Hepatocyte Growth Factor in Normal and Diseased Bone and Joint Tissues

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Abstract: Hepatocyte Growth Factor (HGF) is a multifunctional growth factor which, like its receptor c-Met, is widely expressed in osteoarticular tissues. HGF has profound effects on cell motility and differentiation and tissue morphogenesis and angiogenesis. HGF plays an important role in normal bone and cartilage turnover. Changes in HGF/c-Met have also been linked to pathophysiological changes in several bone and joint disorders. HGF has been implicated in the pathogenesis of inflammatory changes in rheumatoid synovium and in degenerative changes in osteoarthritis. HGF also influences bone remodelling and has significant effects on the proliferation and differentiation of osteoclast precursors, osteoclast activity and survival. A therapeutic role of HGF also has been proposed in the regeneration of osteoarticular tissues.

Keywords: HGF, c-Met, Rheumatoid Arthritis, Osteoarthritis, Osteoclasts, Bone resorption.

HEPATOCYTE GROWTH FACTOR

Hepatocyte Growth Factor (HGF) is a heparin binding glycoprotein also referred to as scatter factor (SF) because it has been shown to influence the motility of cells at picomolar concentrations. HGF regulates the growth and differentiation of many tissues and is synthesized and secreted by a number of cell types including mesenchymal cells in bone and joint such as osteoclasts [1], osteoblasts, [2] chondrocytes [3] and synovial cells [4]. HGF consists of two subunits held by a disulfide bond; the alpha subunit is composed of 440 amino acids and the beta subunit of 234 amino acids. HGF is synthesized as an inactive single chain precursor (approximately 90 kDa) of about 728 amino acids. HGF is converted to a biologically active heterodimer by a specific serine protease termed HGF-activator (HGF-A), as well as by factor XIIa [5], urokinase [6], and tissue-type plasminogen activator [7]**.** The mature form of HGF is produced following proteolytic cleavage and is composed of a 69 kDa α-subunit (containing four kringle domains) and a 34 kDa β-subunit, similar to the catalytic domain of other serine proteases but with amino acid substitutions at the active site [8]. Two natural alternatively spliced transcripts of HGF known as NK1 and NK2 have been identified [9]. These function as competitive inhibitors of HGF by binding to c-Met [10, 11]. In man, the HGF gene is a single copy gene, located on chromosome 7q11.1-21; it consists of 18 exons and 17 introns which span approximately 70 kb [12]. Disruption of the gene for mouse HGF leads to middle-stage embryonic lethality because of a defect in placental development, and no specific osteoatricular abnormalities have been observed [13]. A single injection of HGF at embryonic day 9.5 into the amniotic cavity of HGF(-/-) embryos rescued the placental defect and resulted in the survival of the embryos until term, but again no specific joint abnormalities have been noted [14]. HGF initiates intracellular signalling by binding to its receptor which is encoded by the c-Met proto-oncogene [15]. The phenotype of Met mutants is identical to HGF [16-18], which shows that, during development, Met is the only functional HGF receptor, and HGF is the only Met ligand [19]. The c-Met proto-oncogene product is a transmembrane tyrosine kinase which has a high affinity binding region for HGF. HGF also interacts with heparin proteoglycans and various collagen molecules [20, 21]. Cells that express c-Met include fibroblasts, macrophages, osteoblasts, [22] osteoclasts [22] and synovial lining cells [22]. Downstream transducers of HGF signalling include Src kinase, phosphatidylinositol-3(OH)-kinase (PI3-Kinase) and Cbl, proteins that are all involved in macrophage spreading and migration [23].

A hepatocyte growth factor-like protein termed macrophage stimulating protein (MSP) has also been identified. MSP is an 85 kDa heterodimeric glycoprotein that shares 45% homology with HGF [24]. The 693 amino acid propeptide of MSP is proteolytically cleaved to generate two polypeptide chains, a 55 kDa alpha-chain of 465 amino acids and a 28 kDa beta-chain of 228 amino acids that are linked together by one disulfide bridge [25]. The α-chain contains four triple disulfide loop structures called kringle domains that are identical to those seen in HGF [26]. The human receptor of MSP is termed RON and is a 185 kDa 1400 amino acid transmembrane protein that exhibits intrinsic tyrosine kinase activity [27]. Mouse RON which is termed STK (stem cell-derived tyrosine kinase) has also been cloned and found to show 74% homology to human RON at the amino acid sequence level. Cells known to express RON/STK include peritoneal macrophages [28] and osteoclasts [29, 30]. The expression of RON is induced by 1,25-Dihydroxyvitamin D_3 [30].

HGF AND ARTICULAR CARTILAGE

HGF and c-Met have been identified in murine and rodent embryos at sites of future joint development. This is in keeping with a role for HGF in skeletal morphogenesis [31]. HGF and c-Met are synthesized by chondrocytes and HGF has been shown to stimulate the migration of cultured articular chondrocytes. As HGF is found in the matrix of articular cartilage, and is known to influence cell motility,

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1573-3971/06 $50.00+.00

adhesion and cell-cell interaction, it is envisaged that HGF plays a significant role in normal cartilage homeostasis and the pathophysiology of OA.

HGF stimulates chondrocyte proliferation and proteoglycan synthesis *in vivo*. Both HGF and c-Met have been identified in situ in the territorial matrix of chondrocytes in articular cartilage. HGF expression is most prominent in the deep zone of normal articular cartilage, including the zone of calcified cartilage [32]. c-Met expression shows a similar distribution, although a small number of chondrocytes in the superficial zone are also positive for c-Met. HGF m-RNA is produced by chondrocytes in the deep zone and deeper mid zone regions of articular cartilage. Increased expression of HGF protein and m-RNA is found in OA cartilage, particularly in the deep zone; isolated chondrocytes in the superficial zone in OA are also positive for HGF [32]. Territorial or interterritorial HGF expression is positively correlated to the proteoglycans content of OA cartilage. In severe OA where there is extensive loss of matrix proteoglycan there is little or no HGF expression [32]. Reboul *et al*. have also shown that HGF induces collagenase 3 synthesis in OA chondrocytes by a kinase cascade involving SAPK/JNK [33]. In contrast Bau *et al*., [34] concluded that HGF appears to play little role in cartilage turnover; they found that proteoglycan, collagenase and aggrecanase synthesis in adult human articular cartilage was not stimulated by HGF. Although they identified c-Met m-RNA in both normal and OA cartilage, they found only low levels of HGF in these tissues [34].

These findings indicate that both HGF and its receptor c-Met are expressed by chondrocytes in normal and OA cartilage, and that their expression is upregulated in early OA. Synthesis of HGF and its receptor may be related to proteoglycans production, an important factor in the pathogenesis of OA. As HGF can also increase type II collagen synthesis and bind to thrombospondin, chondroitin and hyaluronic acid [21, 35], it has been hypothesized that HGF may be involved in cartilage regeneration in OA. Intra-articular injection of HGF in rabbits has been shown to promote the healing of osteochondral defects and HGF has been proposed as a possible anabolic factor to promote cartilage synthesis and matrix production [36].

HGF AND SYNOVIAL TISSUES

HGF and its receptor c-Met have been identified in synovial tissues of rheumatoid arthritis (RA) and osteoarthritis (OA). c-Met is mainly expressed by synovial fibroblasts and synovial macrophages as well as by cells in the synovial lining [22], whereas HGF is expressed mainly by subintimal synovial fibroblasts and synovial macrophages [37]. Several studies have shown that HGF, HGF-A, and c-Met m-RNA are expressed in synovial tissues in RA and OA. As synovial macrophages are more numerous in RA than in OA it has been proposed that HGF may play a role in the pathogenesis of RA. Immunohistochemical staining has shown that HGF-A, which converts inactive HGF to its biologically active form, and c-Met are strongly expressed in synovial lining cells, subintimal fibroblasts, macrophages, and endothelial cells. HGF is weakly expressed by subintimal macrophages and fibroblasts and by some but not all endothelial cells in RA and OA synovial tissues.

It has been proposed that HGF-A activation may play a role in the induction of angiogenesis in inflammatory arthritis by promoting the binding of HGF to c-Met on endothelial cells [22]. Vascular proliferation is important in the pathogenesis of RA and HGF has been shown to promote angiogenesis in many tissues; it has been shown that the level of synovial tissue immunoreactive HGF correlates positively with the number of synovial tissue blood vessels [22]. Further support for a role for HGF in the vasculoproliferative phase of RA, is provided by the finding that anti-HGF neutralizes the chemotactic activity of endothelial cells in rheumatoid joints [37]. HGF also activates the transcription factor nuclear factor NF-κB mimicking the prototypical NF-κB activators TNFα and IL1α *via* activation of extracellular signal regulated kinase 1/2 (ERK1/2) and p38 mitogen activated protein kinase (MAPK p38) cascades, independently of PI3-Kinase activation [38]. NF-κB activation by HGF may be an additional pathway by which angiogenesis is achieved [38, 39].

Elevated levels of HGF have also been found in RA synovial fluid and serum [40]. Although the pathogenetic mechanism is not fully understood, it is postulated that HGF plays a role in the propagation of rheumatoid synovitis and pannus formation by promoting the invasive properties of rheumatoid synovial cells, endothelial cell migration and synovial neovascularization [37]. HGF release by adherent synovial cells and synovial fluid cells derived from RA patients has also been correlated with increased levels of IL-6 in synovial fluid and disease activity. This is of some interest as it has been shown that this cytokine stimulates osteoclast formation and bone resorption, possibly indicating a role for HGF in periarticular resorption in RA [41].

HGF AND BONE

Bone remodelling occurs throughout life and involves a coordinated sequence of osteoclastic bone resorption and osteoblastic bone formation on the surface of trabecular bone and Haversian canals. HGF is considered to be a regulator of bone remodelling [17, 42] and, by inducing cell mitogenesis [43] and tissue angiogenesis [44, 45], is thought to play a role in normal skeletogeneis and bone turnover. It has also been shown to be involved in several pathological conditions of bone such as osteoporosis, bone tumour formation and fracture repair [46, 47].

Bone resorption is carried out by osteoclasts which are large 20-100μm multinucleated cells formed by fusion of monocyte/macrophage precursors [48] that are of haematopoietic origin [49]. Osteoclast formation requires the presence of macrophage colony stimulating factor (M-CSF) and occurs when marrow and circulating precursors, which express the receptor for nuclear factor kappa B (RANK), come into contact with osteoblasts/bone stromal cells which express RANK ligand (RANKL). Osteoprotegerin (OPG) is a soluble decoy receptor for RANKL that inhibits osteoclast formation and bone resorption. RANKL and OPG m-RNA expression by osteoblastic cells is regulated by osteoclastogenic factors such as $1,25(OH)_2D_3$. Recently other activated signalling pathways involving TNFα/IL-1 [50-52] IL-6 [41] and TGF-β [53] have also been reported to promote osteoclastogenesis. TNFa, IL-1, IL-6, and TGF-β

have been shown to markedly influence levels of c-Met m-RNA in human tissues [54]; it is therefore possible that c-Met may indirectly influence the regulation of osteoclast differentiation through these cytokines.

The effects of HGF on osteoclast formation and activity are complex. Biologically active HGF is secreted by osteoblasts and co-operatively stimulates the growth and survival of haematopoietic stem cells [2] from which osteoclast precursors are derived [55-57]. HGF has been shown to stimulate the formation of osteoclasts in the presence of the osteoblastic cell line UMR 106. The addition of anti-HGF neutralizing IgG [58] inhibited the formation of osteoclasts and stimulation of osteoclastic resorption in co-cultures of haemopoietic blast cells and calvaria-derived osteoblast-like cells [59]. This is most likely due to HGF stimulation of the proliferation of mononuclear phagocyte osteoclast precursors by enhancing DNA replication of activated monocytes [60]. An indirect role of HGF in monocyte/macrophage differentiation is further supported by the fact that compounds that promote monocyte/macrophage differentiation, such as 12-O-tetradecanolyphorbol-13-acetate (TPA) and 1,25-Dihydroxyvitamin D_3, induce changes in the amount of HGF receptor mRNA.

TPA acts through Protein Kinase C in a dose dependent manner and induces HGF receptor mRNA expression up to 10-fold [61]. Monocytic cells (THP-1) do not normally express c-Met mRNA and protein. However upon incubation with TPA, IL-6 and TNFα, or interferon gamma and TNFα, a pronounced increase in the amount of Met mRNA and protein is seen in THP-1 cells; these changes correlate with the onset of differentiation in these monocytic cells [62]. These findings indicate that the HGF and Met act as physiological regulators of monocyte-macrophage differentiation. HGF also upregulates monocyte expression of its receptor c-Met, HGF convertase (uPa), and IL-6 production [60]. HGF is also known to induce IL-11 secretion by osteoblasts [63]. HGF stimulation of cytokine production is of interest as it has recently been shown that IL-6 and IL-11 support human osteoclast formation by a RANKL independent mechanism [41]. We also have some preliminary data to suggest that HGF can induce osteoclast formation from monocyte precursors in the absence of M-CSF (Adamopoulos & Athanasou unpublished observations).

Osteoclasts are highly motile cells. Osteoclast motility is a factor in bone resorption as osteoclasts are thought not to resorb bone while migrating [64]. In osteoclast cultures the addition of HGF has been shown to inhibit osteoclastic bone resorption [59]. This has led to the suggestion that it may do so by modulating the balance between resorption and migration. Osteoclast motility on bone depends on regulated generation of force against substrate through adhesion receptors known as integrins. Integrins are transmembrane non-covalently linked heterodimeric receptors consisting of α and β subunits. Osteoclasts highly express the vitronectin receptor (integrin $\alpha_v\beta_3$) and, when its function is disrupted, osteoclast adhesion and bone resorption is inhibited [65]. HGF has been shown to modulate $\alpha_v\beta_3$ conformational states required for osteoclast polarization and resorption through activation of PI3-Kinase, [66] a protein involved in podosome formation by osteoclasts [67]. Podosomes are small cylindrical structures that consist of an F-actin core surrounded by the actin-binding proteins vinculin, talin, and α-actinin [68]. Osteoclasts use their unique speed of podosome assembly and disassembly to generate high rates of motility. HGF is known to induce tubulogenesis in many cell types and is therefore likely to regulate the assembly of tubular structures such as podosomes in osteoclasts [69, 70]. During bone resorption calcium is released and it is possible that an increase in intracellular calcium may signal termination of bone resorption. Non resorbing osteoclasts that possess pseudopodia have twice as much intracellular calcium compared with resorbing osteoclasts that do not have pseudopodia [71]. As HGF can induce a rapid rise in the cytosolic free calcium [72] it is possible that HGF may regulate podosome formation and motility by both calcium regulation and PI3-Kinase activation in osteoclasts.

Terminally differentiated osteoclasts produce and secrete HGF suggesting that other pathways independent of differentiation may be regulated by HGF [1]. One of the proteins that become phosphorylated by HGF and is highly expressed in osteoclasts is Src kinase. This non receptor tyrosine kinase has been implicated as a modulator of cell proliferation, spreading and migration, and osteoclasts from *Src*-/- mice are severely osteopetrotic [73]. Recently Src was shown to act as a regulator of the respiratory enzyme cytochrome C oxidase (Cox) where Src-induced phosphorylation of Cox is required for maintaining high levels of ATP to meet the cells high energy requirement for bone resorption [74]. Src may also be important for regulating the localization of certain proteins or stabilizing signalling complexes that are necessary for formation of the ruffled border and bone resorption. Consistent with this hypothesis, Cbl, a scaffolding protein that binds to multiple signalling proteins, is not localized properly in Src-deficient osteoclasts reviewed by [75]. HGF treatment of osteoclasts increases Src kinase activity and alters osteoclast cell shape [1]. Moreover MSP treatment of osteoclast-like cells induces formation of ruffled border, and causes rapid redistribution of Src to the borders of cytoplasm. These phenomena are associated with increased bone resorption [30]. Osteoclasts depend critically on vitronectin-receptor function for substrate adhesion, and it has been postulated that Src regulates integrin function [76]. The precise site of action of Src and its location in the signalling cascade that regulates vitronectin-receptor function remains unclear.

HGF and MSP activation of downstream signalling proteins is also associated with antiapoptotic effects that would increase the survival of osteoclasts (and osteoclast precursors) and hence the extent of bone resorption. Although the exact mechanism by which HGF promotes cell survival is unknown, it appears to involve PI3-Kinase activation of serine/threonine Protein Kinase B (PKB/Akt); the latter plays an important role in the regulation of anti- or pro-apoptotic members of the Bcl-2 family, such as BAG-1, Bcl-xL or Bad [77-80]. From experiments in $SHIP^{-/-}$ mice, it has also been shown that the PKB/Akt pathway is vital for the survival of osteoclast precursors. Src homology 2–containing inositol-5-phosphatase (SHIP) specifically recognizes and cleaves the 5'-phosphate group from phosphatidylinositol-3,4,5-trisphosphate (PIP_3), the major product of PI3-Kinase and in this way it blunts PI3-Kinase signalling. In $SHIP^{-/-}$ mice, osteoclasts are increased two-fold

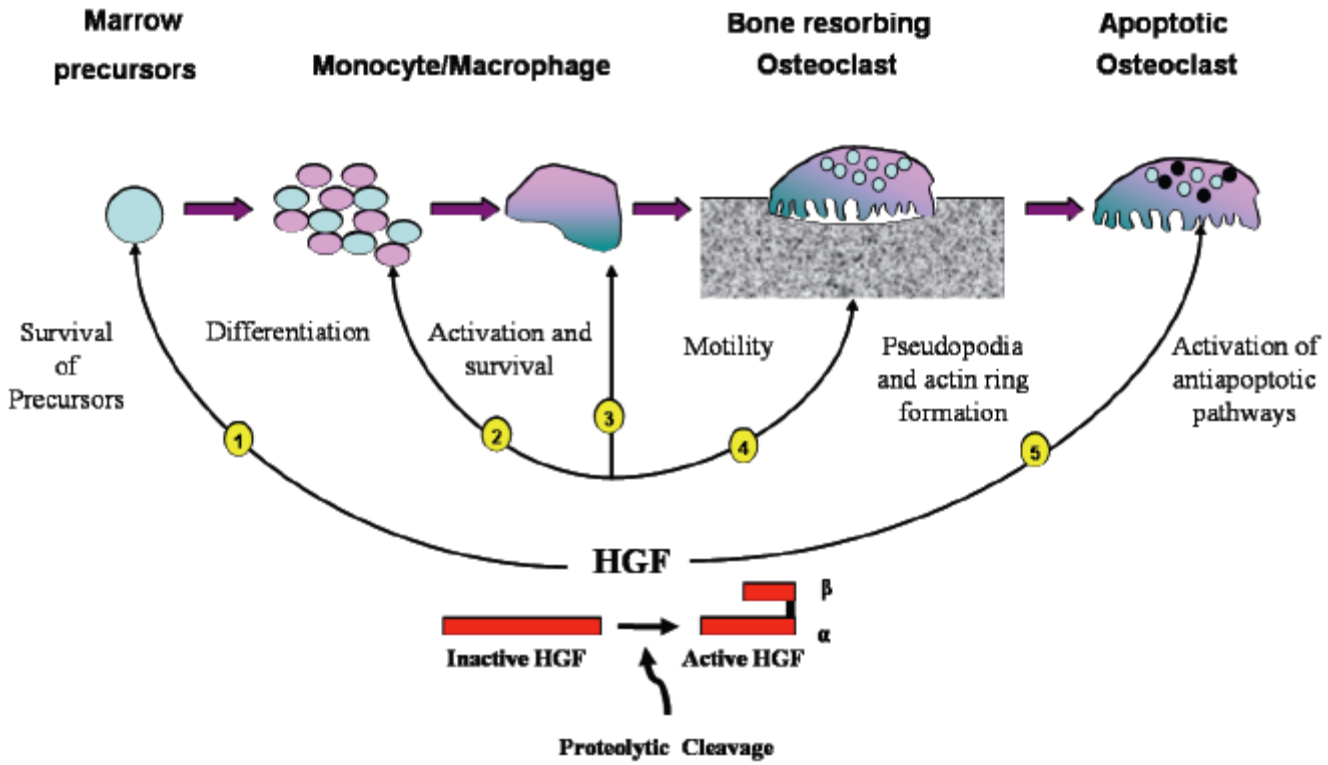

Fig. (1). Multiple actions of Hepatocyte Growth Factor on the osteoclast formation and function. HGF induces the survival of marrow osteoclast precursors (1), stimulates the differentiation of mononuclear phagocytes (2), activates and promotes the survival of macrophages (3) induces the motility of osteoclasts by enhancing pseudopodia and actin ring formation (4) and stimulates osteoclast survival (5).

in number mainly due to increased survival of osteoclast precursors [81]. The osteoclasts formed in SHIP$^{-/-}$ mice are similar in morphology to osteoclasts in Paget's disease, [82] being larger in size and containing more nuclei than normal osteoclasts, and showing increased resorptive activity. RON signalling also exhibits antiapoptotic effect and has been shown to support survival of activated macrophages during inflammation [83]. Taken together these findings indicate that HGF and MSP are likely to stimulate osteoclast survival by inhibiting apoptosis. The actions of HGF on osteoclast formation and function are summarised in Fig. **1**.

HGF is also known to influence the function and differentiation of osteoblasts and other stromal cells in bone. Inhibition of HGF expression by osteoblasts may play a role in glucocorticoid induction of osteoporosis [84]. In osteoblasts, fibroblast growth factor 2, one of the major factors stimulating bone formation, [85] is believed to exert its effects partly through stimulation of HGF expression [86]. It has been shown that OA osteoblasts produce five times more HGF than normal osteoblasts and almost no HGF truncated isoforms such as HGF/NK1 [87]. HGF activity may in this way contribute to the subchondral bone sclerosis that is such a prominent feature of OA. HGF is also known to induce IL-11 secretion from osteoblasts, this is of interest as it has recently been shown that IL-6 and IL-11 support human osteoclast formation by a RANKL independent system [41].

Benign and malignant bone tumours showing fibroblastic, osteoblastic or chondroblastic differentiation such as desmoplastic fibroma, osteoblastoma, osteosarcoma and chondroblastoma as well as giant cell tumour have been shown to expresses c-Met receptor [88, 89]. Expression of c-Met has been correlated with more aggressive tumour behaviour and overexpression of c-Met has also been linked to malignant transformation of mesenchymal tumours and tumour invasion.

SUMMARY

HGF is a ubiquitous growth factor which profoundly influences cell differentiation and function as well as tissue morphogenesis. In bone and joint tissues, HGF plays a role in normal cartilage and bone metabolism. It has been implicated in the pathogenesis of OA and RA and clearly acts at various stages of the osteoclastic bone resorption process. Further work is needed to define the precise role of HGF in normal and diseased bone and joint tissues in order to determine the full potential of this fascinating growth factor.

ACKNOWLEDGEMENTS

IA is the recipient of a Research into Ageing DPhil studentship. Current work in the Laboratory is supported by Research into Ageing Grants.

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Received: November 1, 2004 Accepted: January 12, 2005 Revised: January 26, 2005