Vol. 153, No. 1, 1988 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 31, 1988 Pages 39-45

EVIDENCE OF AUTOCRINE REGULATION IN HUMAN HEPATOMA CELL LINES

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Received April 4, 1988

Summary Human hepatoma cell lines were studied for the expression of platelet-derived growth factor (PDGF), insulin-like growth factor-I (IGF-I) and their receptors at the mRNA level. Transcripts of PDGF were consistently detected in these cell lines. In addition, some cell lines also expressed PDGF receptor RNA. Moreover, RNA of IGF-I and its receptor were detected in every cell line examined. These results suggest that autocrine regulation may be an important mechanism for the maintenance of the transformed state of human hepatoma cells.

The recent development of oncogene and growth factor studies supports the hypothesis that the growth advantage of cancer cells is mediated through autocrine regulation (1). Several studies have shown that tumor-derived cell lines secrete polypeptide growth factors and in many cases release these cell lines of serum requirements (2,3,4). Therefore, in transformed cells, the requirements for specific growth factors is lost or decreased, while nontransformed cells show an absolute requirement for growth factors to proliferate in culture.

Study of the growth of fibroblast cells led to the discovery of two types of growth factors to be required for a cell proliferation. One is the factor that induces cells to become competent to enter G1 phase of the cell cycle (5). Examples are platelet-derived growth factor (PDGF) and fibroblast growth factor. Another is the factor that allows competent cells to undergo progression through the G1 phase to enter the S phase of the cell cycle (6). Examples are insulin-like growth factors (IGFs), insulin and epidermal growth factor. It has been shown that NIH 3T3 cells can progress into the S phase of

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Abbreviations: PDGF, platelet-derived growth factor; IGF-I, insulin-like growth factor-I; hepatoma, hepatocellular carcinoma; DMEM, Dulbecco's modified Eagle medium; FCS, fetal calf serum; kb, kilobases; SDS, sodium dodecyl sulfate.

0006-291X/88 $1.50

the cell cycle in a serum-free medium supplemented with PDGF and IGF-I. Another study also demonstrates that PDGF and IGF-I have an in vivo coordinate influence on wound healing (7). These studies illustrate the importance of the synergistic effects of the competence factor and progression factor in promoting the growth of fibroblasts.

Hepatocellular carcinoma (hepatoma) is the liver cancer most common in Asia and Africa (8). To attempt to understand the mechanism of the uncontrolled growth of human hepatoma, human hepatoma cell lines were studied for the expression of PDGF, IGF-I and their receptors at the RNA level.

Materials and Methods

Cell lines --- Human hepatoma cell lines were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS). The origins of the cell lines are as described (9,10).

Isolation of RNA from cell lines --- Cell lines were grown to about 80% confluency in DMEM supplemented with 10% FCS. Total cellular RNA was extracted by the guanidium/cesium chloride method (11). Poly(A^+) RNA was purified by fractionation using oligo(dT)-cellulose chromatography as described by Aviv and Leder (12). The concentrations of RNA were determined by A_{260} reading and by the intensity of nucleic acid on the gel by ethidium bromide staining.

Northern blot analysis of RNA --- Five μg of poly(A^+) RNA from human hepatoma cell lines was denatured with glyoxal and applied to a 1.2% agarose gel for electrophoresis (13,14). The RNA was transferred to nitrocellulose paper and hybridized with nick-translated [^{32}P] labeled DNA probes under the conditions described (13,14). The blots were washed in 15 mM NaCl/1.5 mM sodium citrate plus 0.1% SDS at 50-55°C and exposed to Fuji X-ray film at -70°C using a Kyokko intensifying screen. The sizes of the transcripts were determined relative to human 28S and 18S RNA. The following cloned DNA fragments were used as probes: human PDGF A-chain cDNA (15), v-sis (16), human PDGF receptor cDNA (17), human IGF-I cDNA (18) and human IGF-I receptor cDNA (19).

Slot blot analysis of PDGF A-chain and B-chain RNA --- Three μg of poly(A^+) RNA denatured by formaldehyde was filtered through a slot blot apparatus (Schleicher & Schuell, W. Germany) (20). The filters were prepared in duplicate; one filter was hybridized to nick-translated [^{32}P] labeled cDNA probes of PDGF A-chain (15), and the other was hybridized to v-sis (16) under the condition as described in the Northern blot analysis.

Growth of human hepatoma cell line HA22T/VGH --- HA22T/VGH was grown in DMEM in the presence or in the absence of FCS supplement. Cells were plated at a density of $5x10^4$ cells/well of 24-well tissue culture plates in DMEM supplemented with 10% FCS. On the following day (day 1), media were removed and cells were washed 3 times with Hank's balanced salt solution. Afterward, one set of cells was grown in DMEM in the presence of 10% FCS supplement and another set was in DMEM alone. Fresh medium was given every third day and cell numbers were counted at 24 hour intervals using a hemocytometer.

Results

Expression of PDGF and its receptor mRNA in human hepatoma cell lines --- Human PDGF molecule is a dimer of two chains: PDGF A-chain and B-chain (21). In this study, the expression of both A-chain and B-chain transcripts were examined in 10 human hepatoma cell lines. Four species of PDGF A-chain RNA,

2.8, 2.4, 2.1 and 1.9 kilobases (kb) respectively in size, were observed in most of the cell lines examined (Fig. 1A). An exception is the cell line Tong/HCC which showed no detectable A-chain transcript by this analysis. However, when RNA of c-sis, which corresponds to the RNA of PDGF B-chain was examined, every cell line showed various degrees of expression of the 3.8 and 2.8 kb RNA (Fig. 1B). Moreover, an aberrant transcript of 3.4 kb was detected in cell lines PLC/PRF/5, HA22T/VGH, Mahlavu while in the Hep G2 cell line, a 2.0 kb RNA was transcribed. The relative abundance of PDGF A-chain and B-chain RNA was studied by slot blot analysis. Fig. 2 clearly shows that a relatively higher PDGF A-chain RNA expression was detected in a majority of the cell lines examined. In contrast, in two normal tissue controls, the B-chain had a higher level of steady state RNA expression than the A-chain.

Using human PDGF receptor cDNA as a probe, cell line HA22T/VGH showed a relatively high level of PDGF receptor RNA, while cell lines HuH-6c15, HuH-7, HCC36 and Tong/HCC also have some level of expression (Fig. 3).

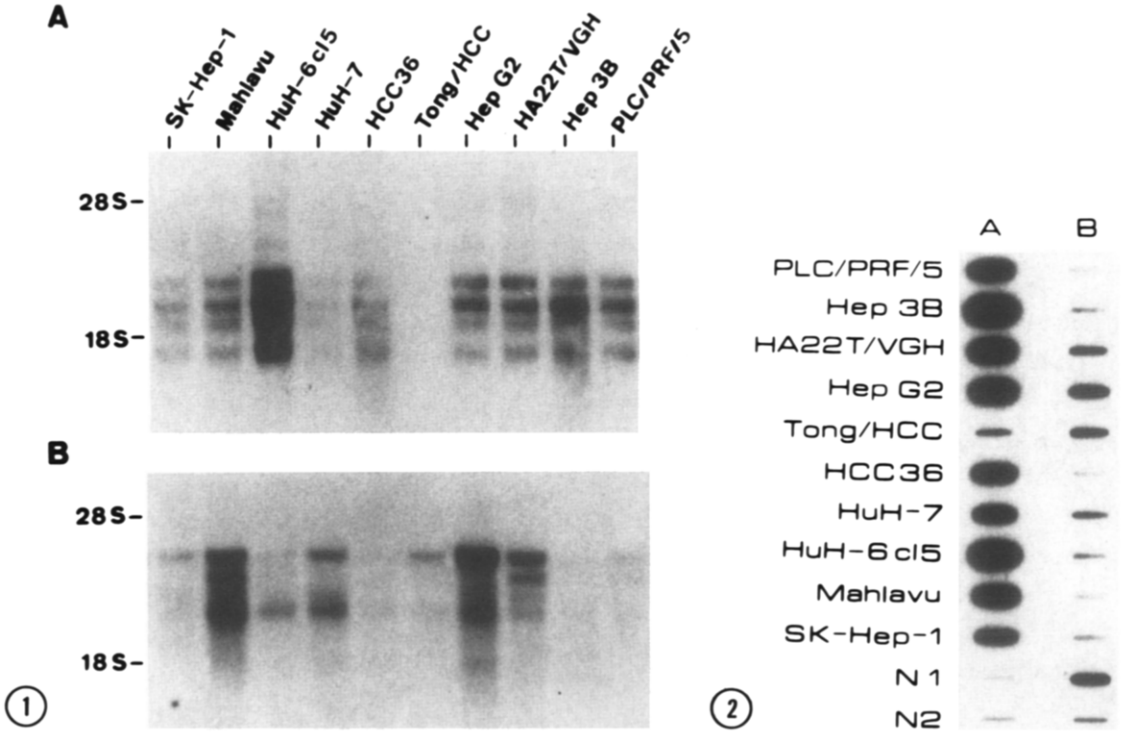

Figure 1. Autoradiograph of a Northern blot analysis of PDGF RNA from human hepatoma cell lines. Panel A: filter was hybridized to nick-translated [^{32}P] labeled PDGF A-chain probe (1.5×10^8 dpm/μg). Panel B: filter was hybridized to v-sis probe (1.7×10^8 dpm/μg). Autoradiography was for 4 days in (A) and 15 days in (B).

Figure 2. Autoradiograph of a slot blot analysis of PDGF RNA from human hepatoma cell lines and normal tissue controls (N1, N2). Lane A: filter was hybridized to nick-translated [^{32}P] labeled PDGF A-chain probe (2.0×10^8 dpm/μg). Lane B: filter was hybridized to v-sis probe (1.8×10^8 dpm/μg). Autoradiography was for 7 days in both lanes.

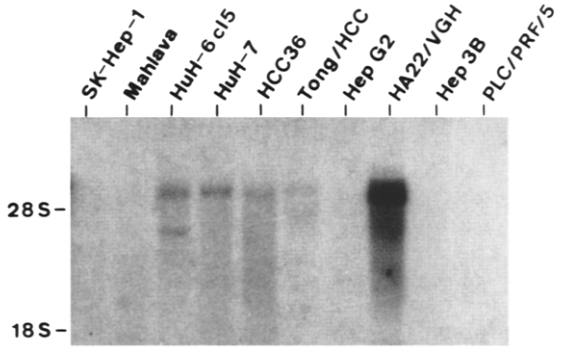

Figure 3. Autoradiograph of a Northern blot analysis of PDGF receptor RNA from human hepatoma cell lines. RNA filter was hybridized to nick-translated [^{32}P] labeled PDGF receptor probe ($2x10^8$ dpm/μg) and exposed to X-ray film for 5 days.

Expression of IGF-I and its receptor RNA in human hepatoma cell lines --- Using IGF-I cDNA as a probe, a major transcript, 5.0 kb in size, was detected in all the hepatoma cell lines studied (Fig. 4A). Besides the expression of IGF-I transcripts, IGF-I receptor RNA was also expressed in all cell lines examined although the level of expression was rather low in Hep 3B cell line (Fig. 4B). Figure 4B shows that three species of RNA, 10.0, 7.0 and 5.6 kb in size, can be detected by human IGF-I receptor cDNA probe.

Growth of human hepatoma cell line in serum-free medium --- The ability of the human hepatoma cell line HA22T/VGH to grow in a serum-free medium was demonstrated in Fig. 5. At a plating density of $5x10^4$ cells/well in a 24-well tissue culture plate, HA22T/VGH can be grown in DMEM alone without serum supplement. Under this condition, the doubling time was about 22 hours; which is similar to that in medium with 10% FCS supplement.

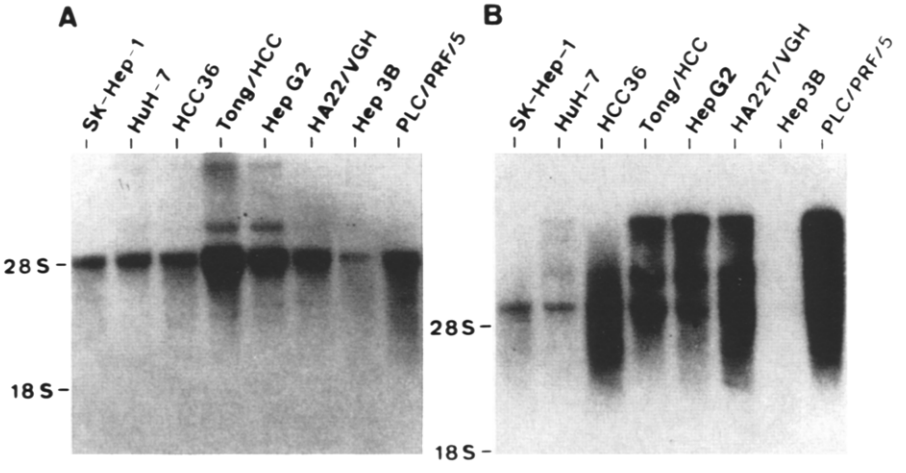

Figure 4. Autoradiograph of a Northern blot analysis of IGF-I and its receptor from human hepatoma cell lines. Panel A: filter was hybridized to nick-translated [^{32}P] labeled IGF-I probe ($5x10^8$ dpm/μg). Panel B: filter was hybridized to IGF-I receptor probe ($1.2x10^8$ dpm/μg). Autoradiography was for 7 days in (A) and 1.5 days in (B).

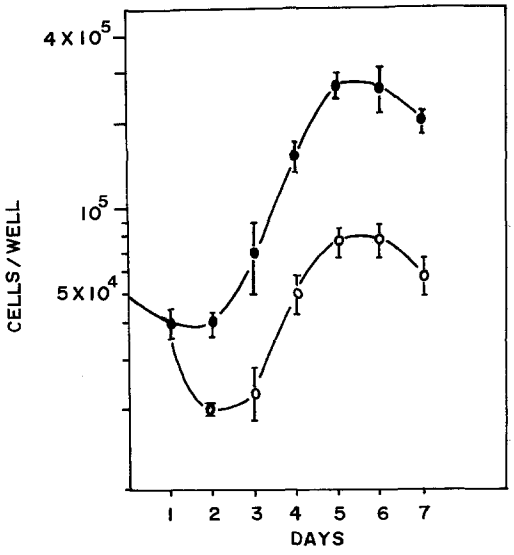

Figure 5. Growth curve of human hepatoma cell line HA22T/VGH. Cells were plated at a density of $5x10^4$ cells/well of a 24-well tissue culture plate. Cells were cultured either in DMEM supplemented with 10% FCS (•) or in DMEM with no supplement (o). Each point is the mean ± SE of four determinations.

Discussion

This study shows that in most of the human hepatoma cell lines examined, both PDGF A-chain and B-chain RNA are expressed. Furthermore, the expression of PDGF A-chain RNA is much higher than that of B-chain RNA. A relative abundance of A-chain RNA has also been reported in human melanoma cell lines and some other tumor cell lines (22). In addition, this study shows that multiple species of A-chain and B-chain RNA were expressed, and some had abnormal sizes as compared to that in other studies (15). Whether any of the aberrant transcripts observed in human hepatoma cells affect its function as has been reported in the study of PDGF A-chain of human glioma cell line (23) remains to be studied.

The PDGF gene is generally transcribed in cells of mesenchymal origin and its targets are also limited to those tissues derived from mesenchyme (24). Thus, PDGF receptors are generally found on fibroblasts, glial cells, arterial smooth muscle cells and other connective tissue cells (24).This study shows that PDGF RNA is expressed in all human hepatoma cell lines studied. Moreover, some also express PDGF receptor RNA. This observation is unusual since hepatocyte is of ectodermal origin and is believed not to express either PDGF or its receptor. The prevalence of PDGF RNA in human hepatoma cell lines suggests that the expression of this factor may play an important role in hepatocarcinogenesis. The possible modes of action are: the secreted PDGF may act on hepatoma cells which also express the PDGF receptor to promote tumor growth through an autocrine mechanism. Or they may act through a paracrine mechanism (1) to support the growth of neighboring cells to facilitate tumor

establishment. In this regard, the occurrance of liver fibrosis or cirrohosis that is commonly associated with the progression of hepatoma is worth noting. The outgrowth of the fibroblasts may be partly promoted by PDGF that is secreted by hepatoma cells.

The expression of IGF-I and its receptor in all hepatoma cell lines studied suggests that cancer cells can progress from G1 phase to S phase of the cell cycle without growth factor supplements. The co-expression of both PDGF and IGF-I in hepatoma cell lines suggests that in the presence of their receptors such as in hepatoma cell line, HA22T/VGH, the cells can complete their cell cycle without growth factor supplements. Indeed, the fact that HA22T/VGH can be grown in a serum-free medium suggests that autocrine regulation may be an important mechanism for the uncontrolled growth of human hepatoma.

Acknowledgments

We thank Drs. C. Betsholtz, M. Jansen and A. Ullrich for cDNA clones and Drs. C.-K. Ho and P. Denlinger for editing the manuscript.

This work was supported by research grant NSC76-0610-B075-73 from the National Science Council, Taiwan, Republic of China.

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