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N-Methyl Pyrrolidone Promotes Osteoblast Differentiation Impaired by Tumor Necrosis Factor-alpha

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Introduction

TNF-alpha is a pro-inflammatory cytokine that has a profound role in many skeletal diseases. Since it is known for its bone resorptive action and inhibition of osteoblast differentiation (1) new therapeutic methods to antagonize these effects are needed. NMP enhances bone formation induced by BMP and inhibits RANKL-induced bone resorption (2, 3). In the present study we investigated the effect of NMP on BMP-2-induced osteoblast differentiation in the presence of TNF-α.

Material and Methods

Pluripotent mesenchymal precursor C2C12 cells were exposed to BMP-2, TNF-α and NMP for various time periods. Cell differentiation was determined by monitoring expression of key osteoblastic markers. BMP-2, TNF-α and NMP signalling pathways were examined using Western blot analysis and different MAPK inhibitors. RT-PCR was used to determine Runx2 and TNF-α receptors mRNA levels.

Results

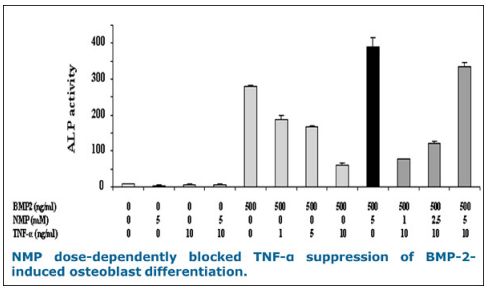

Fig. 1: Dose response of TNF-α and NMP on BMP-2 induced ALP-activity

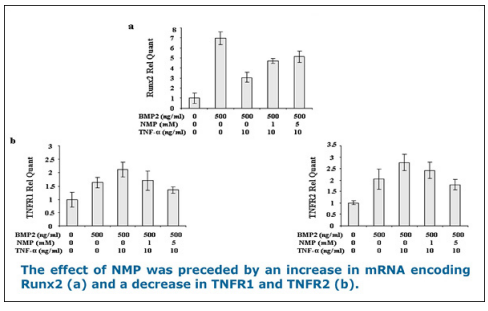

Fig. 2. NMP reverses the effect of TNF-α on Runx2, TNFR1 and TNFR2 mRNA expression

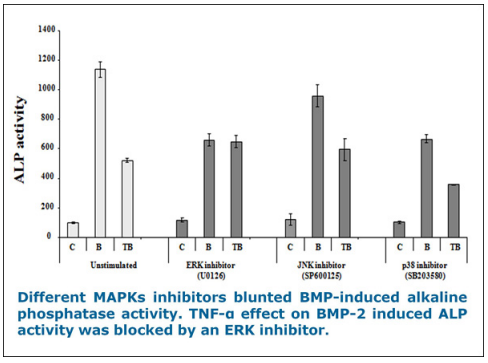

Fig. 3: Effect of MAPK inhibitors on TNF-α suppresion of BMP-2 induced ALP-activity

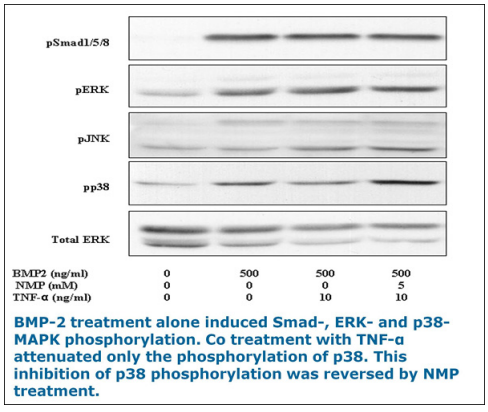

Fig. 4: Effect of NMP on Smad1/5/8 and MAPK activation

Conclusions

NMP represses the inhibitory effect of TNF-α on osteoblast differentiation through decrease in expression of TNFR1 and TNFR2. The involved inhibiting mechanisms of NMP are ERK and p38-MAPK dependent. However, further in vitro and in vivo studies are needed for verification.

Literature

1. Gilbert L, He X, Farmer P, Boden S, Kozlowski M, Rubin J, et al.: Inhibition of osteoblast differentiation by tumor necrosis factor-alpha. Endocrinology. 2000;141(11):3956-64.
2. Miguel BS, Ghayor C, Ehrbar M, Jung RE, Zwahlen RA, Hortschansky P, et al.:N-methyl pyrrolidone as a potent bone morphogenetic protein enhancer for bone tissue regeneration. Tissue Eng Part A. 2009;15(10):2955-63.
3. Ghayor C, Correro RM, Lange K, Karfeld-Sulzer LS, Graetz KW, Weber FE.: Inhibition of osteoclast differentiation and bone resorption by N-methyl pyrrolidone. J Biol Chem. 2011 Jul 8;286(27):24458-66.

Abbreviations

ALP = alkaline phosphatase
BMP = bone morphogenetic protein
ERK = extracellular-signal regulated kinase
GAPDH = glyceraldehyde 3-phosphatase dehydrogenase
JNK = C-Jun N-terminal kinase
MAPK = mitogen-activated protein kinase
mRNA = messenger ribonucleic acid
NMP = N-methyl pyrrolidone
RANKL = receptor activator of nuclear factor kappa-beta ligand
RT-PCR = quantitative real time reverse transcription polymerase chain reaction
TNF = tumor necrosis factor
TNFR = TNF-α receptor

This Poster was submitted by M Dent Med et MD Johann Malina-Altzinger.

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NMP Promotes Osteoblast Differentiation Impaired by TNF-Alpha

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Oral Biotechnology & Bioengineering, Division of Cranio-Maxillo-Facial and Oral Surgery

Introduction

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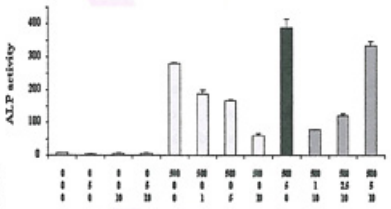
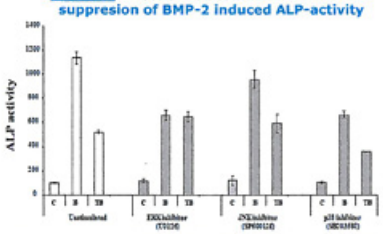
Results

Figure 1. Dose response of TNF-α and NMP on BMP-2 induced ALP-activity



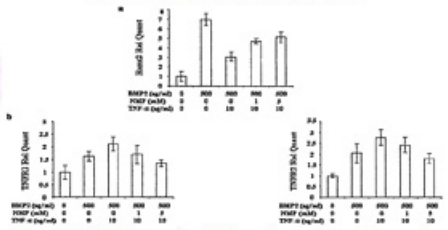
NMP dose-dependently blocked TNF-α suppression of BMP-2-induced osteoblast differentiation.

Figure 3. Effect of MAPK inhibitors on TNF-α suppresion of BMP-2 induced ALP-activity



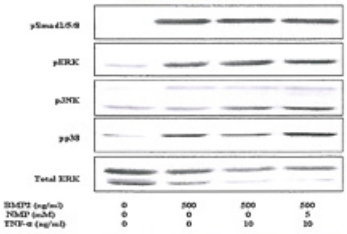
Different MAPKs inhibitors blunted BMP-induced alkaline phosphatase activity. TNF-α effect on BMP-2 induced ALP activity was blocked by an ERK inhibitor.

Figure 2. NMP reverses the effect of TNF-α on Runx2, TNFR1 and TNFR2 mRNA expression



The effect of NMP was preceded by an increase in mRNA encoding Runx2 (a) and a decrease in TNFR1 and TNFR2 (b).

Figure 4. Effect of NMP on Smad1/5/8 and MAPK activation



BMP-2 treatment alone induced Smad-, ERK- and p38-MAPK phosphorylation. Co treatment with TNF-α attenuated only the phosphorylation of p38. This inhibition of p38 phosphorylation was reversed by NMP treatment.

Conclusions

NMP represses the inhibitory effect of TNF-α on osteoblast differentiation through decrease in expression of TNFR1 and TNFR2. The involved inhibiting mechanisms of NMP are ERK and p38-MAPK dependent. However, further *in vitro* and *in vivo* studies are needed for verification.

References

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Abreviations

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