RAPID COMMUNICATION

Hepatocyte Growth Factor Is a Synergistic Factor for the Growth of Hematopoietic Progenitor Cells


Bone marrow (BM) stromal cells, which include macrophages, fibroblasts, endothelial cells, and adipocytes, have been shown to produce several factors that modulate the growth of BM progenitors. Hepatocyte growth factor (HGF) is a fibroblast-derived factor and has recently been shown to be a ligand for the c-met proto-oncogene, a member of the receptor class of tyrosine kinases. c-met messenger RNA (mRNA) is predominantly expressed in epithelial cells, but has been detected in several murine hematopoietic progenitor cell lines, suggesting that HGF and met might function during hematopoiesis. Here, BM cells were found to express both met mRNA and protein. Moreover, HGF was shown to synergize with interleukin-3 and granulocyte-macrophage colony-stimulating factor to stimulate colony formation of hematopoietic progenitor cells in vitro. These results show that, in addition to its activity on epithelial cells, HGF is a new member of the functionally related group of factors that modulate hematopoiesis. This is a US government work. There are no restrictions on its use.

Hepatocyte growth factor (HGF) has been shown to be a critical mitogen for liver regeneration. In addition to its activity on hepatocytes, it has been found to promote the growth of melanocytes, epithelial cells, and endothelial cells. HGF is predominantly produced by fibroblasts and has recently been shown to be identical to scatter factor, an activity that causes epithelial cells to dissociate and become motile. HGF is unique among the peptide growth factors in showing 38% homology to plasminogen. HGF and plasminogen both contain kringle domains, a conserved motif found in several proteins that are involved in coagulation and wound repair. These observations suggest that HGF might function in the growth and renewal of epithelial cells that would be required in wound repair. Moreover, HGF has been shown to bind to the extracellular matrix, which would allow it to function in the hematopoietic microenvironment, as has been shown for granulocyte-macrophage colony-stimulating factor (GM-CSF).

We and others have recently shown that HGF is a ligand for met. The c-met gene encodes a member of the tyrosine kinase receptor family and is expressed in a wide variety of tissues, most predominantly in tissues of epithelial origin. It is synthesized as a 170-Kd precursor, which is then proteolytically processed to 140-Kd and 45-Kd disulfide-linked chains. We have shown that c-met messenger RNA (mRNA) is expressed in several murine myeloid progenitor cell lines that are blocked in differentiation, raising the possibility that HGF might be mitogenic for these cell lines. One of these cell lines, NFS-60, expresses significant levels of c-met mRNA and requires interleukin-3 (IL-3) for growth in vitro. We examined the ability of HGF and IL-3 to stimulate [3H]thymidine incorporation into NFS-60 cells. As previously shown, IL-3 stimulated [3H]thymidine incorporation into NFS-60 cells in a dose-dependent manner. To determine whether HGF might synergize with IL-3, we examined the effect of HGF on [3H]thymidine incorporation using a concentration of IL-3 (2.5 U) (Fig 1B) that was suboptimal for growth stimulation. Under these conditions HGF stimulated a threefold increase in [3H]thymidine uptake. The half-maximal level of [3H]thymidine incorporation was obtained with approximately 50 ng/mL of HGF, which is similar to the amount of HGF needed to stimulate [3H]thymidine incorporation into epithelial cells growing at subconfluence. Comparable HGF/IL-3 synergy was observed in another IL-3-dependent myeloid cell line, Da-i, that expresses high levels of c-met mRNA (data not shown).

The expression of met in myeloid progenitor cell lines raised the question of whether unfractionated and progenitor enriched murine bone marrow (BM) cell populations expressed met mRNA and protein. The progenitor enriched (lineage negative [lin-]) cells were prepared by removing committed myeloid, B, and T cells from total BM cells using magnetic beads coated with antibodies specific for antigens present on differentiated cells. Total RNA was prepared using the RNAzol reagent and 10 µg of each RNA was run on a formaldehyde-agarose gel. NFS-60 was used for comparison (Fig 2A, lane 1). All three samples showed a 7-kb mRNA that corresponds in size to the major met transcript. Similar levels of met mRNA were detected in unfractionated BM and lin- BM cells (Fig 2A, lanes 2 and 3).
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Fig 1. [3H]thymidine uptake of NFS-60 cells. (A) Cells (5 x 10^3) were plated in 100 µL media. Ten units of IL-3 (Genzyme, Cambridge, MA) was added to the first well, with decreasing amounts of IL-3 in successive wells as indicated. After 48 hours, 1 µCi [3H]thymidine was added per well. The cells were incubated another 7 hours and then harvested onto filters and processed for scintillation counting. Points shown are the average of two wells. (B) The lower curve shows [3H]thymidine uptake from the addition of HGF alone. The first well received a 1:4,000 dilution of HGF, with further dilutions as shown. The upper curve shows the effect of HGF when added in combination with IL-3. IL-3 at 2.5 U was added per well. HGF levels were as above.

These results show for the first time that met is expressed in BM cells.

Having detected met mRNA in BM cells, we then tested for expression of met proto-oncogene product. Again, NFS-60 cells were used for comparison. Unfractionated BM cells were labeled for 45 minutes with [35S]methionine and cysteine (labeling conditions optimized for detection of the 170-Kd precursor of met in these cells; T. Kmiecik, unpublished data). Cells were lysed and c-met protein was immunoprecipitated with a rabbit antiserum (SP-301) directed against amino acids 965 to 981 of the intracellular domain of the murine met protein. Fractionation of the immunoprecipitates on a sodium dodecyl sulfate (SDS)-acrylamide gel showed that the precursor 170-Kd met protein is present on NFS-60 cells and BM cells (Fig 2B).

The expression of the met proto-oncogene in BM cells led us to examine whether HGF would affect the growth of these cells in soft agar colony assays. Similar to the results obtained in [3H]thymidine incorporation assays performed with the progenitor cell line NSF-60 (Fig 1), HGF added alone to lin^- cells showed no effect in any colony formation assays (data not shown). However, when HGF was added to a concentration of IL-3 (1 ng/mL) suboptimal for colony growth, we obtained a 50% increase in colony formation (Fig 3A). HGF also showed a synergistic effect with GM-CSF and enhanced colony formation by 60%. With both IL-3 and GM-CSF, the resultant colonies contained macrophages and granulocytes in similar proportion to that obtained with IL-3 or GM-CSF alone, indicating that HGF did not alter the pattern of differentiation (data not shown). Although the above analyses were performed with recombinant human HGF, we observed the same pattern of stimulation of colony formation of BM cells with murine scatter factor (data not shown). Thus, in addition to its previously documented effects upon hepatocytes, epithelial cells, endothelial cells, and melanocytes, HGF synergizes with IL-3 and GM-CSF to stimulate proliferation of myeloid progenitor BM cells. In this regard, there is some overlap with IL-6, which has been shown to induce proliferation of hepatocytes and also to act as a synergistic factor for IL-3-dependent colony formation. HGF has been shown to be produced by the myelomonocytic cell line HL-60 and may be produced by monocytes and alveolar macrophages. A feedback mechanism may therefore exist whereby HGF production by differentiated cells of the myeloid lineage would stimulate the production of monocytes and macrophages from BM progenitors. A similar proposal has recently been made for tumor necrosis factor. While the effect of HGF on hematopoietic progenitors may represent a direct proliferative stimulus, it is also...
Fig 2. (A) Northern blot of RNA from NFS-60 myeloid leukemia line, unfractonated BM, and lin- BM. Total RNA (10 µg) was run on a 1.2% formaldehyde-agarose gel, transferred to Duralose-UV, and probed with a restriction fragment containing the entire coding region of murine met. The membrane was prehybridized in 5× SSPE, 5× Denhardt’s, 50% formamide, 0.1% SDS, and 100 µg/mL salmon sperm DNA for 4 hours and then hybridized in the above solution containing probe labeled by random priming. The membrane was washed twice for 10 minutes each in 2× SSC, 0.1% SDS at room temperature and then twice for 10 minutes each in 0.2× SSC, 0.1% SDS at 53°C. The membrane was exposed to Kodak X-OMAT AR film with an intensifying screen at -70°C.

(B) Immunoprecipitation of met protein from NFS-60 and BM cells. Cells were labeled for 45 minutes with [35S]methionine and [35S]cysteine in methionine, cysteine-free RPMI 1640 supplemented with 10% fetal calf serum. Cells were lysed in NP-40 lysis buffer. Lysates were centrifuged 15 minutes at 15,000 RPM. Each lysate containing 3 x 10⁵ cpm was added per immunoprecipitation reaction. Supernatants were incubated in the absence (-) or presence (+) of competing peptide with antisera 301 (directed against the peptide ELVRYDARVHTPHLDRLVSAR). Proteins were precipitated with protein G beads (Bethesda Research Laboratories, Gaithersburg, MD). Immunoprecipitates were fractionated on 3% to 17% SDS-acrylamide gels. The gels were treated with Amplify (Amenham, Arlington Heights, IL) and fluorography was performed. Exposure time was 1 week for the BM lanes and overnight for the NFS-60 lanes to compensate for the much lower specific activity of the BM lysate (6.8 x 10³ cpm/µg) compared with the NFS-60 lysate (6.4 x 10⁴ cpm/µg).

Fig 3. Colony formation in agar of BM cells. (A) HGF was added in combination with IL-3 (1 ng/mL). (B) HGF was added in combination with GM-CSF (1 ng/mL) [gift of T. Boone, Amgen, Thousand Oaks, CA). A modification of the method of Stanley et al was used to measure colony formation of lin- cells. Lin- BM cells were suspended in 1 mL RPMI 1640, 10% fetal calf serum, and 0.3% Seaplaque agarose (Rockland, ME), and incubated in 35-mm Lux Petri dishes (Miles Scientific, Naperville, IL) at 37°C in 5% CO₂ and scored for colony growth. Data shown represent an average of two experiments ± SD.

possible that HGF functions by suppressing apoptosis and increasing the population of cells that can respond to other factors. Given the ability of HGF to mediate epithelial cell motogenesis, there may be an additional role for HGF in modulating the interaction of BM progenitor and stromal cells and/or the extracellular matrix.

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