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Anti-microRNA-378a enhances wound healing process by up-regulating integrin beta-3 and vimentin

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One criterion for microRNA identification is based on their conservation across species, and prediction of miRNA targets by empirical approaches using computational analysis relies on the presence of conservative mRNA 3'UTR. Because most miRNA target sites identified are highly conserved across different species, it is not clear whether miRNA targeting is species-specific. We aligned all 3'UTRs of fibronectin and observed significant conservation of all 20 species. Twelve miRNAs were predicted to target most fibronectin 3'UTRs, but rodent fibronectin showed potential binding sites for five different miRNAs. One of them, the miR-378, contained a complete matching seed-region for all rodent fibronectin, which could not be found in any other species. We have previously demonstrated that expression of miR-378 promoted tumorigenesis and angiogenesis by targeting human Fus-1 and Sufu. Consistent with this result, we found that ectopic expression of miR-378 inhibited cell differentiation and promoted cell invasion. To understand the specific targeting of miR-378 on fibronectin, we expressed miR-378 in mouse breast cancer cells and found that overexpression of miR-378 enhanced cancer cell proliferation, migration, invasion, and colony formation, resulting in inhibition of tumor growth. Induced expression of fibronectin produced opposite results, while silencing fibronectin displayed similar effects as miR-378. To understand how miR-378 works, we generated transgenic mice expression miR-Pirate378 can not only arrest the functions of mature miRNAs by binding to them but it can also induce the "mis-processing" of the target miRNA producing a non-functional truncated miRNA. This approach involves generating an expression construct that produces a RNA fragment with sixteen repeat sequences. The construct is named miR-Pirate or microRNA-interacting RNA producing imperfect RNA and tangling endogenous miRNA. The transcript of the construct contained mismatches to the seed region, and thus it would not target the potential targets of the miRNA under study. The homology of the construct is sufficiently high allowing the transcript to block miRNA functions. The functions of the construct were validated in cell cultures, in tumor formation assays, and in transgenic mice stably expressing this construct. We showed that miR-Pirate378 transgenic mice display enhanced wound healing. Expression of vimentin and β3 integrin, two important modulators of wound healing, is elevated remarkably in the transgenic mice. To explore the possibility of adopting this approach in gene therapy, we transfected cells with synthetic miR-Pirate and obtained the results we expected. The miR-Pirate, expressed by the construct or synthesized chemically, was found to be able to specifically pirate and silence a mature miRNA through its dual roles and thus could be clinically applied for miRNA intervention. Migration assays showed a greater mobility in the miR-Pirate378-transfected cells, which was due to up-regulation of vimentin and β3 integrin. Both molecules were confirmed as targets of miR-378, and thus their expression could be rescued by miR-Pirate378. Overexpression of vimentin also contributed to fibroblast differentiation, and up-regulation of β3integrin was responsible for increased angiogenesis. Treatment with miR-Pirate378 conjugated to nanoparticle enhanced wound healing in mice. Thus, we have demonstrated that knockdown of miR-378 could increase the expression of its target proteins, vimentin and β3 integrin, which accelerated fibroblast migration and differentiation *in vitro* and enhanced wound healing *in vivo*.

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