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Bone morphogenetic proteins, which includes growth / differentiation factor-5 (GDF-5), are multifunctional cytokines. Recent studies of intracellular signal transduction mechanism, for transforming growth factor- β superfamily have focus on Smad proteins, but have paid little attention to the mechanism by which GDF-5 exert its negative growth effect on immunological competent cell. In present study, we demonstrated that GDF-5 induced cell cycle arrest in the G1 phase before appearance of apoptosis in mouse B cell hybridoma HS-72. Moreover, we found that Smad6 and Smad7 suppressed GDF-5-induced apoptosis in HS-72 cells.

13. 骨芽細胞に対する PRP (Platelet rich plasma) の効果について

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PRP (Platelet Rich Plasma) 中に高濃度に含まれる, 血小板由来の各種成長因子には, 組織細胞の走化性や細胞増殖を促進し, 血管の増生, 骨芽細胞の活性化に影響するといわれている. そこで, ヒト由来培養細胞である, HOS, SaOS-2 の 2 種の細胞に PRP を投与して, 骨芽細胞の分化マーカーである ALP (alkaline phosphatase) 活性と細胞増殖活性に対する評価を行ったところ, PRP は培養初期では活性を抑制した. しかし, その後培養を続けたところ, ALP 活性の増強が認められた. また, 細胞増殖に関して MTT assay を用いて, 評価をおこなったところ, PRP の投与により細胞の増殖活性が認められた.

Effects of PPR (Platelet Rich Plasma) on human osteoblast like cells

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PRP has recently applied clinically in the field of oral and maxillofacial surgery for the efficacy of bone regeneration, bone augmentation, wound healing. In PRP various kinds of growth factors and cytokines derived from platelets such as TGF- β , PDGF, IGF, bFGF have been reported to be included a lot by some researchers. In this study we evaluated the effects of PRP on osteoblast like cells, HOS and SaOS-2 examining Alkaline phosphatase activity and cell viability using MTT assay. PRP seemed to enhance the cell viability soon after addition of PRP and have inhibitory effects of ALP activity in the initial periods.

14. グリコサミノグリカンによる破骨細胞誘導系の抑制作用についての研究

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細胞外マトリックスの主成分のひとつであるグリコサミノグリカン (glycosaminoglycan; GAG) は蛋白質と共有結合してプロテオグリカンを形成し, 細胞同士, あるいは細胞と細胞外マトリックスとの相互作用に重要な調節機構を営んでいる.

今回, 破骨細胞の前駆細胞である RAW 細胞およびマウス骨髄細胞の破骨細胞誘導系において, GAG であるコンドロイチン硫酸 A, B, D, E およびヘパリンを添加して培養を行った. TRAP 染色を行い, TRAP 陽性細胞数を計測したところ, RAW 細胞培養系ではヘパリンに, 骨髄細胞培養系ではコンドロイチン硫酸 B, E とヘパリンにおいて, TRAP 陽性細胞の形成が顕著に抑制された. 現在, その抑制メカニズムを検討しているところである.

Inhibitory effect of glycosaminoglycan on the formation of osteoclasts

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Glycosaminoglycan (GAG) is a component of extracellular matrix, and plays an important role in bone formation, resorption and mineralization in vivo and in vitro. In the present study, we examined the effects of GAG, chondroitin sulfate A, B, D and E, and heparin on the osteoclast formation in mouse marrow culture. Interestingly, chondroitin sulfate A, B, D and E, and heparin enhanced multinucleated osteoclast-like cell formation induced by receptor activator of NF- κ B ligand (RANKL). Studies are under way to define the GAG-activated pathways in osteoclastogenesis by RANKL.

15. ウサギ下顎骨仮骨延長中に発現した軟骨組織の免疫組織化学的観察

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仮骨延長法は整形外科領域で長管骨延長に用いられて発展し、現在では顎顔面領域においても盛んに臨床応用されている。長管骨の仮骨延長法における骨化過程に関する実験的研究では、膜性骨化のみであるとする報告と膜性骨化と軟骨内骨化が共に認められるとする報告がある。我々はウサギ下顎骨の仮骨延長法において膜性骨化、軟骨内骨化が共に認められ、待機期間を置かなければ、仮骨延長中に軟骨組織が多く認められることを報告した。しかしこの軟骨組織が軟骨として残るのか、また骨化するなどのような過程で骨化していくのかは不明である。そこで今回、仮骨延長中に発現した軟骨組織の動態を明らかにするために実験的研究を行った。ウサギ下顎骨を骨切りし、待機期間をおかずに1日0.5 mm、10日間延長し、計5 mmの延長を行った。延長終了直後、5日後、10日後の連続切片標本をそれぞれ作製し、H-E染色およびI, II型コラーゲンの免疫染色およびTRAP, ALPの酵素染色を行った。また、TUNEL法を用いて、仮骨延長法で生じた軟骨組織におけるアポトーシスを検出した。

An immunohistochemical study of cartilage during distraction osteogenesis in rabbits.

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Ossification during distraction osteogenesis is generally classified into two types: intramembranous and endochondral. The development of intramembranous or endochondral bone formation is influenced by different conditions. It is unknown whether the cartilage in the distraction gap is transformed into the new bone or not.

The purpose of our experimental work was to investigate the morphological features of the ossification that occurs during the transition of cartilage to bone in mandibular distraction osteogenesis using the rabbit model. The cortical osteotomy were performed, and the custom-made devices were applied. Immediately after surgery, the devices were lengthened 0.25 mm every 12 hours for 10 days. After the lengthening was completed, 4 rabbits were sacrificed and then same number at 5- and 10-day. Histological staining techniques together with immunohistochemistry were performed. Apoptotic cells were identified by means of an in situ detection assay for nuclear DNA fragmentation using a modified TUNEL procedure.

The cartilage in the distraction gap ossifies in the following two patterns: endochondral ossification and trans-chondroid bone formation. Apoptotic cells were discerned in the hypertrophic chondrocytes. The apoptosis is implicated to play a role in the process of the transition of the cartilage to bone in mandibular distraction osteogenesis.