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sdRNAs Participate in the Maintenance and Onset of Prostate Cancer

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Introduction

What is an sdRNA?

- Noncoding RNA (ncRNA) is a class of RNA that includes microRNA (miRNA) and small nucleolar RNA (snoRNA)
- Fragments excised from ncRNAs are known as noncoding-derived RNA (ndRNA)
- A certain class of ndRNAs that are derived from snoRNAs have been denoted as Small Nucleolar Derived RNAs (sdRNAs)
- Our lab has previously hypothesized and demonstrated that sdRNAs display regulatory functions on target genes, similar to the functions of miRNAs
- As seen in **Figure 1**, sdRNAs are almost identical to microRNAs and differ mainly in their source of origin

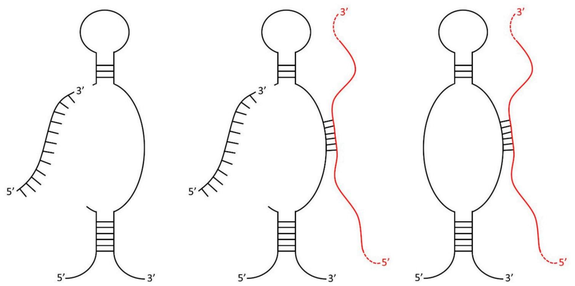

Figure 1. sdRNAs are excised fragments derived from snoRNAs.

Diagnostic and Prognostic Relevance of sdRNAs

- Our lab has shown that sdRNA-93 is frequently misexpressed in breast cancer cell lines and directly contributes to the cellular pathology present in breast cancer
- We showed that sdRNAs play a role in the molecular events responsible for malignancy and that sdRNAs may represent entirely new tools for diagnostic and prognostic determination

Why Study Prostate Cancer?

- In 2021, prostate cancer was the most prevalent malignant cancer type in American men
- Castration resistant prostate cancer (CRPC) lacks effective treatment options, leading to prostate cancer's second place standing as a leading cause of cancer death in American men
- Our lab identified two distinct sdRNAs (sdRNA-D19b and sdRNA-A24) that are overexpressed in prostate cancer samples compared to normal tissues
- We aimed to evaluate the role these sdRNAs play in the maintenance and/or onset of prostate cancer

Project Aims

Overall Aim

- To highlight the characteristic overexpression of sdRNA-D19b and sdRNA-A24 in prostate cancer to further explore their phenotypic effects.

Specific Aims

- To display how the overexpression of these sdRNAs leads to increased cell proliferation.
- To examine the impact of these sdRNAs on cell migration and the ability of cancer cells to metastasize.
- To explore the roles of these sdRNAs on specific drug resistances

Results

1. sdRNA-D19b and sdRNA-A24 are overexpressed in prostate cancer (PCa) samples

- The SURFR program was used to determine sdRNA expressions in 489 PCa and 52 normal prostate TCGA patient RNA-seq datasets. This produced a ranked catalogue of significantly differentially expressed sdRNAs in PCa.
- As seen in **Table 1**, sdRNA-D19b and sdRNA-A24 stood out as: sdRNA-D19b is expressed in 91.6% of 489 TCGA PCa samples versus only 42.3% of normal tissue controls and sdRNA-A24 is expressed in 97.5% of 489 TCGA PCa samples versus only 30.8% of normal tissue control.

	Prevalence (%) in 489 PCa Samples	Average Expression (RPM) in PCa Samples	Prevalence (%) in 52 Tissue Controls	Average Expression (RPM) in Tissue Controls	Differential Expression Fold Change (Cancer/Control)
sdRNA-A24	97.5	711	30.8	150	4.74x
sdRNA-D19b	91.6	384	41.3	162	2.4x
sdRNA-D30	99.6	31067	100.0	19719	1.6x
sdRNA-D61	53.2	215	17.3	119	1.9x

Table 1. Significant overexpression of sdRNA-D19b and sdRNA-A24.

2. sdRNA-D19b and sdRNA-A24 levels significantly impact PC3 cell proliferation

- To manipulate sdRNA expression a custom mimic/inhibitor system was utilized
- RNA sequences identical to sdRNA-D19b and sdRNA-A24 were commercially synthesized and used to simulate sdRNA overexpression through transfecting PC3 cells (a well-established model of castration-resistant prostate cancer (CRPC)) with these specific sdRNA mimics.
- RNAs complementary to sdRNA-D19b or sdRNA-A24 were similarly synthesized and employed as sdRNA inhibitors through transfecting PC3 cells with these specific sdRNA antagomiRs.
- As seen in **Figure 2**, The overexpression of sdRNA-D19b increased PC3 cell proliferation by 24% and 32% at 24 and 72 h. sdRNA-D19b inhibition reduced PC3 cell proliferation by 22% and 32% at 24 and 72 h. Similarly, sdRNA-A24 overexpression enhanced PC3 proliferation by ~25% at both 24 and 72 h, and sdRNA-A24 inhibition decreased proliferation by 14% and 40% at 24 and 72 h, respectively (as compared to cells transfected with scrambled controls).
- These results indicate functional involvements for both sdRNA-D19b and sdRNA-A24 in PC3 proliferation

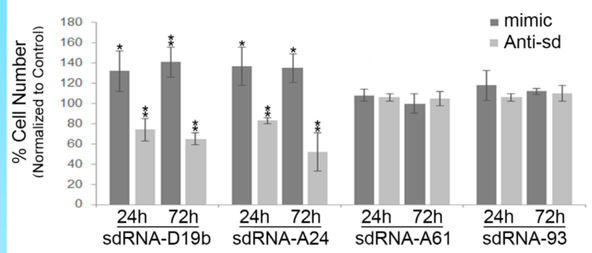

Figure 2. sdRNA-D19b and sdRNA-A24 levels significantly impact PC3 cell proliferation.

Results

3. Overexpression of sdRNA-D19b markedly increases the migration of PC3 cells

- Aside from proliferation, migration is another vital hallmark of cancer which enables primary tumors to exit their local environments and give rise to metastases responsible for patient mortality.
- As seen in **Figure 3**, the wound-healing assay was used to assess whether sdRNAs-D19b and –A24 contribute to PC3 cell migration. A "scratch" was introduced to bisect confluent cells in a culture dish following sdRNA mimic, inhibitor, or scrambled control transfection.
  - Images were taken every 3 hours using an EVOS XL Core inverted microscope to assess the rate of migration.
- **Figure 4** visually depicts how sdRNA-D19b overexpression markedly increased the migration (avg 86.8%) between 6 h and 24 h.
- **Figure 5** summarizes this data graphically

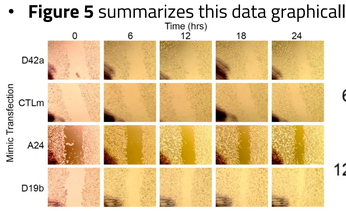

Figure 3. Effects of sdRNA mimic transfection on PC3 cell migration.

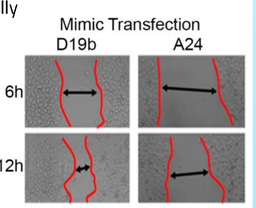

Figure 4. sdRNA-D19b overexpression markedly increases cell migration.

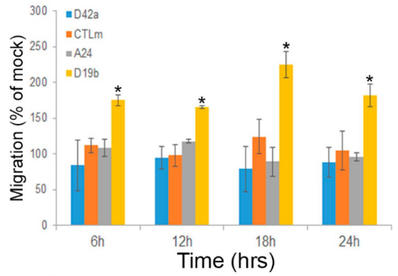

Figure 5. Quantification of PC3 migration assays.

4. Altering the levels of sdRNA-D19b and sdRNA-A24 changes drug sensitivities in vitro

- Paclitaxel and dasatinib are both common drug therapies employed in the treatment of prostate cancer.
- **Figure 6** shows how manipulating levels of sdRNA-D19b did not significantly alter the effect of paclitaxel on PC3 cells, but sdRNA-A24 overexpression improved PC3 resistance to paclitaxel, increasing cell viability between 28.9% and 70.3% at all of the measured time points
- **Figure 7** shows how sdRNA-D19b overexpression markedly desensitized PC3 cells to dasatinib treatment, increasing cell viability by over three times at 24 h as compared to controls.
- These results depict a complex, significant role for sdRNAs in relation to PC3 drug resistance. They imply that sdRNA-D19b and sdRNA-A24 occupy different mechanistic roles in greater drug resistance.

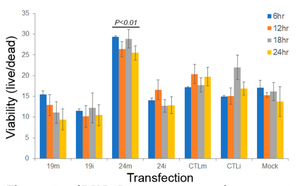

Figure 6. sdRNA-A24 overexpression protects PC3 cells from paclitaxel.

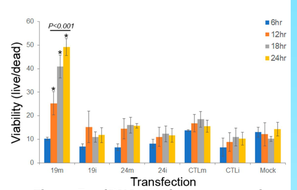

Figure 7. sdRNA-D19b overexpression protects PC3 cells from dasatinib.

Conclusions

- We have identified sdRNA-D19b and sdRNA-A24 to be overexpressed in Prostate Cancer
- Upon further investigation, we have revealed their functions in promoting PC3 cell proliferation
- We have also shown how sdRNA-D19b specifically contributes to increased cell migration
- We have described how altering the levels of these sdRNAs changes drug sensitivities in vitro

Future Directions

- Examine the differential expression of sdRNA-D19b and sdRNA-A24 in different tumor grades to define or rule out a potential linkage
- Define whether identifying sdRNA presence can guide which treatment options to employ/avoid
- Conduct future research opportunities regarding the effects of sdRNAs on a variety of other cancers and diseases
- Determine if these sdRNAs can be used as prognostic biomarkers and/or novel therapeutics in clinical settings

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