Development of Prostate Cancer Personalized Medicine

Advancing analysis of prostate tumor molecular heterogeneity by combined immunohistochemistry and novel RNA *in situ* hybridization

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Working towards the future of refined techniques for prostate cancer personalized medicine, Dr Nallasivam Palanisamy discusses his research examining fusion genes as biomarkers for tumor classification.

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Can you give us a brief summary of your research?

My current research is centered on addressing genetic differences in the changing landscape of tumors. We are striving to develop refined approaches for molecular classification of tumors to replace morphological assessment as the future norm in cancer diagnosis and treatment. There is a real need for this: identifying the correct drug target helps to select the correct method of treatment, making a real difference in the life of a cancer patient. However, molecular analysis is dynamic, and must incorporate new discoveries. Working towards this goal, I focus on the discovery of new molecular markers in cancer – particularly recurrent gene fusions, and understanding their role in cancer development. This has included the discovery of several recurrent gene fusions in lymphoma and solid cancers, and my recent work identified "druggable" *RAF* kinase gene fusions in a subset of high-grade prostate cancers. Further screening also identified *RAF* kinase gene fusions in gastric cancer and melanoma.

With the increasing availability of targeted compounds, how does this highlight the need for research tools to keep up with this?

As new tumor-specific molecular markers are identified to classify cancers into distinct molecular subtypes, robust methods for their reliable detection are essential.
For example, *ETS* family gene fusions are common in prostate cancer, yet distinct members of this family of genes, including *ERG* and *ETV1*, have distinct molecular functions in terms of disease progression and response to treatment. With the development of small molecule inhibitors for *ETV1* and indirect targeting of *ERG* gene fusion with *PARP* inhibitors, molecular stratification of tumors is critical to direct appropriate treatment. In this regard, it is vital to develop robust tools for accurate detection of these markers with high specificity and sensitivity, both in the clinical and research settings.

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Which techniques are currently employed for biomarker analysis, and what are their limitations?

Antibody-based detection is the current standard for the majority of disease biomarkers. These techniques are easy to use, can be analyzed under brightfield microscopy and quantified using software tools. However, limitations are associated with the sensitivity and specificity of the antibodies. Moreover, antibody performance varies under different detection platforms, and suitable antibodies are often unavailable. With the lack of specific antibodies for most of the transcription factor genes, RNAscope is a method of choice for researching biomarkers.

What do you consider the utility of looking at protein and RNA in one sample?

Development of combined protein and RNA detection methods may alleviate many concerns for accurate detection of biomarkers, and I can see this being the standard practice in future molecular cancer profiling. I say this because rapid advances are being made in understanding tumor molecular heterogeneity, revealing the "personalized molecular landscape" and in turn driving the "personalized medicine" option in the treatment of cancer.

Are you able to combine IHC and RNA ISH on the same slide? What are the advantages of this?

Yes, in my research we have demonstrated the molecular heterogeneity of prostate cancer by both duplex IHC and IHC combined with RNA ISH. Given the limited availability of tissue from a small biopsy, it is important to develop methods to detect more than one type of marker on the same slide.
In the research setting, we are now able to do both IHC and RNA ISH on the same biopsy material. We perform this in a sequential manner by doing RNA ISH first followed by IHC, but the reverse is not recommended. With this approach we were the first to report the presence of *ERG* and *ETV1* rearrangement in two independent tumor foci of a multifocal prostate cancer specimen with the same Gleason grade.

Do you consider it advantageous to use RNA as a biomarker? Have there been any instances where it was beneficial to detect RNA instead of protein?

Yes, in our biomarker discovery projects we use transcriptome sequencing as an unbiased approach to characterize most, if not all, of the expressed transcripts in a given sample. From this we have identified biomarkers in both protein coding and non-coding genes. For subsequent biomarker detection, when looking at non-coding genes we must detect RNA, and even some of the markers based on protein-coding genes do not have good antibodies, and once more our only option is to detect the expression of the gene at the RNA level only. For example, *ETV1*, *ETV4* and *ETV5* genes are overexpressed in a small subset of prostate cancer, and in order to assess the tissue level expression of these genes RNA based screening is the method of choice. Even for the genes with good antibodies, if the protein level is variable or always too low for detection, it will be beneficial to support protein analysis with information on RNA expression to get an unequivocal assessment.

What features influenced your decision to choose RNAscope?

We tried several RNA detection platforms prior to RNAscope, and became frustrated with inconsistent and poor quality results. Given the unique probe design and detection chemistry of the RNAscope method, we were able to get results with high specificity and sensitivity for many probes that we have used in my laboratory. To date we don't have any comparable technology for the reliable detection of RNA in FFPE tissues other than RNAscope.

To discover how RNAscope® technology can unveil tumor heterogeneity in your cancer research, visit acdbio.com/cancer.

Evaluation of tissue *PCA3* expression in prostate cancer by RNA *in situ* hybridization - a correlative study with urine *PCA3* and *TMPRSS2-ERG.*

Warrick JI, Tomlins SA, Carskadon SL, Young AM, Siddiqui J, Wei JT, Chinnaiyan AM, Kunju LP, Palanisamy N. Mod Pathol. 2014 Apr;27(4):609-20.

Novel RNA hybridization method for the *in situ* detection of *ETV1*, *ETV4*, and *ETV5* gene fusions in prostate cancer.

Kunju LP, Carskadon S, Siddiqui J, Tomlins SA, Chinnaiyan AM, Palanisamy N.

Appl Immunohistochem Mol Morphol. 2014 Sep;22(8):e32-40.

"RNAscope is a unique technology with many advantages due to its consistency in yielding reproducible results."

What have you found to be the main advantages RNAscope?

RNAscope offers me the opportunity to address important questions in my research. Based on my experience in working with other platforms for RNA ISH, I found RNAscope a unique technology with many advantages due to its consistency in yielding reproducible results. Technically speaking, it is easy to perform – especially the automated approach – while the availability of suitable positive and negative control probes is a plus.

Options to use manual and automated procedures in single or multiplex assay format is an added advantage, especially for screening a large cohort of specimens with multiple markers. Recent development of software tools for quantitation of the RNA ISH signature also enables researches to get unbiased quantitative results, thus avoiding observation bias between samples.

Above all, the ready availability of technical support and even an on-site visit to troubleshoot issues with setting up assays and using new probes or protocols is certainly an advantage for anyone who would want to adopt this technology.

How have you found RNAscope with FFPE samples?

For my research, I use FFPE samples from needle biopsy, prostatectomy tissues, cell blocks, and tissue microarray. We get consistently good RNA ISH results using FFPE slides cut fresh (up to three months old) rather than older slides. We've also found that it is ideal for specimens to be preserved in FFPE blocks under optimal storage conditions rather than on slides.

How important do you consider the spatial gene expression information that RNAscope provides?

Assessment of distinct molecular differences by spatial RNA or protein expression analysis may enable clinicians to predict the clinical course of the disease and select appropriate treatment options; so I'd say it's very important. My group has pioneered such approaches to reveal the hidden molecular differences in prostate tumors and the correlation of these differences forms our current focus of research.

What do you consider the importance of analyzing non-coding and pseudogenes in tumor samples?

With the emerging role of non-coding RNA and pseudogenes in cancer, I believe that many of these biomarkers will be identified for each cancer type, with utility for assessing the clinical course of the disease and predicting treatment response. Given these markers are non-protein coding genes, RNAscope will be an essential tool for the identification of these markers.

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

With the many advantages provided by RNAscope in my research, I can see it becoming an increasingly important tool. Analyzing multiple RNA species alongside protein biomarkers, it is sure to play a central role in meeting future demands of molecular tumor characterization and personalized medicine.

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