

DETECTION OF MICRORNA EXPRESSION IN FORMALIN-FIXED PARAFFIN-EMBEDDED MATERIALS WITH FLUORESCENT *IN SITU* HYBRIDIZATION

Zonggao Shi (Postdoctoral Fellow)

(M. Sharon Stack, PhD)

School of Medicine, Department of Pathology and Anatomical Sciences

Non-coding regulatory RNAs designated microRNAs (miRNA) are small single-stranded RNAs that may suppress the protein outcome of target messenger RNAs and thereby regulate gene expression networks. To facilitate delineation of the role of microRNAs in cancer pathology, the goal of this study was to demonstrate the feasibility of detecting microRNA expression using commercially available formalin-fixed paraffin-embedded (FFPE) tissues. Using FFPE materials, we have compared fluorescent *in situ* hybridization (FISH) procedures with (a) different synthetic probes: regular custom DNA oligos vs. LNA incorporated DNA oligos complementary to mature microRNA sequence; (b) different tracers for the probes: biotin vs. digoxigenin; (c) different visualization: direct vs. TSA amplification; and (d) different blocking reagents for endogenous peroxidase. Finally, we performed mir-146a FISH on a commercially available oral cancer tissue microarray (TMA), which contains 40 cases of oral squamous cell carcinoma (OSCC) and 10 cases of normal epithelia from the human oral cavity. Spiny cells in most normal oral squamous epithelia were positive for mir-146a, while basal cells stained negative. In OSCC tissues, a correlation between a decrease in mir-146a and an increase in histological grade was observed. In summary, we have established reliable *in situ* hybridization procedures for detecting the expression of microRNA in FFPE oral cancer tissue. This detection is useful for studies on the participation of microRNA in oral cancer pathology, and may have potential prognostic or diagnostic value.