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ORIGINAL ARTICLE

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Expression of transforming growth factor-alpha and its receptor during human liver development and maturation

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Abstract We investigated the expression of transforming growth factor-alpha (TGF-α) and its receptor during human liver development and maturation, using immunohistochemistry. In the fetal liver, strong immunoreactivity for TGF-α and its receptor was noted in intrahepatic bile duct cells of various developmental stages; moderate immunoreactivity for TGF-α and mild immunoreactivity for TGF-α receptor were found in immature hepatocytes. In the postnatal liver, reactivity for TGF-α in hepatocytes decreased gradually and was negative or only weakly positive in the adult liver, while reactivity for TGF-α receptor in hepatocytes increased gradually and was strongly positive in the adult liver. In contrast, immunoreactivity of TGF-α and its receptor in intrahepatic bile duct cells persisted in the postnatal liver and was positive in the adult liver. These data suggest that the system of TGF-α and its receptor has an important role in the proliferation and differentiation of intrahepatic biliary cells and hepatocytes in the fetal liver. The decreasing expression of TGF-α in hepatocytes in the postnatal liver may indicate that proliferative activity of hepatocytes gradually decreases with liver maturation. The presence of TGF-α and its receptor in intrahepatic bile ducts in the postnatal liver suggests that the system of TGF-α and TGF-α receptor is operative postnatally.

Key words Human liver development · TGF-α Intrahepatic bile ducts · Hepatocytes

Introduction

Transforming growth factor alpha (TGF-α) is a 50-amino-acid polypeptide that binds to the epidermal growth factor (EGF) receptor (EGF-R), a protein tyrosine kinase that mediates signal transduction in a variety of cells and stimulates their proliferation (Derynck 1988: Massague 1990). TGF-α is present in many cell types (Yasui et al. 1992) and cancer cells (Goustin et al. 1986), and is believed to play an important part in cell proliferation and differentiation via an autocrine mechanism (Madtes et al. 1988; Mead and Fausto 1989). It shares 35% sequence homology and a nearly identical spectrum of biological activities with EGF (Derynck 1988).

TGF-α is also involved in cell proliferation of parenchymal cells of the liver (Fausto and Mead 1989; Michalopoulos 1990). TGF-α and its mRNA have been demonstrated in parenchymal and nonparenchymal cells of the liver of experimental animals (Burr et al. 1993; Liu et al. 1988) but rarely examined in the human liver. TGF-α and its mRNA have been investigated during development in several other organs in experimental animals (Brown et al. 1990; Lee et al. 1985; Perez-Tomas et al. 1993; Twardzik 1985; Wilcox and Derynck 1988), and Evarts et al. (1992) have described TGF-α expression during the development of the rat liver. However, expression of TGF-α and its receptor has not been investigated in the development of the human liver. We have examined the expression of TGF-α, EGF-R and EGF during human liver development and maturation using immunohistochemical methods.

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Materials and methods

We collected 32 human embryonic and fetal livers of various gestational ages [8 weeks, 9 weeks (2 cases), 10 weeks (3 cases), 11 weeks (2 cases), 12 weeks (2 cases), 13 weeks (2 cases), 14 weeks (2 cases), 15 weeks (2 cases), 16 weeks (2 cases) 17, 18, 19, 20, 22, 24, 26, 28, 29, 32, 35, 37, 38, and 40 weeks), 4 neonatal livers (age<4 w), 5 infant livers (4 weeks to 1 year), 15 child livers (1–5 years, 5; 6–10 years, 5; 11–15 years, 5), 7 adolescent livers (16–19 years), and 7 adult livers (20–30 years). The fetal and embryonic livers were obtained from aborted or autopsied fetuses at our laboratory and affiliated hospitals. The other livers were obtained from autopsies at our laboratory. Abortion was spontaneous in all cases, and informed consent was obtained from the mother in every case. None of the livers had significant pathological changes. Each was sliced frontally at the hepatic hilum. One or two specimens, including the hepatic hilum, were obtained from each fetal liver. In the case of each neonate, infant, child, adolescent or adult liver,

one specimen containing large bile ducts was taken from the hilum and one specimen from the subcapsular part. The liver specimens were fixed in 4% neutral formaldehyde and embedded in paraffin. Several 5-μm serial sections were obtained from each paraffin-embedded block; one of these was stained with haematoxylin and eosin, and the rest were subjected to immunohistochemical staining.

Four sections from each block were stained immunohistochemically for TGF-α, EGF-R, EGF and cytokeratins using the avidin-biotin-peroxidase complex method of Hsu et al. (1981). Cytokeratin immunostaining was performed to differentiate immature intrahepatic biliary cells from immature hepatocytes (Shah and Gerber 1989; Van Eyken et al. 1988). In brief, after deparaffinization and elimination of endogenous peroxidase activity, the sections were treated with normal serum. The sections were then treated for 18 h at 4° C with the monoclonal antibody against human TGF-α (Ab-2, Oncogene Science, Uniondale, N.Y. diluted 1:16 (6 μg/ml) in polyclonal antibody against human EGF-R (Oncogene Science) diluted in 1:100, polyclonal antibody against human EGF (Oncogene Science diluted in 1:100, or monoclonal anti-cytokeratin antibody AE1 (ICN Immunobiochemicals, Costa Mesa, Calif.) diluted in 1:100. AE1 is positive in immature and mature bile duct cells, but negative in immature and mature hepatocytes (Terada and Nakanuma 1993). Then, biotinylated secondary antibodies (Vector Lab, Burlingame, Calif.) were applied to the sections for 1 h. The sections were treated with avidin-biotin-peroxidase complex (Vectastain ABC Kit, Vector Lab). Reaction products were developed by immersing the sections in 3,3′-diaminobenzidine tetrahydrochloride solution (Sigma Chemicals, St. Louis, M.) containing 0.1% hydrogen peroxide. Nuclei were lightly counterstained with haematoxylin.

Specificity of TGF-α immunostaining was examined by the absorption test; the primary antibody solution was absorbed by excess recombinant TGF-α (Biomedical Technologies, Stoughton, Mass.) at 4° C overnight and then centrifuged for 1 h, after which the supernatant was used as primary antibody. In addition, nonimmune serum or phosphate-buffered saline was substituted for the primary antibodies, followed by the immunostaining.

Previously reported studies on intrahepatic bile duct development in humans (Desmet 1992; Shah and Gerber 1989; Terada and Nakanuma 1993; Van Eyken et al. 1988) propose division of the cells of developing intrahepatic bile ducts in the fetal liver into the following categories: cells of the ductal plate, biliary cells migrating into the mesenchyme, and newly-formed bile duct cells.

Results

The absorption test of TGF-α produced almost no staining (Fig. 1). No staining was found when nonimmune serum or phosphate-buffered saline was used as the first layer in each immunostaining.

Fetal livers

TGF-α was moderately expressed in the immature hepatocytes with a diffuse cytoplasmic pattern throughout the fetal period (Figs. 2–4). It was strongly expressed with a diffuse cytoplasmic pattern in the ductal plate cells (Fig. 2), in the biliary cells migrating into the mesenchyme (Fig. 3), and in the newly formed intrahepatic bile ducts (Fig. 4). Nerve bundles, smooth muscles of blood vessels, and some nonparenchymal cells and vascular endothelial cells of portal tracts were also positive with a difuse cytoplasmic pattern (Figs. 2–4). None of the TGF-α-positive nonparenchymal cells showed the characteristic stellate morphology of Ito cells or Kupffer cells (Figs. 2–4). EGF-R was weakly expressed in the immature hepatocytes, with a granular or diffuse pattern (Figs. 5–7). It was strongly expressed with a granular cytoplasmic pattern in the ductal plate cells (Fig. 6), in the biliary cells migrating into the mesenchyme (Fig. 7), and

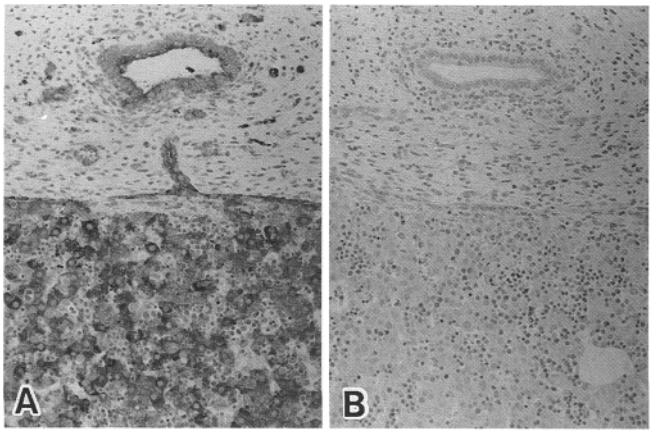

Fig. 1A, B Specificity of transforming growth factor-alpha (TGF-α) immunostaining. **A** TGF-α is expressed in both immature bile ducts and hepatocytes in a fetal liver of 15 weeks of gestation. Immunostaining of transforming growth factor-alpha. (× 250) **B** Expression of TGF-α is almost abolished when primary antibody absorbed by recombinant TGF-α is used as the first layer. (× 250)

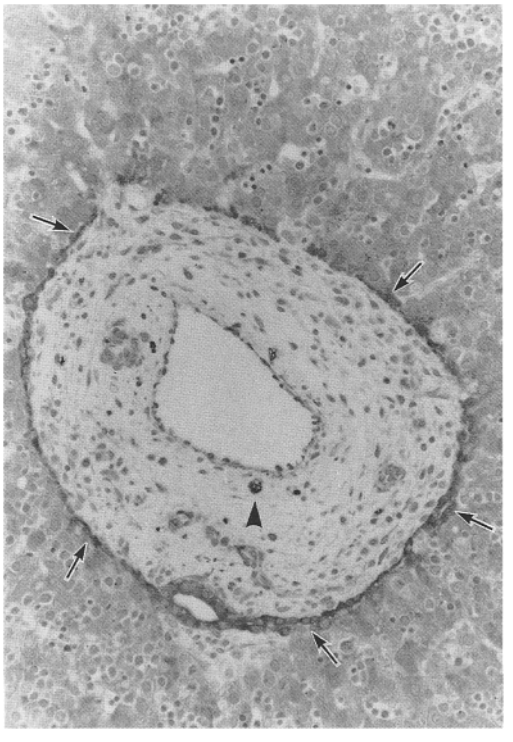

Fig. 2 Expression of TGF-α in a fetal liver of 10 weeks of gestation. The immature hepatocytes are moderately positive for TGF-α. The ductal plates *(arrows)* are strongly positive for TGF-α. Nerve bundles are positive for TGF-α *(arrowhead)*. (Immunostaining for TGF-α, × 200)

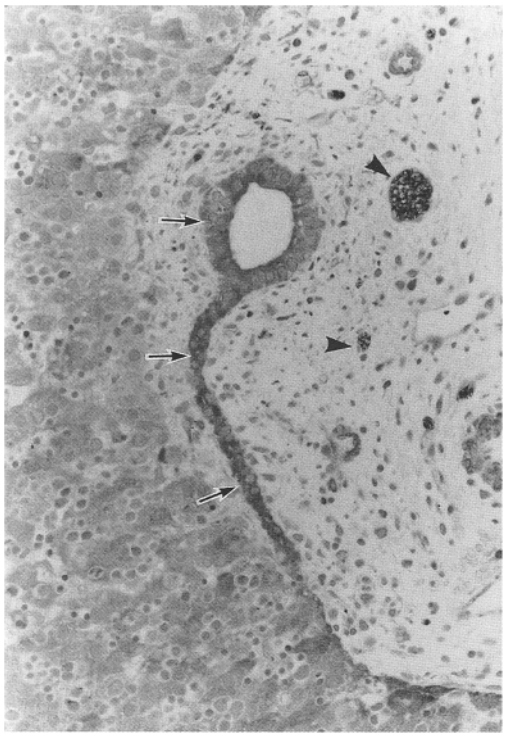

Fig. 3 Expression of TGF-α in a fetal liver of 14 weeks of gestation. The immature hepatocytes are moderately positive; biliary cells migrating into the mesenchyme *(arrows)* are strongly positive; nerve bundles are positive *(arrowheads)*. (Immunostaining for TGF-α, × 250)

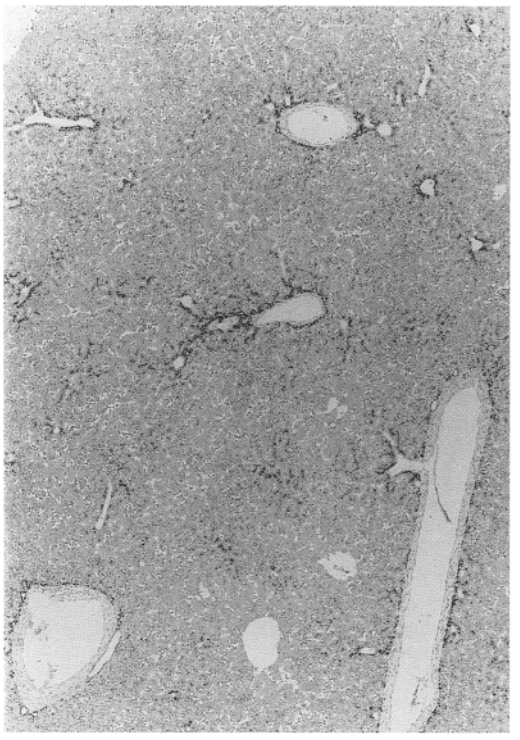

Fig. 5 Expression of epidermal growth factor (EGF) receptor (EGF-R) in a fetal liver of 12 weeks of gestation. The immature hepatocytes are weakly positive for EGF-R. Its expression is accentuated in the ductal plate around the immature portal vein. (Immunostaining for EGF-R, × 40)

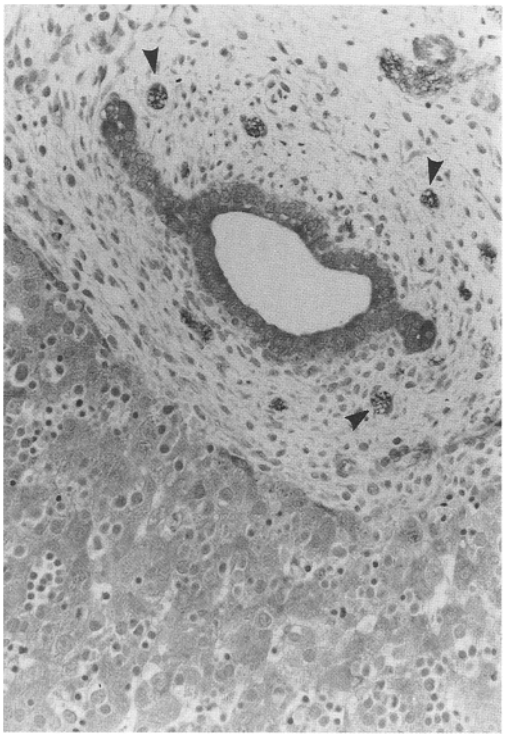

Fig. 4 Expression of TGF-α in a fetal liver of 20 weeks of gestation. The immature hepatocytes are moderately positive; the newly formed intrahepatic bile duct is strongly positive; nerve bundles are positive *(arrowheads)*. (Immunostaining for TGF-α × 250)

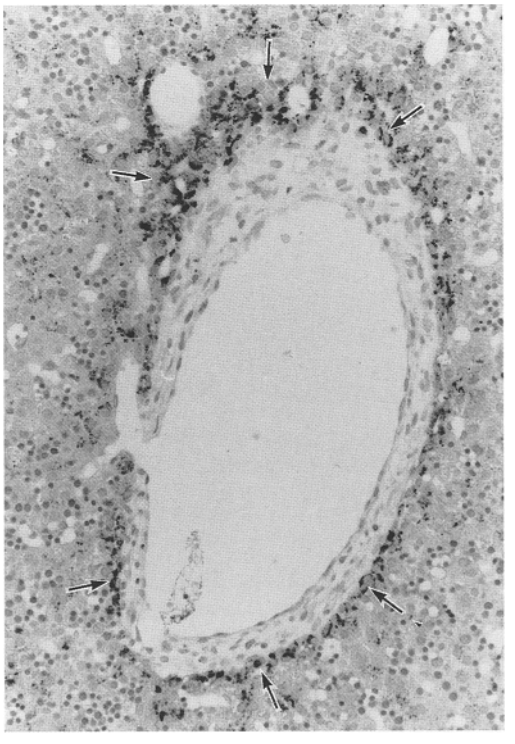

Fig. 6 Expression of EGF-R in a fetal liver of 13 weeks of gestation. The immature hepatocytes are weakly positive and the cells of ductal plate, strongly positive *(arrows)* for EGF-R. (Immunostaining for EGF-R, × 200)

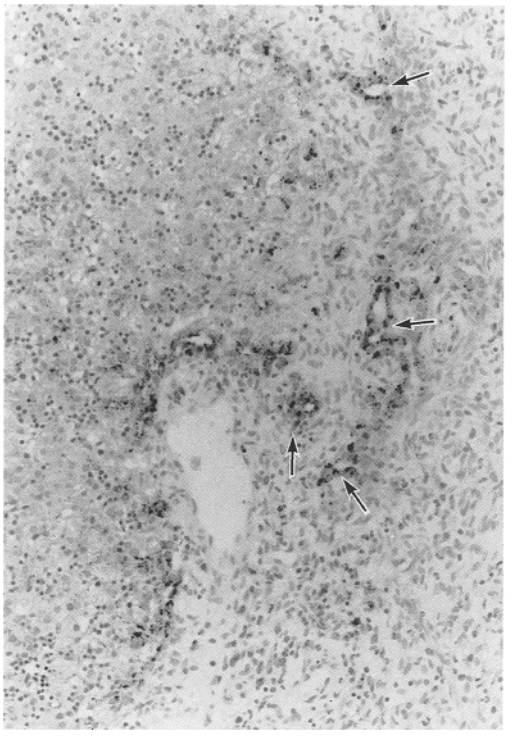

Fig. 7 Expression of EGF-R in a fetal liver of 15 weeks of gestation. The immature hepatocytes are weakly positive and the biliary cells migrating from the ductal plate into the mesenchyme strongly positive *(arrows)* for EGF-R. (Immunostaining for EGF-R, × 200)

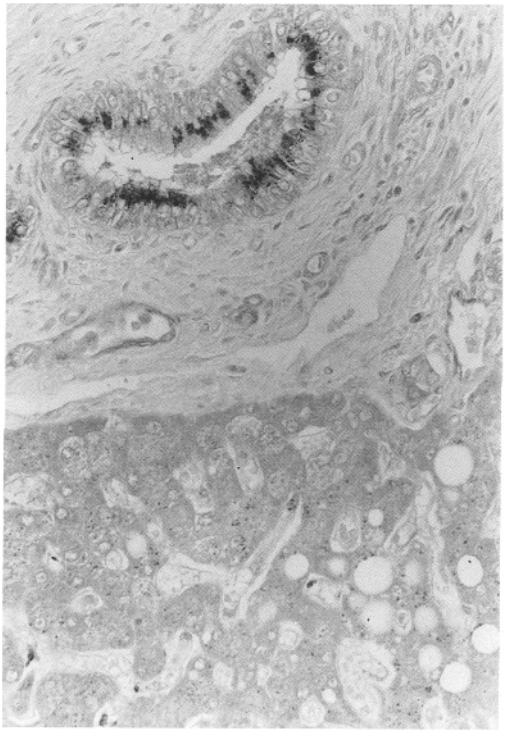

Fig. 8 Expression of TGF-α in the liver of a 3-year-old child. Hepatocytes are weakly positive and the intrahepatic bile ducts strongly positive for TGF-α. (Immunostaining for TGF-α, × 250)

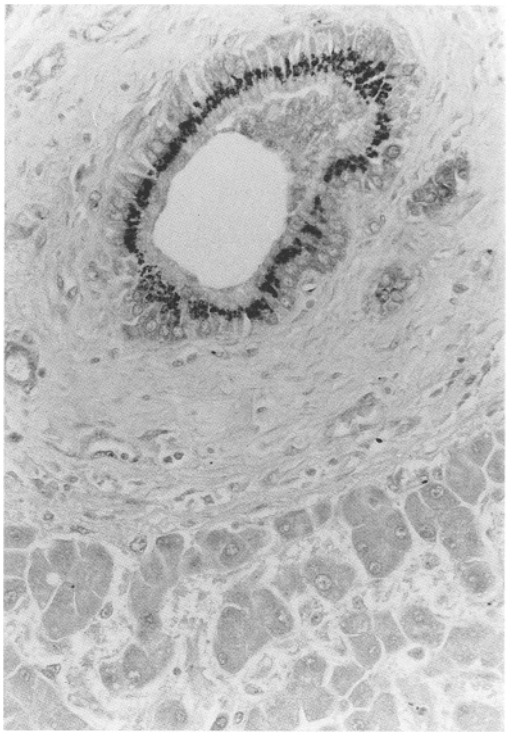

Fig. 9 Expression of TGF-α in the liver of a 20-year-old man. Hepatocytes are negative, the intrahepatic bile ducts strongly positive for TGF-α. (Immunostaining for TGF-α, × 250)

in the newly formed intrahepatic bile ducts. EGF expression was not found in any cell types in the liver.

Neonate and infant livers

TGF-α was moderately expressed in the maturing hepatocytes with a diffuse cytoplasmic pattern. It was also strongly expressed in maturing intrahepatic bile ducts, with a granular pattern. Nerve bundles, smooth muscles of blood vessels and some endothelial cells were also positive. None of the TGF-α-positive nonparenchymal cells showed the characteristic stellate morphology of Ito cells or Kupffer cells. EGF-R was moderately expressed with a granular cytoplasmic pattern in the maturing bile duct cells and hepatocytes. No cell type expressed EGF.

TGF-α was weakly expressed in the hepatocytes of children with a diffuse cytoplasmic pattern (Fig. 8). Expression in hepatocytes became weak as the maturation of the liver progressed TGF-alpha was strongly expressed in the maturing intrahepatic bile ducts, with a granular pattern (Fig. 8). Nerve bundles and smooth muscles of blood vessels were positive. No stellate cells in the liver lobules were positive for TGF-α (Fig. 8). EGF-R was strongly expressed in the hepatocytes with a diffuse cytoplasmic pattern. EGF-R expression in hepatocytes became stronger as the maturation of the liver progressed. EGF-R was moderately expressed in the maturing intrahepatic bile ducts with a diffuse cytoplasmic pattern. Expression was not found in any cell type in the liver.

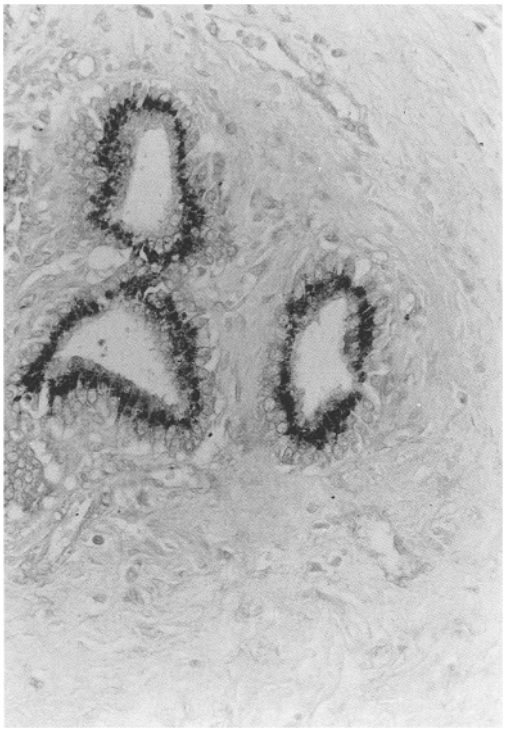

Fig. 10 Expression of TGF-α in the liver of a 18-year-old woman. Intrahepatic peribiliary glands are strongly positive for TGF-α. (Immunostaining for TGF-α, × 250)

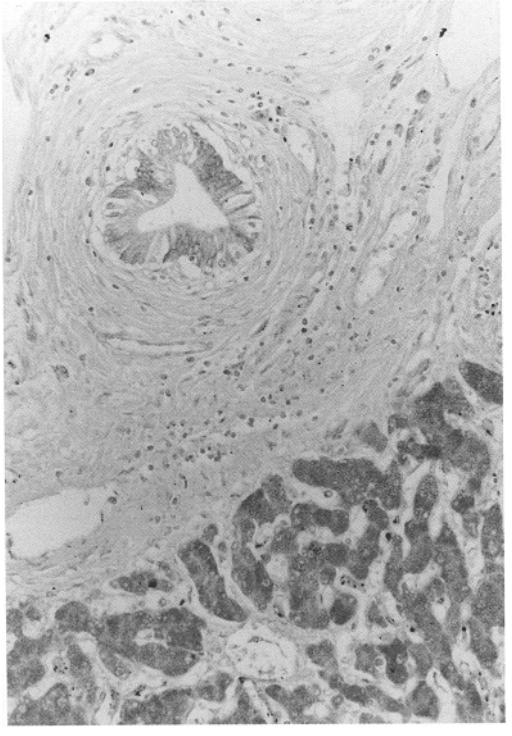

Fig. 11 Expression of EGF-R in the liver of a 20-year -old man. Hepatocytes are strongly positive, intrahepatic bile ducts moderately or weakly positive for EGF-R. (Immunostaining for EGF-R, × 200)

Adolescent and adult livers

TGF-α was negative or only weakly expressed in the hepatocytes of adolescents and adults (Fig. 9). It was strongly expressed in mature intrahepatic bile ducts, with a granular pattern (Fig. 9). Intrahepatic peribiliary glands (Kida et al. 1992; Terada and Nakanuma 1992, 1993) were also positive for TGF-α with a granular pattern (Fig. 10). Nerve bundles and smooth muscles of blood vessels were positive. No stellate cells in the liver lobules were positive for TGF-α (Fig. 9). EGF-R was strongly expressed in the hepatocytes with a diffuse cytoplasmic pattern (Fig. 11). EGF-R was moderately or weakly expressed in the mature intrahepatic bile ducts with a diffuse cytoplasmic pattern (Fig. 11). EGF expression was not found in any cell type in the liver.

Discussion

Growth factors play a pivotal role in liver regeneration, acting either as potent mitogenic or as mito-inhibitory regulators of hepatocyte proliferation (Fausto and Mead 1989; Michalopoulos 1990). Several polypeptide growth factors, such as EGF, TGF-α, TGF-β, acidic fibroblast growth factor and hepatocyte growth factor, are involved in cell proliferation in liver regeneration (Armendaris-Borunda et al. 1993; Fausto and Mead 1989; Masuhara et al. 1992; Michalopoulos 1990; Webber et al. 1993). Some of these growth factors may also be involved in the development and differentiation of the liver in experimental animal models (Evarts et al. 1992; Marsden et al. 1992; Nagy et al. 1989) However, the expression of TGF-α, EGF-R and EGF in human liver development and maturation has not been investigated.

Immature hepatocytes in fetal, neonatal and infant livers were moderately positive for TGF-α and were weakly or moderately positive for EGF-R. These results suggest that hepatocytes in these periods express TGF-α and EGF-R and that TGF-α stimulates hepatocytes to proliferate and differentiate, probably via an autocrine mechanism using the system of TGF-α and EGF-R in the fetal, neonatal and infant periods. In contrast, in the livers of children TGF-α expression in hepatocytes became weak as maturation progressed and was negative or only weakly positive in adolescent and adult livers, while EGF-R expression of hepatocytes became strong as the maturation of the liver progressed. These results may indicate that hepatocyte proliferation by the system of TGF-α and EGF-R decreases with liver maturation. Evarts et al. (1992) demonstrated that nonparenchymal cells (Ito cells and Kupffer cells) and hepatocytes express TGF-α and its mRNA in rat liver development. In the present study, however, none of the TGF-α-positive nonparenchymal cells showed the characteristic stellate morphology of Ito cells or Kupffer cells, raising the possibility that its role in liver development varies according to species. Burr et al. (1993) also failed to demonstrate TGF-α expression in Ito cells and Kupffer cells in rat liver regeneration or in the normal rat liver.

The present study showed that TGF-α and EGF-R were strongly positive during intrahepatic bile duct development in the fetal liver. Precursor biliary cells express TGF-α and EGF-R; TGF-α may stimulate these cells to proliferate and differentiate via an autocrine mechanism. It has been suggested that epithelial–mesenchymal interactions are important in the morphogenesis of intrahepatic bile ducts during their development and in the morphogenesis of other organs (Madri and Bassan 1992; Martinez-Hernandez et al. 1992; Shah and Gerber 1990; Terada and Nakanuma 1994). The presence of TGF-α and EGF-R during intrahepatic bile duct development may suggest that this system is also important in cell migration and morphogenesis of intrahepatic bile ducts during their development, as is the case with hepatocyte growth factor in several organs (Johnson et al.1993; Fujiwara et al. 1993; Michalopoulos and Zarnegar 1992; Montesato et al. 1991). In contrast to hepatocytes, intrahepatic bile ducts were positive for TGF-α and EGF-R in postnatal livers, including adult livers, suggesting that mature intrahepatic bile ducts express these proteins and that the system may be involved in proliferation of mature bile ducts. Burr et al. (1993) also found that intrahepatic the ducts were strongly positive for TGF-α in the adult normal rat liver.

Although EGF is present in the liver (Marti 1993), its localization is unclear. However, it has been reported to be present in perisinusoidal cells (Yasui et al. 1992). In the present study, EGR expression was not found in any of the cell types of the liver. This may be because the present study used formalin-fixed, paraffin-embedded materials. In this respect, immunohistochemical procedures using fresh-frozen sections seem mandatory.

The present study disclosed that nerve bundles and smooth muscles of blood vessels were positive for TGF-α throughout development and maturation. Although the biological significance of TGF-α in these cells remains unclear, it seems possible that Schwann cells of nerve and smooth muscle cells of vessels produce TGF-α and that it may play a role in proliferation of these cells. Similar observations have been reported by Yasui et al. (1992), who demonstrated that Schwann cells and smooth muscle cells are positive for TGF-α in human tissues of both the adult and the fetus.

This study, however, has not determined whether these cytokines and receptors are produced by hepatocytes and bile duct cells. For this purpose, an in situ hybridization technique is mandatory, but it was not possible with our materials.

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