Microrna Biomarkers For Identification Of Chondrosarcoma

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Disclosures:

Introduction: Despite being the most common skeletal neoplasm affecting bone\(^1\) cartilaginous tumors present a diagnostic and treatment dilemma. Discrimination between benign enchondroma and low grade chondrosarcoma and between grades of malignancy in chondrosarcomas is difficult and subject to high inter-observer variability. To date attempts to identify reliable molecular biomarkers that would distinguish stages in chondrosarcoma development have been unsuccessful. Chondrosarcomas are also notoriously resistant to conventional radiation and chemotherapies. As a consequence surgery is the only treatment strategy. MicroRNAs play critical roles in endochondral differentiation but their value as biomarkers or their contribution to chondrosarcoma development has not been investigated. MicroRNAs are small (~21 nucleotide) non-coding RNA molecules that provide an overarching regulation of gene expression at the post-transcriptional level. They play a critical role in almost all biological process, including cell division, differentiation, growth and apoptosis. It is not surprising then that changes in microRNA expression have been associated with many diseases, including neurological diseases, cardiovascular disease and particularly cancer\(^5\). Extensive studies have shown that microRNA signatures can provide biomarkers for the diagnosis of many types of tumor and predict tumor progression. Recent improvements in RNA isolation have facilitated the analysis of microRNAs in formalin fixed paraffin embedded (FFPE) tissue sections and provide the potential to analyze a wide range of less common tumors, including chondroid lesions, that have been archived over many years. We have performed array analysis of microRNA expression in archived enchondroma and chondrosarcoma to develop a microRNA signature for discrimination of chondrosarcoma from enchondroma and for better definition of the grades of chondrosarcoma. In addition analysis of the rat chondrosarcoma showed strong parallels with that seen in human chondrosarcoma suggesting the rat model as an excellent system to investigate microRNA function and therapeutic approaches based on targeting microRNAs.

Methods: Hematoxylin-eosin stained FFPE sections of human enchondroma and chondrosarcoma were obtained from the Henry Ford tumor archive, the grade confirmed and new curls of approximately 10 paraffin sections used for RNA extraction. RNA was also extracted normal human articular cartilage from a large series of donors, ages 18 to 80; and from rat chondrosarcoma developed by subcutaneous injection of rat chondrosarcoma cells using the miRNeasy mini kit (Qiagen, CA) with modifications adapted in our laboratory. MicroRNA expression was profiled using Low Density TaqMan microRNA arrays using an Applied Biosystems 7900 HT thermocycler. Raw cycle threshold (CT) values were calculated using SDS 2.3 and RQ manager 1.2 software (Applied Biosystems) applying automatic baselines and threshold settings. The CT values were imported into StatMiner 4.2 (Integromics Inc., Philadelphia, PA) for global normalization of each sample with microRNAs ordered by hierarchical clustering. These analyses identified several microRNAs that showed differences in expression between normal tissues, enchondromas and chondrosarcomas and importantly several microRNAs that showed very consistent expression across all samples analyzed. Array analyses were confirmed by independent RT-PCR. MicroRNAs selected as invariant across groups were used to normalize expression values.

Results: Good-quality RNA for array analysis was isolated from FFPE tissues and analysis showed very similar array profiles with RNA isolated from newly dissected tissue from the same chondrosarcoma. Extraction of RNA from the FFPE section was performed approximately one year after isolation of RNA from the newly dissected tissue demonstrating that storage of the paraffin embedded tissue did not affect microRNA levels. Array analysis identified approximately 250 microRNAs expressed by chondrosarcomas and enchondromas. As shown in Figure 1 expression levels in of selected microRNAs in normal cartilage were generally similar to that in enchondromas. Several microRNAs showed much higher expression levels in chondrosarcomas compared with enchondromas. This was statistically significant for miR-181a. MiR-320 showed a higher expression level in enchondromas than chondrosarcomas. However, expression levels in enchondroma were highly variable and will require analysis of additional samples.

The relative expression of the selected microRNAs in rat chondrosarcoma compared with normal rat cartilage was very similar to the relative expression in human chondrosarcoma and normal cartilage (Figure 2).

Discussion: Sufficient RNA can be extracted from archived paraffin sections of chondrosarcomas and enchondromas to enable analysis of microRNA profiles and overcomes the difficulty in obtaining fresh tissues. The data presented suggest microRNA analysis can provide very valuable biomarkers able to distinguish enchondromas from low grade chondrosarcoma. The data presented are consistent with published analysis demonstrating the value of microRNA analysis for characterizing tumor progression. The increased expression levels of miR-21 seen with chondrosarcomas have been previously associated with the development of a variety of tumors, such that miR-21 has been termed and oncoMir. Expression levels of microRNAs frequently
show apparently contradictory changes; increased expression indicative of an oncogenic effect, in some tumors and decreased expression indicative of a tumor suppressive effect in others. MiR-181a is an example of these, suggested to be a tumor suppressor for leukemia but highly expressed and predictive of pancreatic cancer in published studies and chondrosarcoma development in our analysis.

The consistency in microRNA analyses observed between the rat and human chondrosarcomas suggest the rat model can be used to characterize the function of microRNAs in chondrosarcomas and to investigate the potential of therapeutic strategies targeting microRNAs such as miR-181a.

Figure 1. MicroRNA analysis in normal articular cartilage, enchondroma and low (c’sarc G1) and high (c’sarc G2). MicroRNAs were analyzed by TaqMan RT-PCR using commercially available primers. Values indicated are fold change and standard error compared with average value in normal articular cartilage. Expression levels of miR 181a in low grade chondrosarcoma compared to enchondroma were statistically significant (p=0.0005, two tailed t-test). N=number of patient samples analyzed.

Figure 2. Analysis of miRs 181a and 320 in rat normal cartilage and chondrosarcoma. Values are average and standard error compared with normal cartilage.

Significance: MicroRNA signatures could provide the differential diagnosis between benign and malignant chondroid lesions that is currently well recognized as difficult. The ability to sample chondroid lesions, with a reliable marker to distinguish benign vs. malignant cartilage would provide an extremely important tool to the clinician in the treatment of these relatively common lesions.

Acknowledgments: none

References: