Final riport: Examination of the regulatory SMARCB1 gene microRNA in soft tissue sarcomas

The importance of miRNAs in cancer is well recognized and an increasing number of reports have shown that miRNAs are differentially expressed in many cancers. Indeed, miRNAs have been shown to be relevant to the biology of many soft tissue sarcomas, including rhabdomyosarcoma, liposarcoma, osteosarcoma, angiosarcoma, Ewing sarcoma, and synovial sarcoma. Our aim was to reveal the role of miRNAs as epigenetic regulators in different soft tissue sarcomas. Our results can be found and is summarized in our article: Sápi Z, Papp G, Szendrői M, Pápai Z, Plótár V, Krausz T, Fletcher CD: Epigenetic regulation of SMARCB1 By miR-206, -381 and -671-5p is evident in a variety of SMARCB1 immunonegative soft tissue sarcomas, while miR-765 appears specific for epithelioid sarcoma. A miRNA study of 223 soft tissue sarcomas, Genes Chromosomes Cancer. 2016 Oct;55(10):786-802., 2016

Complete/partial loss of SMARCB1 nuclear-immunopositivity is characteristic of a certain subset of soft tissue sarcomas. A miRNA study was conducted using 51 epithelioid sarcomas, 20 rhabdoid tumors, 20 synovial sarcomas, 15 malignant peripheral nerve sheath tumors, 11 myoepithelial carcinomas, and 10 extraskeletal myxoid chondrosarcomas with complete/partial loss of SMARCB1 nuclear immunostain, in contrast to controls (SMARCB1-immunopositive) of 96 soft tissue sarcomas, 13 melanomas and 10 sarcomatoid carcinomas. Methodology included as follows: Immuno-histochemistry, FISH, SMARCB1 MLPA, qRT-PCR, miRNA in Situ Hybridization and miRNA Next-Generation Sequencing. A subset of epithelioid sarcomas showed biallelic deletion of SMARCB1 with no overexpression of any miRNA, suggesting these tumors could be the counterpart of pediatric rhabdoid tumor, at least genetically. Another subset was genetically either intact or monoallelic deleted with at least threefold overexpression of one of miR-206,-381,-671-5p, suggesting epigenetic regulation only. 39/51 epithelioid sarcomas had a biallelic deletion, but with overexpressed miR-206,-381, and 671-5p, suggesting intratumoral heterogeneity, i.e., both genetic and epigenetic regulation. At least threefold overexpression of one of miR-206,-381, and 671-5p was detected in all MPNSTs, EMCSs, SSs and 7 MCs. There was no event above threefold overexpression of miR-765 among all 195 tested tumors. Our results suggest a general role of miR206,-381, and 671-5p in SMARCB1 gene silencing of ES, MC, EMCS, MPNST and SS. In the future, miR-765 could possibly be a diagnostic tool for ES because of its 97% specificity and 80% sensitivity.

Further investigating the most important miR-206 we conducted another study; results can be found and is summarized in our second article: The oncomir face of microRNA-206: A permanent miR-206 transfection study. Dora Mihaly, Gergo Papp, Zsolt Mervai, Andrea Reszegi, Peter Tatrai, Gabor Szaloki, Johanna Sapi and Zoltan Sapi

MiR-206 is a remarkable miRNA because it functions as a suppressor miRNA in rhabdomyosarcoma while at the same time, as previously showed, it can act as an oncomiRNA in SMARCB1 immunonegative soft tissue sarcomas. The aim of this study was to investigate the effect of miR-206 on its several target genes in various human tumorous and normal cell lines. In the current work, we created miR-206-overexpressing cell lines (HT-1080, Caco2, iASC, and SS-iASC) using permanent transfection. mRNA expression of the target genes of miR-206 (SMARCB1, ACTL6A, CCND1, POLA1, NOTCH3, MET, and G6PD) and SMARCB1 protein expression were examined with quantitative real-time polymerase chain reaction, immunoblotting, immunocytochemistry, and flow cytometry. MiRNA inhibition was used to validate our results. We found a diverse silencing effect of miR-206 on its target genes. While an overall tendency of downregulation was noted, expression profiles of individual cell lines showed large variability. Only CCND1 and MET were consistently downregulated. MiR-206 had an antiproliferative effect on a normal human fibroblast cell line. A strong silencing effect of SMARCB1 in miR-206 transfected SS-iASC was most likely caused by the synergic influence

of the SS18-SSX1 fusion protein and miR-206. In the same cell line, a moderate decrease of SMARCB1 protein expression could be observed with immunocytochemistry and flow cytometry. In the most comprehensive analysis of miR-206 effects so far, a modest but significant downregulation of miR-206 targets on the mRNA level was confirmed across all cell lines. However, the variability of the effect shows that the action of this miRNA is largely cell context-dependent. Our results also support the conception that the oncomiR effect of miR-206 on SMARCB1 plays an important but not exclusive role in SMARCB1 immunonegative soft tissue sarcomas so it can be considered important in planning the targeted therapy of these tumors in the future.