Roles of Epidermal Growth Factor Family in the Regulation of Postnatal Somatic Growth

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Ligands of the epidermal growth factor receptor (EGF-R), known to be important for supporting tissue development particularly in the gut and brain, have also been implicated in regulating postnatal somatic growth. Although optimal levels of both milk-borne and endogenous EGF-R ligands are important for supporting postnatal somatic growth through regulating gastrointestinal growth and maturation, supraphysiological levels of EGF-R ligands can cause retarded and disproportionate growth and alter body composition because they can increase growth of epithelial tissues but decrease masses of muscle, fat, and bone. Apart from their indirect roles in influencing growth, possibly via regulating levels of IGF-I and IGF binding proteins, EGF-R ligands can regulate bone growth and modeling directly because they can enhance proliferation but suppress maturation of growth plate chondrocytes (for building a calcified cartilage scaffold for bone deposition), stimulate proliferation but inhibit differentiation of osteoblasts (for depositing bone matrix), and promote formation and function of osteoclasts (for resorption of calcified cartilage or bone). In addition, EGF-like ligands, particularly amphiregulin, can be strongly regulated by PTH, an important regulatory factor in bone modeling and remodeling. Finally, EGF-R ligands can regulate bone homeostasis by regulating a pool of progenitor cells in the bone marrow through promoting proliferation but suppressing differentiation of bone marrow mesenchymal stem cells. (Endocrine Reviews 28: 284–296, 2007)

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Abbreviations: AR, Amphiregulin; BTC, betacellulin; EGF, epidermal growth factor; EGF-R, EGF receptor; EPR, epiregulin; HB-EGF, heparin-binding epidermal growth factor; IGFBP, IGF binding protein; MMPs, matrix metalloproteases; MSCs, mesenchymal stem cells; 1,25(OH)2D3, 1,25-dihydroxyvitamin D3; RANKL, receptor activator of nuclear factor-κB ligand.

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I. Introduction

It has been clear since the early and mid 1990s that the epidermal growth factor (EGF) family of growth factors is important in regulating growth, maturation, function, and maintenance in epithelial tissues (particularly the mammary gland and gastrointestinal tract) and the nerve system. However, roles of this family of growth factors in regulating somatic growth, particularly skeletal tissue growth, have been less clear. In the last few years, several studies have appeared that have demonstrated some important functions of EGF and EGF-like ligands in regulating bone growth and modeling. This review has provided a summary of studies reported in the last two decades focusing on physiological roles of the EGF family of growth factors and their possible indirect and direct ways in regulating postnatal somatic growth, body composition, bone growth, modeling, and maintenance.

II. EGF Family of Growth Factors and Receptors

A. EGF family of growth factors

The EGF family comprises six known structurally related polypeptides, namely EGF, TGF-α, amphiregulin (AR), heparin-binding EGF (HB-EGF), betacellulin (BTC), and epi-
regulin (EPR). This family of growth factors is distinguished by the fact that their soluble forms are proteolytically derived from their integral membrane precursors and that they all contain a conserved three-loop compact structure, known as the EGF-like domain (1). These growth factors, produced by various cell types such as the mammary gland and gut epithelial cells and cells in the nerve system, have been implicated in growth, differentiation, maintenance, and repair of various tissues (2). Related to the EGF family of growth factors is another subfamily of growth factors collectively termed neuregulins, which will not be included in this review because they function mainly in regulating proliferation and cell fate determination of pluripotent neural crest cells and to be survival and/or differentiation factors for Schwann cells and oligodendrocytes in the nerve system (3–6).

B. ErbB receptors and signal transduction pathways

The EGF family ligands all bind and activate the common tyrosine kinase receptor, EGF receptor (EGF-R or ErbB1) (7), which is a transmembrane glycoprotein with intrinsic tyrosine kinase activity (8). Once bound by a ligand, EGF-R functions as a part of a network, forming heterodimers with one of the three related proteins, ErbB2/c-neu, ErbB3, and ErbB4. The ErbB receptors are activated by binding the ligands of the EGF family or the neuregulin family and can signal through homodimerization or through heterodimerization with other ErbB molecules. Although all of the six EGF-R ligands bind EGF-R, three of them (EGF, TGF-α, and AR) only bind EGF-R, and three of them (HB-EGF, BTC, and EPR) also directly bind ErbB4 (Fig. 1) (7, 9–11). Neuregulins bind directly to the receptors ErbB3 and/or ErbB4 (4). The bound homo- or heterodimerized ErbB receptors undergo auto- or trans-phosphorylation on specific tyrosine residues in their intracellular tails, resulting in subsequent initiation of multiple intracellular signaling pathways, including the Ras-Raf-MAPK pathway and the phosphatidylinositol 3-kinase-Akt pathway (10, 12, 13).

Activation of ErbB receptors plays an important role in the regulation of cell proliferation, differentiation, migration, and survival in different tissues, and thus in maintaining homeostasis and regulating injury responses in various tissues including the mammary gland (1, 14), the nerve system (15, 16), and the gastrointestinal tract (1, 17–20). Although there are excellent reviews on different aspects of these physiological roles (1, 14–18), roles of the EGF family of growth factors in regulating somatic growth, particularly skeletal tissue development and maintenance, are less clear.

III. Roles of EGF Family Ligands in Regulating Postnatal Growth and Body Composition

A. Roles in regulating maternal milk supply for neonate growth

Evidence has suggested that EGF-R ligands can regulate postnatal development and growth indirectly by influencing the supply of maternal milk, which is the only complete source of nutrients for neonatal development and growth (21). Genetic studies or studies on ligand expression and receptor activation have revealed that EGF-R signaling is required in normal growth, ductal morphogenesis, and homeostasis of the mammary gland, and may play a role in regulating lactation (22–25). Mutant mice with genetic knockout of three EGF-R ligands (AR, TGF-α, and EGF) showed an aggravated defect in lobuloalveolar development and lactation, revealing poorly organized and differentiated alveoli and lowered milk protein gene expression in the triple null glands (23, 25). Consistent with the observation of the possible supporting roles for EGF and TGF-α in lactogenesis (23), it has been shown that TGF-α can support the capacity of mammary ductal epithelium to proliferate and differentiate in the absence of estrogenic stimuli (26). Treatment with TGF-α of the virgin mammary glands from estrogen receptor-α knockout mice stimulated ducts to undergo localized branching, growth, and secretory differentiation as evidenced by the production of milk proteins casein and whey acidic protein (26). Furthermore, suggesting possible roles of EGF-like ligands and ErbB receptors in lactation, ErbB2 and sialomucin complex/MUC4 (a membrane mucin and an intracellular ligand for ErbB2) (27) have differential expression patterns in the developing rat mammary glands, but both are maximally expressed during late pregnancy and lactation (28).

In addition, studies have also suggested that endocrine-derived EGF may also contribute to the mammary gland development and lactation. EGF mRNA is found highly expressed in the salivary gland, and the salivary gland serves as a major source of circulating EGF (29). Consistently, sialoadenectomy lowered concentration of circulating EGF (30) and affected mammary development and the capacity of the lactating mothers to nurse pups (31, 32). Sialoadenectomy induced a lower production of casein in response to lactogenic stimuli during pregnancy and caused reductions in size and in milk production of lactating mammary glands, all of which could be rescued when EGF was injected daily into sialoadenectomized pregnant mice (31, 32). These studies suggested that the submandibular salivary gland contributes to the regulation of mammary gland developmental via secretion of EGF, and that EGF family of growth factors is important for the optimal quality and quantity of milk, which is critical for ensuring optimal postnatal growth of neonates.
B. Roles in regulating neonate gut development and somatic growth

Genetic studies showed that EGF-R and its ligands are important for optimal growth. Depending on the genetic background of mice used, mutants with EGF-R knockout had either embryonic lethality or severe growth retardation (50–70%) in neonates, suggesting that the EGF-R is important for somatic growth (33–35). Similarly, homozygous mice with a conditional knock-in of human EGF-R (replacing mouse endogenous EGF-R and thus resulting in a reduced EGF-R signaling) showed growth retardation (35). Although knock-out of TGF-α, EGF, or BTC alone affected postnatal growth only slightly (23, 36, 37), probably due to redundancy and compensation mechanism by other EGF-R ligands, triple null mice lacking TGF-α, EGF, and AR simultaneously showed significantly impaired postnatal growth of the pups (23, 38). The triple null mutants had a 40% reduction in body weight compared with the wild-type littermates by 3 wk of age (23, 38). Furthermore, cross-fostering experiments have revealed that growth factors from maternal milk and from the neonate endogenous sources were important for postnatal growth (38). Body weights of triple null mice were only partially restored when these pups were nursed by wild-type dams; conversely, wild-type pups fed triple null milk had reduced body weights, relative to the wild-type control pups. In addition, growth of triple hemizygous pups born to triple null homozygous dams was retarded (38). Thus, optimal postnatal growth requires both maternal and neonatal sources of EGF-R ligands, and loss of either is deleterious to postnatal growth.

It is possible that the majority of the effects on somatic growth seen in EGF-R null or ligand triple null mice could, as shown in these studies, be due to the multiple organ maldevelopment. Importantly, optimal growth, development, and functional maturation of the gastrointestinal tract are vital for nutritional supply for optimal postnatal growth. It is now clear that EGF-R ligands of both maternal and endogenous sources are critical for supporting the gut growth and development, and the impaired postnatal growth of the above triple null mice or wild-type pups nursed by triple null dams was mostly contributed by impairment of gut development in these young mice (38). Due to the high concentrations of milk-borne growth factors including the EGF family in the colostrums and expression of endogenous EGF-like peptides and EGF-R in the gut tissue, the milk-borne and endogenous EGF-R ligands have been demonstrated to contribute to neonatal intestinal growth and functional development in the early postnatal period (17, 18, 38).

Therefore, the above studies suggest that EGF-R ligands from both maternal and neonatal sources are important for optimal postnatal growth, and that these growth factors can regulate postnatal growth both indirectly by influencing maternal milk supply and by supporting neonate gastrointestinal development and maturation and directly because EGF-R signaling deficiency causes somatic growth retardation.

C. Supraphysiological levels of EGF-R ligands and postnatal growth retardation

On the other hand, pharmacological or transgenic studies have demonstrated that supraphysiological levels of EGF-R ligands have negative effects on somatic growth. Systemic treatment with EGF in neonatal rats caused growth retardation (39, 40). Similarly, an early genetic study has observed that mice overexpressing TGF-α had significant decreases in the size of body and skeleton and their weight was approximately 90% of the weight of control mice (41). Consistently, EGF overexpressing mice showed retarded somatic and bone growth (42). The weights of these mice were about 78% of the body weights of nontransgenic littersmates by adulthood. Similarly, HB-EGF overexpression exhibited a 20% decrease in body weight before 6 wk of age in the mutants compared with wild-type littermates (43), and mice overexpressing BTC ubiquitously exhibited disproportionate growth with reduced body weight gain and impaired longitudinal growth with reduced dimensions of long bones (37). Interestingly, all these transgenic studies demonstrated retarded bone growth, suggesting that EGF family ligands negatively regulate postnatal bone growth.

D. Roles of EGF-R ligands in regulating body composition

It is known that gonadal sex steroids and GH/IGF-I are the two main determinants of evolving body composition in infancy, childhood, puberty, and adulthood, because signals from the gonadotropic and somatotropic axes singly and jointly govern accrual and depletion of muscle, fat, and bone mass across a lifetime (44). Several pharmacological and genetic studies have shown that EGF-R ligands can also modify body composition. In newborn rats, systemic administration of EGF for 10 d dose-dependently resulted in body weight loss and a decrease in fat pad weight, although it did not alter or slightly increased the weights of other organs, such as liver and intestine (45). In adult rats, systemic infusion of recombinant rat BTC stimulated growth of intestine by enlarging crypt epithelial area, but it reduced body weight and food intake and caused a diuretic condition characterized by a reduced serum insulin concentration, increased water intake and urine output, suggesting that BTC stimulates gastrointestinal growth and regulates fluid homeostasis (46). In an early study, mice overexpressing TGF-α had a disproportionate reduction in carcass weight but an increase in weights of several epithelial organs, resulting in induction of epithelial hyperplasia, pancreatic metaplasia, and mammary carcinoma (47). Consistently, changes in organs and tissues have also been observed in BTC transgenic mice, which exhibited a disproportionately decreased pancreas weight and a significant and disproportionate reduction in absolute and relative carcass weight (comprising a large proportion of total body weight, composed of muscle, bone, fat, and connective tissue) (37).

In mice overexpressing TGF-α, a significant reduction in muscle weight has also been observed, with the distal hindlimb muscle weights being 20% below normal and other skeletal muscles being visibly smaller in size, which was associated with decreases in the number and area of striated
muscle fibers (47). Consistently, administration of EGF peptide enhanced mRNA expression in skeletal muscle of mitochondrial uncoupling protein UCP3, a protein thought to uncouple the respiratory chain and thus to increase energy expenditure (48), suggesting that EGF-like peptides can influence skeletal muscle growth and metabolism.

The ability of EGF-R ligands to retard adipose tissue development in vivo has been relatively well characterized. Adipose tissue growth occurs by both hyperplasia and hypertrophy. Because EGF-R has been detected in normal adipose tissue (47), EGF-R ligands may have physiological roles in regulating adipocyte differentiation, adipogenesis, and fat mass. Consistently, administration of EGF to newborn rats for 10 d resulted in decreased fat pad weight in a dose-dependent fashion (45), and mice overexpressing TGF-α had significantly reduced fat contents, with weights of epididymal fat pads decreased by 40–80% and total body fat by 50% compared with control (47). From previous studies, several mechanisms could account for effects of EGF-R ligands in fat content. Firstly, EGF-like ligands suppress adipocyte differentiation and maturation. Although the number of adipocyte precursors in inguinal fat pads of EGF-treated animals was higher, the number of mature adipocytes and the amount of triglyceride accumulated per fat pad were concomitantly lower, and adipocyte precursors isolated from EGF-treated animals displayed reduced differentiation ability in culture compared with normal controls (45). Consistently, reductions in fat weights in TGF-α transgenic mice were found associated with decreases in the cellularity of fat pads, and TGF-α dose-dependently suppressed adipocyte differentiation of precursor cell line 3T3-F442A and diminished mRNA expression of adipocyte-specific markers adipsin and glycerophosphate dehydrogenase (47). Secondly, EGF-R ligands may induce lipolysis and enhance metabolism in fat tissue. Systemic EGF treatment in rats was shown to reduce amounts of adipose tissue through up-regulation of lipase level and activity and thus induction of lipolysis (49). Furthermore, EGF treatment in vivo was demonstrated to enhance mRNA expression in adipose tissue of mitochondrial uncoupling protein UCP3, a protein thought to uncouple the respiratory chain and thus to increase energy expenditure (48). Therefore, the above studies suggest that EGF-R ligands, apart from being strong stimulators for epithelial tissue growth, reduce body and carcass weights and decrease muscle and fat mass.

IV. Roles of EGF Family Ligands in Regulating Postnatal Bone Growth and Modeling

A. Mechanisms of children’s bone growth and modeling

Growth of the skeletal system is the major component or driver for the postnatal somatic growth and development. During childhood and adolescence, bone lengthening and acquisition of peak bone mass and its trabecular organization are achieved mainly through the process called endochondral ossification, involving production of calcified cartilage and its conversion and modeling into trabecular bone. The growth plate cartilage, located at the ends of children’s long bones (Fig. 2A), is responsible for the production of the intermediate cartilaginous template through chondrocyte activation, proliferation, maturation, hypertrophy, synthesis of
cartilage matrix, its calcification and vascularization (Fig. 2B) (50, 51). Conversion of calcified hypertrophic cartilage into primary trabecular bone with partially modeled cores of calcified cartilage occurs at the adjacent metaphyseal primary spongyosa region (Fig. 2B), which involves bone-forming cells (osteoblasts) lining and synthesizing new bone matrix on trabecular surface and resorptive cells (osteoclasts and chondroblasts) binding to the surface of calcified tissues and secreting acid and proteolytic enzymes to undergo resorption and modeling of calcified cartilage or bone (Fig. 2C) (50, 51). Through coordinated action of osteoclasts and osteoblasts, the bony trabeculae in the primary spongyosa (greater in number but thinner in size) are further modeled into the more mature and mechanically sound bone trabeculae (thicker in size but smaller in number) located in the secondary spongyosa of the metaphyseal bone (Fig. 2B) (52).

B. Regulation of bone growth and modeling

Longitudinal bone growth occurs at a higher rate during the first year of life and during pubertal growth spurt, but at a more modest rate during the decade of life between these two periods (53). The process of endochondral bone formation is tightly regulated by endocrine/paracrine/autocrine factors such as hormones, vitamins, transcriptional factors, and growth factors, and involves coordinated and sequential expression of regulatory factors (54). Many factors have now been shown to have important roles in regulating various processes of the endochondral ossification and modeling, including hormones (such as GH, thyroid hormone, sex hormones, glucocorticoids, calcitonin); vitamins (vitamin D3, ascorbate, retinoic acid); morphogens such as Indian hedgehog; growth factors such as IGF-I, TGF-β, bone morphogenic proteins, fibroblast growth factors, PTHrP, platelet-derived growth factor, and vascular endothelial growth factor; and cytokines such as TNF-α and IL-1 (52, 53, 55–62). Roles of many regulatory factors (such as GH/IGF-I, bone morphogenic proteins/TGF-β, fibroblast growth factors, PTHrP/Indian hedgehog, vascular endothelial growth factor, and Wnt families) and some transcriptional factors (such as Sox9 and Runx2) in bone growth and modeling are quite clear or well characterized (51, 61, 63–73). However, expression, roles, and action mechanisms of the EGF family of growth factors in bone growth regulation are less clearly delineated.

C. Roles of EGF-R ligands in regulating development of ossification centers

EGF-R ligands may play a role by negatively regulating development of ossification centers and limbs. EGF-R is expressed in chondroblasts of the developing ossification centers, and TGF-α treatment inhibited cartilage nodule formation from mesenchymal cells derived from rat mandibles and from rat embryonic limb bud, suggesting a role of TGF-α in inhibiting chondrogenesis during embryonic development (74). During limb development, epithelial cells in the apical ectodermal ridge keep the underlying mesenchymal cells in a proliferative state, preventing their differentiation possibly by secreting signaling molecules including TGF-α and EGF (75, 76). Consistently, in vitro studies have shown that EGF negatively regulates chondrogenesis from chick limb bud mesenchymal cells by inhibiting precartilage condensation and by modulating signaling pathways including those of protein kinase C-α, Erk-1, and p38 MAPK (77).

D. Roles of EGF-R ligands in regulating chondrocyte proliferation and maturation

The growth plate functions to produce calcified hypertrophic cartilaginous scaffold for bone deposition. Studies have suggested that EGF-R ligands can regulate postnatal bone growth and bone mass by their direct functions among growth plate chondrocytes. Because chondrocytes in the growth plate express EGF and bear EGF-R (42), EGF-R ligands may directly regulate chondrocyte proliferation and maturation during postnatal bone growth in an autocrine or paracrine manner. In vitro studies have suggested that chondrocytes respond to EGF by increased proliferation (78, 79). Furthermore, a genetic study has demonstrated that in EGF-R null mice, the region of hypertrophic chondrocytes in the growth plate was significantly increased (80), indicating premature differentiation of chondrocytes and suggesting that EGF-R signaling prevents terminal differentiation of chondrocytes. Consistent with the mitogenic effects and differentiation inhibitory effects of EGF-R ligands on chondrocytes, defects in bone lengthening were observed in EGF transgenic mice, which were associated with an expansion of the proliferative zone and a delay in chondrocyte hypertrophic maturation in the growth plate (42).

Interestingly, EGF can augment responsiveness of IGF-I stimulation by increasing the expression of the IGF-I receptor in growth plate chondrocytes (81). Treatment of cultured growth plate chondrocytes with EGF produced an increase in cell surface density of IGF-I receptor and specific binding of IGF-I, resulting in an enhanced protein synthesis, and pretreatment with EGF increased the responsiveness of chondrocytes to IGF-I, resulting in augmentation of IGF-I-stimulated mitotic activity and proteoglycan synthesis (81). Given the important role of IGF-I in bone growth (see Section V) and the presence of EGF and receptor in the growth plate, this in vitro study suggests a role for the interaction between these two growth factors in the regulation of bone lengthening and bone growth.

E. Roles of EGF-R ligands in regulating osteoblast proliferation and maturation

Bone matrix is synthesized by active osteoblasts on bone surface. Within an appropriate signaling environment, committed mesenchymal osteoprogenitor cells in the bone marrow first differentiate into the intermediate preosteoblasts before they mature into bone matrix-synthesizing osteoblasts, and osteoblasts finally become inactive bone-lining osteoblasts or osteocytes embedded in mineralized bone (82–84). Studies have suggested that growth and development of osteoblasts are regulated by EGF-R ligands. Early studies demonstrated expression of EGF-R in osteoblasts and osteocytes in vivo (85, 86), and recent studies showed that EGF-R and all its ligands are expressed in osteoblastic cells (87). Consistently, EGF and EGF-R were found widely distributed...
in growing antler, with expression of both molecules identified in osteoblastic cell lineage (including osteoprogenitor cells, osteoblasts, and osteocytes) (88). Functionally, as shown in in vitro studies using osteogenic cell lines (89), EGF-R in osteogenic cells is important in maintaining their proliferation potential and in suppressing osteoblast differentiation. Consistently, EGF (90, 91), and more recently AR and TGF-α (87), strongly stimulated osteoblastic cell proliferation, and EGF (92, 93) and AR (87) inhibited osteoblast differentiation as they decreased alkaline phosphatase levels and collagen-1 production. Concordant with the mitogenic role, blocking the EGF-R by using specific EGF-R inhibitors significantly inhibited basal DNA synthesis of osteoblastic cells (87). Furthermore, the roles of EGF-R in osteoblastic cells have been confirmed with in vivo genetic studies. In EGF-R null mice and mice humanized for EGF-R, accelerated osteoblast differentiation and hindered osteoblast proliferation were observed, confirming that signaling of EGF-R positively influences bone cell proliferation but negatively regulates their differentiation (80). Consistently, calvarial osteoblasts isolated from these mutant mice proliferated more slowly and displayed an increased differentiation capacity in vitro. Conversely, in EGF transgenic mice, there was an accumulation of irregular osteoblasts along trabecular bone surfaces (42). These in vitro and in vivo studies demonstrate that EGF-R ligands stimulate proliferation but suppress differentiation of osteoblastic cells.

**F. Regulation of EGF-R ligand production by PTH**

PTH plays an important role in calcium and phosphate homeostasis and bone remodeling (94–96). Depending on ways of administration or patterns and duration of PTH elevation in circulation, PTH has two opposite effects on bone. Although continuous administration causes bone loss, intermittent injection increases bone formation and improves trabecular architecture and bone density (95). To date, PTH is the only valid treatment option for osteoporosis and for preventing osteoporosis-induced fractures that works by predominantly promoting bone anabolism (97). PTH stimulates osteoblast accumulation and bone formation possibly via several means. It can promote osteoblast recruitment, maturation, and activity (possibly mediated by increased IGF-I production), increase bone remodeling rate as well as the amount of bone deposited in each remodeling cycle, and/or prevent osteoblasts from undergoing apoptosis (95, 98–100). Although the “anabolic” effects of PTH require brief exposures to higher than average PTH concentrations, its "catabolic" and bone destruction effects result from continuous infusion or continuous secretion of PTH (as in chronic renal disease and primary hyperparathyroidism) (100). It has been shown that continuous PTH exposure inhibits osteoblast differentiation (101) and results in an increase in expression of the osteoclastogenic cytokine [receptor activator of nuclear factor-κB ligand (RANKL)] with an associated inhibitory effect on expression of osteoclastogenic inhibitor osteoprotegerin (61, 102, 103).

An early study has shown that PTH, in a dose- and time-dependent manner, increased levels of EGF-R mRNA expression, EGF-R numbers, and EGF binding in osteoblast-like UMR 106-01 cells, which was associated with an enhanced biological effect (104–106), suggesting that effects of PTH on osteoblasts may be mediated indirectly or at least partially by signaling through EGF-R. Consistently, activation of the MAPK ERK1/2 by PTH treatment in differentiating transgenic murine osteoblasts was shown to be mediated by transactivation of EGF-R (107).

More recently, EGF-R ligand AR has been identified to be a general immediate response gene following PTH action in bone. Although expression of EGF-R ligands TGF-α and BTC was shown to be stimulated by PTH (87), AR has been found to be the major member of the EGF-R ligands that is highly induced and regulated by PTH (108). AR has profound effects on osteoblastogenesis because it strongly stimulates proliferation of preosteoblasts but inhibits their differentiation and mineralization of mature osteoblasts (87). Consistently, AR null mice had significantly less tibial trabecular bone than wild-type mice and displayed an osteoporotic phenotype (87). Similarly, AR expression was also induced by other osteotropic agents such as the active metabolite of vitamin D₃, 1,25-dihydroxyvitamin D₃ [1,25-(OH)₂D₃], and prostaglandin E₂ (87). These studies suggest an important role for AR in bone development and metabolism and implicate it as the major EGF-R ligand that is regulated by osteotropic hormones such as PTH in bone (87). Because intermittent PTH mainly functions to facilitate the final differentiation of the osteoblasts and inhibit their apoptosis, thus increasing their bone formation activities, the biological significance of AR production as regulated by PTH remains to be further characterized. However, due to its possible role in controlling osteoblastogenesis (increasing preosteoblast proliferation but inhibiting its maturation), AR could be one of the means for PTH to manipulate the microenvironment in bone to control remodeling (87).

**G. Roles of EGF family ligands in regulating osteoclast formation and bone modeling**

Bone laid down on the calcified growth plate cartilaginous scaffold at the metaphysis is woven bone, which, through coordinated action of bone synthesis cells (osteoblasts) and resorptive cells (osteoclasts), is to be modeled to become more mature and mechanically sound lamellar bone (Fig. 2B) (52). Osteoclasts, produced and activated from the marrow hematopoietic pathway under the influence of osteoclastogenic signals produced by osteoblast lineage cells, bind to the surface of calcified tissues and secrete acid and proteolytic enzymes to undergo resorption and modeling of calcified cartilage or bone (109). It is now known that two hematopoietic factors, namely RANKL and macrophage colony-stimulating factor, together are necessary and sufficient for osteoclast formation (61, 64, 110). Several factors that can modulate osteoclastogenesis have also been identified. Although vitamin D₃ active metabolite, PTH, PTHrP, prostaglandin E₂, cytokines IL-1, IL-6, TNF-α, and IL-11, and corticosteroids have been shown to induce the expression of RANKL in stromal/osteoblastic cells and thus stimulate osteoclast formation, osteoprotegerin (also produced by osteoblastic lineage cells and functioning as a decoy receptor for RANKL), blocks the RANKL interaction with its receptor...
RANK (receptor activator of nuclear factor-κB) and inhibits osteoclastogenesis (61, 64, 110).

It has long been demonstrated that EGF has catabolic effects on bone. In an organ culture study, high concentrations of EGF stimulated bone resorption in fetal rat long bone (111, 112). Administration of EGF at high dose to rats caused an elevation of osteoclastic cell density on trabecular bone surface (113). Consistently, in EGF transgenic mice, thinner bones were observed (42), suggesting an imbalance in bone modeling or remodeling. Evidence has also demonstrated that differentiation and function of osteoclasts are also regulated by EGF-R ligands. An early in vitro study showed that addition of TGF-α or EGF peptide in the first week of culture followed by treatment with 1,25-(OH)2D3 for 2 wk increased formation of osteoclast-like multinuclear cells from cultured human bone marrow cells (114). Interestingly, further studies showed that, whereas TGF-α promoted proliferation of osteoclast precursor cells, 1,25-(OH)2D3 increased the rate of precursor cell fusion into osteoclasts and that treatment of these cultures with TGF-α without later addition of 1,25-(OH)2D3 did not increase osteoclast-like cell formation (114). These findings suggest that TGF-α and EGF may stimulate bone resorption by increasing the proliferation of osteoclast precursors, which leads to increased numbers of osteoclasts. Consistently, early during tooth eruption, which involves accumulation of mononuclear osteoclast precursor cells in the dental follicle, the apical part of the follicle has been shown to be a site that binds EGF and contains intense cell proliferation, suggesting that EGF may play a role in increasing numbers of mononuclear osteoclast precursor cells for osteoclast differentiation (115, 116).

More recently, it has been shown that EGF-R plays a role in mediating the ability of human bone marrow stromal cells to induce osteoclast differentiation (117, 118). Human primary bone marrow stromal cells and mesenchymal stem cell-like HDS cells were shown to express immunoreactive EGF-R, and treatment of HDS cells with EGF increased EGF-R activation. Interestingly, treatment of HDS cells with EGF-R inhibitor decreased levels of secreted osteoclastogenic factors (macrophage colony-stimulating factor and RANKL) by these cells, and reduced the ability of the conditioned medium from treated HDS cells to sustain the differentiation of preosteoclasts (117). These findings suggest that EGF-R regulates the ability of bone marrow stromal cells to induce osteoclast differentiation.

In addition, mature osteoclasts have been shown to express EGF-R (85), and their resorptive function may be stimulated by locally acting agents such as TGF-α (119). Furthermore, EGF-R signaling has been shown to be important for secretion of matrix metalloproteases (MMPs), enzymes that are important for tissue degradation. Like IL-1a, EGF was shown to be potent in inducing production of MMPs, including gelatinase A (MMP-2) and gelatinase B (MMP-9), and enhanced bone resorption in cultured rabbit calvarial bone explants (120).

Moreover, also possibly through controlling MMP production, EGF-R signaling is necessary for normal craniofacial development because EGF-R null mutation caused facial maldevelopmental defects in newborn mice, and cultured mandibulomental processes from EGF-R null embryos had deficient morphogenesis of Meckel’s cartilage (121). Consistent with the ability of exogenous EGF to increase MMP secretion and with the decreased MMP expression after blockade of EGF-R signaling in wild-type explants, the secretion of MMPs was diminished in explants from EGF-R null embryos. These studies suggest that the role of EGF-R signaling for normal craniofacial development is mediated in part by its down-stream target, i.e., increased secretion of MMPs (121). Furthermore, EGF, particularly in synergy with cytokine IL-1, increased release of collagenase in soft tissues as shown in periosteal explants from rabbit calvaria, suggesting that concomitant presence of IL-1 and EGF may play a role in collagenase-mediated degradation of collagen (122, 123). The above studies suggest that EGF and related ligands have catabolic effects on bone by influencing the differentiation and activity of osteoclasts as well as through increasing production of MMPs.

V. Roles of EGF-R Ligands in Regulating Levels of Growth Modulators IGF-I and IGFBP-3

It is well known that GH and IGF-I are two major factors regulating postnatal somatic and, particularly, bone growth. The critical role of IGF-I in normal bone growth has been confirmed by the severe growth retardation in null mice lacking IGF-I or its receptor (124, 125), and genetic evidence has suggested that GH has a dual, IGF-I-independent and IGF-I-dependent, role in promoting longitudinal bone growth (70). It is now clear that both circulating and locally expressed IGF-I are important in longitudinal bone growth and the maintenance of bone mass, and IGF-I plays an essential role in longitudinal bone growth in response to GH exposure (126, 127). IGF-I stimulates proliferation of chondrocytes at the proliferative zone of the growth plate (128) and stimulates osteoblast proliferation, matrix protein synthesis, and bone formation (126, 129). Furthermore, skeletal expression of IGF-I is critical for osteoblast differentiation (130).

Studies have suggested that EGF-R ligands may indirectly cause postnatal growth retardation by regulating levels of IGF-I and IGF-I binding proteins (IGFBPs). Systemic treatment with EGF reduced circulating levels of IGF-I in neonatal rats (131, 132) and in adult rats and minipigs (133–135). The rapid decline in IGF-I concentration after EGF administration, particularly in neonates, implies that changes in IGF-I levels could be involved in mediating EGF-induced growth retardation (131).

IGFBPs carry IGF-I in the circulation or in the local tissue environment. Although the bioactivity of IGF-I in the tissue cellular microenvironment is modulated by both inhibitory and stimulatory IGFBPs, the stability or half-life of IGF-I in the circulation is maintained by IGFBPs, predominantly IGFBP-3. In EGF transgenic mice, as a possible explanation for their stunted growth, serum IGFBP-3 level was found significantly reduced, suggesting a decreased level of circulating IGF-I for skeletal growth (42). Consistently, there was a lowered serum IGFBP-3 level in normal rats that received exogenous EGF injection for 4 wk (133). Similarly, HB-EGF overexpression decreased expression of IGFBP-3 and -4 mRNA, particularly in the kidney, and the transgenic mice...
exhibited a 20% decrease in weight before 6 wk of age compared with wild-type littermates (43), suggesting that over-expression of HB-EGF affected growth possibly through a pathway involving IGFBP-3 and IGFBP-4. In addition, accompanying growth retardation, EGF administration caused a rapid increase in the serum concentration of inhibitory IGFBP-1 and hepatic IGFBP-1 mRNA levels in newborn rats, suggesting that elevated IGFBP-1 concentrations may restrict IGF bioactivity in the neonatal rat (132).

Since IGF-I and IGFBPs are major endocrine factors that regulate bone growth, the above evidence suggests that EGF-R ligands may indirectly regulate postnatal growth by their effects on the IGF-I and IGFBP levels in the circulation. However, because both IGF-I and IGFBPs are expressed in growth plate chondrocytes and osteoblasts (136–138), and because the locally expressed IGF-I and IGFBPs are also important in regulating bone growth (126), it will be interesting to find out whether EGF-R ligand treatment or over-expression would modulate expression levels of IGF-I or IGFBPs in the growth plate and metaphyseal bone, the two regions that make up the active bone “growth unit.” Recently, in vitro studies showed that IGF-I modulates its activity in cultured rat growth plate chondrocytes by modulating the synthesis of IGFBP-3 and IGFBP-5 (139), and IGFBP-5 has been shown to potentiate chondrocyte differentiation (138). To investigate potential interaction of EGF-R ligands and IGF-I on bone growth, it would be interesting to investigate any potential regulation of levels of both stimulatory (e.g., IGFBP-5) and inhibitory IGFBPs (e.g., IGFBP-4) (136, 137) in the growth plate or bone in vivo by EGF-R ligands. IGFBP-3 has recently been identified as an EGF-R downstream target molecule in primary and immortalized human esophageal epithelial cells to regulate bioavailability of free IGF-I for growth of these cells through EGF activation of EGF-R and MAPK (140), and IGFBP-3 has been shown to have IGF-independent growth inhibitory effects in chondroprogenitor cells inducing their apoptosis and antagonizing TGF-β chondroinductive effects (141, 142). Therefore, it would also be interesting to find out whether IGFBP-3 could be a downstream target molecule of EGF-R signaling in growth plate chondrocytes, bone cells, or bone marrow mesenchymal cells.

VI. Roles of EGF-R Ligands in Biology of Bone Marrow Mesenchymal Stem Cells

Pluripotent mesenchymal stem cells (MSCs) in the bone marrow stroma give rise to osteoblastic precursors (osteoprogenitor cells) in addition to precursor cells for adipocytes, muscle cells, chondrocytes, and endothelial cells and are important in maintaining bone homeostasis and regeneration (143, 144). Human primary bone marrow stromal cells and MSCs express EGF-R (117,145), and MSC-like HDS cells express immunoreactive EGF-R and respond to EGF with an elevated level of EGF-R activation (117). Functionally, studies suggest that EGF-R ligands can regulate MSC proliferation and differentiation. MSCs can proliferate and form colonies to give rise to colony-forming unit fibroblasts in a serum-free medium in the presence of EGF (143). Through activating EGF-R, ERK, and protein kinase B/akt, EGF stimulated motility and proliferation of human bone marrow MSCs; however, it did not modify their differentiation potential into adipogenic or osteogenic lineages (146). Similarly, HB-EGF stimulated MSC ex vivo proliferation without inducing spontaneous differentiation, and interestingly, HB-EGF reversibly prevented adipogenic, osteogenic, and chondrogenic differentiation from MSCs induced by specific differentiation culture conditions, maintaining their self-renewal divisions (147). In addition, AR and TGF-α have been shown to stimulate growth of primary bone marrow stromal cells (148, 149). Although the in vivo role of EGF-R expression by MSCs is not clear, the above studies have demonstrated that the primary bone marrow stromal cells or MSCs can proliferate, maintain their self-renewal potential, and prevent their multilineage differentiation ex vivo in response to EGF-like ligands. These studies suggest that EGF-R ligands (such as EGF, TGF-α, and HB-EGF) could be used as mitogens for ex vivo expansion of bone marrow MSCs for the purposes of tissue engineering and regenerative medicine (144,146). Interestingly, a recent study has shown that there was a greater growth factor production (including EGF) by bone marrow stromal cells in response to exposure to wound microenvironment, suggesting that bone marrow stromal cells themselves might augment wound healing through the responsive secretion of growth factors (including EGF) (150).

Although the above studies suggest that EGF-R and ligands are relevant to biology of bone marrow MSCs, further studies are required to investigate the sources of EGF-R ligands in the bone marrow and whether the EGF-R signaling indeed contributes to maintain a broad, proliferating pool of undifferentiated MSCs in vivo. Interestingly, an ex vivo study has shown that, compared with healthy controls, cultured bone marrow stromal cells from lung and breast cancer patients were found to have a diminished percentage of cells expressing growth factor receptors including EGF-R, and such alterations could help to explain a deficient cloning capacity of the bone marrow MSCs, an inefficient confluence capacity of bone marrow stromal cells, and a lower content of colony-forming unit fibroblasts in the bone marrow of these cancer patients (151, 152). These findings suggest that EGF-R ligands may have a role in regulating the MSC pool in the bone marrow in vivo.

VII. Concluding Remarks

Gene expression, pharmacological, and genetic studies have suggested or/and demonstrated that the EGF family of growth factors plays a role in regulating postnatal somatic growth, and that EGF-R ligands can regulate postnatal somatic growth by several means (Fig. 3). Although optimal levels of both milk-borne and endogenous EGF-R ligands are important for ensuring optimal maternal milk supply for neonates and supporting postnatal somatic growth by regulating growth and maturation of the gastrointestinal tract, supraphysiological levels of EGF-R ligands induce retarded and disproportionate growth and alter body composition because they can increase epithelial tissue growth but decrease muscle, fat, and bone proportions. Although EGF-R
signaling can indirectly cause growth retardation by negatively regulating levels of IGF-I and IGFBP-3, EGF-R ligands can also directly regulate bone growth and modeling. EGF-R signaling promotes proliferation but prohibits differentiation or maturation of growth plate chondrocytes and metaphyseal bone osteoblasts, and EGF-R ligands also promote formation and function of bone resorptive osteoclasts. Therefore, through controlling these three types of bone cells, EGF-R ligands act to allow proper development of long bones with appropriate bone mass accumulation and trabecular organization attainment. In addition, EGF-R signaling also appears to regulate bone homeostasis and regeneration potential by promoting proliferation but suppressing differentiation of bone marrow mesenchymal stem cells.

Additional studies are needed to evaluate roles of the EGF family of growth factors in regulating postnatal growth and body composition. In particular, more in vivo studies will be needed to investigate potential synergistic or inhibitory interactions and their mechanisms between EGF-R ligands and other important growth regulatory factors (such as IGF-I, IGFBPs, PTH, and sex hormones). For example, whereas PTH has been shown to influence expression of EGF-like ligands (particularly AR) strongly, the biological significance of this regulation remains to be investigated. In addition, whereas EGF-R ligands can stimulate proliferation but prevent differentiation of bone marrow MSCs ex vivo, their in vivo role in bone marrow MSC biology remains to be investigated. Due to the potential compensatory redundancy among members of the EGF family, analysis of roles and action mechanisms of EGF-R would be more informative with mutant mice with multiple member gene knockout (such as EGF-AR-TGF-α triple null) or receptor mutant mice.

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