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Integrated analysis of the prostate cancer small-nucleolar transcriptome reveals *SNORA55* as a driver of prostate cancer progression

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ABSTRACT

Metastasis is the primary cause of death in prostate cancer (PCa) patients. Small nucleolar RNAs (snoRNAs) have long been considered "housekeeping" genes with no relevance for cancer biology. Emerging evidence has challenged this assumption, suggesting that snoRNA expression is frequently modulated during cancer progression. Despite this, no study has systematically addressed the prognostic and functional significance of snoRNAs in PCa.

We performed RNA Sequencing on paired metastatic/non-metastatic PCa xenografts derived from clinical specimens. The clinical significance of differentially expressed snoRNAs was further investigated in two independent primary PCa cohorts (131 and 43 patients, respectively). The snoRNA demonstrating the strongest association with clinical outcome was quantified in PCa patient-derived serum samples and its functional relevance was investigated in PCa cells via gene expression profiling, pathway analysis and gene silencing.

Our comparison revealed 21 differentially expressed snoRNAs in the metastatic *vs.* non-metastatic xenografts. Of those, 12 were represented in clinical databases and were further

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analyzed. *SNORA55* emerged as a predictor of shorter relapse-free survival (results confirmed in two independent databases). *SNORA55* was reproducibly detectable in serum samples from PCa patients. *SNORA55* silencing in PCa cell lines significantly inhibited cell proliferation and migration. Pathway analysis revealed that *SNORA55* expression is significantly associated with growth factor signaling and pro-inflammatory cytokine expression in PCa.

Our results demonstrate that *SNORA55* up-regulation predicts PCa progression and that silencing this non-coding gene affects PCa cell proliferation and metastatic potential, thus positioning it as both a novel biomarker and therapeutic target.

1. Introduction

Prostate cancer (PCa) is a heterogeneous disease that constitutes the most frequently diagnosed neoplasm in North American males (Siegel et al., 2012). Patients with localized PCa can be treated by radical prostatectomy or radiotherapy, often resulting in complete disease remission. Conversely, the vast majority of PCa-related deaths result from progression of localized disease to metastatic castration-resistant PCa (mCRPC) (Kirby et al., 2011). Despite recent therapeutic advancements (Bishr and Saad, 2013), mCRPC remains an incurable disease. Novel insights into PCa progression might therefore identify more effective therapeutic targets. In addition, clinico-pathological factors such as T (tumor) stage, Gleason score and prostate-specific antigen (PSA) are still the gold standard for PCa prognostication. However, it has been shown that their discriminatory value for identifying potentially lethal disease is limited (D'Elia et al., 2014). As a result, the discovery of more effective and potentially non-invasive prognostic biomarkers is of paramount importance.

Recent analyses have revealed that most RNA molecules produced in human cells are not translated into proteins (Kapranov et al., 2007). Included among these non-coding transcripts are microRNAs, which have emerged as critical mediators of cancer progression (Di Leva et al., 2014) and potentially useful biomarkers in oncology (Watahiki et al., 2013, 2011), as well as long non-coding RNAs (lncRNAs) and small nucleolar RNAs (snoRNAs). Many research groups, including ours, have identified numerous PCa-associated lncRNAs (Chakravarty et al., 2014; Crea et al., 2014b; Iyer et al., 2015; Prensner et al., 2014). Indeed it has been shown that the PCA3 lncRNA is detectable in biological fluids and discriminates PCa patients vs. healthy subjects (Wei et al., 2014), suggesting that the latter two classes of non-coding RNAs may also be useful as biomarkers.

On the contrary, snoRNAs are a poorly investigated class of non-coding RNAs that are encoded by at least 400 distinct genomic regions. They have traditionally been considered "housekeeping" genes, mainly involved in alternative splicing and ribosomal RNA (rRNA) modifications (Martens-Uzunova et al., 2013). In light of their presumptive stable expression, some snoRNAs have been selected as reference genes in studies investigating the expression of microRNAs in human neoplasm (Gee et al., 2011). However, recent findings have challenged these assumptions, revealing that snoRNAs can migrate to the cytoplasm and modulate a wide range of cellular functions (Taulli and Pandolfi, 2012). At the same time, it has become evident that snoRNA expression patterns are deregulated in cancer cells and in serum samples from cancer patients (Baraniskin et al., 2013). In addition, some snoRNAs are emerging as critical mediators of cancer progression. For example *SNORA42* is often amplified in lung cancer, and its silencing leads to cancer cell apoptosis, thereby impairing *in vivo* cancer growth (Mei et al., 2012). *SNORD50A* is frequently deleted and acts as a tumor suppressor in breast cancer and PCa (Dong et al., 2009, 2008). This gene also represents, to date, the only functionally characterized snoRNA in PCa.

We have utilized novel patient tumor tissue-derived xenograft models to identify snoRNAs that were differentially expressed in non-metastatic versus metastatic xenografts derived from the primary tumor of one patient. Differentially expressed snoRNAs were then queried in a test cohort of PCa patients to identify their correlation with disease progression and prognosis. These analyses revealed that *SNORA55* was reproducibly associated with worse PCa outcome. In addition, we found that *SNORA55* is required for PCa proliferation and migration. Taken together our studies support the hypothesis that *SNORA55* may be a novel prognostic biomarker, as well as a novel therapeutic target, in PCa.

2. Patients and methods

2.1. *Patient-derived prostate cancer xenografts*

The generation and propagation of the LTL313B and LTL313H tumor tissue xenograft models, as well as their characterization and their use in identification of differentially expressed transcripts, has been described previously (Watahiki et al., 2011) (Wang et al., 2005) (Crea et al., 2014b). Tumor tissue was accrued under UBC/BCCA REB protocol #: H04-60131 and engraftment was performed according to the University of British Columbia Animal Care Committee protocol: A10-0100.

2.2. *RNA sequencing*

Total RNA extraction, RNA Sequencing and transcript annotation have been described previously, and have been performed on one sample per model (Crea et al., 2014b). Importantly for

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