The Epigenetic Regulation Of SOX9 By MiR-145 In Human Chondrosarcoma

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Introduction: Chondrosarcoma is a malignancy of cartilage and is the most common primary bone malignancy in the adult population [1]. Chondrosarcoma is a unique bone sarcoma in that it does not respond to chemotherapy or radiation treatment. Thus, survival rates in this patient population are low with a high chance of developing lung metastases [1]. Chondrosarcoma is treated with surgical excision where feasible, often carrying a very high morbidity rate, and an attempt at reconstruction [1]. There exists a dire need to develop systemic treatments to target chondrosarcoma cells and prevent metastatic spread. Our lab has previously shown that the ability of malignant cartilage cells in chondrosarcoma to invade calcified bone matrix is due, at least in part, to matrix metalloproteinase-2 (MMP-2), which is in turn regulated by its upstream transcription factor, ETS transcript variant 5 (ETV5) [2]. It has been shown that the SRY-related high mobility group-Box gene 9 (SOX9) is an upstream regulator of ETV5 in kidney development [3], as well as the master regulator of chondrogenesis [4]. Epigenetic regulation of SOX9 may involve microRNAs (miRNAs), specifically miRN-145 [5]. In this study, we hypothesized that the lack of miR-145 epigenetic suppression of SOX9 in chondrosarcoma cells results in SOX9 overexpression and downstream upregulation of ETV5.

Methods: Cell cultures: The human chondrosarcoma cell line JJ012 (JJ) was provided as a generous gift by Dr. Joel Block (Rush University, Chicago, IL), and human chondrocyte cells CHON-002 (CHON) were used as a control cell line. Four chondrosarcoma patient samples were collected at the time of surgery.

Transfection of miR-145 lentivirus: The miR-145 CMV 3rd generation lentiviral vector containing GFP was amplified in bacteria, packaged in 293T cells, and infected into chondrosarcoma cells.

GFP fluorescent images: Fluorescent images were taken from the miR-145-transfected chondrosarcoma cells.

Real time PCR analysis: Cells were lysed and total RNA was extracted for real time PCR analysis using the TaqMan MicroRNA with hsa-miR-145 TaqMan probes on the mature miR-145 sequence to quantitate only mature miRNAs. The expression levels of SOX9, ETV5 and MMP-2 in chondrosarcoma cells transfected with miR-145 lentivirus were determined using real-time PCR as previously described [6]. miRNA expression levels in chondrosarcoma patient samples were also determined by real time PCR. GAPDH & RNU48 (stably expressed snRNA) were used for normalization.

Results: The expression of miR-145 in all four chondrosarcoma patient samples was significantly down-regulated compared to the chondrocyte control cell line, CHON (Figure 1). Using stable lentiviral transfection of miR-145, the effect of miR-145 expression on the subsequent mRNA expression of SOX9, ETV5, and MMP-2 in chondrosarcoma cells was examined. In chondrosarcoma cells, GFP expression was observed at day 1 (Figure 2) following infection with the miR-145 recombinant lentivirus (titer of 1×108 TU/ml; MOI of 5), confirming that the lentiviral infection of the chondrosarcoma cells was effective and stable. MiR-145 expression was significantly upregulated (approximately 3-fold) in the lentiviral-
transfected chondrosarcoma cells Conversely, the mRNA expression levels of SOX9, ETV5 and MMP-2 were significantly downregulated compared to the negative plasmid-transfected control (Figure 3).

Discussion: The clinical challenge for chondrosarcoma is to identify treatment options for unresectable or metastatic disease. SOX9 plays a pivotal role in chondrocyte differentiation [4], and it is over-expressed in chondrosarcoma. Since our lab has previously shown that ETV5 has a significant role in regulating MMP2 expression [2], it is important to determine if SOX9 regulates ETV5. From our previous work (data not shown) we found that SOX9 up-regulates ETV5 activities at the promoter region in chondrosarcoma. In this study, the baseline expression of miR-145 was found to be down-regulated in chondrosarcoma cell lines (data not shown) and patient samples. After stable miR-145 lentiviral transfection, the subsequent mRNA expression levels of SOX9, ETV5 and MMP-2 were significantly decreased in chondrosarcoma cells. The results generated by this study may have important clinical significance in the treatment of patients with chondrosarcoma in that targeted miRNA may have the potential to downregulate the upstream activators of proteases such as MMP-2.

Significance: Results from this study would close the loop between the invasive properties of chondrosarcoma and the upstream epigenetic regulation of SOX9. The expression of miR-145 is aberrantly low in chondrosarcoma, hence the lack of inhibition of SOX9, leading to downstream activation of ETV5 and MMP2, and subsequent bone matrix invasion. Several diseases are currently being approached with therapeutic miRNA [7] and therefore, the information generated by this project is likely to have important clinical significance in the treatment of patients with chondrosarcoma in that targeted miRNA may have potential to decrease chondrosarcoma cell invasion and metastasis.
Figure 1: The intrinsic expression level of miR-145 in four chondrosarcoma patient samples was significantly lower (decreased by 55-95%) compared to the chondrocyte control cell line CHON.
Figure 2: Representative photos taken using fluorescent microscopy showed successful miR-145 recombinant lentivirus infection in the chondrosarcoma cells.
Figure 3: mRNA expression of SOX9, ETV5 and MMP-2 genes were depleted in miR-145-overexpressed chondrosarcoma cells. Normalized fold changes of three repeats with $p < 0.05$ were plotted (*).