Effects of the antagomirs 15B and 200B in the altered healing pattern of diabetic wounds

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Diabetic subjects with non-healing ulcers have a reduced expression of vascular endothelial growth factor (VEGF). Genetically diabetic mice are an useful model for studying VEGF alterations in wound healing, because they also display an altered expression pattern of VEGF and of its receptor VEGF-R2. In diabetic wounds, miR-15b and miR-200b which respectively inhibit VEGF and VEGF-R2 expression are up-regulated, potentially reducing even further the impaired angiogenesis in diabetes. Therefore, we investigated whether antagomiRs directed toward miR-15b and miR-200b could improve wound repair in genetically diabetic mice (C57BL/KsJ-$m^{+/+}$ $Lept^{db}$).

Incisional and excisional wound models were produced on the back skin of female diabetic mice (db^{+}/db^{+}) and their normal littermates (db^{+}/m^{+}). After the incision, animals received in the wound edge, the antagomiRs (15b, 200b, or 15b+200b) at the dose of 10mg/Kg, or the vehicle. Mice were killed on day 7 and at time of complete wound closure. At day 7, we measured protein levels of VEGF, transglutaminase II, Angiopoietin-1, Cyclin D1, Cdk 6 and p-NF-kB. Furthermore, we evaluated mRNA expression of VEGF, VEGF-R2, Angiopoietin-1, its receptor TEK and. At 7 days and at time of completing healing, we also examined histological features of the healed wound tissue and the time to achieve a complete wound closure.

Diabetic mice showed reduced expression of VEGF, Cyclin D1, transglutaminase II, Angiopoietin-1 and p-NF-kB. Treatment with anti miR-15b and anti miR-15b+miR-200b increased mRNA expression of VEGF and VEGF-R2, and the protein levels of VEGF, p-NF-kB and Angiopoietin-1. These results suggested an improved regenerative pattern in the treated wounds, and in agreement with these observations also the histological evaluation indicated that antagomiRs improved the impaired healing pattern, along with a decreased time to complete skin healing.

In conclusion, these data suggest for the first time that the inhibition of miR-15b and miR-200b may have a potential application in diabetes-related wound disorders.