The role of PTHrP in chondrocyte differentiation.
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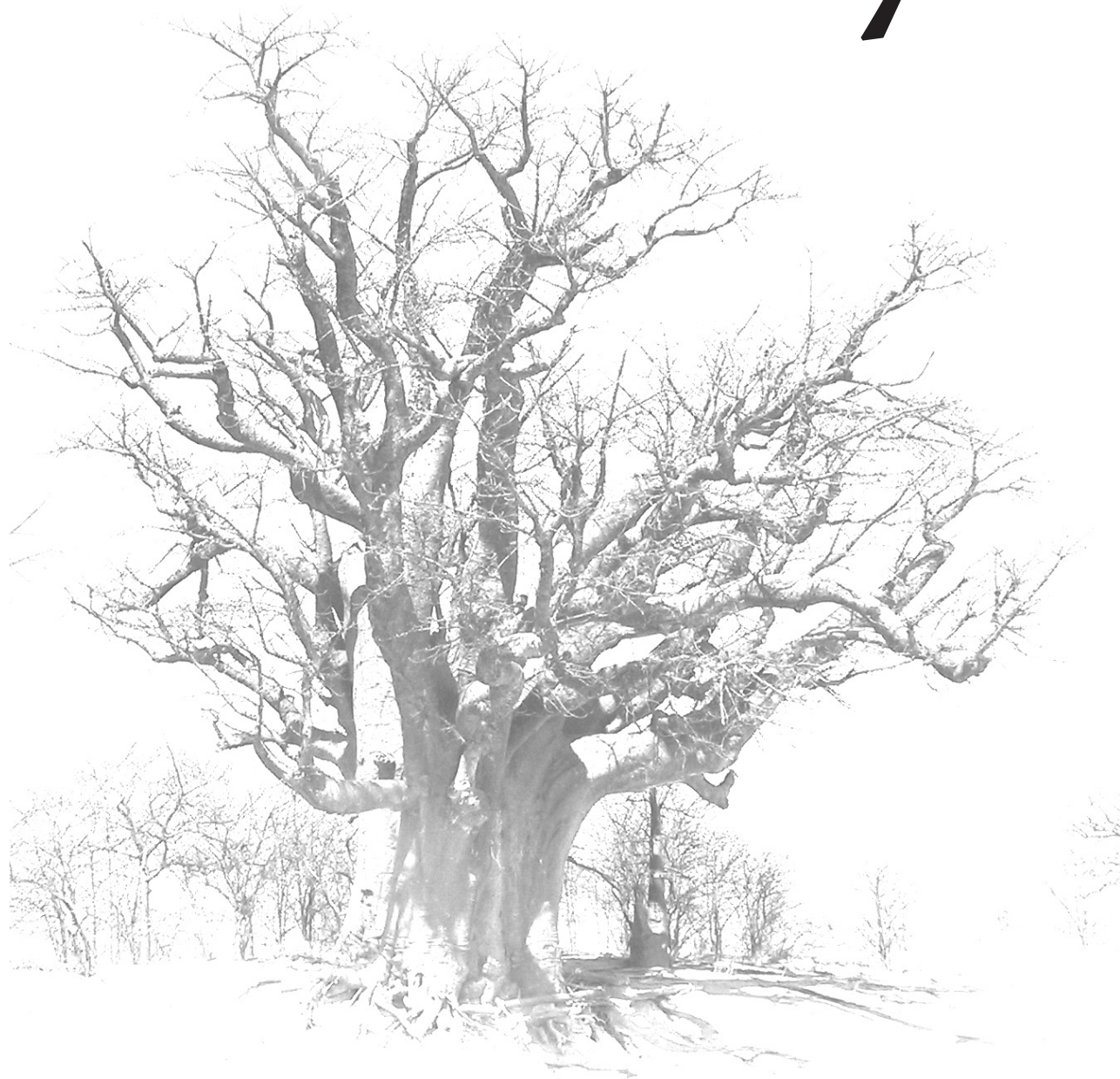Hoogendam, J. (2006, December 6). *The role of PTHrP in chondrocyte differentiation*. Ponsen & Looijen b.v., Wageningen. Retrieved from https://hdl.handle.net/1887/5422

General Discussion

7

Introduction

A network of endocrine and paracrine factors regulates the process of chondrocyte proliferation and differentiation in the growth plate. One of the key regulators involved in this process is Parathyroid Hormone (PTH) related peptide (PTHrP). This has been underlined by several studies, in human and mice, in which type 1 PTH/PTHrP receptor (PTHR1) signalling is interrupted or augmented[1-11]. The exact working mechanism of PTHrP signalling, alone or in combination with other growth factors or systemic hormones, in endochondral bone formation is not completely understood. In this thesis, we further addressed the actions of PTHrP in the complex network of endocrine and paracrine regulation of endochondral bone formation.

The first major result described in this thesis was that the heterogeneity of Blomstrand Osteochondrodysplasia (BOCD) is caused by the impact of a mutation in the PTHR1 gene on the receptor function. In addition, we demonstrated that the PTHR1 adaptor proteins, Na+/H+ exchanger regulatory factor 1 (Nherf1) and Nherf2, are expressed during endochondral bone formation and that they may play a role in matrix mineralization during osteoblast and chondrocyte differentiation. Furthermore, we identified novel early and late PTHrP target genes using cDNA microarray and quantitative PCR (qPCR) analysis. Finally, we showed for the first time interaction between PTHrP and the janus kinase (Jak)/signal transducer and activator of transcription (Stat) pathway.

In this chapter, these major findings will be summarized and critically reviewed.

Genotype-phenotype correlation in Blomstrand Osteochondrodysplasia

BOCD is a lethal osteochondrodysplasia characterized by severe skeletal malformations, due to accelerated endochondral bone formation[12-19]. It is caused by disruption of PTHR1 signalling[2;7-9]. BOCD can be divided into two types, type I and type II, depending on the severity of the skeletal abnormalities, with type I as the most severe phenotype[12]. The molecular basis for this heterogenic clinical presentation is unknown and, therefore, we have addressed the underlying causative factors in chapter 2. For this purpose, we performed mutation analysis in 2 families with type I BOCD and in 3 families with the less severe form, type II BOCD. The latter included 1 case that has not been described before.

Theoretically, there are two possible explanations of the variation in severity of the skeletal abnormalities. First, it is possible that the milder presentation of type II BOCD patients is caused by the genetic background. Support for this explanation comes from two sources. We have identified in two of the type II BOCD families the P132L mutation, which has already been found before in another family with type II BOCD. These families lived in the same region of England and originated from the same ethnic population[7;8]. In addition, it has been shown that the presentation of the phenotype of PTHR1 knockout mice depends on the genetic background[1]. For example, C57BL/6 mice die at mid-gestation, while Black Swiss mice die perinatally.

An alternative explanation is that the clinical severity depends on the degree of inactivation of the PTHR1. We have presented a novel family of different genetic origin, with another PTHR1 mutation causing type II BOCD, providing evidence that type II BOCD does not

depend on the genetic composition. Both mutations resulted in partial inactivation of the receptor with some residual activity. This is in contrast with the mutations identified in type I BOCD. These mutations, including the R104X described in chapter 2, resulted in a complete inactivation of the PTHR1[2;9]. Therefore, we concluded that type I BOCD resulted from a complete inactivation of the PTHR1, whereas a near complete inactivation of the PTHR1, resulting in low levels of residual activity, caused the relatively milder presentation observed in type II BOCD.

This phenomenon is not unique for BOCD. Gradations in the severity of a disease are also found in another syndrome arising from mutations in the PTHR1, namely Jansen's metaphyseal chondrodysplasia (JMC)[20]. This type of osteochondrodysplasia is caused by a constitutively activating mutation in the PTHR1 gene[4;6;11;21]. The T140P mutation caused the classical form of JMC[11]. Another mutation at the same amino acid position, T140R, resulted in ligand independent cAMP formation, which was less pronounced than observed with the T140P mutations[21]. This became clinically evident in a less severe presentation of JMC[21].

Heterogeneity in clinical manifestations due to the nature of the mutation is not only found in diseases arising from the PTHR1 gene. Several types of chondrodysplasia, including hypochondroplasia, achondrodysplasia and thanatophoric dysplasia (from less severe to most severe phenotype) arise from heterozygous mutations in the same gene, namely the Fibroblast Growth Factor (FGF) Receptor 3 (FGFR3)[22]. In these diseases a strong correlation between genotype and phenotype has been found[23].

Genotype-phenotype correlations have also been reported for campomelic dysplasia, a disease characterized by skeletal and also extra-skeletal abnormalities[24]. This disease is caused by mutations in the Sox9 gene, the main transcription factor for chondrocyte development. Both homozygous and heterozygous mutations have been described[25;26]. It has been stated that mutations, resulting in residual transactivation capacity of the transcription factor, may account for a milder phenotype and longer survival of patients[25].

Taken together, these data indicate that the impact of a mutation on the capacity of the receptor to activate downstream signalling pathways, critically determines the clinical presentation.

Regulation of PTHR1 signalling

In chapter 2 we showed that either the type or position of mutations in the PTHR1 resulted in distinct clinical features, due to complete or partial inactivation of the PTHR1. Partial inactivation resulted in residual activity of the PTHR1 and depending on the impact of the mutations, this residual activity could vary. This is also pronounced in patients with an activating mutation in the FGFR3, which can cause hypochondroplasia, achondrodysplasia and thanatophoric dysplasia[11;22].

Two pathways are activated by FGFR signalling in chondrocytes, the Jak/Stat pathway and the extracellular signal regulated kinase (ERK) pathway[27;28]. FGF is a potent inhibitor of chondrocyte proliferation and hypertrophic differentiation[29]. The dominant pathway after FGFR signalling influencing chondrocyte proliferation is the Jak/Stat pathway. FGFR signalling results in the activation of Stat1. Subsequently, Stat1 induces the expression of the cell cycle inhibitor p21$^{\text{waf1/cip1}}$, thereby inhibiting chondrocyte proliferation[29-31].

The ERK pathway appears to be more important in the inhibition of hypertrophic

differentiation. Transgenic mice that express a constitutively active mutant of ERK kinase (Mek1) in chondrocytes, displayed incomplete hypertrophy of chondrocytes, but showed no effect on chondrocyte proliferation[32]. Therefore, it has been hypothesized that FGFR signalling inhibits chondrocyte differentiation through the ERK pathway. Thus, the balance between the Jak/Stat pathway and the ERK pathway may adjust the effects of FGFR signalling on chondrocyte proliferation and differentiation[33]. Diverse mutations in the FGFR3 may have distinct impacts on the activity of the two main signalling pathways, which may result in the heterogeneity of the clinical presentation of chondrodysplasia.

Signalling through two pathways is also important in PTHR1 signalling[3;34]. Mouse knockout models have been generated to identify the functions of the two pathways that are activated by PTHR1 signalling in chondrocyte proliferation and differentiation. The two pathways are the adenylate (AC)/protein kinase A (PKA) and the phospholipase C beta (PLCβ)/protein kinase C (PKC) signalling pathway[3;34]. Recently, it has been shown that chondrocyte specific knockout mice, carrying a mutation in the Gαs and thereby disrupting AC/PKA signalling, displayed a phenotype comparable to the PTHrP knockout mice[34;35]. These mice had severe growth plate defects with reduction of the proliferative zone and accelerated chondrocyte differentiation. The opposite was demonstrated in mice carrying a mutant form of the PTHR1 (DSEL mice), which specifically interrupted signalling via the PLCβ/PKC pathway and signalled normally via the AC/PKA pathway[3]. These mice showed an increase in chondrocyte proliferation and a decrease in chondrocyte differentiation. These results indicated that the AC/PKA is the dominant pathway after PTHR1 signalling in stimulating chondrocyte proliferation and inhibiting chondrocyte differentiation. In addition, these results showed that chondrocyte differentiation is stimulated by the activation of the PLCβ/PKC pathway. Thus, the two pathways had opposite effects on chondrocyte proliferation and differentiation. In addition, these results showed that a disbalance between these pathways after PTHR1 activation can result in altered chondrocyte proliferation and differentiation and can cause heterogeneity of diseases, which are caused by mutations in the PTHR1.

The phenotype of the DSEL mice, in which PTHR1 signalling via the PLCβ/PKC pathway was specifically interrupted, was remarkably similar to the phenotype displayed by a consanguineous family with a rare growth disorder, the Eiken syndrome[3;36]. Eiken syndrome is caused by a homozygous mutation in the PTHR1 gene, resulting in a truncated PTHR1[5]. The truncated protein only missed a small part of the C-terminus. Because of the comparable phenotype of the mice model, it is hypothesized that PTHR1 signalling through the PLCβ/PKC pathway is disrupted. Eiken patients were considered normal at birth, but after a few months they developed a skeletal dysplasia, characterized by delayed ossification, principally of the epiphyses, the pelvis, the hands and the feet. Taken together, this indicates that the role of the PLCβ/PKC pathway becomes more pronounced after birth, while the AC/PKA pathway is the dominant pathway during prenatal growth, which has also been suggested previously[3;34].

Normal development of the growth plate requires a balanced signalling between the two main signalling pathways of PTHR1. This balance could be influenced by adapter proteins, like the PDZ domains containing proteins, Nherf1 and Nherf2. Nherf1 and Nherf2 were first found in renal tubuli as regulators of ion channels[37]. Further experiments revealed that the Nherf proteins not only bind to ion channels, but also to transcription factors, signalling molecules,

structural proteins and receptors[38;39]. The binding of Nherf1 and Nherf2 to the PTHR1 and PLCβ is best studied in renal tubuli[38].

In chapter 3 we showed that both Nherf1 and Nherf2 mRNAs were expressed in several organs using in situ hybridizations, which is in line with other studies[40;41]. In addition, we demonstrated Nherf1 and Nherf2 mRNA expression during endochondral bone formation by growth plate chondrocytes and osteoblasts in the bone collar. To establish whether Nherf1 and Nherf2 play a role in osteoblast and chondrocyte differentiation, Nherf1 and Nherf2 overexpressing KS483 cell lines were generated. Nherf1 and Nherf2 overexpression had distinct effects on the differentiation of osteoblasts and chondrocytes. While Nherf1 overexpression inhibited terminal osteoblast differentiation, Nherf2 overexpression stimulated terminal osteoblast and chondrocyte differentiation using matrix mineralization as a read out. The negative actions of Nherf1 in bone formation have been shown before in Nherf1 knockout mice[41]. Some, but not all female Nherf1 knockout mice were dwarfs and they displayed severe osteoporosis and bone fractures. This is most likely a secondary effect. Nherf1 regulates phosphate transport by binding to the sodium/phosphate co-transporter type IIa (Npt2a)[41]. In addition, Nherf1 null mice showed mild hypophosphatemia, which probably affected bone formation. However, our data suggests that the bone phenotype may also be explained partly by direct effects on osteoblastic matrix mineralization. Others have reported distinct functions for Nherf1 and Nherf2 in renal tubuli in the regulation of the Npt2[41-43]. The different functions for Nherf1 and Nherf2 might be explained by the recruitment of different molecules to form a membrane/submembrane bound complex, displaying diverse actions[40;44-46].

In chapter 3 we also showed that Nherf1 and Nherf2 overexpression did not change the actions of PTHR1 signalling on osteoblast en chondrocyte differentiation. We demonstrated, however, overexpression of Nherf1 and Nherf2 mRNA and it is likely that the Nherf1 and Nherf2 proteins are overexpressed as well, because both overexpressing cell lines displayed altered biological responses on osteoblast differentiation. Functional overexpression will probably induce a shift in the balance of PTHR1 signalling, since it has previously been shown that Nherf redirects PTHR1 signalling from the AC/PKA to PLCβ/PKC pathway in renal tubuli[38]. An explanation for the fact that we did not demonstrate an effect of Nherf1 or Nherf2 overexpression on PTHR1 signalling, could be inefficient coupling of Nherf1 and Nherf2 to the PTHR1 in osteoblasts and chondrocytes, in contrast to renal kidney cells. In addition, levels of PLCβ could be too low to affect the dominant AC/PKA pathway. The measurements of the activation of the AC/PKA pathway and the PLCβ/PKC pathway are currently underway.

Microarray and qPCR analysis

In chapter 4 and 5 we identified PTHrP target genes by performing microarray analysis. The microarrays, on which the NIA 15k mouse cDNA clone set[47] was spotted, were custom-made. The quality of the spots of the cDNA microarray was low, due to heterogeneous spot morphologies ("doughnuts"), deposition inconsistencies, and oversized spots. In addition, the quality control of cDNA microarrays is a well-known problem[48;49].

Halgren et al. reported a very high error rate of nearly 38% in the spots on cDNA microarrays[50]. The spots did not contain the cDNAs expected, because of contamination. Also in our study,

all spots containing the regulated cDNAs were sequenced and even a larger error rate was found. Approximately 55% of the cDNAs were not the cDNAs expected (unpublished data). The contamination is most likely introduced during multiple rounds of replication of the bank by PCR, as previously suggested[50;51]. Because of the uncertainty of the identity of the spots cDNA microarray analysis is not the ideal technique for pathway screening or genome wide analysis. For this purpose, commercially available oligonucleotide microarrays are much more reliable. cDNA microarray analysis is, however, a suitable technique for the identification of a subset of target genes, but only by applying very stringent selection criteria, by sequencing the regulated cDNAs, and by validation of regulated genes.

Since the cDNA microarray is used extensively in nearly all areas of biomedical research, investigators should always be aware of any indications that there may be serious unreliability in the microarray data. In addition, a major focus of microarray data appears to be centred on the statistical treatment of microarray data, such as image analysis, normalization, and background subtraction. However, more attention should be given to artefacts generated by amplification of the cDNAs and investigators should be especially cautious when interpreting data obtained from cDNA microarrays.

Microarray data, especially derived from cDNA microarrays, should be validated before jumping to conclusions. Bio-informatic analysis and techniques like Northern blot and qPCR analysis are mainly used for this purpose[52;53]. In our study we used bio-informatic analysis and qPCR analysis in different cell models and we thereby showed the validity of a part of the microarray data. However, due to the distinct methodologies and the relative poor quality of the custom cDNA microarrays, the overlap was not 100%, which is in line with other studies[52-54]. In our study the overlap in expression pattern between microarray and qPCR analysis was 57%. The expression pattern of the remaining genes differed only slightly between microarray analysis and qPCR analysis. In contrast to the expression patterns between microarray and qPCR analysis, which were comparable, the fold changes were dissimilar. A decrease, as well as an increase in fold change were demonstrated, which has also been reported by others[52-54]. cDNA microarray analysis is not a quantitative method in contrast to qPCR and Northern blot. Therefore, direction of changes, but not fold changes, can be determined with cDNA microarray analysis.

While the technical approach of qPCR is straightforward, the statistical analysis of qPCR data is less clear. A model providing computations for expression ratios and p-values in experiments in which all samples are run in triplicate in a single qPCR experiment, well organized in a single spreadsheet, is not available. For this purpose, we generated the double delta model (DDM), which is described in chapter 6. This model is derived from the $2^{-\Delta\Delta Ct}$ method and the variance of the gene of interest as well as the variance of the reference is taken into account[55]. The DDM is particularly useful when working with many samples, because the calculations of the ratios and the calculations of the p-values are well organized in a single spreadsheet. Because the DDM does not include corrections for qPCR efficiency, it can only be used when the amplification efficiencies of the target and reference gene are close to 1. The DDM may also be applied to other data sets, in which experimental values are correlated with reference values.

PTHrP target genes

To unravel how PTHrP exerts its effects on growth plate chondrocytes, we have identified early and late PTHrP target genes, using cDNA microarray analysis. By applying very stringent criteria for data generation and by performing validation studies we have produced a small list of early (12) and late (9) PTHrP response genes. The list of early PTHrP response genes included 7 upregulated (RGS2, SGK, Upar, IER3, Ptp4a1, Stat3, and Csrp2) and 5 downregulated (Sf3a2, Gab1, DYM, LamRI and Acvr2b) genes and the list of late response genes included 3 upregulated (IGFBP4, Csrp2, and Ecm1) and 6 downregulated (Col9a1, Col2a1, Agc, Hmgn2, Calm1, and Mxd4) genes. The majority of the early PTHrP response genes, 83%, were involved in signal transduction and regulation, compared to only 44% of the late PTHrP target genes. In addition, none of the early PTHrP response genes were structural proteins, compared to 44% of the late target genes. This is in agreement with the notion that the majority of early target genes are implicated in signal transduction and regulation and that most of late response genes code for structural proteins belonging to the extracellular matrix[54;56].

In chapter 4 we showed that the majority of early PTHrP effects were part of a more generalized response, not only restricted to chondrocytes, but also present in osteoblasts. In chapter 5 we demonstrated that the expression of most of the late response genes showed temporal and directional changes between the different cell models. This indicates that for studying the actions of PTHrP, the early response genes are most relevant. The late targets of PTHrP are not only manipulated by PTHrP treatment, but have also endured other influences, which are dependent of the cell type and environmental effects. This suggest that between early and late effects mechanisms must be operational that translate the PTHrP responses from a more generalized effect into a cell type and cellular context dependent effect. Results of other studies also indicate such a mechanism[54;56]. The factors involved in this process are not known.

Transcription factor binding sites in promoters of PTHrP target genes

To identify common conserved regulatory elements involved in the expression regulation of early PTHrP target genes, we analyzed their promoter regions. We found several conserved transcription factor binding sites in various early target genes. Among these transcription factor binding sites were cAMP response elements (CRE) and AP-1 response elements. CREs can be bound by CRE binding proteins (CREB). CREB and AP-1 are the main transcription factors activated by the AC/PKA pathway after PTHR1 signalling[3;57]. Remarkably, 6 out 7 of the upregulated genes in chondrocytes contained CREB transcription factor binding sites, 4 times in combination with an AP-1 response element, and only 2 out of 5 of the down regulated genes in chondrocytes contained CREB transcription factor binding sites. In addition, these 2 downregulated target genes were upregulated in osteoblasts. Comparable to our study were the predictions of CREB and AP-1 transcription factor binding sites predominantly in upregulated genes in a study by Qin et al.[58]. They used a statistical approach to identify transcription factor binding sites used by PTH-signalling in osteoblasts instead of enrichment for evolutionary conserved binding sites applied in this paper. This suggests that CREB and AP-1 are predominantly involved in upregulation of transcription of PTHrP target genes.

Among downregulated genes by CREB after PTHR1 signalling is the transcription factor for osteoblast differentiation and hypertrophic chondrocyte differentiation, the runt related transcription factor 2 (Runx2)[59;60]. The suppression of Runx2 transcription is primarily regulated through the AC/PKA signalling pathway[61]. This effect is only partially mediated via CREB, suggesting that other transcription factors are involved in the negative regulation of Runx2 transcription and probably also in the transcription of other downregulated genes[61].

PTHrP and the interactions with other paracrine and endocrine signals

Endochondral bone formation is under the control of endocrine (systemic hormones) and paracrine (growth factors) factors[29;62-64]. From the systemic hormones, the dominant regulator of endochondral bone formation is Growth Hormone (GH)[65]. GH can act directly on the growth plate, via its receptor, which is expressed by growth plate chondrocytes[66]. In addition, GH influences chondrocyte proliferation and differentiation indirectly via the induction of Insulin-like Growth Factor 1 (IGF-1)[67-69]. It is likely that GH also controls the expression of other locally produced growth factors, for instance components of the Indian Hedgehog (IHh)/PTHrP negative feedback loop[62]. In this thesis we provide evidence for the opposite, namely, the regulation of GH actions by PTHrP. Thus, the interaction between GH and PTHrP might be reciprocal.

Besides GH, another important regulator of chondrocyte differentiation in the postnatal growth plate is estrogen. Its main actions are inducing the growth spurt at the beginning of puberty and stimulation of growth plate fusion at the end of puberty[70;71]. While we demonstrated possible interactions between PTHrP and GH, no indications for interactions between PTHrP and estrogens were found in our limited data set.

In chapter 4 we demonstrated for the first time a crosstalk between PTHrP and members of the Jak/Stat family. PTHrP induced the mRNA expression of Stat3, Stat5a, Stat5b, and Csrp2. Csrp2 is a binding partner of PIAS1, which is an inhibitor of Stat1[72]. In addition, several conserved Stat transcription factor binding sites were predicted in the early PTHrP response genes. Furthermore, PTHrP influenced posttranslational activation of Stat3, Stat5a, and Stat5b. Interestingly, activation of Stat3 was increased, while the activation of Stat5a and Stat5b was decreased. Both the increased activity of Stat3 and the decreased activity of Stat5a and Stat5b after AC\PKA signalling have already been demonstrated before in other cell types[73;74].

Stat5b is the most important Stat protein in GH signalling, with respect to longitudinal growth[75;76]. GH induces IGF-1 expression through Stat5b and IGF-1 stimulates chondrocyte proliferation and differentiation[69;77]. By inhibiting Stat5b activity, PTHrP may diminish the positive actions of GH on chondrocyte proliferation and differentiation. Thus, we found that paracrine factors, in our study PTHrP, may influence the actions of endocrine signals, in our study GH. Our experiments were not set up to investigate whether endocrine signals influenced the actions of paracrine factors. This suggests that if indeed endocrine signals exert their effects by modulating paracrine factors, like PTHrP, it is not a one-way direction, but a reciprocal interaction.

Most Stat proteins are also involved in FGFR signalling. The negative regulation of chondrocyte proliferation by FGFR signalling is mediated through Stat1, by inducing the

expression of cell cycle inhibitor p21[waf1/cip1(30;31)]. Stat1 activation is inhibited by PIAS1, which is a binding partner of Csrp2[72]. By inducing the expression of Csrp2 (chapter 4 and 5), PTHrP could alleviate the inhibitory actions of PIAS1 on Stat1 and thereby increasing the negative regulation of chondrocyte proliferation. The inhibitory actions of FGFR signalling on chondrocyte differentiation is mediated through the ERK pathway[32]. No indications for interactions between PTHrP and the ERK pathway were found in our limited data set.

The role of PTHrP in the growth plate is regulating the transition of proliferating into hypertrophic chondrocytes and thereby keeping the chondrocytes in a proliferative competent stage[78]. It has been shown that p57, a member of the CIP/KIP family of inhibitors of cyclin-dependent kinases, is one of the major mechanism used by PTHrP to maintain chondrocyte proliferating and delay their differentiation[79;80]. Studies with transgenic mice demonstrated that the level of PTHrP is very important in normal endochondral bone formation. Both mice with ablation of the PTHrP gene or ectopic expression of PTHrP showed severe dwarfism[35;81]. The expression of PTHrP is tightly controlled. This is accomplished by IHh[62].

In this study we provide evidence that the biological actions of PTHrP might also be controlled by other regulators of endochondral bone formation. PTHrP may influence the signalling pathways of FGF and GH, thereby probably counteracting its positive effect on chondrocyte proliferation, but possibly enhancing its negative effect on chondrocyte differentiation (fig. 1). PTHrP might affect the actions of FGF signalling on chondrocyte proliferation and the actions of GH signalling on both chondrocyte proliferation and differentiation, through influencing Jak/Stat signalling. Through inhibition of the activity of Stat5b by PTHrP, IGF-1 expression may be diminished, presumably resulting in a decrease of chondrocyte proliferation and differentiation. In addition, by inducing Csrp2 expression, PTHrP is potentially able to alleviate the inhibition of PIAS1 on Stat1 activity. This may result in a decrease of chondrocyte proliferation. Experiments to establish whether Stat1 is directly activated by PTHR1 signalling are currently underway. In addition, conflicting data have been reported about Stat3 phosphorylation after FGF activation. Hart et al. found that FGFR signalling could activate Stat3 in the fibroblast cell line NIH3T3, however, another study using rat chondrosarcoma cells (RCS), revealed that FGF treatment had no effect on Stat3 phosphorylation[30;82]. Therefore, the role of Stat3 after FGF activation in this model is unclear.

The transition of proliferating chondrocytes into hypertrophic chondrocytes is under the control of the IHh/PTHrP negative feedback loop[62]. It has been proposed that FGF and BMP signalling affects the negative feedback loop. They show opposite effects on the expression of IHh, on chondrocyte proliferation and on terminal differentiation[29;63]. These actions are believed to be independent of the IHh/PTHrP pathway[29]. By showing that PTHrP influenced the Jak/Stat pathway, which signalling molecules are intracellular mediators of FGF signalling, we provide evidence that a cross talk between PTHrP and FGF signalling may exist.

Final remarks

PTHrP is an essential growth factor in the regulation of growth plate chondrocyte development. It works, however, in a complex environment of other growth factors and endocrine signals, which can influence either their own or each others expression and activity. Thus, the overall effect of the actions of systemic hormones and locally acting growth factors on chondrocyte

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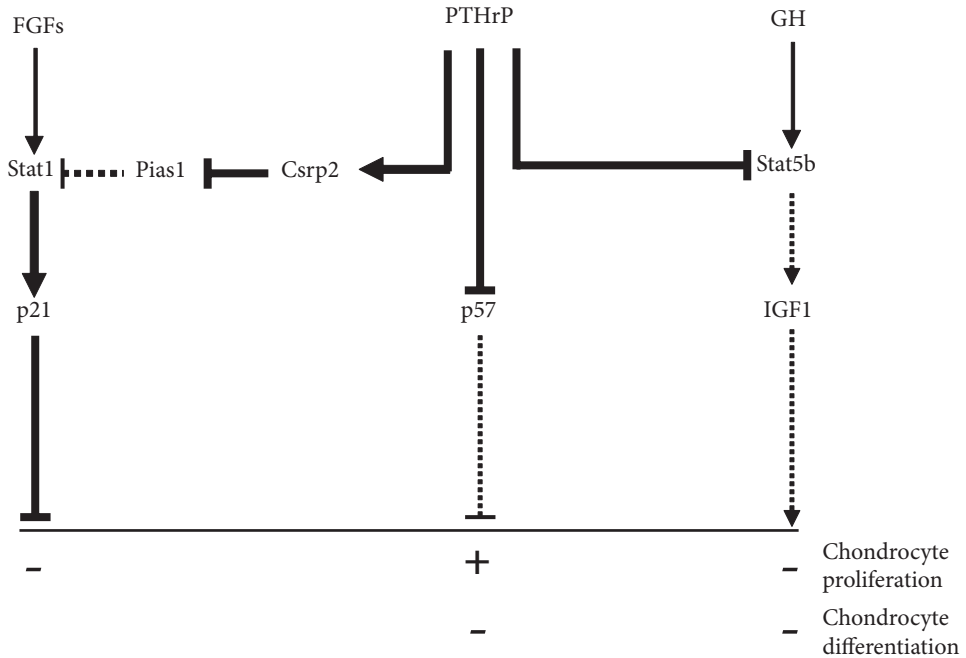
Figure 1: positive and negative actions of PTHrP on chondrocyte proliferation and differentiation.
PTHrP exerts its positive effects on chondrocyte proliferation through the suppression of p57 expression. We hypothesize that PTHrP may also have negative effects on chondrocyte proliferation, through influencing the signalling pathways of FGF and GH and may enhance its negative effect on chondrocyte differentiation, through influencing the GH signalling pathway. PTHrP is potentially able to alleviate the inhibition of PIAS1 on Stat1 activity by inducing the expression of Csrp2. The negative actions of Stat1 on chondrocyte proliferation after FGF activation may be enhanced through inhibition of p21. GH induces IGF-1 expression through Stat5b activation. PTHrP decreases posttranslational activation of Stat5b, thereby probably inhibiting the positive actions of IGF-1 on chondrocyte proliferation and differentiation.

proliferation and differentiation is the result of the activation of signalling pathways and the interactions between them. The occurrence and intensity of specific signalling responses depend upon many factors that may be controlled directly or indirectly by the environment of the chondrocyte. These may include the level of receptor expression, the abundance of adaptor or scaffolding proteins, for instance Nherf, or the expression and activity of downstream kinases and their substrates. In addition, interactions with adjacent cells or with the extracellular matrix could also influence the response to systemic hormones or growth factors.

In the past, most studies have focussed on the actions of a single gene. With the rapid advancements in technology, a growing number of studies are using techniques to determine a wide range of target genes. Among these techniques is genome wide analysis, but another increasing field of interest is the field of bioinformatics and the use of computer models. By using these techniques, the connections between the target genes will lead to new insights.

Reference list

1. Lanske B, Karaplis AC, Lee K, Luz A, Vortkamp A, Pirro A, Karperien M, Defize LH, Ho C, Mulligan RC, Abou-Samra AB, Juppner H, Segre GV, Kronenberg HM 1996 PTH/PTHrP receptor in early development and Indian hedgehog-regulated bone growth. Science 273:663-666

2. Karperien M, van der Harten HJ, van Schooten R, Farih-Sips H, den Hollander NS, Kneppers SL, Nijweide P, Papapoulos SE, Lowik CW 1999 A frame-shift mutation in the type I parathyroid hormone (PTH)/PTH-related peptide receptor causing Blomstrand lethal osteochondrodysplasia. J Clin Endocrinol Metab 84:3713-3720

3. Guo J, Chung UI, Kondo H, Bringhurst FR, Kronenberg HM 2002 The PTH/PTHrP receptor can delay chondrocyte hypertrophy in vivo without activating phospholipase C. Dev Cell 3:183-194

4. Schipani E, Kruse K, Juppner H 1995 A constitutively active mutant PTH-PTHrP receptor in Jansen-type metaphyseal chondrodysplasia. Science 268:98-100

5. Duchatelet S, Ostergaard E, Cortes D, Lemainque A, Julier C 2005 Recessive mutations in PTHR1 cause contrasting skeletal dysplasias in Eiken and Blomstrand syndromes. Hum Mol Genet 14:1-5

6. Schipani E, Langman C, Hunzelman J, Le Merrer M, Loke KY, Dillon MJ, Silve C, Juppner H 1999 A novel parathyroid hormone (PTH)/PTH-related peptide receptor mutation in Jansen's metaphyseal chondrodysplasia. J Clin Endocrinol Metab 84:3052-3057

7. Karaplis AC, He B, Nguyen MT, Young ID, Semeraro D, Ozawa H, Amizuka N 1998 Inactivating mutation in the human parathyroid hormone receptor type 1 gene in Blomstrand chondrodysplasia. Endocrinology 139:5255-5258

8. Zhang P, Jobert AS, Couvineau A, Silve C 1998 A homozygous inactivating mutation in the parathyroid hormone/parathyroid hormone-related peptide receptor causing Blomstrand chondrodysplasia. J Clin Endocrinol Metab 83:3365-3368

9. Jobert AS, Zhang P, Couvineau A, Bonaventure J, Roume J, Le Merrer M, Silve C 1998 Absence of functional receptors for parathyroid hormone and parathyroid hormone-related peptide in Blomstrand chondrodysplasia. J Clin Invest 102:34-40

10. Hopyan S, Gokgoz N, Poon R, Gensure RC, Yu C, Cole WG, Bell RS, Juppner H, Andrulis IL, Wunder JS, Alman BA 2002 A mutant PTH/PTHrP type I receptor in enchondromatosis. Nat Genet 30:306-310

11. Schipani E, Langman CB, Parfitt AM, Jensen GS, Kikuchi S, Kooh SW, Cole WG, Juppner H 1996 Constitutively activated receptors for parathyroid hormone and parathyroid hormone-related peptide in Jansen's metaphyseal chondrodysplasia. N Engl J Med 335:708-714

12. Oostra RJ, van der Harten JJ, Rijnders WP, Scott RJ, Young MP, Trump D 2000 Blomstrand osteochondrodysplasia: three novel cases and histological evidence for heterogeneity. Virchows Arch 436:28-35

13. Den Hollander NS, van der Harten HJ, Vermeij-Keers C, Niermeijer MF, Wladimiroff JW 1997 First-trimester diagnosis of Blomstrand lethal osteochondrodysplasia. Am J Med Genet %19;73:345-350

14. Blomstrand S, Claesson I, Save-Soderbergh J 1985 A case of lethal congenital dwarfism with accelerated skeletal maturation. Pediatr Radiol 15:141-143

15. Leroy JG, Keersmaeckers G, Coppens M, Dumon JE, Roels H 1996 Blomstrand lethal osteochondrodysplasia. Am J Med Genet 63:84-89

16. Loshkajian A, Roume J, Stanescu V, Delezoide AL, Stampf F, Maroteaux P 1997 Familial Blomstrand chondrodysplasia with advanced skeletal maturation: further delineation. Am J Med Genet 71:283-288

17. Spranger J, Maroteaux P 1990 The lethal osteochondrodysplasias. Adv Hum Genet 19:1-103, 331-2.:1-2

18. Young ID, Zuccollo JM, Broderick NJ 1993 A lethal skeletal dysplasia with generalised sclerosis and advanced skeletal maturation: Blomstrand chondrodysplasia? J Med Genet 30:155-157

19. Galera MF, Silva Patricio FR, Lederman HM, Porciuncula CG, Lopes M, I, Brunoni D 1999 Blomstrand chondrodysplasia: a lethal sclerosing skeletal dysplasia. Case report and review. Pediatr Radiol 29:842-845

20. Ozonoff MB 1969 Metaphyseal dysostosis of Jansen. Radiology 93:1047-1050

21. Bastepe M, Raas-Rothschild A, Silver J, Weissman I, Wientroub S, Juppner H, Gillis D 2004 A form of Jansen's metaphyseal chondrodysplasia with limited metabolic and skeletal abnormalities is caused by a novel activating parathyroid hormone (PTH)/PTH-related peptide receptor mutation. J Clin Endocrinol Metab 89:3595-3600

22. Horton WA 1997 Fibroblast growth factor receptor 3 and the human chondrodysplasias. Curr Opin Pediatr 9:437-442

23. Horton WA, Lunstrum GP 2002 Fibroblast growth factor receptor 3 mutations in achondroplasia and related forms of dwarfism. Rev Endocr Metab Disord 3:381-385

24. Wagner T, Wirth J, Meyer J, Zabel B, Held M, Zimmer J, Pasantes J, Bricarelli FD, Keutel J, Hustert E, . 1994 Autosomal sex reversal and campomelic dysplasia are caused by mutations in and around the SRY-related gene SOX9. Cell 79:1111-1120

25. Pop R, Zaragoza MV, Gaudette M, Dohrmann U, Scherer G 2005 A homozygous nonsense mutation in SOX9 in the dominant disorder campomelic dysplasia: a case of mitotic gene conversion. Hum Genet 117:43-53

26. Meyer J, Sudbeck P, Held M, Wagner T, Schmitz ML, Bricarelli FD, Eggermont E, Friedrich U, Haas OA, Kobelt A, Leroy JG, Van Maldergem L, Michel E, Mitulla B, Pfeiffer RA, Schinzel A, Schmidt H, Scherer G 1997 Mutational analysis of the SOX9 gene in campomelic dysplasia and autosomal sex reversal: lack of genotype/phenotype correlations. Hum Mol Genet 6:91-98

27. Ebong S, Yu CR, Carper DA, Chepelinsky AB, Egwuagu CE 2004 Activation of STAT signaling pathways and induction of suppressors of cytokine signaling (SOCS) proteins in mammalian lens by growth factors. Invest Ophthalmol Vis Sci 45:872-878

28. Kanai M, Goke M, Tsunekawa S, Podolsky DK 1997 Signal transduction pathway of human fibroblast growth factor receptor 3. Identification of a novel 66-kDa phosphoprotein. J Biol Chem 272:6621-6628

29. Minina E, Kreschel C, Naski M, Ornitz D, Vortkamp A 2002 Interaction of FGF, Ihh/Pthlh, and BMP Signaling Integrates Chondrocyte Proliferation and Hypertrophic Differentiation. Dev Cell 3:439

30. Sahni M, Ambrosetti DC, Mansukhani A, Gertner R, Levy D, Basilico C 1999 FGF signaling inhibits chondrocyte proliferation and regulates bone development through the STAT-1 pathway. Genes Dev 13:1361-1366

31. Sahni M, Raz R, Coffin JD, Levy D, Basilico C 2001 STAT1 mediates the increased apoptosis and reduced chondrocyte proliferation in mice overexpressing FGF2. Development 128:2119-2129

32. Murakami S, Balmes G, McKinney S, Zhang Z, Givol D, de Crombrugghe B 2004 Constitutive activation of MEK1 in chondrocytes causes Stat1-independent achondroplasia-like dwarfism and rescues the Fgfr3-deficient mouse phenotype. Genes Dev 18:290-305

33. Ozasa A, Komatsu Y, Yasoda A, Miura M, Sakuma Y, Nakatsuru Y, Arai H, Itoh N, Nakao K 2005 Complementary antagonistic actions between C-type natriuretic peptide and the MAPK pathway through FGFR-3 in ATDC5 cells. Bone 36:1056-1064

34. Sakamoto A, Chen M, Kobayashi T, Kronenberg HM, Weinstein LS 2005 Chondrocyte-specific knockout of the G protein G(s)alpha leads to epiphyseal and growth plate abnormalities and ectopic

chondrocyte formation. J Bone Miner Res 20:663-671

35. Karaplis AC, Luz A, Glowacki J, Bronson RT, Tybulewicz VL, Kronenberg HM, Mulligan RC 1994 Lethal skeletal dysplasia from targeted disruption of the parathyroid hormone-related peptide gene. Genes Dev 8:277-289

36. Eiken M, Prag J, Petersen KE, Kaufmann HJ 1984 A new familial skeletal dysplasia with severely retarded ossification and abnormal modeling of bones especially of the epiphyses, the hands, and feet. Eur J Pediatr 141:231-235

37. Yun CH, Oh S, Zizak M, Steplock D, Tsao S, Tse CM, Weinman EJ, Donowitz M 1997 cAMP-mediated inhibition of the epithelial brush border Na+/H+ exchanger, NHE3, requires an associated regulatory protein. Proc Natl Acad Sci U S A 94:3010-3015

38. Mahon MJ, Donowitz M, Yun CC, Segre GV 2002 Na(+)/H(+) exchanger regulatory factor 2 directs parathyroid hormone 1 receptor signalling. Nature %20;417:858-861

39. Shenolikar S, Weinman EJ 2001 NHERF: targeting and trafficking membrane proteins. Am J Physiol Renal Physiol 280:F389-F395

40. Weinman EJ, Steplock D, Wang Y, Shenolikar S 1995 Characterization of a protein cofactor that mediates protein kinase A regulation of the renal brush border membrane Na(+)-H+ exchanger. J Clin Invest 95:2143-2149

41. Shenolikar S, Voltz JW, Minkoff CM, Wade JB, Weinman EJ 2002 Targeted disruption of the mouse NHERF-1 gene promotes internalization of proximal tubule sodium-phosphate cotransporter type IIa and renal phosphate wasting. Proc Natl Acad Sci U S A %20;99:11470-11475

42. Weinman EJ, Steplock D, Shenolikar S 2003 NHERF-1 uniquely transduces the cAMP signals that inhibit sodium-hydrogen exchange in mouse renal apical membranes. FEBS Lett 536:141-144

43. Wade JB, Welling PA, Donowitz M, Shenolikar S, Weinman EJ 2001 Differential renal distribution of NHERF isoforms and their colocalization with NHE3, ezrin, and ROMK. Am J Physiol Cell Physiol 280:C192-C198

44. Mahon MJ, Cole JA, Lederer ED, Segre GV 2003 Na+/H+ exchanger-regulatory factor 1 mediates inhibition of phosphate transport by parathyroid hormone and second messengers by acting at multiple sites in opossum kidney cells. Mol Endocrinol 17:2355-2364

45. Palmada M, Poppendieck S, Embark HM, van de Graaf SF, Boehmer C, Bindels RJ, Lang F 2005 Requirement of PDZ domains for the stimulation of the epithelial Ca2+ channel TRPV5 by the NHE regulating factor NHERF2 and the serum and glucocorticoid inducible kinase SGK1. Cell Physiol Biochem 15:175-182

46. Weinman EJ, Hall RA, Friedman PA, Liu-Chen LY, Shenolikar S 2006 THE ASSOCIATION OF NHERF ADAPTOR PROTEINS WITH G PROTEIN-COUPLED RECEPTORS AND RECEPTOR TYROSINE KINASES *. Annu Rev Physiol 68:491-505.:491-505

47. Tanaka TS, Jaradat SA, Lim MK, Kargul GJ, Wang X, Grahovac MJ, Pantano S, Sano Y, Piao Y, Nagaraja R, Doi H, Wood WH, III, Becker KG, Ko MS 2000 Genome-wide expression profiling of mid-gestation placenta and embryo using a 15,000 mouse developmental cDNA microarray. Proc Natl Acad Sci U S A 97:9127-9132

48. Rickman DS, Herbert CJ, Aggerbeck LP 2003 Optimizing spotting solutions for increased reproducibility of cDNA microarrays. Nucleic Acids Res 31:e109

49. Handley D, Serban N, Peters D, O'Doherty R, Field M, Wasserman L, Spirtes P, Scheines R, Glymour C 2004 Evidence of systematic expressed sequence tag IMAGE clone cross-hybridization on cDNA microarrays. Genomics 83:1169-1175

50. Halgren RG, Fielden MR, Fong CJ, Zacharewski TR 2001 Assessment of clone identity and sequence fidelity for 1189 IMAGE cDNA clones. Nucleic Acids Res 29:582-588

51. Kargul GJ, Dudekula DB, Qian Y, Lim MK, Jaradat SA, Tanaka TS, Carter MG, Ko MS 2001 Verification and initial annotation of the NIA mouse 15K cDNA clone set. Nat Genet 28:17-18

52. Shiffman D, Mikita T, Tai JT, Wade DP, Porter JG, Seilhamer JJ, Somogyi R, Liang S, Lawn RM 2000 Large scale gene expression analysis of cholesterol-loaded macrophages. J Biol Chem 275:37324-37332

53. De Jong DS, van Zoelen EJ, Bauerschmidt S, Olijve W, Steegenga WT 2002 Microarray analysis of bone morphogenetic protein, transforming growth factor beta, and activin early response genes during osteoblastic cell differentiation. J Bone Miner Res 17:2119-2129

54. De Jong DS, Vaes BL, Dechering KJ, Feijen A, Hendriks JM, Wehrens R, Mummery CL, van Zoelen EJ, Olijve W, Steegenga WT 2004 Identification of novel regulators associated with early-phase osteoblast differentiation. J Bone Miner Res 19:947-958

55. Livak KJ, Schmittgen TD 2001 Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402-408

56. Vaes BL, Dechering KJ, Feijen A, Hendriks JM, Lefevre C, Mummery CL, Olijve W, van Zoelen EJ, Steegenga WT 2002 Comprehensive microarray analysis of bone morphogenetic protein 2-induced osteoblast differentiation resulting in the identification of novel markers for bone development. J Bone Miner Res 17:2106-2118

57. Ionescu AM, Schwarz EM, Vinson C, Puzas JE, Rosier R, Reynolds PR, O'Keefe RJ 2001 PTHrP modulates chondrocyte differentiation through AP-1 and CREB signaling. J Biol Chem 276:11639-11647

58. Qin L, Qiu P, Wang L, Li X, Swarthout JT, Soteropoulos P, Tolias P, Partridge NC 2003 Gene expression profiles and transcription factors involved in parathyroid hormone signaling in osteoblasts revealed by microarray and bioinformatics. J Biol Chem 278:19723-19731

59. Inada M, Yasui T, Nomura S, Miyake S, Deguchi K, Himeno M, Sato M, Yamagiwa H, Kimura T, Yasui N, Ochi T, Endo N, Kitamura Y, Kishimoto T, Komori T 1999 Maturational disturbance of chondrocytes in Cbfa1-deficient mice. Dev Dyn 214:279-290

60. Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Deguchi K, Shimizu Y, Bronson RT, Gao YH, Inada M, Sato M, Okamoto R, Kitamura Y, Yoshiki S, Kishimoto T 1997 Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. Cell 89:755-764

61. Li TF, Dong Y, Ionescu AM, Rosier RN, Zuscik MJ, Schwarz EM, O'Keefe RJ, Drissi H 2004 Parathyroid hormone-related peptide (PTHrP) inhibits Runx2 expression through the PKA signaling pathway. Exp Cell Res 299:128-136

62. Vortkamp A, Lee K, Lanske B, Segre GV, Kronenberg HM, Tabin CJ 1996 Regulation of rate of cartilage differentiation by Indian hedgehog and PTH-related protein. Science 273:613-622

63. Minina E, Wenzel HM, Kreschel C, Karp S, Gaffield W, McMahon AP, Vortkamp A 2001 BMP and Ihh/PTHrP signaling interact to coordinate chondrocyte proliferation and differentiation. Development 128:4523-4534

64. Nilsson O, Marino R, De Luca F, Phillip M, Baron J 2005 Endocrine regulation of the growth plate. Horm Res 64:157-165

65. Van der Eerden BC, Karperien M, Wit JM 2003 Systemic and local regulation of the growth plate. Endocr Rev 24:782-801

66. Gevers EF, van der Eerden BC, Karperien M, Raap AK, Robinson IC, Wit JM 2002 Localization and

regulation of the growth hormone receptor and growth hormone-binding protein in the rat growth plate. J Bone Miner Res 17:1408-1419

67. Tsukazaki T, Matsumoto T, Enomoto H, Usa T, Ohtsuru A, Namba H, Iwasaki K, Yamashita S 1994 Growth hormone directly and indirectly stimulates articular chondrocyte cell growth. Osteoarthritis Cartilage 2:259-267

68. Nilsson A, Carlsson B, Isgaard J, Isaksson OG, Rymo L 1990 Regulation by GH of insulin-like growth factor-I mRNA expression in rat epiphyseal growth plate as studied with in-situ hybridization. J Endocrinol 125:67-74

69. Wang J, Zhou J, Cheng CM, Kopchick JJ, Bondy CA 2004 Evidence supporting dual, IGF-I-independent and IGF-I-dependent, roles for GH in promoting longitudinal bone growth. J Endocrinol 180:247-255

70. Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB, Korach KS 1994 Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. N Engl J Med %20;331:1056-1061

71. Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin K 1995 Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. J Clin Endocrinol Metab 80:3689-3698

72. Weiskirchen R, Moser M, Weiskirchen S, Erdel M, Dahmen S, Buettner R, Gressner AM 2001 LIM-domain protein cysteine- and glycine-rich protein 2 (CRP2) is a novel marker of hepatic stellate cells and binding partner of the protein inhibitor of activated STAT1. Biochem J 359:485-496

73. Park YJ, Park ES, Kim MS, Kim TY, Lee HS, Lee S, Jang IS, Shong M, Park DJ, Cho BY 2002 Involvement of the protein kinase C pathway in thyrotropin-induced STAT3 activation in FRTL-5 thyroid cells. Mol Cell Endocrinol 194:77-84

74. Zhang H, Conrad DM, Butler JJ, Zhao C, Blay J, Hoskin DW 2004 Adenosine acts through A2 receptors to inhibit IL-2-induced tyrosine phosphorylation of STAT5 in T lymphocytes: role of cyclic adenosine 3',5'-monophosphate and phosphatases. J Immunol 173:932-944

75. Kofoed EM, Hwa V, Little B, Woods KA, Buckway CK, Tsubaki J, Pratt KL, Bezrodnik L, Jasper H, Tepper A, Heinrich JJ, Rosenfeld RG 2003 Growth hormone insensitivity associated with a STAT5b mutation. N Engl J Med 349:1139-1147

76. Rosenfeld RG, Kofoed E, Buckway C, Little B, Woods KA, Tsubaki J, Pratt KA, Bezrodnik L, Jasper H, Tepper A, Heinrich JJ, Hwa V 2005 Identification of the first patient with a confirmed mutation of the JAK-STAT system. Pediatr Nephrol 20:303-305

77. Woelfle J, Billiard J, Rotwein P 2003 Acute control of insulin-like growth factor-I gene transcription by growth hormone through Stat5b. J Biol Chem %20;278:22696-22702

78. Kronenberg HM 2003 Developmental regulation of the growth plate. Nature 423:332-336

79. MacLean HE, Guo J, Knight MC, Zhang P, Cobrinik D, Kronenberg HM 2004 The cyclin-dependent kinase inhibitor p57(Kip2) mediates proliferative actions of PTHrP in chondrocytes. J Clin Invest 113:1334-1343

80. Zhang P, Liegeois NJ, Wong C, Finegold M, Hou H, Thompson JC, Silverman A, Harper JW, DePinho RA, Elledge SJ 1997 Altered cell differentiation and proliferation in mice lacking p57KIP2 indicates a role in Beckwith-Wiedemann syndrome. Nature 387:151-158

81. Weir EC, Philbrick WM, Amling M, Neff LA, Baron R, Broadus AE 1996 Targeted overexpression of parathyroid hormone-related peptide in chondrocytes causes chondrodysplasia and delayed endochondral bone formation. Proc Natl Acad Sci U S A 93:10240-10245

82. Hart KC, Robertson SC, Kanemitsu MY, Meyer AN, Tynan JA, Donoghue DJ 2000 Transformation and Stat activation by derivatives of FGFR1, FGFR3, and FGFR4. Oncogene 19:3309-3320