Twisting Microrna Expression to Resist Cell Death in MDS

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Myelodysplastic syndromes (MDS) are an array of blood disorders that result in defective maturation of one or multiple blood cell lineages, including white blood cells, red blood cells, and platelets. About one-third of patients with MDS progress to acute myeloid leukemia (AML). MDS is a clonal disorder thought to arise by mutations in bone marrow stem cells, although the precise causes are largely unknown. Both mutations in MDS cells, and interactions with the bone marrow microenvironment contribute to the broad range of clinical manifestations of MDS. Many cytokines and signals are upregulated in the MDS bone marrow microenvironment that can induce apoptosis, including tumor necrosis factor α (TNFα). Clonal MDS cells are less responsive to these signals in early stage MDS and grow increasingly resistant to apoptosis in advanced stages of disease, outcompeting normal cells for space in the marrow. To uncover the mechanism of apoptosis resistance, researchers from Dr. Joachim Deeg's lab in the Clinical Research Division, including lead author and postdoctoral fellow Dr. Xiang Li and Associate in Clinical Research Dr. Mario Marcondes, have elucidated how the interaction between the bone marrow microenvironment and precursor MDS cells modulates expression of the transcription factor TWIST-1 to regulate microRNA expression and apoptotic signaling.

In a previous study (Li et al., 2010), Dr. Li had shown that expression of the transcription factor TWIST-1 is increased in MDS precursor cells. TWIST-1 negatively regulates apoptosis by binding to and inhibiting the pro-apoptotic protein p53. Primary MDS cells or myeloid cell lines with high levels of TWIST-1 expression were co-cultured with a stromal cell line and treated with TNFα to replicate the bone marrow microenvironment. Upon co-culture, TWIST-1 expression was decreased and p53 expression increased in the MDS cells, which in turn increased TNFα-induced apoptosis of the co-cultured MDS cells. Silencing of TWIST-1 expression by RNA interference further enhanced TNFα-induced apoptosis in cells co-cultured with stroma. This effect was partially mediated through p53-dependent expression of the pro-apoptotic protein BAX.

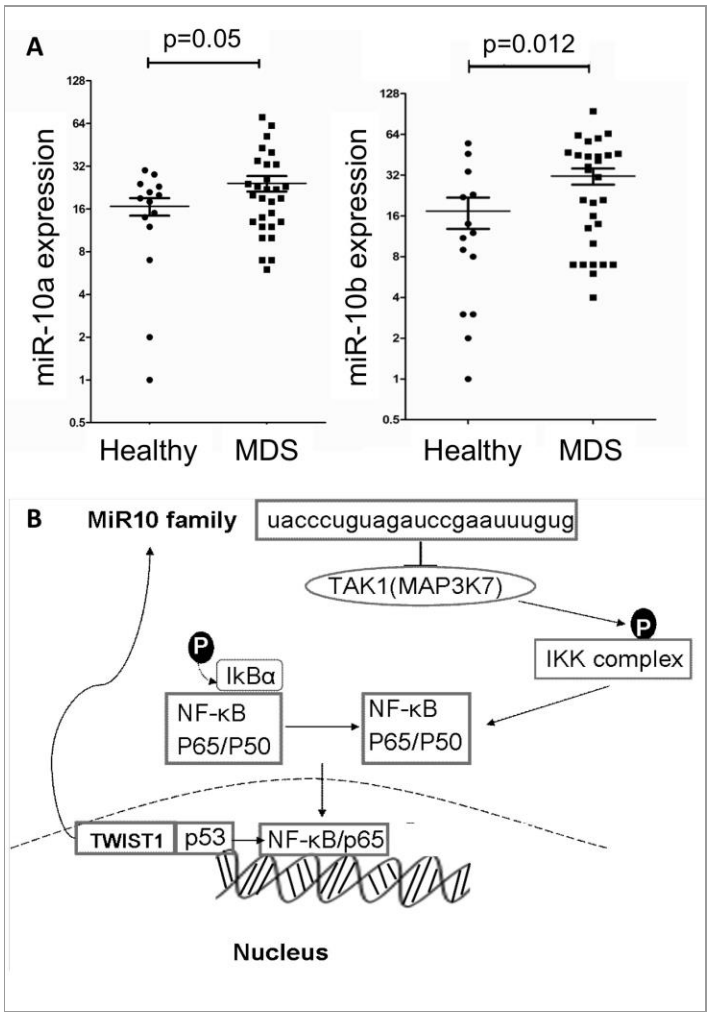
In the current study, Li et al. examined MDS patient cells for changes in expression of microRNAs (miRs), small non-coding RNAs 19-25 nucleotides in length. MiRs regulate a variety of cell processes, including hematopoiesis and apoptosis, by fine-tuning protein expression through mRNA

degradation or inhibition of protein translation. Levels of miR-10a and miR-10b were significantly higher in 28 MDS patient samples compared to normal bone marrow cells sorted for the hematopoietic precursor cell surface marker CD34 (miR-10a p=0.05, and miR-10b p=0.012). TWIST-1 is a known transcriptional regulator of miR-10a/b expression, and the levels of miR-10a/b expression correlated with TWIST-1 protein levels in primary MDS cells (miR-10a, R=+0.69, p<0.0001; miR-10b, R=+0.56, p=0.0008) and in a subset of myeloid cell lines. Furthermore, silencing TWIST-1 expression by RNA interference in myeloid cell lines decreased miR-10a/b expression, confirming that miR-10a/10b expression is transcriptionally controlled by TWIST-1.

To examine the role of miR-10a/b in apoptosis resistance, miR-10a and miR-10b were stably knocked down in the myeloid cell lines KG1a and PL-21 and the cells were co-cultured with stromal cells. Apoptosis was significantly enhanced in the knock-down cells after exposure to TNFα. Importantly, miR-10a/b silencing increased activity of the p53 promoter, as well as p53-dependent expression of the pro-apoptotic proteins BAX and BID. This result was similar to the previous study involving TWIST-1 knockdown. To address how miR-10a/b specifically controlled p53 and apoptosis, the authors examined the transcription factor NF-κB, which modulates the ability of p53 to induce gene expression. They showed miR-10a/b specifically targets and decreases expression of the protein TAK-1, which controls the nuclear translocation and activity of NF-κB by altering phosphorylation of the inhibitor of NF-κB (I-κB). By decreasing TAK-1 levels, miR-10a/b blocks the activity of NF-κB and thus p53 induced apoptotic proteins. Taken together, elevated TWIST-1 expression increases miR-10a/b expression, which in turn inhibits p53 and NF-κB activity to mediate apoptosis resistance in MDS clonal cells. This regulatory network could be targeted therapeutically to treat advanced MDS.

Li X, Xu F, Chang C, Byon J, Papayannopoulou T, Deeg HJ, Marcondes AM. 2012.Transcriptional regulation of miR-10a/b by TWIST-1 in myelodysplastic syndromes. Haematologica. Epub ahead of print. doi: 10.3324/haematol.2012.071753

Also see: Li X, Marcondes AM, Gooley TA, Deeg HJ. 2010. The helix-loop-helix transcription factor TWIST is dysregulated in myelodysplastic syndromes. Blood. 116(13): 2304-14.



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Transcription factor TWIST-1 induces miR-10a/b expression in MDS clonal cells. A) RNA was harvested from CD34+ sorted precursor bone marrow cells from MDS (n=28) and healthy (n=13) patients. MiR-10a and miR-10b expression was significantly increased in MDS versus healthy cells as determined by Nanostring analysis. B) Schematic representation of the proposed regulatory network. TWIST-1 induces miR-10a/b expression, which in turn targets TAK-1 to block NF-KB and p53 activity to mediate apoptosis resistance in MDS cells.