Platelet-derived growth factor receptor-α: a novel therapeutic target in human hepatocellular cancer

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Abstract

Hepatocellular cancer (HCC) is a disease of poor prognosis. Identifying novel molecular aberrations might present opportunities to identify new therapeutic targets. Due to the similarities between the processes of development and cancer, we used early developing livers to identify genes that might play a primary role in HCC. Platelet-derived growth factor receptor-α (PDGFRα) was identified from microarray using early developing mouse livers. Expression of PDGFRα and its upstream effectors, PDGF-AA and PDGF-CC, were examined in HCC tissues (n = 43) by Western blot, real-time PCR, and immunohistochemistry. Finally, effect of anti-PDGFRα antibody (mAb 3G3, ImClone Systems, Inc.) was examined on human hepatoma cells. A high expression of PDGFRα was observed during early liver development. HCCs (17 of 21) revealed cytoplasmic PDGFRα and activated PDGFRα (phospho-Tyr746) by immunohistochemistry. Additional HCCs (14 of 22) showed elevated PDGFRα levels when compared with the adjacent normal livers by Western blots. Of these 14 patients, 3 showed increased PDGFRα gene expression, 3 showed elevated PDGF-AA, and 4 had higher PDGF-CC levels in the tumors compared with adjacent livers. Multiple hepatoma cell lines, when treated with mAb 3G3, showed significant decreases in cell proliferation and survival (P < 0.05). In conclusion, ~70% of HCC tissues had elevated PDGFRα levels due to diverse mechanisms. PDGFRα inhibition in hepatoma cells led to diminution of tumor cell survival and proliferation and thus might be of therapeutic significance. [Mol Cancer Ther 2007;6(7):1932–41]

Introduction

Hepatocellular cancer (HCC) remains a disease of grim prognosis due to poorly understood molecular mechanism. Owing to commonalities between these processes, cancer has also been referred to as recapitulation of development in adults (1). The oncofetal protein α-fetoprotein exists during liver development and reappears only in HCC in adults (2). The proto-oncogene c-kit, a class III receptor tyrosine kinase, was identified during embryonic development and several cancers (3, 4). We investigated early liver development and identified high expression of platelet-derived growth factor receptor-α (PDGFRα), another member of the class III receptor tyrosine kinase family (5).

PDGFRs are related to the CFS-1 receptor/c-fms and the stem cell growth factor/c-kit proto-oncogene family (6). PDGFRs play many critical roles in embryonic and postnatal development (7). In addition, overproduction of PDGFs may be involved in growth stimulation of human tumors (8). In fact, high expression of PDGFRαs is detectable in a variety of tumors, such as the basal cell carcinoma, brain tumor, gastrointestinal stromal tumor, prostatic intraepithelial neoplasia, ovarian cancer, osteosarcoma, and leukemia (3, 9–12). PDGFRβ, an isomer of PDGFR, appears later in liver development and plays a role in hepatic fibrosis (13, 14). PDGFRα undergoes homodimerization of two α-chains following ligand (PDGF-AA, PDGF-AB, or PDGF-CC binding; ref. 15). PDGFRs also play key roles during tissue development and maintenance (16). Specifically, PDGF-CC is known to exhibit greater mitogenic potency than PDGF-AA and comparable or greater mitogenic activity than PDGF-AB and PDGF-BB on several mesenchymal cells (17). Our present study used a novel strategy based on the premise that molecular basis of development and cancer cross paths, which led us to identify PDGFRα as a promising new therapeutic target in HCC. In addition, with the availability of new PDGFRα-specific antibody, this finding might have strong clinical implications (18).

Materials and Methods

Animals

ICR strain mice were obtained from Charles River Laboratories for studies approved by the Institutional Animal Use and Care Committee at the University of Pittsburgh School of Medicine and the NIH.

Collection of Embryos or Tissues

The embryos were obtained from pregnant mice at E10, E11, E12, E14, E15, E16, E17, E18, and E19 stages. For
immunohistochemistry, the isolated embryos (E10–E15) or livers (E16–E19) were fixed in 10% buffered formaldehyde. Livers were also isolated from newborn pups, 2-day-old mice, 35-day-old mice, and adult mice. For RNA and protein isolation, fresh livers from same stage were pooled (n ≥ 4).

Cell Lines and Primary Hepatocyte Cultures
The human hepatoma cell lines, Hep3B, SK-Hep1, HepG2, and Snu449, and mouse hepatoma cells, Hepa 1 to Hepa 6 cells, were purchased from the American Type Culture Collection and grown according to their guidelines. The rat hepatoma cell lines (JM1 and JM2) were cultured as described elsewhere (19). Rat hepatocytes were isolated from male Fisher rats (250 g) by collagenase perfusion as described by Berry and Friend and Seglen with some modifications (20, 21).

HCC Tissue
HCC tissues were used from 43 patients (22 frozen tumors with adjacent livers and 21 paraffin blocks) and obtained under an approved Institutional Review Board protocol (exempt) from the Tissue Bank at the University of Pittsburgh, Medical Centre, Pittsburgh and Cooperative Human Tissue Network, Eastern Division, Philadelphia. Comprehensive patient data are given in Supplementary Data.5

Immunohistochemistry
Sections from the mouse embryos were subjected to immunohistochemistry for PDGFRα to determine stage-specific expression. Twenty-one paraffin sections from resected HCC were obtained from Department of Pathology, University of Pittsburgh (Supplementary Data).5 Immunohistochemistry was done by an indirect immunoperoxidase method as described previously (22, 23). Primary antibodies are discussed later. All secondary antibodies were horseradish peroxidase conjugated (Chemicon). Slides were viewed on a Zeiss upright research microscope (Axioskop 40) and digital images were obtained using a Nikon Coolpix 4500 camera. Collages were prepared using the Adobe Photoshop 7.01 software.

RNA Isolation and Affymetrix Gene Expression
Fresh pooled livers from E11, E14, E17, and E18 and adult mice were used for isolating and purifying RNA by Qiagen RNeasy kit (Qiagen) and examined by Affymetrix gene expression analysis (Affymetrix) as described elsewhere (22). The chips were scanned in an HP ChipScanner (Affymetrix) and analysis was done with Affymetrix Microarray Suite 5.0 software. The data were examined for changes in albumin, α-fetoprotein (AFP), c-kit, and β-actin expression.

Protein Extraction and Western Blots
Whole-cell lysates were prepared from pooled livers (n ≤ 4) from same stages of mice development. Additionally, whole-cell lysates were prepared from 22 frozen human HCC tissue and adjacent or true normal tissue. Protein extraction in radioimmunoprecipitation assay buffer and Western blot analysis has been described elsewhere (22). Blots were visualized by autoradiography. Primary antibodies used were against PDGFRα, phospho-Tyr56 kinase, PDGFRβ, PDGF-β, PDGF-CC (Santa Cruz Biotechnology); PDGFRα (Upstate Biotechnology); and β-actin (Chemicon). Specificity of PDGFRα antibody was confirmed by application of the blocking peptide (Santa Cruz Biotechnology).

Extraction of RNA, Reverse Transcription, and Real-time PCR
Total RNA was extracted from HCC (n = 22) and adjacent normal tissues (10–50 mg) using 1 mL Trizol reagent (Invitrogen). Following RNase-free DNase (Promega) treatment, reverse transcription was done using the First-Strand cDNA Synthesis kit (Fermentas). Assays-on-Demand gene expression products Hs00183486_m1 and Hs99999903_m1 (Applied Biosystems) were used to detect PDGFRα and β-actin, respectively. Real-time PCR was done according to Komoroski et al. (24). The relative cDNA content was determined from standard curves constructed from serially diluted cDNA, normalized to β-actin in each sample.

Proliferation Assay
Human hepatoma cell lines, Hep3B, HepG2, Snu449, and SK-Hep1, and mouse hepatoma cell line, Hepa 1 to Hepa 6 tumor cells, were grown to ~40% confluence. All cultures were done in triplicates for statistical analysis. Cells were treated once with 100 nmol/L monoclonal antibody (mAb) against PDGFRα (3G3, ImClone Systems; ref. 18). One additional treatment was done after 24 h at which time radio-labeled thymidine was added for 24 h and the incorporated thymidine was assayed as described elsewhere (25). The average counts for each cell line (in triplicates) were normalized to the respective untreated controls. The differences were compared for statistical significance by the Student’s t test and P < 0.05 was considered significant. The data were presented as a bar graph depicting fold decrease in thymidine incorporation in response to 3G3 treatment compared with untreated controls (KaleidaGraph, Synergy software).

Terminal Deoxynucleotidyl Transferase–Mediated Nick-End Labeling Assay
Human hepatoma cell lines, Hep3B, HepG2, Snu449, and SK-Hep1, and mouse hepatoma cell line, Hepa 1 to Hepa 6 tumor cells, were grown on coverslips in six-well plates to ~60% confluence. All cultures were done in triplicates for statistical analysis. Cells were treated once with 100 nmol/L 3G3 mAb against PDGFRα. An additional treatment was done after 24 h at which time cells were fixed on coverslips in 4% formaldehyde in PBS. The apoptotic assay was done using the DeadEnd Fluorometric TUNEL system (Promega) according to the manufacturer’s instructions. The apoptotic cells were observed by green fluorescence after the cells were counterstained with 4',6-diamidino-2-phenylindole. The numbers of positive cells were counted in three fields for each cell line (done in triplicates) in the presence and absence of 3G3. The average number of terminal deoxynucleotidyl

5 Supplementary material for this article is available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).
transferase–mediated nick-end labeling–positive cells for each cell line was normalized to the respective untreated cells. Statistical analysis was done using the Student’s *t* test and the *P* < 0.05 was considered significant. The data were presented as a bar graph depicting fold change in the numbers of terminal deoxynucleotidyl transferase–mediated nick-end labeling–positive cells normalized to controls by using the KaliedaGraph software.
Statistical Analysis

For immunohistochemistry, staining was arbitrarily scored from + to +++, based on the intensity and number of positive cells, by a single pathologist. Mean scores from controls and tumors were compared for statistical significance by two-tailed Student’s t test (InStat) and P > 0.05 was considered significant. The paired t test was used to compare the mean scores of the protein expressions in patients with liver cirrhosis SigmaPlot software (Sigma). All graphs were plotted using the KaleidaGraph or Microsoft Excel software.

For Western blots, the autoradiographs were scanned and subjected to densitometric analysis with NIH Imager software. The mean integrated absorbance was compared between the tumors and controls for statistical significance by the Student’s t test or Mann-Whitney test by SigmaPlot software and a two-tailed P < 0.05 was considered significant.

For real-time PCR, due to increased scatter in the observed events, statistical analysis was done using the unpaired Student’s t test as well as Mann-Whitney test by SigmaPlot software. P < 0.05 was considered significant.

Results

Peak PDGFRα Expression during Early Mouse Liver Development

Based on the similarities between the ongoing events, such as regulated proliferation, differentiation, and survival, between the processes of development and cancer, we examined early liver development to identify signaling pathways that may be playing a role in HCC (22). Using Affymetrix gene array, we identified high expression of PDGFRs (Pdgfra) at E11 and E14 compared with all later stages including adult liver. This expression of PDGFRα was 37- and 7-fold higher at these stages than adult liver (Fig. 1A). Expression profiles of previously characterized genes in liver development validated our microarray results by serving as internal controls. C-kit, a known hepatic stem cell marker, was highest at E11 (Fig. 1A; ref. 26). The Afp gene is expressed in embryonic and fetal hepatocytes and was significantly higher in E11 to E18 stages than adults (Fig. 1A; ref. 27). The albumin (Alb1) gene expression, a known marker of differentiated hepatocytes, increased with development (Fig. 1A).

Immunohistochemistry showed cytoplasmic, membranous, and perinuclear localization of PDGFRα in several but not all hepatoblasts and hepatocytes at E10 to E16 stages, with a gradual decrease in staining intensity (Fig. 1B). Some smaller hematopoietic cells were showing nuclear and perinuclear PDGFRα localization at these stages, as well (Fig. 1B). At E18, most hepatocytes showed faint membranous or cytoplasmic localization of PDGFRα, whereas a subset of hematopoietic cells were exhibiting nuclear or perinuclear localization for this protein. Adult mouse as well as donor human liver showed faint membranous localization of PDGFRα (Fig. 1B).

A more accurate and quantitative validation of Pdgfra expression came from Western blot analysis that showed elevated PDGFRα at E12 and E14 followed by a sequential decrease and reappearance in newborn and adult livers (Fig. 1C). Densitometry verified ~5-fold decrease in PDGFRα protein levels from E12 to adult liver (Fig. 1C).

Increased PDGFRα Activation in Hepatoma Cell Lines

Protein extracts from various hepatoma cell lines were used to determine PDGFRα protein levels (Fig. 1D). Significantly higher levels of PDGFRα were evident in rat and human hepatoma cells compared with normal liver or adult hepatocytes in culture (P < 0.01). Additional analysis revealed increased levels of activated PDGFRα (Tyr754; p-PDGFRα), ranging from 5.8- to 65-fold in the hepatoma cells compared with hepatocytes, confirming the importance of PDGFRα in hepatoma cells (Fig. 1D).

Elevated PDGFRα in Predominant HCC Tissues from Patients

Whole-cell lysates from HCC tissues and adjacent livers (n = 22; Supplementary Data) were examined by Western blot analysis for PDGFRα protein (Fig. 2A). Overall, >63% of HCC tissues showed up to 7-fold increase in total PDGFRα levels compared with adjacent controls. Whereas 3 of 22 patients showed no change, 5 of 22 patients showed a decrease in PDGFRα (Table 1). Densitometry on Western blots identified ~3.3-fold increase in total PDGFRα protein expression in tumors compared with their adjacent controls (Fig. 2B), which was statistically significant (P < 0.01).

In addition, expression of PDGFRβ was examined in these samples (Fig. 2A). Increase in PDGFRβ was identified in only 6 of 22 tumors compared with their adjacent controls. In addition, five of six tumors had concomitant increase in PDGFRα levels. Interestingly, when overall expression of

Figure 1. PDGFRα mRNA and protein expression during normal liver development in mice and hepatoma cells. A, results from microarray analysis of pooled multiple livers at E11, E14, E17, and E18, and adult stages. E11 and E14 showed high PDGFRα (Pdgfra) expression (signal), which were 37- and 7-fold higher than adults, respectively. Internal controls, decreasing c-kit expression, high prenatal Afp expression, and loss in adults and increasing Alb1 expression, validated the study. B, PDGFRα localization during mice liver development and in adult liver (Ad). Cytoplasmic or perinuclear PDGFRα is seen in epithelial cells (arrow) at E10, E12, E14, E16, and E18 stages shown at higher magnification (right). Magnification, ×60. Hematopoietic cells show nuclear localization at these times (arrowheads). Adult normal mouse liver (NML) hepatocytes show faint membranous and cytoplasmic localization (arrow). Normal human liver shows faint membranous localization at hepatocytes (arrowhead). C, representative Western blot shows PDGFRα protein expression during normal mouse liver development (top). The protein expression at E12 and E14 was higher than later stages as shown by densitometry (bottom). Equal loading confirmed by β-actin (data not shown). D, normalized integrated absorbance (to normal rat liver) from three representative Western blots was used to plot a graph that showed 3- to 6-fold and significant (P < 0.05) increase in PDGFRα protein expression in hepatoma cells (top). Densitometric analysis from a representative Western blot showed 5.8- to 65-fold increase in activated or p-PDGFRα-Tyr754 in all hepatoma cell lines when compared with NML (bottom). Equal loading for Western blots was confirmed by β-actin (data not shown). Hepatoma cell lines: M1 and JM2 (rat) and Hep3B, HepG2, and SK-Hep1 (human). NRL, normal rat liver; Hep-culture, hepatocyte culture.
PDGFRα and PDGFRβ was compared in tumors with cirrhosis, PDGFRβ was significantly higher in these samples confirming its association with cirrhosis (P < 0.01; Fig. 2C).

Next, 21 additional HCC paraffin sections were examined for total and p-PDGFRα expression by immunohistochemistry (Fig. 3A). In normal donor livers, PDGFRα was observed at the membrane only (Fig. 3A, a and e). In addition, no p-PDGFRα was detected in normal livers (Fig. 3A, b and f). Tumors (17 of 21) showed aberrant PDGFRα protein accumulation in hepatocytes as shown in representative tumors (Fig. 3A, c and g). Simultaneously, these samples displayed cytoplasmic p-PDGFRα staining (Fig. 3A, d and h). The staining was graded based on intensity to obtain an arbitrary immunohistochemistry score as discussed in Materials and Methods (Table 2). Statistical analysis using these scores showed a significant increase in total and activated PDGFRα in tumors compared with normal tissue (P < 0.01; Fig. 3B).

**Mechanism for PDGFRα Protein Up-regulation and Activation in HCC**

To address the mechanism of PDGFRα up-regulation, we examined frozen HCC and control samples for Pdgfra mRNA expression by real-time PCR. Interestingly, an overall significant decrease in Pdgfra mRNA expression was observed in HCC (n = 22) compared with controls (P < 0.05; Fig. 4A). Only 3 of the 22 tumors, which displayed elevated PDGFRα levels, showed a concomitant increase in Pdgfra expression (Table 1). The increase ranged from 1.22- to 1.6-fold and was significant as judged by Mann-Whitney test (P < 0.05).

To investigate additional mechanisms of PDGFRα activation, frozen tumors and controls were examined for its known ligands, PDGF-AA and PDGF-CC (Table 1). HCC samples (8 of 22) showed an up-regulation of PDGF-AA protein, which was coexistent with elevated PDGFRα protein in three patients. In addition, out of these three tumors, one just had simultaneous increases in Pdgfra expression and PDGF-CC protein and the remaining two had a unique increase in PDGF-AA only. Cumulative analysis in 22 tumors and normal samples showed insignificant differences in PDGF-AA levels except in three samples that showed a significant increase (2.39- to 3.63-fold; P < 0.05; Fig. 4B). PDGF-CC, a selective PDGFRα ligand, was statistically increased in four HCC samples.
with elevated PDGFRα protein \((P < 0.05; \text{Table 1})\). Out of these four tumors, one showed concomitant increase in \(Pdgfra\) expression and PDGF-AA levels and one showed a concurrent increase in \(Pdgfra\) expression only. However, overall insignificant difference in PDGF-CC was observed in tumors versus normal adjacent controls (Fig. 4B).

**Anti-PDGFRα mAb Inhibits Hepatoma Cell Proliferation and Induces Apoptosis**

Next, we wanted to examine the effect of mAb anti-PDGFRα at 100 nmol/L PDGFRα on the growth and survival on various human hepatoma cell lines and in mouse hepatoma cells. Efficacy of mAb (3G3) to inhibit PDGFRα activity has been reported recently (18). Tumor cell lines were grown to ~40% confluence and treated with 3G3 as described in Materials and Methods. This treatment affected proliferation of all cell lines significantly \((P < 0.05)\) tested with a decrease in thymidine incorporation ranging from 10% to 40% compared with the untreated cells (Fig. 5A). The greatest difference was observed in the SK-Hep1 cells and the least in mouse HePa 1 to HePa 6 cells.

Next, we examined apoptosis in these cells in response to 3G3 treatment for 2 days at 100 nmol/L as described in Materials and Methods. A significant increase in the number of apoptotic nuclei was observed in response to 3G3 treatment \((P < 0.05)\), which ranged from 4- to 18-fold in various cell lines when normalized to the respective controls (Fig. 5b and C). The most pronounced effect was in Hep3B cells and the least in Snu449.

These results show a modest antiproliferative and a prominent antisurvival role of PDGFRα inhibition on the various hepatoma cells of human and mouse origin. These combinatorial effects show the usefulness of PDGFRα inhibition in HCC.

### Discussion

Despite several advances in understanding the pathogenesis of HCC, its prognosis remains ominous. Although some reports have examined the expression patterns of multiple genes in these tumors, these studies are limited by reproducibility and ability to address ‘cause or effect’ relationship of observed changes (28, 29). Although inclusion of controls and tumors at various stages of progression might assist in addressing the latter issue to an extent, the interpretation might be complicated by heterogeneity in the tumors or patient population. Such variations become apparent as differences in gene expression patterns continue to be observed among various studies (30–32). Thus, these studies have only limited practicability to identify novel molecular targets for therapy.

Instead, we used a novel approach to initiate analysis in early liver development, based on similarities in the processes of development and cancer (1). Cancer has been appropriately called as recapitulation of development in adults, or development and organogenesis might represent “physiologic tumorigenesis.” Examining the molecular

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**Table 1. Summary of PDGFR protein expression in patient samples with corresponding results for PDGFR mRNA expression and the ligands PDGF-AA and PDGF-CC**

<table>
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<th>Patient no.</th>
<th>PDGFRα protein</th>
<th>PDGFRα RNA</th>
<th>PDGFR-AA protein</th>
<th>PDGFR-CC protein</th>
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<td></td>
<td>Regulation</td>
<td>Fold change</td>
<td>Regulation</td>
<td>Fold change</td>
</tr>
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<td>D</td>
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| Abbreviations: I, increase; D, decrease; NC, no change. |
basis of these stages thus might reveal primary pathways of physiologic or, in cancer, more pathologic relevance and thus be more appropriately a primary aberration. This strategy is also of essence in identifying molecular basis of potential cancer stem cells, which might be similar to developmental stem cells as shown in gastrointestinal tract (33, 34). Thus, based on ongoing events at E11 and E14 in developing livers, such as regulated cell proliferation, differentiation, and survival, we examined global gene expression to identify PDGFRα as one of the highly expressed genes and proteins for its possible role in HCC (22).

Involvement of PDGF proteins in liver fibrosis and cirrhosis, especially PDGFRβ, is known (13, 35). Lately, studies are focused on ameliorating in vitro and in vivo hepatic fibrogenesis (36). Hepatic fibrosis, a sequel to infectious, inflammatory, or metabolic disorders of the liver, also predisposes to HCC (37, 38). Although a number of reports have examined the PDGF pathway in fibrosis, its role and expression in HCC remains elusive. More recently,

Table 2. Summary of PDGFR protein expression in patient samples (paraffin sections)

<table>
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<th>Patient no.</th>
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<th>PDGFRα (Tyr745)</th>
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NOTE: ++, moderate; +, mild; 0, no staining.
mice overexpressing (liver specific) PDGF-CC, a ligand for PDGFRα, go on to develop cirrhosis and HCC (39). Getting cues from our developmental analysis, we expected a promising role of PDGFRα in HCC.

PDGFRα plays a role in development in many tissues, assisting in proliferation, morphogenesis, angiogenesis, or epithelial-mesenchymal interactions (40, 41). Activation of PDGFRα secondary to mutations or other causes has been reported in many cancers as well, where it is associated to tumor cell proliferation, progression, or angiogenesis (3, 9). In our study, aberrant increase and activation of PDGFRα was observed in hepatoma cell lines of human and nonhuman origin. Similarly, >70% of all HCC samples displayed a significant increase in total and p-PDGFRα, indicating aberrant activation of this pathway (n = 43). Whereas increase in PDGFRα was reported in endothelial cells in highly metastatic HCCs, we observed an increase in parenchymal liver cells by immunohistochemistry, suggesting that PDGFRα might be playing a role in events critical for tumor cells, such as proliferation and survival (42).

Analysis from frozen tissues revealed 3 out of 22 tumors with elevated PDGFRα levels, showing a significant increase in Pdgfra expression indicating a contributing mechanism for its elevated protein. None of these tumors were examined for mutations in Pdgfra gene as has been reported in gastrointestinal stromal tumor and brain tumors (3, 9, 15). Samples (11 of 14) showed increased PDGFRα protein despite decreased mRNA, reflecting possible posttranscriptional modification of mRNA to increase half-life or a posttranslational event affecting its degradation. Both forms of regulation are known for PDGFRα (43, 44). PDGFRα up-regulation or constitutive activation has been reported as a consequence of fusion, chromosomal translocations, and up-regulation of ligands leading to autocrine or paracrine stimulation (45). PDGF-AA and PDGF-CC are known ligands for this receptor. Tumors (three of eight) showed concomitant elevation in PDGF-AA and PDGFRα proteins. Tumors (four of five) showed simultaneous increase in PDGF-CC and PDGFRα proteins. These observations reveal heterogeneity in the mechanisms of activation of PDGFRα in these tumors. Additionally, this analysis supports the presence of autocrine or paracrine loops, which might be contributing to the disease process. Also of relevance is the recently reported study where PDGF-CC overexpression in hepatocytes leads to the development of HCC (39).

Because PDGF signaling has been associated to hepatic fibrosis and cirrhosis, we investigated any correlation of the observed PDGFRα up-regulation in HCC to cirrhosis. Six of 14 tumors that showed elevated PDGFRα had coexisting cirrhosis and 8 of 14 tumors had no cirrhosis. Out of the eight tumors without cirrhosis but increased PDGFRα levels, three were fibrolamellar carcinomas. In fact, three of four fibrolamellar HCCs presented up-regulated PDGFRα protein without any evidence of cirrhosis, whereas one showed no change. Additionally, out of the 8 of 22 frozen tumors that showed either decrease or no change in PDGFRα protein, 4 had cirrhosis. Thus, any correlation between PDGFRα up-regulation and cirrhosis was coincidental. On the other hand, PDGFRβ protein showed significant expression in cirrhotic livers (P < 0.05), an observation well described previously (13, 46, 47).

Recent therapies against cancers are based on identification of unique molecular aberrations and have shown varying degree of success (48). The presence of PDGFRα, as well as activated PDGFRα in HCC in >70% of patients and its relationship to proliferation in liver development and other tumors, justifies its consideration as a valid therapeutic target in HCC. We used a mAb against PDGFRα to test its effect on multiple human hepatoma cells, which showed aberrant PDGFRα activation. This antibody has been characterized recently as a selective PDGFRα inhibitor (18). This resulted in a modest and significant decrease in the tumor cell proliferation. Additionally, there was a marked increase in tumor cell death in response to PDGFRα antibody. Because high PDGFRα expression was evident in the tumor core compared with the adjacent liver, it suggested a role of this protein in events associated with advanced stages of tumor development, such as tumor cell survival rather than proliferation, which might be critical for early tumors or cells at the tumor margin. Indeed, PDGFRα inhibition dramatically affected hepatoma cell survival, only modestly reducing their proliferation. Nonetheless, this combined effect on tumor cell proliferation and their viability indicates PDGFRα as a promising therapeutic target in HCC. Additional studies examining more cell

Figure 4. Mechanism for PDGFRα protein up-regulation and activation in HCC tissues. A, real-time PCR analysis of PDGFRα mRNA expression in tumor and adjacent normal tissue (n = 22) showed an overall decrease in tumors, which was significant (P < 0.05). B, mean integrated absorbance, from densitometric analysis of PDGF-AA and PDGF-CC Western blots using lysates from all patients, showed no significant differences in their expression levels between tumors and adjacent liver. Columns, mean integrated absorbance; bars, SE.
lines as well as primary cultures from resected human tumors to further evaluate the efficacy of targeting PDGFRα are under way. Interestingly, there are several existing case reports that have shown a favorable therapeutic response to tyrosine kinase inhibitors that also possess anti-PDGFRα in both HCCs and cholangiocarciomas (49, 50). In addition, targeting PDGF in HCC has also been suggested in transforming growth factor β-mediated tumor progression in hepatocytes (51). As mentioned previously, a recent study reporting generation and characterization of PDGF-CC transgenic mice also supports the role of aberrant PDGFRα activation in HCC and validates its therapeutic targeting in this tumor (39).

References

Figure 5. Effects of mAb 3G3 on human hepatoma cell proliferation and survival. A, modest decrease in thymidine incorporation (range, 10–40%), in response to mAb 3G3 in multiple hepatoma cell lines, which was statistically significant (P < 0.05). The values are normalized to the respective untreated controls and presented as fold change. B, terminal deoxynucleotidyl transferase–mediated nick-end labeling (TUNEL) assay identified a significant increase in the number of apoptotic nuclei in response to mAb 3G3 treatment in multiple hepatoma cell lines, which ranged from 4- to 18-fold and was statistically significant (P < 0.05). The values are normalized to respective untreated controls and presented as fold change. C, representative photomicrographs show an increase in apoptotic nuclei (green) in Hep3B and SK-Hep1 cells in the presence of mAb 3G3.
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