HEPARIN-BINDING (FIBROBLAST) GROWTH FACTORS TYPE ONE AND TWO GENES ARE CO-EXPRESSED IN PROLIFERATING NORMAL HUMAN VASCULAR ENDOTHELIAL AND SMOOTH MUSCLE CELLS IN CULTURE

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SUMMARY

Complimentary ribonucleic acid (cRNA) probes were used to detect expression of the genes for heparin binding (fibroblast) growth factor type one (HBGF-1) and two (HBGF-2) in cultured endothelial and smooth muscle cells from normal human blood vessels. Hybridization in situ revealed that transcripts for both HBGF-1 and HBGF-2 are expressed in endothelial cells from both umbilical vein and aorta. Relative intensity of radioactive grains suggest that HBGF-1 gene expression may exceed HBGF-2 expression in aortic smooth muscle cells. Collective expression of both HBGF-1 and HBGF-2 in smooth muscle cells may exceed that in endothelial cells.

Key words: FGF; mRNA; atherosclerosis; wounds; growth factors.

INTRODUCTION

Understanding the factors that regulate human large vessel endothelial (EC) and smooth muscle cells (SMC) is essential to understanding normal regeneration of human large vessels and the abnormalities in EC and SMC proliferation associated with atherosclerosis (Ross and Glomset, 1976; Ross et al., 1984; Ross, 1986; Hoshi et al., 1988). Heparin-binding (fibroblast) growth factors (HBGF) are key mitogens for both human large vessel endothelial (EC) and smooth muscle cells (SMC) in culture (Winkles et al., 1987; Hoshi et al., 1988; Burgess and Maciag, 1989). HBGF appears to be the single most important mitogen for EC while HBGF act together with insulin-like growth factors, epidermal growth factor (or the homolog, TGF-α) and platelet-derived growth factor (PDGF) to support maximal SMC proliferation (Hoshi et al., 1988). Although monocytes may deliver some HBGF to vascular wound sites (Baird, Mormede and Bohlen, 1985), HBGF originate endogenously in many tissues and, therefore, probably act on EC and SMC in an autocrine or paracrine mode. Understanding the factors that regulate EC and SMC proliferation is essential to understanding normal regeneration of human large vessels and the abnormalities in EC and SMC proliferation associated with atherosclerosis.

We suggest, in agreement with Winkles et al. (1987), that expression of HBGF-1 mRNA may predominate in smooth muscle cells, but that both HBGF-1 and HBGF-2 mRNA are co-expressed. In contrast to the report by Winkles et al. (1987), we show that both HBGF-1 and HBGF-2 are also co-expressed in human endothelial cells.

MATERIALS AND METHODS

Materials. Human umbilical cords were obtained from Saranac Lake General Hospital (Saranac Lake, NY). Human large vascular tissues were obtained from the National Diabetes Research Interchange (Philadelphia, PA) and the Comprehensive Cancer Research Center (Birmingham, AL). Culture grade epidermal growth factor (EGF) and bovine hypothalamic extract (H-NEUREXT) were purchased from UBI (Lake Placid, NY).

Cell culture. Endothelial cells were isolated and cultured from human umbilical cord veins and human abdominal aorta and smooth muscle cells from human abdominal aorta from nonatherogenic autopsy specimens as previously described (Hoshi et al., 1988). For in situ hybridization experiments, two-chamber glass slides (Labtech, Nunc, Inc., Naperville, IL) were coated with collagen and cells were inoculated at a density of $2 \times 10^4$ cells per chamber in 2 ml of medium MCDB 107 containing 2% (v/v) fetal bovine serum (FBS), bovine hypothalamic extract (H-NEUREXT) (25 μg/ml), heparin (25 μg/ml), and EGF (10 ng/ml). After incubation for 3 days, the medium was changed to medium MCDB 107 containing 1% FBS and maintained for 24 hours prior to fixation. Cells used for in situ hybridization were secondary cultures prepared from primary cultures of respective cell types.

In situ hybridization. In situ hybridization was performed by modification of described procedures (Singer, Lawrence and Villmave, 1986). Cells prepared on slides as described above were rinsed twice in phosphate-buffered saline (pH 7.4) (PBS) and fixed with fresh 4% paraformaldehyde in PBS containing 5 mM MgCl₂ for 15 min. The slides were stored at 4°C in 70% ethanol. Prior to hybridization the cells were rehydrated in PBS and 5 mM MgCl₂ for 10 min and then placed in 0.20 M Tris-HCl (pH 7.4) and 0.10 M glycine for 10 min. Immediately before hybridization, the slides were transferred to 50% formamide in double-strength 0.15 M sodium chloride and 0.015 M sodium citrate (SSC) and heated at 65°C for 10 min. The probe mixture consisted of 5 μl formamide, 1 μl of 20 X SSC, 1 μl yeast tRNA (10 mg/ml), 1 μl of single-stranded DNA (10 mg/ml), 1 μl bovine serum,
(BSA) albumin (20 mg/ml) and 1 μl of labeled RNA (1 × 10^6 cpm). The hybridization mixture was applied to the slides at 10 μl per 22 mm^2. Radioactive probe concentrations were determined to be in excess using both human umbilical vein endothelial cells and cultured rat hepatocytes which express high levels of HBGF-1 mRNA (Kan et al., 1989). Cover slips were applied and the hybridization was carried out for 3 hr at 50°C in a plastic slide carrier. After hybridization, the slides were washed twice for 5 min and then one time for 20 min in 50% formamide in 2 X SSC at 52°C with constant agitation, then four times for 1 min in 2 X SSC at room temperature, and then treated with 0.10 μg/ml RNase A + T for 30 min at 37°C. The slides were then rinsed two times for 1 min in 2 X SSC at room temperature, then 50% formamide in 2 X SSC at 52°C for 5 min with constant agitation, then in 2 X SSC for 1 min at room temperature, and then stirred gently for 10 min in 2 X SSC. Dehydration of the slides was carried out sequentially in 70, 80 and 95% ethanol with frequent agitation. Air dried slides were dipped into NTB-2 emulsion diluted 1 to 1 with water, exposed for 5 days, developed in Kodak D19 solution and finally stained with hematoxylin and eosin. Radioactive sense- and anti-sense (cRNA) strand probes were synthesized and labeled with [32P]UTP from linearized SK vectors (Stratagene) containing the rat HBGF-1 cDNA (Goodrich et al., 1989) or the bovine HBGF-2 cDNA (Abraham et al., 1986) using T7 and T3 polymerase according to the manufacturers suggestions. The nucleotide coding sequence for rat and human HBGF-1 exhibits 89% homology and bovine and human HBGF-2 exhibit a homology of 95%.

RESULTS

Variability and the large numbers of pure populations of normal human large vessel cells required precluded clear and routine assessment of HBGF gene expression by conventional Northern analysis of poly(A+) RNA. Hybridization parameters were first optimized using endothelial cells (EC) from umbilical vein which can be obtained in much larger numbers relative to EC cultures from small pieces of human adult large vessels (Hoshi et al., 1988). Northern analysis of 5 μg of poly(A+)RNA extracted from a pool of cultured umbilical vein EC at passages 2 to 5 revealed predominately a 1.8 Kb HBGF-2 transcript that hybridized under stringent conditions with the bovine HBGF-2 cDNA (Abraham et al., 1986) (Fig. 1A). A minor transcript at 2.8 Kb was also detectable. No HBGF-1 transcripts were detectable after hybridization of the same filter with the human HBGF-1 cDNA (Mansson et al., 1989) of similar specific activity after the same 11 day exposure of the blot (not shown). This suggested that expression of HBGF-2 may predominate in human umbilical vein EC and that the HBGF-1 gene may not be detectable by Northern blot analysis in EC as suggested by Winkles et al. (1987). Sense- and anti-sense-strand RNA probes for both HBGF-1 and HBGF-2 mRNA were prepared and hybridized to fixed umbilical vein EC as described in Materials and Methods. The hybridization in situ clearly revealed that both HBGF-1 and HBGF-2 transcripts are co-expressed in the umbilical vein-derived EC (Fig. 1 B-E). The indicated fields are representative of over 90% of cells on the slide. It remains unclear why HBGF-1 transcripts are not detectable relative to HBGF-2 transcripts in Northern blot analysis of poly(A+) RNA (Fig. 1A). Hybridization in situ using both HBGF-1 and HBGF-2 RNA probes revealed that both genes are also co-expressed in cultured EC derived from human aorta at levels that appear similar to those in umbilical vein EC (Fig. 2). Smooth muscle cell (SMC) cultures were also established from human adult aorta and analyzed under

Fig. 1. Expression of HBGF in human umbilical vein endothelial cells. A. Northern blot analysis of poly(A+) RNA (5 μg) using human HBGF-1 cDNA (1.7 × 10^6 cpm/μg) and bovine HBGF-2 cDNA (1.3 × 10^6 cpm/μg). The blot was sequentially probed with both cDNA's and exposed for 11 days in each case. Only the indicated 1.8 Kb band was detectable after hybridization with the HBGF-2 probe. B-E. Cells were fixed and probed in situ with [32P]-labeled rat HBGF-1 and bovine HBGF-2 RNA probes and exposed for 5 days as indicated below. B = sense-strand HBGF-1 RNA (3.7 × 10^6 cpm/μg); C = HBGF-1 cRNA (3 × 10^6 cpm/μg); D = sense HBGF-2 RNA (2.19 × 10^6 cpm/μg); E = HBGF-2 cRNA (2.29 × 10^6 cpm/μg).