Realizing the potential of long noncoding RNA as a cancer biomarker

From NGS discovery to validation with RNA *In Situ* Hybridization

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An emerging body of evidence points towards the significance of long noncoding RNA (lncRNA) in cancer development. These oncogenic molecules therefore lend themselves as predictors of disease outcome, but without the option of antibody-based detection, utilizing non-protein coding molecules as biomarkers can be challenging. Speaking with ACD, Clinical Assistant Professor of Pathology at Michigan Center for Translational Pathology (MCTP), Rohit Mehra, M.D., explains how the potential of the lncRNA *SChLAP1* as a biomarker for prostate cancer outcome is being realized through RNA *in situ* hybridization with RNAscope® technology.

Can you provide a brief summary of your research focus?

Our research focus is on the critical patho-genetic events underlying the development of genitourinary cancers, especially prostate cancer. Studying lncRNAs is a core part of this focus. The biology of lncRNAs is a relatively new field of study, however, and we do not yet have a full understanding of their roles in normal and disease states.

Advancing knowledge in this area, our center (MCTP) has discovered several lncRNAs that play an important role in prostate cancer, some of which may have clinical utility as prognostic or diagnostic biomarkers. For this, accessible methods for routine lncRNA detection are vital.

How did you discover that *SChLAP1* was a biomarker for prostate cancer progression?

This involved two main stages: biomarker discovery, utilizing next-gen sequencing of the transcriptome, and the validation of *SChLAP1*, where we used ACD's RNAscope® technology.

- Discovery

Next generation sequencing has been instrumental for identifying novel, disease-associated lncRNAs. Our lab used RNA-seq to comprehensively profile the transcriptome of >100 prostate cancer tissues and cell lines, and found that ~20% of RNA transcripts in prostate cancer represent novel, uncharacterized lncRNA genes[1]. The novel lncRNAs were distributed throughout the genome, occurring in both intergenic and intronic, and in sense and antisense orientations. We assessed lncRNA expression across all samples and nominated 121 candidate lncRNAs that were overexpressed in prostate cancer. These were termed PCATs (Prostate Cancer Associated Transcripts).

- Validation

One of these PCATs was validated and re-named as Second Chromosome Locus Associated with Prostate-1 (*SChLAP1*) because it represented a large ~500kb region of high transcription in prostate cancer.

Importantly, we determined that *SChLAP1* was a promising biomarker. We used a Mayo Clinic cohort of 235 high-risk prostate cancer specimens obtained from radical prostatectomy of localized prostate cancer patients. High risk disease entailed either advanced Gleason score (>=8), high serum PSA (>20), seminal vesicle invasion or extra-prostatic extension upon surgical resection. In this cohort, *SChLAP1* expression measured

To discover how RNAscope® technology can unveil the potentiel of long noncoding RNA, visit acdbio.com/ncrna.

1 - Transcriptome sequencing across a prostate cancer cohort identifies *PCAT-1*, an unannotated lincRNA implicated in disease progression.

Prensner J.R. et al. (2011), *Nat. Biotechnol.* 29(8):742-9

2 - The long noncoding RNA *SChLAP1* promotes aggressive prostate cancer and antagonizes the SWI/SNF complex.

Prensner J.R. et al. (2013), *Nat. Genet.* 45(11):1392-8

3 - A novel RNA In Situ Hybridization Assay for the Long Noncoding RNA *SCHLaP1* predicts poor clincial outcome after radical prostatectomy in clinically localized prostate cancer.

Mehra R. et al. (2014), Neoplasia. 16(12):1121-1127

"We found RNAscope technology fast and easy to use, this technology also allows us to directly visualize gene expression in the target tissue of interest ....Also, only one 4 micron FFPE section is sufficient to give us all this information."

using expression profiling technology effectively stratified patient outcomes by predicting more rapid biochemical recurrence, clinical progression to metastatic disease (defined by a positive bone scan) and prostate cancer-specific mortality.

Through enabling the routine detection of lncRNA, how do you see RNA ISH opening doors in biomarker-based profiling of cancer samples?

To date, the majority of biomarker efforts have focused on protein coding genes, with the protein counterpart detected using antibody-based methodologies. However, these comprise only a subset of all transcribed genes and the potential utility of detecting lncRNAs in clinical assays is becoming increasingly evident.

The routine detection of non-coding RNA presents a particular set of challenges, since they do not have protein counterparts for antibody detection. This is why the ability to detect RNA in cancer biopsy samples is so valuable, opening up the use of lncRNA as a biomarker.

We have several lncRNA candidates with promising diagnostic and prognostic utility, and RNA ISH allows for easy and reproducible *in vivo* detection of these lncRNA biomarkers.

What features influenced your decision to choose RNAscope® technology over other methods?

We found RNAscope® technology fast and easy to use. Compared to qPCR, this technology also allows us to directly visualize gene expression in the target tissue of interest – for example, within the same sample we can tell whether gene over-expression occurs in benign prostate glands, high-grade prostatic intraepithelial neoplasia (HGPIN – a pre-cancerous state) or prostate cancer. With RNAscope® we can look for expression in glands of interest, whereas PCR would allow us detect expression but we would not be able to determine the exact site of origin.

Also, only one 4 micron FFPE section is sufficient to give us all this information, whereas more tissue would be needed for PCR assessment. With just one section per sample, this allowed us to look at a vast number of samples plotted on a tissue microarray. Further increasing throughput, RNAscope® technology is amenable to large-scale automation.

How have you found RNAscope® assay to perform with FFPE samples?

RNA transcript detection can be influenced by several potential issues like tissue fixation, ischemia time, etc. that can promote RNA degradation and compromise its integrity. We have been able to detect RNA in a variety of rapid autopsy samples which originated from University of Michigan Hospital. RNA expression analysis of samples includes comparison to known matched positive controls.

Has RNAscope® assay facilitated your work in uncovering the function of *SChLAP1*?

Our early studies, which involved the use of RNAscope technology[2], found that *SChLAP1* antagonizes the genome-wide localization and regulatory functions of the SWI/SNF chromatin-modifying complex. Furthermore, through antagonizing the tumor-suppressive functions of the SWI/SNF complex, it contributes at least in part to the development of lethal cancer. Further studies to uncover the molecular mechanisms by which *SChLAP1* functions are ongoing.

How has the ability to multiplex RNAscope® assays facilitated your studies?

We are working towards multiplexing, as several lncRNAs are currently being evaluated for their potential as biomarkers. Multiplexing will allow us to simultaneously detect multiple markers of interest that will be able to better stratify aggressive disease than individual markers alone.

What do you see for the future of using RNAscope® technology in your research?

This technology has tremendous potential for realizing the utility of lncRNA biomarkers in stratified cancer medicine[3]. In our research, its application has laid the foundations for developing a *SChLAP1* assay for future use in diagnostic.

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