Pleiotrophin, an extracellular matrix-associated protein, is an accessory protein / co-factor with multiple functions in bone development

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Pleiotrophin (PTN) is a secreted, extracellular matrix-associated, lysine-rich, 136 amino acid peptide. It is also called heparin-binding growth-associated molecule (HB-GAM), heparin affin regulatory peptide (HARP) or osteoblast-stimulating factor-1 (OSF-1). During embryonic and fetal development, *ptn* is widely expressed, but the post-natal expression is predominantly in the nervous system and bone. The cytokine has diverse functions, ranging from stimulating neurogenesis, cell proliferation and chemotaxis to tumor angiogenesis (reviewed by (1)). In contrast to the widely studied biological functions in neuronal tissues or as a tumor promoter, the roles of PTN in bone are not fully understood. Interest in a potential role as a bone-stimulating agent arose because bone matrix contains large amounts of PTN, PTN influenced BMP-induced ectopic osteogenesis and transgenic mice, which overexpressed the human *ptn* gene, had a higher bone mineral content.

To clarify the biological functions of PTN in bone, we combined experimental studies of the effects of exogenous PTN on bone cell and organ cultures with studies of transgenic mice, in which over-expression of *ptn* was targeted to osteogenic cells by the osteocalcin promoter. (The mice were generously provided by Prof. Tam Hashimoto-Gotoh, Kyoto).

**PTN localized to sites of new bone formation and in skeletal muscle**

Immunostaining of paraffin sections of wild-type and transgenic mice demonstrated that PTN was synthesized by osteoblasts, then stored in the bone matrix. PTN was also present in skeletal muscle and syndecan-3, a possible receptor for pleiotrophin, co-localized with the peptide. These results confirmed previous work that PTN, like other bone growth factors, is secreted from osteoblasts into the extracellular matrix, presumably for future use during the remodelling process.

**PTN stimulates chemotaxis of osteoprogenitor cells**

Patterned surfaces were generated by placing EM grids on tissue culture plastic coated with PTN and irradiated with UV light, which destroyed PTN in exposed regions. Human bone marrow cells, seeded onto the patterned surface, migrated to areas of intact PTN after 24 hours, whereas no migration was observed in the absence of PTN. This chemotactic property of PTN would be important in vivo, because matrix-bound PTN could play a role in recruiting osteoprogenitors.

**PTN enhanced proliferation and stimulated osteogenic differentiation of bone marrow cells**

PTN was added to cultures of mouse or human bone marrow cells. Cell proliferation was deduced from the total DNA/well and osteogenic differentiation was inferred from alkaline phosphatase (ALP) specific activity. PTN had a modest, but significant effect on cell proliferation, whereas the effects on differentiation depended on the timing and concentrations of PTN: High concentrations or presence during the first days of culture had no effect, but addition of only 10pg/ml during day 7-12 of culture increased ALP specific activity by 20-50%. These results suggest that PTN, at low concentrations, enhanced bone formation.

**PTN was not osteoinductive and inhibited BMP-2 mediated osteoinduction**

To determine whether PTN has osteoinductive potential similar to the BMPs, we utilised the fact that C2C12 cells, a murine pre-myoblastic cell line, can be driven toward the osteoblast lineage by BMP-2. Addition of 100 ng/ml of BMP-2 to culture of C2C12 cells for 2 days induced osteogenic differentiation, as indicated by the presence of numerous ALP+ cells. However, addition of PTN at concentrations between 5pg/ml and 100 ng/ml failed to induce the switch to the osteogenic pathway, thereby indicating that PTN was not osteoinductive. We then examined whether PTN could influence BMP-2 induced osteo-differentiation. When present together with 100 ng/ml of BMP-2 for
the first 2 days of culture, PTN inhibited osteoinduction by 65-75%, an effect that was apparent at concentrations as low as 0.05 pg/ml. This identified PTN as a previously unknown inhibitor of BMPs – a property that could be important in preventing inappropriate bone formation, which could be of relevance in fibrodysplasia ossificans progressiva (FOP).

**Effects of ptn overexpression on calvaria and long bones in transgenics**

We examined the structure of calvaria in 6-day old mice, which is the time point of most rapid calvarial growth. The width of calvaria of ptn transgenics (46.4 ± 3.5_m) was significantly greater compared with controls (24.3 ±4.6_m). However, no increase in cortical or cancellous bone could be demonstrated. At 15 weeks, the femurs of transgenics were slightly smaller compared to controls.

**Over-expression of ptn affected the bone growth trajectory**

We measured the bone dry weights at 5-weekly intervals up to 30 weeks, from which we determined the bone growth trajectories. There was no difference between transgenics and controls up to 10 weeks. Between 10-15 weeks, bone growth was rapid in control mice, which represented the pubertal growth spurt, but then levelled out. In ptn transgenics, the overall rate of bone growth was slower and there was no marked pubertal growth spurt. However, bone growth continued for a longer duration of time, until 25 weeks.

**Over-expression of PTN in articular cartilage was associated with the synthesis of type I collagen**

PTN was not present in articular chondrocytes in control mice. In these, type I collagen (bone collagen) was also detected in some, but not all, articular chondrocytes and encroachment of subchondral bone into the articular cartilage appeared to take place. PTN also induced the synthesis of type I collagen by chondrocytes during in vitro organ culture of chick nasal cartilage or isolated rat growth plates. Since PTN is not normally present in adult cartilage, the induction of this bone-type collagen probably represented an abnormal side-effect of non-physiological amounts of PTN in post-natal cartilage.

In summary, PTN is not a factor with a single role, but has multiple functions depending on the particular situation and/or other primary factors present. Because of these diverse roles, it is conceptually difficult to fully understand the functions of PTN. However, an emerging concept is that PTN is an accessory protein or cofactor that modulates the effects of primary signals, either enhancing or reducing the responses. After binding of PTN to a membrane receptor, signal transduction depends on the presence of other factors and/or receptors, most of which are still unknown.

(Diagram from ref. 6)