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Cortisol Regulates the Expression of Notch in Osteoblasts

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Abstract Glucocorticoids have important effects on osteoblastic replication, differentiation, and function, and the Notch family of receptors is considered to play a role in osteoblastic cell differentiation. We postulated that cortisol could regulate Notch and Notch ligand expression in osteoblastic cells, providing an additional mechanism by which glucocorticoids could regulate osteoblastic differentiation. We examined the expression and regulation of Notch1, 2, 3, and 4 and their ligands Jagged 1 and 2 and Delta 1 and 3 by cortisol in cultures of osteoblastic MC3T3-E1 cells. Cortisol caused a time-dependent increase in Notch1 and 2 mRNA levels in MC3T3 cells. Notch3 and 4 were not detected in the presence or absence of cortisol. MC3T3 cells expressed Delta 1 and Jagged 1 but not Jagged 2 or Delta 3 mRNAs, and cortisol did not have a substantial effect on the expression of any of these ligands. Cortisol increased the rate of Notch1 and 2 transcription and, in transcriptionally arrested cells, did not modify the decay of the transcripts, indicating a transcriptional level of control. In conclusion, cortisol stimulates Notch1 and 2 transcription in osteoblasts. Since Notch signaling appears to play a negative role in osteoblastic differentiation, its increased expression could be relevant to the actions of cortisol in bone. J. Cell. Biochem. 85: 252–258, 2002.

Key words: glucocorticoids; cell differentiation; signaling; receptors; jagged

Glucocorticoids have complex effects on bone cell function, and when administered for prolonged periods of time, they cause osteoporosis [Canalis, 1996]. This is, to an extent, secondary to an increase in bone resorption. However, the fundamental pathogenetic event is a decrease in bone formation, due to inhibitory actions of glucocorticoids on osteoblastic cell replication, terminal maturation, and function [Canalis, 1996; Dalle Carbonare et al., 2001]. These effects appear to be due to direct actions of glucocorticoids on specific genes important for osteo-blast function, and by indirect actions through the regulation of locally produced growth modulators [Canalis, 1996; Delany et al., 2001].

Under selected conditions, glucocorticoids were reported to increase the differentiation of cells of the osteoblastic lineage, however, this effect is not consistent with their inhibitory actions on the differentiated function of the osteoblast [Leboy et al., 1991; Shalhoub et al., 1992]. Glucocorticoids suppress the expression of core binding factor a1 (Cbfa1), a critical factor for osteoblastogenesis, osteocalcin, an osteoblastic specific gene, and type I collagen, the major component of the bone matrix [Lian et al., 1989; Subramaniam et al., 1992; Delany et al., 1995; Chang et al., 1998]. In addition, they inhibit the expression of insulin-like growth factor (IGF) I, a growth factor that enhances the differentiated function of the osteoblast [Delany et al., 2001]. The negative impact of glucocorticoids on osteoblastic function correlates with their ability to suppress osteogenesis and to induce adipogenesis, a process marked by the stimulation of PPARγ2 transcription [Wu et al., 1996; Lecka-Czernik et al., 1999]. Recently, we re-examined the effects of glucocorticoids on the differentiation of primary cultures of rat calvarial osteoblasts and demonstrated that cortisol repressed

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their differentiation, when cultured under differentiating conditions [Pereira et al., 2001].

Notch1, 2, 3, and 4 are a family of closely related transmembrane receptors that mediate signaling mechanisms controlling cell fate decisions [Weinmaster, 1997; Artavanis-Tsakonas et al., 1999]. The ligands for Notch, Delta and Serrate/Jagged, are single-pass transmembrane proteins that bind and activate the Notch receptor on neighboring cells inducing proteolytic cleavage of the Notch protein at its transmembrane region [Weinmaster, 1997; Artavanis-Tsakonas et al., 1999]. The Notch intracellular domain then mediates signaling following nuclear translocation and complex formation with specific transcription factors [Schroeter et al., 1998; Qi et al., 1999]. Activated Notch receptors have opposite actions to those of bone morphogenetic proteins (BMP), known to induce the differentiation of cells of the osteoblastic lineage [Katagiri et al., 1994; Nofziger et al., 1999]. Furthermore, Notch signaling blocks chondrocyte maturation, resulting in shortened skeletal elements that lack ossification [Crowe et al., 1999]. Notch2 and Kuzbanian, a metallodisintegrin implied in the activation of Notch, were reported to be expressed in skeletal tissue and by osteosarcoma cells [Dallas et al., 1999], and initial studies suggest that overexpression of Notch1 antagonizes osteoblast differentiation [Tezuka et al., 1998]. However, there is no information regarding the expression and regulation of other Notch receptors and their ligands in osteoblasts. The present studies were undertaken to examine the effects of cortisol on Notch and Notch ligand gene expression in cultures of the murine osteoblastic cell line MC3T3-E1.

MATERIALS AND METHODS

Culture Technique

MC3T3-E1 cells (MC3T3), an osteoblastic cell line derived from fetal mouse calvaria, were cultured in α-MEM (Invitrogen, Rockville, MD) supplemented with 20 mM HEPES and 10% fetal bovine serum (FBS) (Atlanta Biologicals, Norcross, GA) in a humidified 5% CO_2 incubator at 37°C until reaching confluence [Sudo et al., 1983]. To test the effects of cortisol on Notch and Notch ligand expression during cell differentiation, confluent MC3T3 cells were switched to α-MEM supplemented with 10% FBS, 50 μg/ml ascorbic acid and 5 mM β-glycerolphosphate (osteoblast differentiation medium) in the presence or absence of 1 μM cortisol (all from Sigma Chemical Co., St. Louis, MO) (12). MC3T3 cells were cultured for up to 4 weeks following confluence, and culture medium was replaced twice a week with fresh solutions with or without cortisol. Cells were harvested 3 or 4 days after the last change of medium.

To test the immediate effects of cortisol on Notch and Notch ligand expression, confluent MC3T3 cells were transferred to serum-free α-MEM supplemented with 50 μg/ml ascorbic acid for 20–24 h and exposed to control or cortisol-containing medium in the absence of serum for 0.5–24 h. To determine changes in mRNA decay, confluent cultures were serum deprived for 24 h, and transcription was arrested with 5,6-dichlorobenzimidazole riboside (DRB, Sigma) in the presence and absence of cortisol [Zandomeni et al., 1983]. For nuclear run-on assays, MC3T3 cells were grown to confluence, serum deprived for 20–24 h, and exposed to test or control solutions for 24 h.

Northern Blot Analysis

Total cellular RNA was isolated with an RNeasy kit following manufacturer's instructions (Qiagen, Valencia, CA). RNA was quantitated by spectrometry and equal amounts of RNA from control or test samples were loaded on a formaldehyde agarose gel following denaturation. The gel was stained with ethidium bromide to visualize RNA standards and ribosomal RNA, confirming equal RNA loading of the samples. The RNA was blotted onto GeneScreen Plus charged nylon (Perkin-Elmer, Norwalk, CT), and uniformity of the transfer was confirmed by revisualization of ethidium bromide stained ribosomal RNA. A 1.2-kb EcoRI/XbaI mouse Notch1 cDNA (U. Lendahl, Stockholm, Sweden), an 800-bp EcoRI/NotI human Notch2 cDNA (American Type Culture Collection, ATCC, Rockville, MD), a 1.4-kb EcoRI/NotI human Notch3 cDNA (ATCC), a 4.2-kb EcoRI/XhoI mouse Notch4 cDNA (Y. Shirayoshi, Yonago, Japan), a 4.2-kb XbaI rat Jagged 1 cDNA (G. Weinmaster, Los Angeles, CA), a 400-bp EcoRI/NotI mouse Jagged 2 cDNA (ATCC), a 2.1-kb EcoRI mouse Delta 1 cDNA (R. Cordes, Hannover, Germany), a 2.1-kb EcoRI mouse Delta 3 cDNA (A. Rana, London, England), and a 752-bp murine 18S ribosomal RNA (ATCC) were purified by aga-

rose gel electrophoresis [Lardelli and Lendahl, 1993; Bettenhausen et al., 1995; Lindsell et al., 1995; Dunwoodie et al., 1997; Shirayoshi et al., 1997]. cDNAs were labeled with [α-^{32}P]-deoxyadenosine 5′-triphosphate (dATP) and [α-^{32}P]-deoxycytidine 5′-triphosphate (dCTP) (50 μCi each at a specific activity of 3,000 Ci/mmol; Perkin-Elmer) using the random hexanucleotide-primed second-strand synthesis method or the Ready-To-Go DNA Labeling Beads (-dCTP) kit, (Amersham Pharmacia Biotech, Piscataway, NJ) in accordance with manufacturer's instructions [Feinberg and Vogelstein, 1984]. Hybridizations were carried out at 42°C for 24–48 h, followed by two post-hybridization washes at room temperature for 15 min in 1 × saline sodium citrate (SSC), and a wash at 65°C for 20 to 30 min in 0.1 to 1 × SSC. The bound radioactive material was visualized by autoradiography on Kodak X-AR5 film (Eastman Kodak, Rochester, NY), employing Cronex Lightning Plus (Perkin-Elmer) or Biomax MS film employing Biomax screens (Eastman Kodak). Relative hybridization levels were determined by densitometry. Northern analyses shown are representative of two to four cultures.

Nuclear Run-On Assay

To examine changes in the rate of transcription, nuclei were isolated by Dounce homogenization in Tris buffer containing 0.5% Nonidet P-40. Nascent transcripts were labeled by incubation of nuclei in a reaction buffer containing 500 μM each of adenosine, cytidine, and guanosine triphosphates, 150 U RNasin (Promega, Madison, WI), and 250 μCi [α^{32}P] uridine triphosphate (UTP) (800 Ci/mM, Perkin-Elmer) [Greenberg and Ziff, 1984]. RNA was isolated by treatment with DNase I and proteinase K, followed by phenol-chloroform extraction and ethanol precipitation. Linearized plasmid DNA containing 1 μg of cDNA was immobilized onto GeneScreen Plus by slot blotting according to the manufacturer's directions. An 800-bp glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (R. Wu, Ithaca, NY) was used to estimate loading of the radiolabeled RNA [Tso et al., 1985]. Equal counts per minute of [^{32}P] RNA from each sample were hybridized to cDNAs at 42°C for 72 h and washed in 1 × SSC 2 × at room temperature for 15 min, then 1 × at 65°C for 30 min. Hybridized cDNAs were visualized by autoradiography.

Statistical Methods

Slopes to determine mRNA decay were compared using a GB-Stat software package (Dynamic Microsystems, Inc., Silver Springs, MD).

RESULTS

Northern blot analysis of total RNA extracted from MC3T3 cells cultured from 1 to 4 weeks following confluence in osteoblastic differentiation medium revealed the expression of Notch1 and 2 transcripts (Fig. 1). Continuous treatment of MC3T3 cells with cortisol at 1 μM for 1–4 weeks following confluence and cultured in the presence of FBS caused an increase in Notch1 and 2 steady state mRNA levels by 3- to 6-fold. Notch1 transcripts were easier to detect than Notch2 transcripts and were more sensitive to the stimulatory effect of cortisol. Notch3 and 4 transcripts were not readily detectable, by Northern blot analysis, in control or cortisol treated cultures (not shown). Expression of Notch transcripts was not examined prior to 1 week of culture in the presence of differentiation medium and serum. To determine whether or not cortisol had a more immediate effect on Notch1 and 2 expression, confluent cultures were serum deprived and exposed to cortisol for up to 24 h. Under these conditions, cortisol increased Notch1 and, to a lesser extent, Notch2 mRNA expression after 24 h of exposure, supporting the effect observed in differentiating MC3T3 cultures (Fig. 2). In MC3T3 cells treated for 24 h, doses of 10 nM, 100 nM, and 1 μM cortisol increased Notch1 and 2 transcripts to a similar extent (not shown). In addition to the expression of Notch1 and 2 transcripts, MC3T3 cells expressed mRNA for the Notch ligands Jagged 1 and Delta 1 (Fig. 3) but did not express Jagged 2 or Delta 3 transcripts (not shown). Northern blot analysis of total RNA showed multiple size transcripts for Jagged 1 and a predominant ~3.9-kb transcript for Delta 1, as reported in extraskeletal tissues [Bettenhausen et al., 1995; Shimizu et al., 1999]. Cortisol at 1 μM did not have appreciable effects on Jagged 1 and Delta 1 mRNA expression in the differentiating cultures over a 4-week period. To determine whether cortisol had immediate effects on Jagged 1 and Delta 1 expression, MC3T3 cells were exposed

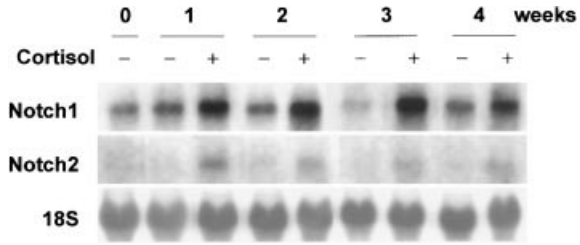

Fig. 1. Effect of cortisol at 1 μM on Notch1 and 2 mRNA expression in MC3T3 cells cultured in osteoblast differentiation medium for 1, 2, 3, and 4 weeks following confluence (0). Total RNA from control (−) or cortisol treated (+) cultures was subjected to Northern blot analysis and sequentially hybridized with [α-^{32}P] labeled Notch1 and 2 and 18S cDNA.

to cortisol for 0.5–24 h (Fig. 4). In these experiments, cortisol did not alter Delta 1 expression and did not increase Jagged 1 transcripts. In fact, a modest and transient decrease was observed.

To determine whether or not cortisol modified the transcription of the Notch1 and 2 genes, a nuclear run-on assay was performed on nuclei from MC3T3 cells treated for 24 h. This assay demonstrated that cortisol increased the rate of Notch1 transcription by about 3-fold when corrected for GAPDH (Fig. 5). While it was difficult to detect the rate of Notch2 transcription in control cultures, cortisol had a slight stimulatory effect. To determine whether or not cortisol modified the stability of Notch1 and 2 mRNAs, confluent cultures of MC3T3 cells were exposed to α-MEM or to α-MEM and cortisol for 4 h, and then treated with the RNA polymerase II inhibitor DRB in the absence or presence of cortisol for 0.5–4 h [Zandomeni et al., 1983]. The half-lives of Notch1 and 2 mRNA were 1.5 and 3.5 h, respectively, in control cultures, and they were not modified

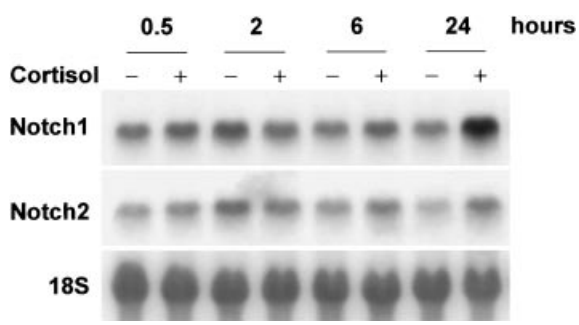

Fig. 2. Effect of cortisol at 1 μM on Notch1 and 2 mRNA expression in cultures of MC3T3 cells treated for 0.5, 2, 6, or 24 h. Total RNA from control (−) or cortisol-treated (+) cultures was subjected to Northern blot analysis and sequentially hybridized with [α-^{32}P] labeled Notch1 and 2 and 18S cDNA.

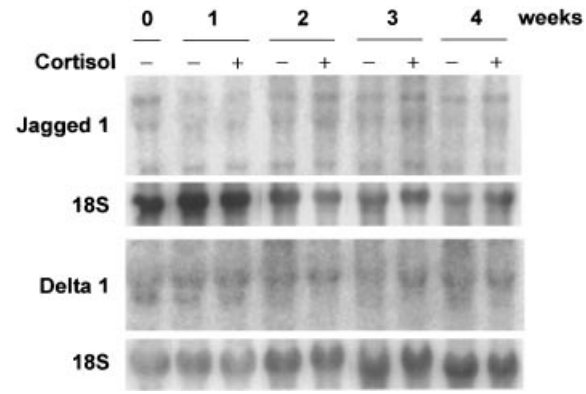

Fig. 3. Effect of cortisol at 1 μM on Jagged 1 and Delta 1 mRNA expression in MC3T3 cells cultured in osteoblast differentiation medium for 1, 2, 3, and 4 weeks following confluence (0). Total RNA from control (−) or cortisol treated (+) cultures was subjected to Northern blot analysis and sequentially hybridized with [α-^{32}P] labeled Jagged 1, Delta 1 and 18S cDNA.

by cortisol (Fig. 6). These experiments indicate that cortisol does not modify Notch1 and 2 transcript decay.

DISCUSSION

Glucocorticoids have significant effects on the differentiation of osteoblastic cells, and Notch1 was reported to inhibit osteoblastic cell differentiation and, like cortisol, to decrease the expression of Cbfa1 in osteoblastic cells [Tezuka et al., 1998; Pereira et al., 2001]. The present investigation was undertaken to determine the pattern of expression of Notch receptors and their ligands in osteoblasts, and to examine their regulation by cortisol. We demonstrated

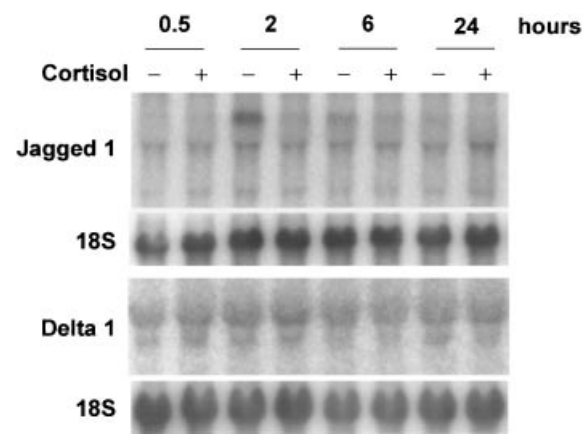

Fig. 4. Effect of cortisol 1 μM on Jagged 1 and Delta 1 mRNA expression in cultures of MC3T3 cells treated for 0.5, 2, 6, or 24 h. Total RNA from control (−) or cortisol-treated (+) cultures was subjected to Northern blot analysis and sequentially hybridized with [α-^{32}P] labeled Jagged 1, Delta 1, and 18S cDNA.

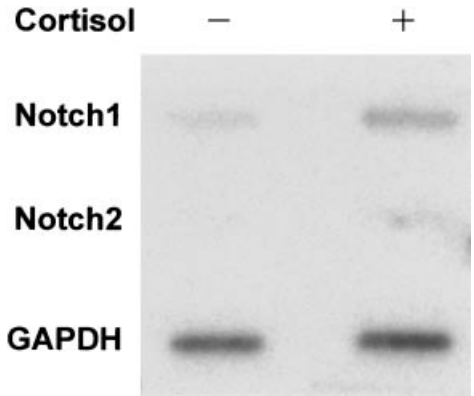

Fig. 5. Effect of cortisol at 1 μM on the rate of Notch1 and 2 mRNA transcription in confluent cultures of MC3T3 cells treated for 24 h. Nascent transcripts from control (−) or cortisol treated (+) cultures were labeled in vitro with [α-^{32}P] UTP, and labeled RNA was hybridized to the immobilized cDNAs indicated on the left of the blot. GAPDH cDNA was used to demonstrate uniformity of loading.

that cortisol increases Notch1 and 2 mRNA levels in MC3T3 cells in a time-dependent manner. Notch3 and 4 transcripts were not readily detectable by Northern blot analysis. Transcripts for the Notch ligands Jagged 1 and Delta 1, but not Jagged 2 or Delta 3, were expressed, and cortisol did not cause significant effects on these mRNAs in MC3T3 cells. Experiments in transcriptionally arrested cells revealed that cortisol did not modify the stability of Notch1 or 2 transcripts. This, in conjunction with an increase in the rate of transcription, indicates that cortisol stimulates Notch1 and 2 expression at the transcriptional level. A shortcoming of the current studies is related to the fact that we examined for changes in Notch1 and 2 mRNA but not protein levels because Notch antibodies were not readily available.

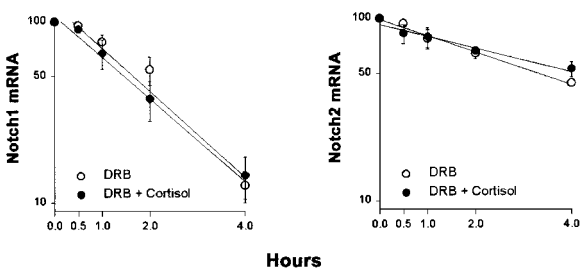

Fig. 6. Effect of cortisol at 1 μM on Notch1 and 2 mRNA decay in transcriptionally arrested MC3T3 cells. Confluent MC3T3 cells were exposed to medium (○) or treated with cortisol (●) 4 h before and 0.5–4 h after the addition of 5,6-dichlorobenzimidazole riboside (DRB). RNA was subjected to Northern blot analysis and hybridized with [α-^{32}P] labeled Notch1 and 2 cDNAs, visualized by autoradiography, and quantitated by densitometry. Values are means ± SEM for three cultures. Values were obtained by densitometric scanning and are presented as percent Notch1 or 2 mRNA levels relative to the time of DRB addition. Slopes from cortisol treated cultures were not statistically different from controls.

The Notch signaling system is highly conserved among species and plays a central role in cell fate decisions, and it is essential for normal post-implantation development of the mouse [Swiatek et al., 1994]. Since Notch1 has the potential to inhibit osteoblastic cell differentiation, its induction by cortisol could be one mechanism by which glucocorticoids modulate osteoblast behavior [Tezuka et al., 1998; Nofziger et al., 1999]. Although glucocorticoids can induce the differentiation of cells of the osteoblastic lineage, this effect is observed only under specific culture conditions. In MC3T3 cells exposure to dexamethasone (100 nM) during the proliferation period blocks their differentiation [Lian et al., 1997]. Recently, we demonstrated that in primary cultures of rat osteoblast enriched cells, cortisol inhibited their differentiation [Pereira et al., 2001]. Using analogous culture conditions, cortisol induced Notch1 and 2 transcripts in MC3T3 cells, and these actions could play a role in the inhibitory effects observed with glucocorticoids on the differentiation and differentiated function of osteoblasts. Although cortisol did not have an effect on the regulation of Notch ligands, the expression of Delta 1 and Jagged 1 by osteoblasts is physiologically significant, since they bind to and activate Notch receptors, a necessary step in the proteolytic processing of Notch. Proteolytic activation of Notch receptors is critical for signal transduction, as the phenotype of mice with mutations at the intramembranous processing site of Notch1 resembles that of Notch1 null mice [Huppert et al., 2000].

Recent studies have demonstrated that misexpression of Delta 1 blocks chondrocyte maturation with resulting shortened skeletal elements lacking ossification [Crowe et al., 1999]. Although there is no information on an induction of Notch or Delta by glucocorticoids in cartilage, the Notch signaling system could mediate the negative effects of glucocorticoids on osteoblastic and chondrocytic differentiation and play a role in developmental as well as adult skeletal changes following glucocorticoid excess. The inhibitory effects of the Notch signaling system on cell differentiation are not limited to skeletal cells. Notch signaling controls devel-

opmental changes of tooth buds, and represses muscle cell and pancreatic cell differentiation [Shawber et al., 1996; Mitsiadis et al., 1997; Apelqvist et al., 1999]. The induction of Notch by glucocorticoids in osteoblasts could play additional roles in skeletal cells. Although glucocorticoids induce the apoptosis of mature osteoblasts, this is not the case during differentiation when glucocorticoids prevent terminal osteoblastic differentiation and cell death [Weinstein et al., 1998; Pereira et al., 2001]. The mechanisms by which this occurs are not known and could involve Notch, which is known to make thymocytes resistant to glucocorticoid induced apoptosis [Deftos et al., 1998; Jehn et al., 1999].

In conclusion, the present studies demonstrate that cortisol stimulates Notch1 and 2 transcripts in osteoblasts through mechanisms that involve increased gene transcription. An induction of Notch may be relevant to the inhibitory actions of cortisol in osteoblastic cell differentiation.

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REFERENCES

Apelqvist A, Li H, Sommer L, Beatus P, Anderson DJ, Honjo T, Hrabe de Angelis M, Lendahl U, Edlund H. 1999. Notch signalling controls pancreatic cell differentiation. Nature 400:877–881.

Artavanis-Tsakonas S, Rand MD, Lake RJ. 1999. Notch signaling: cell fate control and signal integration in development. Science 284:770–776.

Bettenhausen B, Hrabe de Angelis M, Simon D, Guenet JL, Gossler A. 1995. Transient and restricted expression during mouse embryogenesis of Dll1, a murine gene related to *Drosophila* Delta. Development 121:2407–2418.

Canalis E. 1996. Mechanisms of glucocorticoid action in bone: implications to glucocorticoid induced osteoporosis. J Clin Endocrinol Metab 81:3441–3447.

Chang DJ, Ji C, Kim KK, Casinghino S, McCarthy TL, Centrella M. 1998. Reduction in transforming growth factor beta receptor I expression and transcription factor CBFa1 on bone cells by glucocorticoid. J Biol Chem 273: 4892–4896.

Crowe R, Zikherman J, Niswander L. 1999. Delta-1 negatively regulates the transition from prehypertrophic to hypertrophic chondrocytes during cartilage formation. Development 126:987–998.

Dallas DJ, Genever PG, Patton AJ, Millichip MI, McKie N, Skerry TM. 1999. Localization of ADAM10 and Notch receptors in bone. Bone 25:9–15.

Dalle Carbonare LD, Arlot ME, Chavassieux PM, Roux JP, Portero NR, Meunier PJ. 2001. Comparison of trabecular bone microarchitecture and remodeling in glucocorticoid-induced and postmenopausal osteoporosis. J Bone Miner Res 16:97–103.

Deftos ML, He YW, Ojala EW, Bevan MJ. 1998. Correlating notch signaling with thymocyte maturation. Immunity 9:777–786.

Delany A, Gabbitas B, Canalis E. 1995. Cortisol down-regulates osteoblast α1 (I) procollagen mRNA by transcriptional and post-transcriptional mechanisms. J Cell Biochem 57:488–494.

Delany AM, Durant D, Canalis E. 2001. Glucocorticoid suppression of IGF I transcription in osteoblasts. Mol Endocrinol 15:1781–1789.

Dunwoodie SL, Henrique D, Harrison SM, Beddington RS. 1997. Mouse Dll3: a novel divergent Delta gene which may complement the function of other Delta homologues during early pattern formation in the mouse embryo. Development 124:3065–3076.

Feinberg AP, Vogelstein B. 1984. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 137:266–267.

Greenberg ME, Ziff EB. 1984. Stimulation of 3T3 cells induces transcription of the c-fos proto-oncogene. Nature 311:433–438.

Huppert SS, Le A, Schroeter EH, Mumm JS, Saxena MT, Milner LA, Kopan R. 2000. Embryonic lethality in mice homozygous for a processing-deficient allele of Notch1. Nature 405:966–970.

Jehn BM, Bielke W, Pear WS, Osborne BA. 1999. Cutting edge: protective effects of Notch-1 on TCR-induced apoptosis. J Immunol 162:635–638.

Katagiri T, Yamaguchi A, Komaki M, Abe E, Takahashi N, Ikeda T, Rosen V, Wozney JM, Fujisawa-Sehara A, Suda T. 1994. Bone morphogenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage. J Cell Biol 127:1755–1766.

Lardelli M, Lendahl U. 1993. Motch A and motch B—two mouse Notch homologues coexpressed in a wide variety of tissues. Exp Cell Res 204:364–372.

Leboy PS, Beresford JN, Devlin C, Owen ME. 1991. Dexamethasone induction of osteoblast mRNAs in rat marrow stromal cell cultures. J Cell Physiol 146:370–378.

Lecka-Czernik B, Gubrij I, Moerman EJ, Kajkenova O, Lipschitz DA, Manolagas SC, Jilka RL. 1999. Inhibition of Osf2/Cbfa1 expression and terminal osteoblast differentiation by PPARγ2. J Cell Biochem 74:357–371.

Lian J, Stewart C, Puchacz E, Mackowiak S, Shalhoub V, Collart D, Zambetti G, Stein G. 1989. Structure of rat osteocalcin gene and regulation of vitamin D-dependent expression. Proc Natl Acad Sci USA 86:1143–1147.

Lian J, Shalhoub V, Aslam F, Frenkel B, Green J, Hamrah M, Stein G, Stein J. 1997. Species specific glucocorticoid and 1,25-dihydroxyvitamin D responsiveness in mouse MC3T3-E1 osteoblasts: dexamethasone inhibits osteoblast differentiation and vitamin D down-regulates osteocalcin gene expression. Endocrinology 138:2117–2127.

Lindsell CE, Shawber CJ, Boulter J, Weinmaster G. 1995. Jagged: a mammalian ligand that activates Notch1. Cell 80:909–917.

Mitsiadis TA, Henrique D, Thesleff I, Lendahl U. 1997. Mouse serrate-1 (Jagged-1): expression in the developing tooth is regulated by epithelial-mesenchymal interactions and fibroblast growth factor-4. Development 124: 1473–1483.

Nofziger D, Miyamoto A, Lyons KM, Weinmaster G. 1999. Notch signaling imposes two distinct blocks in the differentiation of C2C12 myoblasts. Development 126: 1689–1702.

Pereira RMR, Delany AM, Canalis E. 2001. Cortisol inhibits the differentiation and apoptosis of osteoblasts in culture. Bone 28:484–490.

Qi H, Rand MD, Wu X, Sestan N, Wang W, Rakic P, Xu T, Artavanis-Tsakonas S. 1999. Processing of the notch ligand delta by the metalloprotease Kuzbanian. Science 283:91–94.

Schroeter EH, Kisslinger JA, Kopan R. 1998. Notch-1 signalling requires ligand-induced proteolytic release of intracellular domain. Nature 393:382–386.

Shalhoub V, Conlon D, Tassinari M, Quinn C, Partridge N, Stein GS, Lian JB. 1992. Glucocorticoids promote development of the osteoblast phenotype by selectively modulating expression of cell growth and differentiation associated genes. J Cell Biochem 50:425–440.

Shawber C, Nofziger D, Hsieh JJ-D, Lindsell C, B'gler O, Hayward D, Weinmaster G. 1996. Notch signaling inhibits muscle cell differentiation through a CBF1-independent pathway. Development 122:3765–3773.

Shimizu K, Chiba S, Kumano K, Hosoya N, Takahashi T, Kanda Y, Hamada Y, Yazaki Y, Hirai H. 1999. Mouse jagged1 physically interacts with notch2 and other notch receptors. Assessment by quantitative methods. J Biol Chem 274:32961–32969.

Shirayoshi Y, Yuasa Y, Suzuki T, Sugaya K, Kawase E, Ikemura T, Nakatsuji N. 1997. Proto-oncogene of int-3, a mouse Notch homologue, is expressed in endothelial cells during early embryogenesis. Genes Cells 2:213–224.

Subramaniam M, Colvard D, Keeting PE, Ramussen K, Rigss BL, Spelsberg TC. 1992. Glucocorticoid regulation of alkaline phosphatase, osteocalcin, and proto-oncogenes in normal human osteoblast-like cells. J Cell Biochem 50:411–424.

Sudo H, Kodama HA, Amagai Y, Yamamoto S, Kasai S. 1983. In vitro differentiation and calcification in a new clonal osteogenic cell line derived from newborn mouse calvaria. J Cell Biol 96:191–198.

Swiatek PJ, Lindsell CE, Franco del Amo F, Weinmaster G, Gridley T. 1994. Notch1 is essential for postimplantation development in mice. Genes Dev 8:707–719.

Tezuka K, Iijima N, Miyatani S, Watanabe N, Hozumi N. 1998. Notch inhibits osteoblastic differentiation by suppressing Cbfa1 expression. Bone 23(Suppl):S153.

Tso JY, Sun XH, Kao TH, Reece KS, Wu R. 1985. Isolation and characterization of rat and human glyceraldehyde-3-phosphate dehydrogenase cDNAs: genomic complexity and molecular evolution of the gene. Nucleic Acids Res 13:2485–2502.

Weinmaster G. 1997. Review: the ins and outs of notch signaling. Mol Cell Neurosci 9:91–102.

Weinstein RS, Jilka RL, Parfitt AM, Manolagas SC. 1998. Inhibition of osteoblastogenesis and promotion of apoptosis of osteoblasts and osteocytes by glucocorticoids. Potential mechanisms of their deleterious effects on bone. J Clin Invest 102:274–282.

Wu Z, Bucher NL, Farmer SR. 1996. Induction of peroxisome proliferator-activated receptor gamma during the conversion of 3T3 fibroblasts into adipocytes is mediated by C/EBPbeta, C/EBPdelta, and glucocorticoids. Mol Cell Biol 16:4128–4136.

Zandomeni R, Bunick D, Ackerman S, Mittleman B, Weinmann R. 1983. Mechanism of action of DRB. III. Effect on specific in vitro initiation of transcription. J Mol Biol 167:561–574.