Utility of miR-1 and miR-145 microRNA Probes in Distinguishing Smooth and Skeleton Muscle Differentiation

Ling Xue[1], Krishan Kalra[1], Raju K Pillai[2], Mahul B Amin[2], Daniel J Luthringer[2],
[1]BioGenex Laboratories, Fremont, CA 94538, USA; [2]Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA

Background

Determining specific patterns of differentiation is critical to many aspects of diagnostic pathology, including classification of soft tissue tumors. MicroRNAs (miRNAs) are a group of non-coding RNAs 18-22 base-pairs in length, which play critical roles in a many cellular functions including disease states and cancer. New technology allows miRNA detection by in situ hybridization (ISH) techniques. miR-1 is specifically expressed in adult cardiac and skeletal muscle, and miR-145 is expressed by smooth muscle including bladder, stomach and bowel. The purpose of this study was to explore utility of this evolving technology in the expression of two miRNA probes in hopes that it may find application in solving challenging aspects of tumor differentiation.

Design

Samples of formalin-fixed paraffin embedded (FFPE) tissue were studied, consisting of 3 cardiac muscle, 4 skeletal muscle, 3 colon, 2 small bowel, 2 stomach. Cases were subjected to ISH using FAM-labeled Hsa-miR-1 and Hsa-miR-145 probes (BioGenex) followed by Super Sensitive ISH Detection Kit (BioGenex, DF400-YAX). Slides were heated in Nucleic Acid Retrieval Solution I (NAR-I, BioGenex) for 10 min at 92C, subjected to hybridization buffer for 30 min, incubated with 40 nM of microRNA probe for 60 min at 50 C followed by stringency washes. The signal was amplified with anti-fluorescein antibody and poly-HRP labeled secondary antibody, which can develop brown color with DAB chromogen. Scramble probes served as negative control.

Results

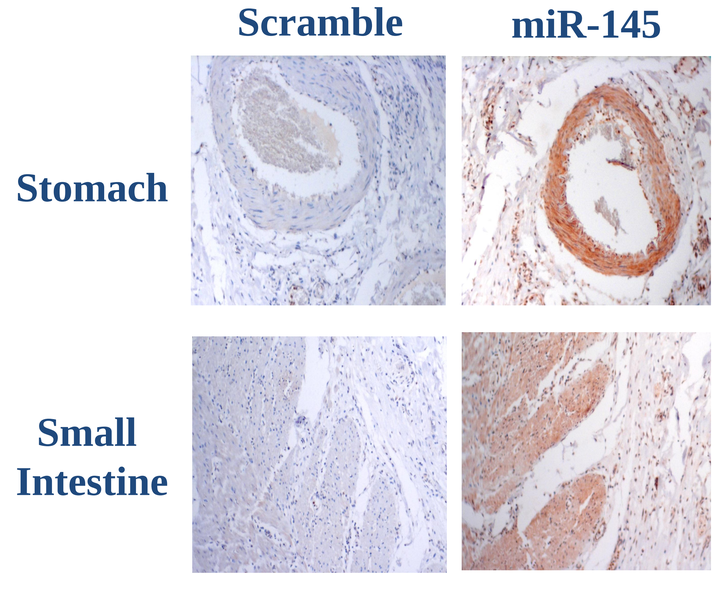

Figure 1. The staining of miR-145 in various tissues. Scramble probe works as the negative control.

Results

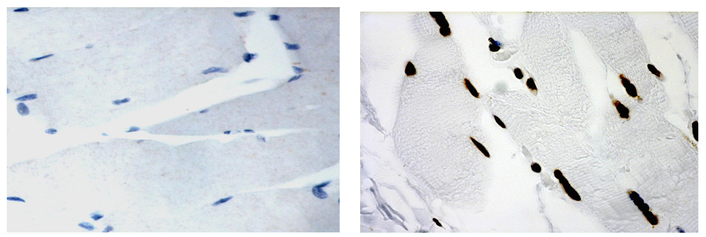

Figure 2: The staining of miR-1 in skeleton muscle. Scramble probe works as the negative control.

In most of cardiac and skeletal muscle tissues, cells demonstrated crisp, nuclear staining of miR-1. But miR-145 staining was observed mainly in cytoplasm of various tissue cells, including stomach, small bowel, and colon. Background staining was minimal. Scramble (negative controls) probes were non-reactive.

Conclusion

In situ hybridization with probes to specific miRNAs can be utilized in diagnostic pathology. miR-1 and miR-145 probes are capable of marking cardiac, skeletal and smooth muscle tissue in formalin fixed paraffin embedded samples. Application of this technology with muscle specific probes to soft tissue tumors may be useful in determination of differentiation and thus aid in classification.