The engineered BMP-2 variant L51P promotes bone formation in vivo via inhibition of Noggin

INTRODUCTION:
Bone morphogenetic proteins (BMP) such as BMP-2 and BMP-7 are increasingly used to treat fracture non-union and to promote lumbar spine fusion. However, these proteins are being used in unphysiologically high concentrations to promote bone formation sufficiently. The necessity for high BMP concentrations may be due to the inhibitory action of BMP regulator proteins such as Noggin, Gremlin, and Dan partially antagonizing BMP mediated osteoinduction. Previously, the synthesis of an engineered BMP-2 variant called L51P has been reported [1]. L51P is deficient in type I receptor binding, whereas its overall structure and its binding to type II receptors and modulator proteins, such as Noggin, Gremlin, and Chordin, is unchanged. The modification in the amino acid sequence makes L51P a modulator proteins, such as Noggin, Gremlin, and Chordin, is insufficient. The necessity for high BMP concentrations may be due to unphysiologically high concentrations to promote bone formation in vivo.

METHODS:
To investigate whether L51P stimulates BMP-2 mediated osteogenesis by blocking the activity of BMP antagonists or by direct osteoinductive activity, primary murine osteoblasts were stimulated with (i) L51P, (ii) BMP-2, (iii) BMP-2+Noggin, and (iv) L51P+BMP-2+Noggin. Alkaline phosphatase (ALP) activity served as a measure for osteoblast differentiation. XTT assays were performed to quantify osteoblast proliferation.

The induction of bone formation by L51P was investigated in vivo. β-tricalciumphosphate (β-TCP) ceramics (cylinders, 5x6mm, 75% porosity, Mathys AG, Bettlach, Switzerland) were adsorbed with (i) 1µg BMP-2 + 1µg L51P, (ii) 1µg BMP-2 +3µg L51P, (iii) L51P, and (iv) L51P+BMP-2+10µg L51P. For positive controls, ceramics were adsorbed with 10µg BMP-2. Materials adsorbed with 1µg BMP-2 and unloaded materials served as negative controls. β-TCP cylinders were implanted into 6mm critical size diaphyseal bone defects in the rat femur (n=36, Wistar rats, female, retired breeders). Defects were stabilized with the RatFix® (AO Foundation, Davos) osteosynthesis system. Bone formation was monitored by x-ray after 1, 4, 8, and 12 weeks. The implants were harvested after 12 weeks. Bone formation was quantified histomorphometrically on 300µm McNeal tetrachrome stained ground sections. In vivo experiments were approved by the local committee for animal experimentation and were conducted in accordance with its regulations.

Data were statistically evaluated by ANOVA using SPSS® software for Mac (Version 16, SPSS Inc., Chicago, IL, USA).

RESULTS:
Murine osteoblasts exhibited high levels of ALP activity when stimulated with 64nM BMP-2. BMP-2 mediated induction of ALP activity was inhibited by addition of equimolar concentrations of Noggin. Additional treatment with 64nM L51P reconstituted the induction of ALP activity. 64nM L51P alone did not induce ALP activity in murine osteoblasts.

In vivo, insufficient bone formation was found with empty β-TCP cylinders and cylinders adsorbed with 1µg BMP-2. 10µg BMP-2 induced a strong stimulation of bone formation. Addition of L51P to 1µg BMP-2 resulted in a dose dependent promotion of bone formation. Carriers loaded with 1µg BMP-2 + 10µg L51P induced bone formation equivalently to carriers loaded with 10µg BMP-2.

DISCUSSION:
Inhibiting BMP antagonists is a promising option to promote bone formation by increasing the biological activity of endogenous and exogenous BMPs. This approach may decrease the amount of exogenous BMPs currently needed to augment bone regeneration. Furthermore, in situations with BMP upregulation such as fracture healing, inhibition of BMP antagonists may promote bone healing by increasing the activity of endogenous BMPs without the need of further BMP administration.

In this study, the engineered BMP-2 variant L51P promoted bone formation by enhancing the activity of exogenously added BMP-2 in vivo. Insufficient concentrations of BMP-2 induced a strong osteoinductive response if administered together with L51P. The in vitro data indicate that L51P stimulates osteoblast activity via inhibition of the BMP-2 antagonist Noggin without exerting a direct osteoinductive effect. It remains to be clarified whether L51P promotes bone formation in vivo by enhancing the activity of endogenous BMPs if administered without exogenous BMP-2. This question is the subject of ongoing experiments.

REFERENCES

Fig. 1: Primary murine osteoblasts were cultured in the presence (+Vit D3) or absence (-Vit D3) 1,25-dihydroxyvitamin D3. Cells cultured in the absence of Vit. D3 were stimulated with 64nM (i) BMP-2, (ii) L51P, (iii) BMP-2 + Noggin, and (iv) BMP-2 + Noggin + L51P. ALP and XTT assays were performed after 6 days of culture. Data are given as the relative optical densities (OD) of ALP/XTT assays. Data are shown as mean ± SD; t*p<0.001.

Fig. 2: β-TCP ceramics were loaded with BMP-2 (1µg, 10µg) or BMP-2 and L51P (1µg BMP-2 plus 1µg, 3µg, or 10µg L51P). Data are shown as mean ± SD. *p<0.001 vs. empty ceramics, BMP_1 (1µg BMP-2), and BMP_1/L51P_1 (1µg BMP-2/1µg L51P); t*p<0.001 vs. BMP_1/L51P_3 (1µg BMP-2/3µg L51P).