Regulators of growth plate maturation

Emons, J.A.M.

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SUMMARY

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Longitudinal bone growth takes place in the epiphyseal growth plates of the long bones and comparable structures in the vertebrae. Regulatory mechanisms within this epiphyseal plate are complex. Only in a minority of children with a growth disorder the aetiology can be definitely established and even in such cases a causal treatment is usually not available.

During puberty longitudinal growth rate first increases, but finally as the growth plate further matures growth decreases and eventually ceases with epiphyseal fusion at the end of puberty. Epiphyseal fusion results in a complete arrest in longitudinal growth ruling out every possible non-surgical treatment option to increase longitudinal growth and thereby adult height. Therefore, modulation of timing of epiphyseal fusion might be beneficial in the treatment of growth disorders; e.g. extending epiphyseal fusion can potentially result in an increase in adult height by allowing more time for growth supporting treatments, while initiating epiphyseal fusion would benefit patients with tall stature. Little is known about the exact mechanisms underlying growth plate maturation and epiphyseal fusion. In this thesis we investigated growth plate maturation and epiphyseal fusion in more detail.

With puberty estrogen levels increase and this is known to play an important role in growth plate maturation and epiphyseal fusion. We detected both estrogen receptor alpha (ERα) and beta (ERβ) in the rat growth plate, predominantly in late proliferating and early hypertrophic chondrocytes and observed a decrease in ERα and ERβ staining with estrogen suppletion. We investigated the genomic and non-genomic pathway by which estrogen can exert its effects on ovariectomized rats with desoxyestrone administration, a synthetic compound that exclusively acts through the non-genomic pathway, and 17β-estradiol administration. Treatment resulted in different degrees of growth inhibiting effects. We concluded that estrogenic effects on longitudinal growth are regulated both through genomic and nongenomic pathways with genomic signalling prevailing.

Estrogen treatment has potential side-effects, that can be circumvented with selective estrogen receptor modulators (SERMs). We investigated the effects of 2-methoxyestradiol and confirmed a tissue-selective effect in the growth plate of sexually immature male rats. However, we were not able to detect an effect on longitudinal growth.

Estrogen and its receptors interact with various growth factors in order to control longitudinal growth. In this thesis we demonstrated that Vascular Endothelial Growth Factor (VEGF) expression in rat chondrocytes was elevated by estrogen treatment *in vivo* and *in vitro*. Removal of estrogens by ovariectomy resulted in a decrease in VEGF expression. Moreover, pubertal human growth plate maturation resulted in an increase in VEGF expression, suggestive for a role of VEGF in the process of epiphyseal maturation as well as epiphyseal fusion.

Another effect of estrogen is acceleration of growth plate senescence, a term for structural changes like a decrease in height of different zones in the growth plate, but also for the decline in chondrocyte proliferation. We hypothesized that senescence might influence proliferation and the cell cycle of chondrocytes by orderly inactivation and activation of cyclin-dependent kinases and kinase inhibitors like p27Kip1 (p27). We did detect p27 mRNA in the growth plates of 5-wk-old mice by real-time PCR, but found no significant difference in p27 expression between the separate zones. In addition, we studied p27-deficient mice and found that p27 negatively modulates growth plate chondrocyte proliferation, but p27 was not required for the conditional regulation of chondrocyte proliferation as induced by dexamethasone in the growth plate of rats. In the human growth plate we did find a possible role for p27 in pubertal maturation of the growth plate, since microarray results showed an up-regulation in expression of the p27 gene with progression of puberty.

After a period of growth inhibition, the linear growth rate usually exceeds the normal range, which is known as catch-up growth. Evidence from animal studies suggests that catch-up growth is due, at least in large part, to a delay in growth plate senescence. We investigated catch-up growth in celiac patients and concluded that the pattern of catch-up growth in these patients is consistent with the hypothesis of delayed growth plate senescence.

Structural growth plate changes associated with senescence as described before in the rabbit and rat growth plate are in line with our data in a longitudinal study of two human growth plates obtained from one patient. Histological investigations showed a clear decrease in width of the total growth plate, more widely spaced columns with less cells and consequently more extracellular matrix in the more mature growth plate. Microarray results on human growth plates revealed for the first time the expression profile of human growth plate cartilage. Results from these experiments also confirmed our histological findings, since many genes and pathways related to the extracellular matrix significantly changed with maturation. In addition, progression of puberty affected many genes in the cell cycle pathway, hormonal pathways and programmed cell death. Moreover in this study we identified for the first time a set of 394 genes involved in growth plate maturation. The promoter regions of these genes contained transcription factor binding motifs for the Estrogen receptor, androgen receptor, STAT5B, ELK-1 and RUNX2. Particularly, ELK-1 and RUNX2 binding sites were enriched compared to controls suggestion a role for these transcription factors in pubertal maturation of the human growth plate. Overall changes in gene expression with pubertal maturation were small, indicating that there maturation is associated with small changes in multiple factors. This is in line with the senescence theory, suggesting that stem-like cells in the resting zone have a finite proliferative capacity, which is gradually exhausted resulting eventually in epiphyseal fusion.

The exact mechanism by which epiphyseal fusion occurs is not yet completely understood. We performed a detailed study on apoptosis in terminal hypertrophic chondrocytes in pubertal female growth plates and found no signs of classical apoptosis at the chondro-osseous junction. In addition, we studied a unique tissue specimen of a late pubertal human growth plate in the process of epiphyseal fusion and found clear evidence that apoptosis is not likely to be involved in the end phase of growth plate fusion. We did observe a dense border of thick bone surrounding the growth plate remnant and signs of hypoxia. We also found a significant increase in expression of the hypoxia-inducible factor 2 alpha gene from prepuberty to early and late stage puberty. Microarray results revealed that many genes of which the expression changed with pubertal maturation are involved in programmed cell death, e.g. proapoptotic and anti-apoptotic genes, but also genes involved in the regulation of autophagy. From these findings we speculate that growth plate fusion is a hypoxia related process eventually leading to a non-classical and perhaps intermediate mechanism of different types of cell death.

We investigated a promising and relatively new human model for the growth plate based on the use of human mesenchymal stem cells (hMSCs). hMSCs can differentiate into the chondrogenic lineage and bone marrow derived MSCs appeared to have the best chondrogenic potential, with fetal bone marrow prevailing over adult bone marrow. Fetal bone marrow-derived hMSCs showed to be an excellent model for the epiphyseal growth plate as during differentiation towards chondrocytes they gradually obtain a gene expression profile that was overlapping the fingerprint of epiphyseal growth plate cartilage. Many genes and pathways were identified as being important in different stages of chondrocyte differentiation. The hyaline cartilage formed by differentiating hMSCs resembled the profile of epiphyseal growth plate cartilage rather than of articular cartilage.