a role in other cellular processes including maintaining the boundaries between heterochromatin and euchromatin [7], mediating proper chromosome segregation [8], and in estrogen receptor signaling [9]. Furthermore, several large-scale gene expression analyses in different cancers have identified H2A.Z as being associated with cancer progression [10–13]. Our group and others have shown that two H2A.Z isoforms that differ by three amino acids and are encoded by separate genes exist in vertebrates (H2A.Z-1 and H2A.Z-2) and exhibit a similar but non-identical distribution within chromatin [14,15]. In addition, the N-terminal tail region of both isoforms can be acetylated at multiple lysine residues [14]. The function of H2A.Z acetylation is unclear but it has been shown to be essential for viability in *Tetrahymena* [16] and to be present at the 5′ region of actively transcribing genes in chicken [3].

The androgen receptor (AR), crucial for male development and in the biology of prostate cancer [17], is a member of the steroid hormone receptor family of nuclear transcription factors. AR is normally held in an inactive state in the cytoplasm where it is complexed with heat shock protein chaperones, but upon binding to androgen the AR translocates to the nucleus where it binds as a homodimer to androgen response elements (ARE), usually located within enhancer sequences of androgen responsive genes [18]. Hormone-bound AR can recruit coactivator proteins, many of which have chromatin remodeling or histone modification abilities, as well as components of the basic transcriptional machinery that leads to increased gene expression. Most prostate cancers are dependent on a functional AR signaling pathway; this is exemplified by the fact that anti-androgen therapies are combined with chemotherapy and radiation therapy to treat disease that has extensive local spread or has developed distant metastases [19–21]. However, these cancers inevitably progress to an androgen-independent (AI) or castration-resistant (CR) stage where they no longer require circulating androgen for growth and hence no longer respond to anti-androgen therapy [22,23]. The AR is expressed in the vast majority of both androgen-dependent and -independent cancers and AR signaling pathways play an important role in both since knockdown of AR protein levels reduces the growth of both tumor types in model systems [24,25]. Notably, it has been shown that in androgen-independent prostate cancer the androgen receptor regulates a distinct transcriptional and independent program when compared to that of androgen-dependent prostate cancer [26].

Two known coactivators of AR include the Tip60 enzyme which has been shown to acetylate AR itself [27] as well as lysine residues within histones [28], and SRCAP (SNF2-related CBP activator protein). SRCAP is one of the human orthologs of the *S. cerevisiae* Swr1 protein and both have been shown to catalyze the ATP-dependent incorporation of H2A.Z/H2B dimers into chromatin [29–31]. Furthermore, the human Tip60 complex is generally considered to be an ortholog of the yeast NuA4 histone acetyltransferase complex [1]. The expression of the prostate specific antigen (PSA) gene is dependent on androgen and its protein product has long been used as a serum biomarker for the monitoring and detection of prostate cancer [32]. We hypothesized that H2A.Z could be involved in AR-mediated transcription of the PSA gene in prostate cancer cells. Furthermore, given the frequency of epigenetic alterations in prostate cancer [33,34] and the association of H2A.Z with cancer progression [10], we also hypothesized that H2A.Z could be involved in the development of castration resistant prostate cancer. To this end, we have employed an *in vivo* mouse LNCaP tumor model of prostate cancer progression as well as a neo-adjuvant hormone therapy (NHT) human prostate cancer tissue microarray and examined H2A.Z expression levels.

Here we show that H2A.Z and N-terminally acetylated H2A.Z are mainly present at the PSA enhancer and proximal promoter when the gene is inactive. Furthermore, we provide evidence that the H2A.Z-1 isoform gene is specifically upregulated in response to androgen treatment, while the H2A.Z-2 isoform gene is not. The upregulation of the H2A.Z-1 gene is due at least in part to increased binding of MYC transcription factor at the H2A.Z-1 promoter that occurs in the presence of androgen. Finally, our results indicate that total H2A.Z protein levels are increased in castration resistant LNCaP xenograft tumors suggesting that H2A.Z may play a role in the androgen-independent phenotype of these tumors.

2. Materials and methods

2.1. Cell culture

PC3, LNCaP and C4-2 cells were from Dr. Rennie's lab at the Vancouver Prostate Centre. LNCaP and C4-2 cells were maintained in RPMI 1640 medium (Invitrogen) supplemented with 10% FBS (PAA laboratories), 1% penicillin/streptomycin (Invitrogen) while PC3 cells were maintained in DMEM (Invitrogen) with 10% FBS and 1% penicillin/streptomycin. All cells were transferred to phenol red-free medium supplemented with 5% charcoal-stripped FBS (PAA laboratories) for 3 days prior to induction with 10 nM of synthetic androgen R1881 or ethanol control.

2.2. Histone acid extraction

Nuclei were isolated from PC3, LNCaP and C4-2 cells or from LNCaP tumors by washing three times in Buffer A (250 mM sucrose, 60 mM KCl, 15 mM NaCl, 10 mM MES pH 6.5, 5 mM MgCl2, 1 mM CaCl2, 0.5% Triton X-100) and twice in Buffer B (50 mM NaCl, 10 mM Pipes pH 6.8, 5 mM MgCl2, 1 mM CaCl2) supplemented with 1:100 protease inhibitor cocktail (Roche) with all centrifugations performed at 3000*g*. The histones were then acid extracted with 0.4 N HCl and the supernatant was precipitated with acetone overnight at −20 °C. The histones were dried under vacuum.

2.3. Western blotting

Acid extracted histones were resolved on 15% acrylamide SDS gels normalized with respect to total H4 and transferred to PVDF membrane for 3 h at 100 V. The membranes were probed with anti-H2A.Z (1:5000, Abcam),