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How to cite:
Crea, Francesco; Quagliata, Luca; Michael, Agnieszka; Liu, Hui Hsuan; Frumento, Paolo; Azad, Arun A.; Xue, Hui; Pikor, Larissa; Wathaiki, Akira; Morant, Rudolf; Castori-Eppenberger, Serenella; Wang, Yuwei; Parolia, Abhijit; Lennox, Kim A.; Lam, Wan L.; Gleave, Martin; Chi, Kim N.; Pandha, Hardev; Wang, Yuzhuo and Helgason, Cheryl D. (2016). Integrated analysis of the prostate cancer small-nucleolar transcriptome reveals SNORA55 as a driver of prostate cancer progression. Molecular Oncology, 10(5) pp. 693–703.

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Version: Version of Record

Link(s) to article on publisher’s website:
http://dx.doi.org/doi:10.1016/j.molonc.2015.12.010

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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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ARTICLE INFO

Article history:
Received 28 August 2015
Received in revised form 13 November 2015
Accepted 7 December 2015
Available online 22 December 2015

Keywords:
Prostate cancer
SNORA55
Non-coding RNAs
Patient-derived xenograft
Next generation sequencing
Antisense oligonucleotide

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

Our results demonstrate that SNORASS 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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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
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 genetics, Oto-Rseq2-20N). In addition, transcript annotation (DNA Nexus) was performed using 36 nt alignment sequences, which are much shorter than the typical snoRNA length (70–120 nt) (Stepanov et al., 2013). Gene expression was quantified through the RPKM value reads per kilobase of transcript per million mapped reads (Mortazavi et al., 2008). RPKM values were normalized to the root mean square (RMS) for each sample. All genes annotated as “non-coding” were further filtered to exclude microRNAs, ribosomal RNAs, transfer RNAs, piwi-interacting RNAs and long non-coding RNAs. The remaining transcripts contained 372 typical small nucleolar RNAs and 24 small Cajal body RNAs (scaRNAs), which were selected for further characterization. Collectively, classical small nucleolar RNAs and scaRNAs are denoted as “snoRNAs” in this manuscript. Differential expression of SNORA55 was confirmed on an independent microarray experiment (LTL-313H vs. LTL-313B), which is publicly available: http://www.livingtumorcentre.com/GeneExpression/search form.php.

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 29 normal prostate, 131 primary and 19 metastatic PCa samples (Taylor et al., 2010). Samples containing more than 70% neoplastic cells based on histological assessment were used for RNA extraction. Metastatic cases were defined as those having clinical or pathological evidence of cancer dissemination to any of the following: lymph nodes, bones or soft tissues (lung, brain, spine, testis). Annotated clinical data from patients who underwent radical prostatectomy was available including PSA every 6 months for the first year post-prostatectomy, semi-annually for the second year and annually thereafter. Recurrence-free survival was calculated as the time between prostatectomy and the occurrence of two, consecutive rising plasma PSA values ≥ 0.2 ng/ml (Mottet et al., 2011).

Gene expression data were downloaded from the portal as log2 whole transcript normalized RNA expression values (Affymetrix Human Exon 1.0 ST arrays). For survival analysis (log-rank test), Z score vs. normal values were employed, since this is the only option available in the portal. The optimal Z score threshold was determined as the one giving the lowest p value (Z score range: 2 ± 0.25). Based on these criteria, a Z score > 2.25 was indicated as “high” SNORA55 expression.

Significance Analysis of Microarray data (SAM) was performed in R using the MSKCC database and clinical and gene expression information available through the cBio portal. We identified 663 positively associated and 220 negatively associated genes (Q < 0.001%, fold change higher than 2). Genes positively associated with SNORA55 were then uploaded in Ingenuity Pathway Analysis (Ingenuity® Systems, www.ingenuity.com).

2.4. Clinical samples

2.4.1. Validation dataset

Samples from 43 PCa patients treated with primary radical prostate-vesiculectomy with pelvic lymph node sampling were collected at the Stephanshorn Clinic in St. Gallen Switzerland, following study protocol approval by the local ethics committee (Protocol number: ZeTuP19/04). Resected specimens were immediately transferred on ice to the Institute for Pathology of the Kantons Hospital, St. Gallen for examination. Small tissue samples from macroscopically visible tumor and non-tumor prostate tissue were dissected, snap frozen in liquid nitrogen and cyro-preserved at −80 °C. Samples were cut in a cyro-microtome and a slide of each probe was stained with hematoxylin-eosin for histological verification. Localized PCa cases were defined as those with no clinical or pathological evidence of metastatic diffusion. PSA measurements were performed pre-operation, and after operation every 6 ± 2 months. Recurrence-free survival was calculated as the time between prostatectomy and the occurrence of two, consecutive rising plasma PSA values ≥ 0.2 ng/ml. RNA was isolated from frozen materials using the TRI-reagent (Ambion) method according to the manufacturer’s guidelines. cDNA was synthesized from 1 µg of total RNA using Superscript II RNase H-reverse transcriptase and DNase digestion (In Vitrogen).

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 infrequent in males younger than 45 (Aubert et al., 1991).

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 expression assays and performed the PCR reaction as previously described (Crea et al., 2011). SNORA55, TNF and GHRHR expression (Applied Biosystems assays: Hs03298696_s1, Hs01113624_g1, Hs00173457_m1, respectively) was normalized to HPRT1 (Applied Biosystems assay: Hs01003267_m1) and quantified through the 2−ΔΔCT 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 obtained 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 us-
ing the NCode VILO cDNA Synthesis kit (Applied Biosystems).
For quantification, we employed the SNORASS5 TaqMan assay
as described above. Mir-30e (assay number: 000602) 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 transfe-
tion 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 Auto-
mated 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,
pictures were taken till full “healing” of the NC-treated cells
(20 h). Cell fractionation and dihydrotestosterone (DHT) treat-
ment of LNCaP cells have been previously described (Parolia
et al., 2015).

2.7. Statistical analysis

Unless otherwise specified, all statistical analyses were per-
formed using Graph Pad Prism 6 software (La Jolla, CA).
MSKCC database clinical annotation and gene expression
levels (available through Cbio portal) were used to dichoto-
mize all patients in 2 groups: high SNORASS5 expression
(threshold as described in “Database analysis”) vs. low
SNORASS5 expression (all other patients).

3. Results

3.1. Small nucleolar RNA transcriptome analysis of
matched non-metastatic and metastatic PCa xenografts

Identification of metastasis-associated genes in primary PCa
using patient samples is a major challenge due to the heter-
ogeneity of such specimens. They typically consist of subpop-
lations with different metastatic abilities, making it difficult to
identify genes with critical roles in the development of metas-
tasis (Fidler, 2002; Lin et al., 2010). For these reasons, molecu-
lar “signatures” for metastatic PCa have been extremely
difficult to identify. To overcome these hurdles, we developed
a multi-step profiling strategy to identify snoRNAs associated
with PCa progression (Figure 1A). First, we profiled snoRNAs in
a paired localized/metastatic tumor tissue PCa xenograft
model (Watahiki et al., 2011) that has already been exploited
to successfully identify miRNAs (Watahiki et al., 2011) and
long-non-coding RNAs (Crea et al., 2014b) associated with
PCa progression. Our analysis showed that the global expres-
sion profiles of protein-coding and non-coding RNAs were
very similar in Metastatic vs. Non-Metastatic PCa xenografts,
indicating that no general alteration in the synthesis of these
two kinds of RNAs was associated with PCa progression
(Suppl. Figure 1). Notably, snoRNAs and ribonuclear-
associated RNAs were the most abundantly expressed tran-
scripts in both tumor tissue lines. We identified a total of
396 snoRNAs expressed in at least one PCa xenograft. Of those,
15 were up-regulated and 6 down-regulated in Metastatic vs.
Non-Metastatic PCa xenografts (Table 1). These transcripts
were further analyzed in clinical samples.

3.2. Expression profile of snoRNAs in clinical samples

In order to validate and establish the clinical relevance of our
RNA Sequencing data, we analyzed differentially expressed
snoRNAs though the Cbio MSKCC database, which holds
gene expression and clinical data from a collection of 131
localized and 19 metastatic PCa samples, as well as 29 benign
prostatic tissues (Cerami et al., 2012; Taylor et al., 2010). We
found that 12 out of 21 differentially expressed snoRNAs
were represented in this database (Table 1). We hypothesized
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 snoR-
NAs should also be down-regulated in the clinical database
samples studied. Those transcripts were therefore investi-
gated 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 per-
formed on this dataset. We identified 6 snoRNAs with statisti-
cally 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 modu-
lated in accordance to our pre-clinical model’s predictions.
For example SNORAS6, 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 (SNORAS31), which was also down-
regulated in Metastatic vs. Non-Metastatic xenografts (Table
1). These results corroborate the clinical relevance of the tran-
scription profiles derived from our pre-clinical model.

3.3. Diagnostic and prognostic value of SNORASS5

In light of our initial findings, we set out to test whether snoR-
NAs 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 ¼ 0.015, median survival ¼ 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. Table 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 ¼ 0.0095). We also confirmed that SNORA55 up-regulation was associated with shorter RFS after prostatectomy (Figure 1D; log-rank p value ¼ 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-
Table 1 – Differentially expressed snoRNAs (Metastatic vs. Non-Metastatic PCa xenograft). RMS, root mean square. RPKM, reads per kilobase of transcript per million mapped reads. We report transcripts with RMS-normalized RPKM ratio > 2 or < 0.5 (Metastatic 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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<tr>
<td>SNORA6</td>
<td>52.24</td>
<td>17.85</td>
<td>3.383431953</td>
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<td>SNORA42</td>
<td>28.86</td>
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<td>SNORA62</td>
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<td>12.75</td>
<td>2.349627175</td>
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<td>10.8</td>
<td>3.559</td>
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<tr>
<td>SCARNA20</td>
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<td>3.751</td>
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<tr>
<td>SNORA11B</td>
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<td>3.166</td>
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<td>SNORA18</td>
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<td>SNORA45</td>
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<td>SNORA72</td>
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<td>SNORA26</td>
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<td>3.736551323</td>
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<td>233.5</td>
<td>70.91</td>
<td>0.551062867</td>
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<td>13.94</td>
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<td>4.432</td>
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<tr>
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<td>2.015</td>
<td>0.7235</td>
<td>0.415312008</td>
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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 multiclinal 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). Our results indicate that SNORA55 is required for PCA cell proliferation and migration. These findings may explain the
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

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**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-10–3.91E-2</td>
<td>130</td>
</tr>
<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>
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<tr>
<td>Drug metabolism</td>
<td>3.89E-4–3.91E-2</td>
<td>4</td>
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<tr>
<td>Molecular transport</td>
<td>3.89E-4–3.91E-2</td>
<td>50</td>
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</tbody>
</table>

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


Funding
Canadian Institutes of Health Research (YW, MG), Terry Fox Research Institute (YW, MG), BC Cancer Foundation (YW), Canadian Cancer Society Research Institute (CDH, YW). FC holds a Michael Smith Foundation for Health Research postdoctoral fellowship award and a Prostate Cancer Foundation-BC award. AAA is supported by a CJ Martin Biomedical Overseas Fellowship from the National Health & Medical Research Council.

Contribute to prostate carcinogenesis and neoplastic progression (De Marzo et al., 2007). Notably, high TNFα expression has been associated with poor PCa prognosis (Rodriguez-Berrigue 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 preclinical 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.


