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Integrated analysis of the prostate cancer small-nucleolar transcriptome reveals \textit{SNORA55} as a driver of prostate cancer progression

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\textsuperscript{e}Oncology, FHMS, School of Biosciences and Medicine, University of Surrey, UK
\textsuperscript{f}Karolinska Institutet, Institute of Environmental Medicine, Unit of Biostatistics, Stockholm, Sweden
\textsuperscript{g}Medical Oncology, BC Cancer Agency Vancouver Centre, Vancouver BC, Canada
\textsuperscript{h}Genetics Unit, Integrative Oncology, BC Cancer Agency Cancer Research Centre, Vancouver BC, Canada
\textsuperscript{i}Cancer Center ZeTuP AG St.Gallen, St.Gallen, Switzerland
\textsuperscript{j}Integrated DNA Technologies, Coralville, IA, USA

\textbf{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...
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., 2011, 2013), 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 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.

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this project, total RNA was extracted using Trizol, a method that preserves small RNAs (Rio et al., 2010). Reverse transcription was performed using random hexamers (Oto- 

cellular RNAs, ribosomal RNAs, transfer RNAs, pwi-

to measure (Z scores from MSKCC database) we dichotomized

Selected snoRNAs were analyzed through the cBio cancer

genomic portal (Cerami et al., 2012). This database includes

clinico-pathological and gene expression information from

2.3. Database analysis

Selected snoRNAs were analyzed through the cBio cancer

genomic portal (Cerami et al., 2012). This database includes

clinico-pathological and gene expression information from

2005). Since we intended to validate gene expression data ob-

2.4. Clinical samples

2.4.1. Validation dataset

Samples from 43 PCa patients treated with primary radical

2.4.2. Serum samples from PCa patients

Serum samples from patients with newly diagnosed prostate
cancer were collected at the Royal Surrey and Frimley Park
Hospital, Guildford, UK. All samples were collected prior to
any prostate cancer treatment. Patients donated samples
following a process of informed consent and an ethical
approval granted by the NHS Ethics Committee number 08/
H1306/115. Healthy controls (males 45 years old or younger)
were recruited and assessed for absence of clinical signs of
prostatic neoplasms, including normal PSA levels, as
described (Crea et al., 2014b). Control subjects’ age was chosen

to minimize the possibility of occult PCa, which is very infre-

2.5. Quantitative PCR (qPCR)

qPCR was performed on RNA extracted and reverse-
transcribed from the validation and serum sample datasets.
For the validation dataset, we employed TaqMan gene expres-
sion assays and performed the PCR reaction as previously
described (Crea et al., 2011). SNORA55, TNF and GHRHR expres-
sion (Applied Biosystems assays: Hs03298696_s1, Hs01113624_g1, Hs00173457_m1, respectively) was normal-
lized to HPRT1 (Applied Biosystems assay: Hs01003267_m1) and quantified through the $2^{-\Delta\Delta C_T}$ method. HPRT1 was chosen because its expression was identified as the most stable
among a panel of putative reference genes tested in normal
and neoplastic tissues (including PCa samples) (de Kok et al.,
2005). Since we intended to validate gene expression data ob-
tained in a different platform and expressed in different unit
of measure (Z scores from MSKCC database) we dichotomized
SNORA55 expression based on the expression level that most
efficiently predicted relapse-free survival (lowest p value, log rank-test; at least 5 patients in the smaller group). RNA derived from the serum samples was reverse transcribed using the NCode VILO cDNA Synthesis kit (Applied Biosystems). For quantification, we employed the SNORASS5 TaqMan assay as described above. Mir-30e (assay number: 000620) was used as the internal reference, since we have previously described this gene as the most stably expressed circulating small RNA in PCa patients (Watahiki et al., 2013).

2.6. In vitro experiments

PCa cell lines were grown in appropriate cell culture media and screened for SNORASS5 expression, as previously described (Crea et al., 2014b). Second-generation (2’OMe/PS gapmers) antisense oligonucleotides (ASOs) specifically targeting SNORASS5 were designed and produced by Integrated DNA Technologies, based on the mature sequence of SNORASS5 (Gene ID: 677834). The negative control ASO was designed to avoid silencing of any known human transcript. ASO transfection was performed as previously described (Narita et al., 2008). Cell proliferation and percentage of cell death were measured on Trypan Blue stained cells using a TC20 Automated Cell Counter (Bio-Rad). Cell migration was measured using a wound healing assay, as previously described by our laboratory (Chiang et al., 2014). The following modifications were introduced to the protocol: cells were transfected with ASO1 or NC; pictures were taken 0, 12, 18, 30, 36 and 48 h post transfections. For the highly migrating DU145 cell line, that snoRNAs up-regulated in the metastatic xenograft could contribute to PCa progression, and should be therefore up-regulated in neoplastic vs. normal and/or in metastatic vs. localized PCa samples. Similarly, the down-regulated snoRNAs should also be down-regulated in the clinical database samples studied. Those transcripts were therefore investigated for correlations with cancer progression and for their prognostic value. In order to reduce our false discovery ratio, we applied the Bonferroni correction to all analyses performed on this dataset. We identified 6 snoRNAs with statistically significant differential expression in normal prostate vs. localized PCa and/or in metastatic vs. localized PCa (Suppl. Figure 2; corrected p < 0.05, ANOVA and Tukey’s post-test). Notably, all differentially expressed transcripts were modulated in accordance to our pre-clinical model’s predictions. For example SNORA6, which was the most highly up-regulated snoRNA in our RNA Sequencing database (Table 1), was progressively up-regulated in normal prostate, localized PCa and metastatic PCa samples (Suppl. Figure 2A). Similarly, SNORASS5 expression was higher in metastatic PCa xenografts, and progressively increased in normal prostate, primary PCa and metastatic PCa samples (Suppl. Figure 2C). The only snoRNA significantly repressed in metastatic vs. normal PCa samples was snoRNA31 (SNORA31), which was also down-regulated in Metastatic vs. Non-Metastatic xenografts (Table 1). These results corroborate the clinical relevance of the transcription profiles derived from our pre-clinical model.

3. Diagnostic and prognostic value of SNORASS5

In light of our initial findings, we set out to test whether snoRNAs were able to predict recurrence-free survival (RFS) when measured in primary PCa samples at the time of surgery.
Among the 12 selected snoRNAs (Table 1), only SNORA55 was associated with clinical outcome. Univariate analysis (Figure 1B) indicated that PCa samples with SNORA55 up-regulation displayed a significantly shorter RFS after surgery (corrected log-rank \( p = 0.015 \), median survival 35.35 vs. >140 months, 6/11 vs. 21/120 events). Subgroup analysis revealed no correlation between SNORA55 expression and ERG fusion status, Gleason grade, T stage or circulating PSA levels (Suppl. Figure 3A-D).

To further strengthen our analysis, we explored the diagnostic and prognostic value of SNORA55 in an independent dataset including benign prostatic hyperplasia (BPH) and localized PCa samples with long-term follow-up (patient characteristics summarized in Suppl. Tab. 1). In this case, SNORA55 expression was measured by qPCR, using RNA extracted from primary PCa biopsies at the time of diagnosis. Our analysis confirmed significant up-regulation of SNORA55 in PCa vs. BPH samples (Figure 1C; mean expression: 8.345 ± 2.555 vs. 1.342 ± 0.3729, \( p = 0.0095 \)). We also confirmed that SNORA55 up-regulation was associated with shorter RFS after prostatectomy (Figure 1D; log-rank \( p \) value = 0.0054, Hazard Ratio 95% Confidence interval: 1.134–8.230).

Recent reports indicate that snoRNAs can be detected in serum samples from cancer patients and that those findings might have a diagnostic value (Baraniskin et al., 2013). In light of the prognostic role of SNORA55, we investigated its expression in a small but homogenous cohort of 9 PCa patients treated with brachytherapy vs. 5 healthy subjects (Suppl. Table 2). Our results indicated that circulating SNORA55 is significantly up-regulated in PCa patients vs. healthy controls (Figure 1E).

### 3.4. Functional characterization of SNORA55

Our results to this point indicate that SNORA55 is increasingly up-regulated during PCa progression, and that higher SNORA55 expression is associated with poorer prognosis and worse response to therapy. SNORA55 locus maps on chromosome 1p34 and co-localizes with an intron of the PABPC4 protein-coding gene (Figure 2A). Notably, clinical data indicate that SNORA55 and PABPC4 expression profiles are not correlated, and that PABPC4 has no prognostic value in PCa (Suppl. Figure 4). To investigate the specific role of SNORA55 in PCa biology, we conducted functional experiments in PCa cell lines. First we screened the expression of this gene in a panel of human prostatic cell lines (Figure 2B). SNORA55 was easily detectable (Ct value < 30) in all the investigated cell lines, with highest expression in LNCaP, an androgen-
negative cells expressing relatively lower levels of SNORA55 significantly increases cell death in both cell lines. Our results indicate that the percentage of cell death in DU145 and LNCaP cells exposed to ASO1 vs. NC. Since our findings revealed that SNORA55 is predominantly nuclear (Figure 2C). Our results suggest that SNORA55 silencing induced a dose-dependent inhibition of cell proliferation (Figure 2D). Subsequently, we designed three ASOs specifically targetting SNORA55 and measured their gene silencing efficacy in LNCaP cells. ASO1 induced a dose-dependent silencing reduced the expression of TNF and, to a lesser extent, of GHRHR (Suppl. Figure 6). Silencing induced a dose-dependent inhibition of cell proliferation (Figure 2D). Since this gene had never been associated with cancer before, we investigated the molecular pathways associated with its expression. To this end, we performed SAM analysis and identified 663 genes significantly correlated with SNORA55 expression. We designated those genes as SNORA55-Related Signature (55-RS) and analyzed them through Ingenuity Pathway Analysis. The 55-RS was significantly associated with several pathways, including “Cell-to-Cell Signaling and interaction” and “Cell death and Survival” (Table 2). The top molecular network associated with 55-RS was “Protein Synthesis, Cell-to-Cell Signaling and Interaction, Lipid metabolism” (Suppl. Figure 5). Notably, tumor necrosis factor (TNF) and growth hormone releasing hormone receptor (GHRHR) occupied a central role in this molecular network and were highly correlated with SNORA55 expression. In keeping with these predictions, SNORA55 silencing reduced the expression of TNF and, to a lesser extent, of GHRHR (Suppl. Figure 6).

### Table 1 – Differentially expressed snoRNAs (Metastatic vs. Non-Metastatic PCa xenograft). RPKM, read per kilobase of transcript per million mapped reads. We report transcripts with RMS-normalized RPKM ratio $> 2$ or $< 0.5$. Met. vs. Non-Metastatic xenograft, RNA Seq. data. Transcripts up-regulated in the metastatic xenograft are in red, transcripts down-regulated in the metastatic xenograft are in blue. Transcripts up-regulated in the metastatic xenograft are ranked based on absolute expression level in LTL313H cells. Transcripts down-regulated in the non-metastatic xenograft are ranked based on absolute expression in LTL313B cells.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Met.</th>
<th>Non-met.</th>
<th>RMS-normalized RPKM ratio</th>
<th>Represented in MSKCC database?</th>
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<td>SNORA6</td>
<td>0.524</td>
<td>0.175</td>
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<td>SNORA42</td>
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<td>SNORA62</td>
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<td>0.1275</td>
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<td>SNORD88A</td>
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<td>2.947147325</td>
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<td>0.108</td>
<td>0.3559</td>
<td>3.511472772</td>
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<tr>
<td>SCARNA20</td>
<td>0.844</td>
<td>0.3751</td>
<td>2.065070423</td>
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<tr>
<td>SNORA11B</td>
<td>0.837</td>
<td>0.3166</td>
<td>3.045045054</td>
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<tr>
<td>SNORA18</td>
<td>0.504</td>
<td>0.6716</td>
<td>8.721094856</td>
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<td>SNORA45</td>
<td>0.474</td>
<td>1.692</td>
<td>2.90603856</td>
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<tr>
<td>SNORA72</td>
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<td>0.3538</td>
<td>6.228373702</td>
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<td>SNORA26</td>
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<td>0.3633</td>
<td>3.736551323</td>
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<td>SNORD33</td>
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<td>0.534</td>
<td>2.49063363</td>
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<td>SNORA22</td>
<td>0.077</td>
<td>0.3333</td>
<td>3.736925515</td>
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<td>SCARNA18</td>
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<tr>
<td>SNORA76</td>
<td>0.205</td>
<td>0.7235</td>
<td>0.451312008</td>
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</table>

3.5. Pathway analysis

Our results indicate that SNORA55 is up-regulated during PCa progression and that it is functionally relevant for PCa cell proliferation and migration. Since this gene had never been associated with cancer before, we investigated the molecular pathways associated with its expression. To this end, we performed SAM analysis and identified 663 genes significantly correlated with SNORA55 expression. We designated those genes as SNORA55-Related Signature (55-RS) and analyzed them through Ingenuity Pathway Analysis. The 55-RS was significantly associated with several pathways, including “Cell-to-Cell Signaling and interaction” and “Cell death and Survival” (Table 2). The top molecular network associated with 55-RS was “Protein Synthesis, Cell-to-Cell Signaling and Interaction, Lipid Metabolism” (Suppl. Figure 5). Notably, tumor necrosis factor (TNF) and growth hormone releasing hormone receptor (GHRHR) occupied a central role in this molecular network and were highly correlated with SNORA55 expression. In keeping with these predictions, SNORA55 silencing reduced the expression of TNF and, to a lesser extent, of GHRHR (Suppl. Figure 6).

4. Discussion

In the present study, we identified SNORA55 as a previously uncharacterized gene that is potentially associated with PCa progression. Our results were generated through a multi-step strategy that takes advantage of unique pre-clinical models developed at the Living Tumor Lab (www.livingtumorlab.com). These patient-derived, PCa xenografts accurately reproduce clinico-pathological and molecular features of the original neoplasm (Lin et al., 2014). snoRNAs identified as differentially expressed in this pre-clinical model were further investigated through 2 independent clinical databases (193 total samples). Our experimental strategy is based on the observation that PCa samples are generally composed of multi-clonal subpopulations, each with a peculiar transcriptional landscape and metastatic potential (Lin et al., 2010; Liu et al., 2009). PCa heterogeneity often masks the expression profile of truly metastatic cells, thereby hampering the identification of an effective prognostic signature. As a consequence, several multi-genic PCa prognostic algorithms have been described (Cooperberg et al., 2013; Glinsky et al., 2004; Irshad et al., 2013; Varambally et al., 2005) but none of them have been currently integrated in the clinical practice. The only risk stratification method that is commonly employed is still based on plasma PSA levels, T stage and Gleason grade (Heidenreich et al., 2014). The lack of more accurate and easy-to-detect
prognostic markers hinders an optimal exploitation of the therapeutic armamentarium against lethal PCa (Cersosimo, 2012).

Our results from two independent clinical datasets demonstrated that SNORA55 is: 1) easily detectable in PCa specimens, including serum samples; 2) significantly up-regulated in neoplastic vs. normal prostatic tissue; and 3) a predictor of post-prostatectomy outcome. While the first two features are shared with other investigated snoRNAs, the third is a distinctive feature of SNORA55. A previous study mainly focusing on miRNAs also reported that some snoRNAs are differentially expressed in primary vs. lymph node-derived lesions from PCa patients (Martens-Uzunova et al., 2012). In accordance with our findings, SNORA6 was listed as up-regulated in metastatic lesions. However, this study did not address the prognostic and functional value of snoRNAs in PCa. Another very recent study showed that snoRNA expression profiles are deregulated during PCa initiation and progression, and suggested that they can be employed as prognostic markers (Martens-Uzunova et al., 2015). However, our report is the first one showing that the expression levels of a snoRNA (measured in primary PCa specimens) efficiently predict post-operative PCa prognosis. Notably, the prognostic value of SNORA55 was confirmed in two clinical datasets with different characteristics: the MSKCC dataset comprises relatively younger men (median age = 58.3) and lower-grade neoplasms (percentage of Gleason grade > 7 = 9%) (Taylor et al., 2010), compared to our validation cohort (median age = 68, percentage of Gleason Grade > 7 = 23%).

SnoRNAs have been long considered “housekeeping” genes, whose only function was to regulate basic cellular processes in the nucleus. Recent evidence indicated that snoRNAs also contribute to cancer progression through nucleolar and extra-nucleolar functions (reviewed in Crea et al. (2014a)). For example, some snoRNAs are able to translocate into the cytoplasm, where they control reactive oxygen species production, thereby fueling chemoresistance and metastatic progression (Chu et al., 2012; Crea et al., 2014a). Other snoRNAs generate miRNA-like transcripts, which in turn regulate the expression of several cancer-related genes (Ono et al., 2011). Our study shows that SNORA55 is specifically expressed and functionally relevant in cancer cells. Our gene expression analysis revealed that, unlike other disease-associated snoRNAs, SNORA55 is mainly retained in the nucleus. Due to its sub-cellular localization, we could not employ small interfering RNAs (siRNAs) to target this gene. For this reason, we silenced its expression via ASOs, which proved effective in silencing nuclear RNAs (Gutschner et al., 2013).

Figure 2 — A, Chromosomal localization of SNORA55 (data from Gene Cards). B, SNORA55 expression in prostate cell lines. C, Subcellular localization of SNORA55 in LNCaP cells (MALAT1 is a nuclear non-coding RNA, actin mRNA is prevalently cytoplasmic). D, Effects of DHT treatment on SNORA55 expression (LNCaP cells). HPRT1 is an AR-independent gene (negative control), PSA and TMPRSS2 are AR-dependent genes (positive controls). The experiment was performed as previously described (Parolia et al., 2015).
strong correlation between SNORA55 expression and PCa prognosis.

In keeping with those findings, our Pathway Analysis sheds new light on the molecular mechanisms by which snoRNAs may contribute to cancer progression. We found that SNORA55 expression is significantly associated with known oncogenic pathways and particularly with the expression of TNF- and GHRH-dependent signaling (Suppl. Figure 5). The pro-inflammatory cytokine TNFα is over-expressed in PCa specimens (Rodriguez-Berriguete et al., 2013), and it may

Figure 3 — A, SNORA55 expression in LNCaP cells exposed to different ASO concentrations (48 h). ***p < 0.001 vs. control (1 way ANOVA and Tukey multiple comparison post-test, highest concentration of each treatment). B, Relative LNCaP cell count after treatment with different ASO concentrations (48 h). **p < 0.01 (2 way ANOVA and Sidak post-test). C, Relative DU145 cell count after treatment with ASOs (250 nM, 48 h). ***p < 0.001 (unpaired T test). D, Percentage of cell death in LNCaP and DU145 cells exposed to NC or ASO1 (250 nM, 48 h). *p < 0.05; **p < 0.01 (unpaired T test).

Figure 4 — LNCaP (A) and Du145 (B) cell migration in cells exposed to control or ASO1 (250 nM). *p < 0.05; **p < 0.01 (2 way ANOVA and Sidak post-test).
Table 2 – Top 5 Molecular and Cellular Functions associated with SNORA55 expression. Ingenuity Pathway Analysis was used for this analysis. Genes significantly associated with SNORA55 (in red) were identified through SAM analysis (see “Methods” for details).

<table>
<thead>
<tr>
<th>Function name</th>
<th>P value</th>
<th>Number of Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell-to-Cell Signaling and interaction</td>
<td>9.04E-18–3.91E-2</td>
<td>130</td>
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<tr>
<td>Cell death and survival</td>
<td>1.46E-4–3.91E-2</td>
<td>35</td>
</tr>
<tr>
<td>Cell Signaling</td>
<td>2.52E-4–2.07E-2</td>
<td>36</td>
</tr>
<tr>
<td>Drug metabolism</td>
<td>3.89E-4–3.91E-2</td>
<td>4</td>
</tr>
<tr>
<td>Molecular transport</td>
<td>3.89E-4–3.91E-2</td>
<td>50</td>
</tr>
</tbody>
</table>

Contribute to prostate carcinogenesis and neoplastic progression (De Marzo et al., 2007). Notably, high TNFα expression has been associated with poor PCa prognosis (Rodriguez-Berriguete et al., 2013). GHRH was first discovered as a hypothalamic neuropeptide (Bowers, 2012). The GHRH-FSH/LH axis is primarily responsible for triggering testosterone production by the testis. Recent evidence showed that GHRH receptors are also expressed by PCa cells, and that their inhibition is an effective therapeutic strategy in PCa pre-clinical models (Rick et al., 2012). Therefore, the GHRH-dependent signal transduction pathway is functionally relevant for PCa cells and might mediate the functional role of SNORA55.

5. Conclusions

The non-coding transcriptome represents a vast and mainly uncharted region of the human genome whose relevance in neoplasms has been overlooked for many years. snoRNAs are one of the most abundant non-coding RNA classes and some of them show deregulated expression and/or epigenetic alterations in human neoplasms (Esteller, 2011; Martens-Uzunova et al., 2013). Notably, snoRNAs are also detectable in biological fluids (Baraniskin et al., 2013), a feature that makes them particularly attractive cancer biomarkers. For these reasons, some authors have predicted that snoRNAs will emerge as novel diagnostic and prognostic tools in the near future (Mannoor et al., 2012). We anticipate that our findings, along with similar studies performed on other neoplasms (Gee et al., 2011; Valleron et al., 2012), will pave the way to the discovery of novel clinically relevant biomarkers and therapeutic targets in oncology.

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Conflict of interest

The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.molonc.2015.12.010.

References


