Immunohistochemical Localization of Transforming Growth Factor Alpha in Regenerating Rat Liver

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ABSTRACT. Immunohistochemical localization of TGF-alpha and cell proliferation kinetics during liver regeneration after two-thirds partial hepatectomy (PH) were investigated. Twenty-four to 72 hr after PH, appreciable increase in the number of TGF-alpha-positive hepatocytes was observed in zones 1 and 2. At the peak at 36 hr, almost all positive cells were stained in their nuclei. Considerable increase in the BrdU labeling index was observed 24–36 hr after PH with a peak at 24 hr in zones 1 and 2. These results indicated an association between TGF-alpha expression and hepatocyte regeneration. It is suggested that immunohistochemical localization of TGF-alpha may be a useful marker of cell proliferation activity in rat liver.

KEY WORDS: immunohistochemistry, liver, TGF-alpha.

Transforming growth factor alpha (TGF-alpha), a member of the epidermal growth factor (EGF) family, is a 50 amino acid polypeptide [12]. It shares about 30% structural similarity with EGF and binds to the EGF receptor [3]. Several reports have suggested TGF-alpha to play important roles during hepatic development and regeneration as a potent stimulator of hepatocyte [2, 8]. However, there are few reports describing TGF-alpha expression and its intracellular localization in relation to cell proliferation in the liver.

In the present immunohistochemical study, we investigated localization of TGF-alpha in different zones of the liver after partial hepatectomy (PH), and analyzed its links with cell kinetics.

Male Fischer 344 rats weighing around 200 g were purchased from Charles River Japan (Yokohama, Japan) and acclimatized for 1 week in an air-conditioned animal room at 22°C with a 12-hr light/dark cycle. They were given Oriental MF diet (Oriental Yeast Co., Tokyo, Japan) and tap water ad libitum.

All animals underwent two-thirds partial hepatectomy under light ether anesthesia. For the cell proliferation kinetic study, the rats were given an intraperitoneal injection of BrdU (100 mg/kg i.p.) (Sigma Chemical Co., St. Louis, MO) 1 hr before sacrifice at 6, 12, 24, 36, 48 or 72 hr after PH under ether anesthesia. Number of rats in each group was 9.

Livers were excised, fixed in 10% neutral-buffered formalin, embedded in paraffin, cut into 3–4 µm sections and examined immunohistochromically using anti-BrdU (Dako Japan Co., Kyoto) and anti-TGF-alpha (Oncogene Research Products, Cambridge, MA, U.S.A.) antibodies by avidin-biotin-peroxidase complex method. For quantitative comparisons, 1,000 nuclei were counted for each zone of the liver, and the percentages of BrdU-positive nuclei at different time points after PH were calculated. Similarly, 1,000 cells were counted for each zone and the percentages of TGF-alpha-positive hepatocytes with staining of their nuclei or cytoplasm were calculated.

Representative immunohistochemical localization of TGF-alpha and BrdU labeling in each zone of the liver are illustrated in Fig. 1. Data for the numbers of hepatocytes with TGF-alpha-positive nuclei or cytoplasm are summarized in Fig. 2. Six to 12 hr after PH, TGF-alpha-positive hepatocytes were mainly observed immediately around the central veins (zone 3) and most of them were only stained in their cytoplasm with only a small proportion having positive nuclei. After 24–72 hr, in addition to zone 3, abundant TGF-alpha-positive hepatocytes were also observed in the peripheral and intermediate regions (zones 1 and 2), and majority of them demonstrated nuclear staining. The peak was at 36 hr. The localization of TGF-alpha also shifted from the cytoplasm to the nucleus in zone 3 hepatocytes after 24 hr, so that nuclear staining predominated from 24–72 hr. Whereas there were only a few hepatocytes with cytoplasmic staining in zones 1 and 2 at any time point, appreciable number of such cells were evident throughout in zone 3.

The data for cell proliferation kinetics in each zone of the liver are summarized in Fig. 3. In zones 1 and 2, BrdU labeling index after PH was at a maximum at 24 hr after the treatment (about 27% and 25%, respectively). The labeling index was also prominent at 24–36 hr after PH. In zone 3, a slightly increased BrdU labeling index (about 2–7%) was induced during 24–72 hr after PH.

The present study demonstrated a close association between TGF-alpha expression and BrdU labeling index of rat hepatocytes after PH, suggesting that TGF-alpha may play a role in liver regeneration. These results are in line with the previous report describing increased TGF-alpha
mRNA expression in proliferating hepatocytes [8] and neoplastic rat liver lesions [5, 6, 10].

The most important finding in the present study is correlation of TGF-alpha expression and its subcellular localization with proliferating activity in liver. TGF-alpha tended to be positive and localized in the nucleus in highly proliferating areas zones 1 and 2, with peaks at 24–36 hr after PH. Kobayashi et al. [6] demonstrated that expression of TGF-alpha and its immunohistochemical localization was linked to cell proliferation in chemically induced rat hepatocellular carcinoma. Likewise, we recently reported TGF-alpha expression in the nucleus to be associated with malignancy.

Fig. 1. Immunohistochemical staining of TGF-alpha (A, C, and E) and BrdU (B, D, and F). (A) Six hours after PH: expression of TGF-alpha in the cytoplasm in hepatocytes of zone 3 (arrowhead). (B) Six hours after PH: BrdU labeling at the control level. (C) Twenty-four hours after PH: prominent nuclear expression of TGF-alpha in zones 1 and 2. (D) Twenty-four hours after PH: note considerable increase in BrdU labeling in zones 1 and 2. (E) Forty-eight hours after PH: expression of TGF-alpha in nucleus in zones 1 and 2. (F) Forty-eight hours after PH: decreased BrdU labeling in zones 1 and 2.
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and cell proliferation in chemically induced mouse hepatocellular tumors [10]. Taken together, there is a tendency that highly proliferating lesions exhibit a nuclear staining pattern of TGF-alpha expression.

In the liver, EGF is normally internalized on plasma membrane after binding receptor and is destroyed in lysosomes. In the regenerating liver, however, the EGF is diverted to hepatocyte nuclei prior to the initiation of DNA synthesis [7]. A number of reports have indicated the presence of EGF [11] and its receptor in the nucleus of target cells [1]. Furthermore, EGF induces translocation of its phosphorylated receptor into the nuclei of a cell line of squamous cell carcinoma [4]. Recently, Saga and Jimbw [9] reported that nuclei of the secretory cells of sweat glands showed a positive reaction to anti-activated EGF receptor antibody. These reports indicate that EGF may act directly to influence nuclear events such as transcription and DNA replication. Since, TGF-alpha is produced in the cytoplasm, then released into the exterior for eventual binding to EGF-receptors and exerts its biological effects [3], it is speculated that similar scenario might also be the case for TGF-alpha from the present observation, while EGF receptor was not studied. Further immunohistochemical study on subcellular localization of EGF receptor is in progress in our facility.

The cytoplasmic TGF-alpha expression in the centrilobular zone 3 in the early phase of hepatic regeneration is also very interesting. Kaufman et al. [5] previously described liver cell foci in carcinogen-treated rat livers displaying a heterogeneous distribution of reaction product of anti-TGF-alpha antibody. Briefly, cells facing onto blood vessels on margin were strongly stained in cytoplasm while the remainder of each focus displayed weak staining near the background. The heterogeneity of TGF-alpha expression and its role on hepatocyte proliferation after PH is not clear. However, TGF-alpha clearly tended to be positive and located in the nucleus in highly proliferative areas zone 1.

Fig. 2. Rates for TGF-alpha-positive hepatocytes stained in the nucleus and cytoplasm in each zone of liver at various times after PH. □ Nuclear and □ cytoplasmic staining. Data are means +/- SD values of 9 animals in each group.

Fig. 3. BrdU labeling indices for each zone of liver at various times after PH. Data are means +/- SD values of 9 animals in each group.
and 2. Further studies are needed to clarify the mechanisms of the change in localization pattern of TGF-alpha.

In conclusion, immunohistochemical localization of TGF-alpha, especially a nuclear staining pattern, may be a useful marker of cell proliferation activity in rat liver.

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REFERENCES