Antisense now makes sense: dual modulation of androgen-dependent transcription by *CTBP1-AS*

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Recent advances in genomic approaches for exploring the transcriptome have led to the identification of thousands of novel non-coding RNA (ncRNA) transcripts (Djebali *et al*, 2012). While some of these ncRNAs have been implicated in the development and progression of cancer and other human diseases, their underlying mechanisms of action remain largely unknown (Esteller, 2011). Takayama *et al* (2013) now report the identification of *CTBP1-AS*, a novel androgen-regulated long ncRNA that promotes prostate cancer growth through sense–antisense repression of the transcriptional co-regulator CTBP1, as well as through the global epigenetic regulation of tumour suppressor genes.

The androgen receptor (AR), a member of the nuclear hormone receptor superfamily of transcription factors, has long been established as a critical driver of prostate cancer and remains functionally important even in advanced stages of the disease (Chng and Cheung, 2012). Previous work from the Inoue lab showed that AR regulates the expression of transcripts located in the intergenic and antisense regions of genes in prostate cancer (Takayama *et al*, 2011). In the present study, the group identified an antisense transcript, *CTBP1-AS* at the *CTBP1* locus whose androgen-regulated expression is

inversely correlated with the expression of CTBP1. Takayama *et al* (2013) found that when they depleted *CTBP1-AS* transcript levels *in vitro* using siRNA they could abolish the androgen-dependent reduction in CTBP1 protein levels, indicating *CTBP1-AS* represses the expression of the CTBP1 sense transcript. Notably, the sense–antisense regulation of CTBP1 by *CTBP1-AS* appears to be clinically relevant in prostate cancer, given the upregulation of *CTBP1-AS* and downregulation of CTBP1 were detected in primary tumours and metastatic prostate cancer samples, but not in benign prostate tissues. To determine the functional significance of *CTBP1-AS*-mediated repression of CTBP1 in prostate cancer, the authors examined the role of CTBP1 in androgen-regulated transcription. They showed that CTBP1 acts as a co-repressor of AR by opposing the androgen-mediated demethylation of the repressive histone mark H3K9 and its recruitment to AR-binding sites. More importantly, silencing of *CTBP1* resulted in increased proliferation of AR-positive cell lines, indicating that this factor is important in prostate cancer progression. Conversely, the silencing of *CTBP1-AS* suppressed androgen-dependent cell proliferation *in vitro* and brought about a remarkable reduction in tumour growth in a xenograft mouse model.

Figure 1 Proposed model of *CTBP1-AS* regulation of androgen-dependent gene expression. Ligand-bound androgen receptor (AR) induces the expression of *CTBP1-AS*, which binds and recruits PSF and HDAC/Sin3A complex to the promoter of CTBP1, resulting in histone deacetylation and inhibition of CTBP1 expression. The decrease in CTBP1 level brings about H3K9 demethylation at AR binding sites and the de-repression of androgen-induced genes. *CTBP1-AS* also cooperates with PSF and HDAC/Sin3A complex to mediate the global repression of androgen-regulated genes, such as *p53* and *SMAD3*.

While the effects of *CTBP1-AS* knockdown might have been in part due to a relief of *CTBP1-AS*-mediated repression on *CTBP1*, Takayama *et al* (2013) provided evidence which suggests that *CTBP1-AS* may also function as a *trans*-acting regulator of androgen-regulated genes by recruiting the histone deacetylase (HDAC)/Sin3A repressor complex via the RNA-binding PTB-associated splicing factor (PSF). Finally, to assess the extent to which *CTBP1-AS* and PSF function to repress androgen-dependent transcription in prostate cancer cells, the authors carried out simultaneous knockdown of these two factors and found that over 35% of androgen-repressed genes were de-repressed. Taken together, these findings implicate *CTBP1-AS*/PSF complex-mediated histone deacetylation as a significant mechanism in androgen-dependent transcriptional repression (Figure 1). However, whether the regulation of androgen-dependent transcription by the *CTBP1-AS*/PSF complex is direct or indirect is unclear. Future experiments using chromatin isolation by RNA purification (ChIRP) followed by deep sequencing will allow one to map the genome-wide occupancy of

CTBP1-AS and identify genes that are direct targets of *CTBP1-AS*/PSF transcriptional regulation.

Several ncRNAs including *PCGEM1*, *PCA3*, *PRNCR1* and *PCAT1* have been previously reported in prostate cancer (Petrovics *et al*, 2004; Chung *et al*, 2011; Prensner *et al*, 2011; Ferreira *et al*, 2012). However, we know little about the functional and mechanistic roles of these ncRNAs. To our knowledge, this is the first study that has provided a precise mode of action of an ncRNA in prostate cancer. In addition, while most antisense transcripts have been proposed to act in *cis* and regulate the expression of neighbouring genes, *CTBP1-AS* appears to be an atypical antisense ncRNA in that it can serve as both *cis*- and *trans*-regulators of gene expression. Most importantly, the work by Takayama *et al* (2013) highlights the significance of a previously overlooked portion of the transcriptome in prostate cancer biology and offers new potential therapeutic targets for managing the disease.

Conflict of interest

The authors declare that they have no conflict of interest.

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