A new GDF-5 mutant with improved osteoinductivity combines positive features of GDF-5 and BMP-2 for bone repair in vitro.

INTRODUCTION:
Bone fractures still lead to a high number of complications, such as delayed fracture healing, non-union and infection. Since the autologous cancellous bone graft as the gold standard in pseudarthrosis therapy involves difficulties, like second and third interventions, prevalent revisions and acute pain, osteoinductive proteins are promising candidates for therapeutic intervention. Growth and differentiation factor (GDF)-5 and bone morphogenetic protein (BMP)-2 are members of the transforming growth factor (TGF)-β super family. Both proteins are osteoinductive mediators during bone repair. GDF-5 has a lower affinity to the osteogenesis mediating BMP type IA receptor (BMPR-IA) than BMP-2, but is assumed to support neovascularisation1-3, which is a major prerequisite for fracture healing.

As BMP-2 and GDF-5 are similar in their BMPR binding domain - varying only in 13 positions - we hypothesized that the exchange of distinguishing amino acids in the BMPR binding sequence of GDF-5 will enhance its receptor binding affinity and osteogenesis and therefore combine positive features for non-union therapy of both molecules.

METHODS:
We designed the mutant protein GDF-5M453V/M456V (GDF5mt) by exchanging methionine in GDF-5 versus valine, which is located at the corresponding positions in BMP-2. The biological activity of GDF-5, BMP-2 and GDF5mt was measured by quantification of alkaline phosphatase (ALP) activity on mouse premyoblastic C2C12 cells and human mesenchymal stem cells (MSC) of n=6 donors that were cultured under osteogenic conditions. Matrix mineralization was quantified by alizarin red staining of MSC monolayers and compared to MSC that were cultured in a dexamethasone, β-glycerol phosphate and ascorbic-2-phosphate free medium. The angiogenic inductivity of different GDF5mt concentrations was investigated by quantifying the secretion of the angiogenesis mediating proteins vascular endothelial growth factor (VEGF) and angiopoietin 1 (ANGPT1) by MSC under osteogenic conditions and compared to GDF-5 and BMP-2. Furthermore, effects of different concentrations of GDF-5, BMP-2 and GDF5mt on the proliferation of human umbilical vein endothelial cells (HUVEC) were examined by WST-1 assay.

RESULTS:
GDF5mt induces a dose dependant increase of ALP activity.
The ALP activity was increased in C2C12 cells and MSC treated with BMP-2 or GDF-5mt (p<0.02) while wild type GDF-5 did not effect ALP-levels. In lower concentration ranges of up to 50 ng/ml for MSC and up to 400 ng/ml for C2C12 cells, BMP-2 mediated ALP-activity levels were still higher (p>0.05) than those induced by equal GDF5mt doses. Though, in higher concentrations ALP induction of GDF5mt reached BMP-2 mediated levels in C2C12 cells (Fig. 1) and in MSC. The BMP-2 mediated ALP effect on MSC was not significant in the higher dosage of 500 ng/ml compared to untreated control cells. All MSC monolayers that received osteogenic differentiation medium had significantly higher alizarin red levels than cells cultured in basal medium without dexamethasone and β-glycerol phosphate (p<0.01). The growth factors did not have an additional effect on matrix mineralization.

High doses of 500 ng/ml BMP-2 reduce the secretion of angiogenic transmitter proteins. 50 ng/ml GDF-5, BMP-2 or GDF-5mt respectively did not influence the VEGF and ANGPT1 secretion levels of MSC. With a 10 fold higher dosage of 500 ng/ml BMP-2 the secretion levels of VEGF (p<0.05) and ANGPT1 (p<0.02) were significantly reduced in MSC monolayers compared to untreated control cells. In contrast, GDF-5 and GDF-5mt did not affect VEGF and ANGPT1 secretion levels of MSC independent from their dosage.

HUVEC proliferation is reduced by high doses of 500 ng/ml BMP-2. Low doses of 5 and 20 ng/ml GDF-5, BMP-2 or GDF5-mt respectively did not mediate effects on WST-1 reduction by HUVEC. With 500 ng/ml BMP-2 the proliferation of HUVEC was significantly decreased (p<0.05) compared to untreated control cells, while equal doses of GDF-5 or GDF5mt had no such effects.

DISCUSSION:
Groundwork for the present study were multiple experimental and clinical reports proving the high osteoinductive potential of BMP-2 in fracture healing1 and results allocating osteogenic2 and particularly angiogenic3-5 effects of GDF-5. Neovascularisation is eminent for bone regeneration and an affected angiogenesis may be a possible reason for the genesis of non-unions. In contrast to the studies cited above3,1 that worked with rodent cells and animal models, we could not approve an increase of angiogenic parameters by GDF-5 on human MSC and human endothelial cells, but present that 500 ng/ml BMP-2 decrease the VEGF and ANGPT1 secretion of these cells. Therefore, we conclude that high doses of BMP-2 may have negative effects on MSC regarding the secretion of angiogenesis mediating proteins and the metabolic activity of endothelial cells which are not observed after GDF-5 and GDF5mt stimulation. Since the newly-created GDF5mt mediated an enhanced osteogenicity of human MSC, but no suppression of the secretion of angiogenic factors by these cells, it seems a promising new molecule for application in bone repair - combining positive features of GDF-5 and BMP-2.

REFERENCES: