Multiplexed Bioimaging of Cancer Biomarkers in Human Thyroid Lesions

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Statement of Purpose: We seek to reduce the number of unnecessary surgical excisions in thyroid lesions by correlating high content biomarker imaging that can be integrated in a typical pathology assessment to proteomic and genomic signatures exhibited in various stages of thyroid cancer.

Background. Advances in nanotechnology are pushing into high-end medical applications such as custom imaging probes for in vivo detection and diagnosis.[1] However, these same advances have been slow to transcend the full spectrum of clinical providers and institutions where their advantages could have the most impact on patient care. Improved, quantum dot based histochemical methods for measuring low copy number biomarkers would aid in the sub-type classification of indeterminate lesions and expedite the translation of biomarkers for expanded use in routine diagnostic and clinical applications.[2] Our first target was to develop an aptamer conjugated quantum dot reporter that was functionalized with a nucleic acid sequence specific for the RNA template region (hTR) of the telomerase holoenzyme. Telomerase is a ribonucleo protein complex that is responsible for maintaining chromosome stability and cell life span by preserving the integrity of telomeres. Telomerase activity (TA) has been shown previously to correlate with poor clinical outcome in various tumor entities. It is expressed during development and in 85-90 % of all human cancers, but not in normal adult, non-stem cell somatic tissue, which makes it an attractive tumor diagnostic marker.[3] However, the use of telomerase as an early detection biomarker for cancer has been hindered by a lack of reliable *in situ* histochemical measurement methods and standards.[4]

Results. We recently developed a scalable two-step microwave-based synthetic procedure that sidesteps the ligand exchange process and required polymeric stabilizing coatings that plague the commercially available quantum dots.[5] We have further developed robust derivatization methods to attached controlled numbers of DNA molecules to the QD surfaces for the purpose of in situ hybridization molecular detection strategies. We have demonstrated that the detection of (hTR) is specific, and correlates with measured mRNA levels (Figure 1).

Specifically, we aim to measure the affinity and specificity of anti-hTERT antibodies and a series of hTERT targeted DNA-derivatized quantum dots. We will assess their ability to bin telomerase expression on well-defined cultured cells in vitro, on paraffin fixed tissue samples and on fine needle aspiration (FNA) samples. We compare these imaging profiles of telomerase activity to currently accepted methods including flow cytometry and reverse transcriptase polymerase chain reaction (RT-PCR). Using pre-selected and blinded thyroid samples from sets of previously classified lesions we will be able to evaluate benign, malignant and more importantly intermediate tissues with the presence of lymphocytic infiltration. Our ultimate goal is to outline a framework for correlating imaging results of in situ hybridization (ISH) and immunohistochemistry (IHC) of telomerase on paraffin fixed and fine needle aspiration (FNA) samples with genetic data and clinical outcomes. This information will serve as a guide to future investigations seeking to develop robust research and clinical tools for the early detection of cancer while minimizing or potentially eliminating unnecessary surgical excisions. Furthermore, the developed methods will be applied to other systems including MSC and in vitro analysis of model systems.

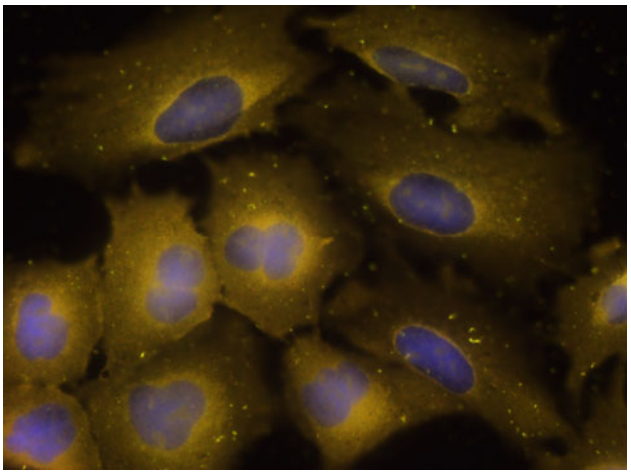

Figure 1. Fluorescent images indicating the specificity of the DNA derivatized quantum dots in the A549 (hTR +) human lung cells.

References

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