Acta Oncologica Turcica 2009; 42: 8-12

The TGFβR-1 Expressions in Different Steps of Liver Injury and Hepatocellular Carcinoma*

Karaciğer Hasarının Farklı Basamaklarında ve Hepatoselüler Karsinomda TGFβR-1 Ekspresyonları

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* This research was supported by Istanbul University research fund (project no: T-707/30062005).

SUMMARY

Hepatic stellate cell (HSC) is the key fibrogenic cell type in liver. Transforming growth factor-β (TGFβ), using mostly transforming growth factor-β receptor-1 (TGFβR-1), generated by the activated HSC is the most significant fibrogenic molecule of cirrhosis. We planned this study to observe the expressions of TGFβR-1 in different steps of liver injury. We immunostained 65 viral hepatitis, 58 cirrhosis and 21 hepatocellular carcinoma (HCC) cases with TGFβR-1 antibody and scored the intensity and the distribution of the stainings. Biostatistical analysis were done to see, if there are any meaningful results between age, sex, etiology, hepatic activity index, fibrosis scores; tumor diameter, vascular invasion, multifocality, presence of cirrhosis and receptor expressions. In the hepatitis group the intensity of TGFβR-1 staining was positively correlated with the increasing fibrosis score ($p< 0.05$). In the cases which cirrhosis was inactive, there were no TGFβR-1 expressions. The only positive result in HCC group was the inverse correlation between tumor diameter and TGFβR-1 expression. TGFβR-1 is an important mediator in liver wound healing. But its' effect is important in the early stages of hepatocarcinogenesis. In complete cirrhosis or HCCs greater than 3 cm, anti-TGFβR strategies will not be useful alone.

Key Words: *Cirrhosis, hepatocellular carcinoma, TGFβ, TGFβR-1.*

ÖZET

Hepatik yıldızsı hücre (HYH) karaciğerde fibrozisten sorumlu temel hücredir. Transforme edici büyüme faktörü-β (TGFβ) ise, aktive HYH'lerden salınan ve çoğunlukla transforme edici büyüme faktörü-β reseptörü-1 (TGFβR-1) üzerinden etkilerini gösteren, siroz gelişiminde fibrozisten sorumlu olan temel moleküldür. Bu çalışma, karaciğer hasarının farklı aşamalarında ve hepatoselüler karsinom (HSK)'da TGFβR-1 ekspresyonlarını değerlendirmek, ve de tedaviye yönelik çıkarımlar yapmak üzere planlanmıştır. Altmış beş viral hepatit, 58 siroz ve 21 HSK olgusu TGFβR-1 primer antikoru ile immünhistokimyasal olarak boyandı. Boyanma yaygınlığı ve yoğunluğu skorlandı. Rutin boyalardaki histolojik aktiviteler ve fibrozis skorları ile immünhistokimyasal boyanma paternleri ve boyanan hücreler karşılaştırıldı. Ardından yaş, cinsiyet, etyoloji, histolojik aktivite indeksleri, fibrozis skorları; tümör çapı, vasküler invazyon, multifokalite ve siroz varlığı ile TGFβR-1 boyanmasındaki yaygınlık ve yoğunluklar arasındaki ilişkileri saptamak üzere biyoistatistiksel çalışmalar uygulandı. Hepatit grubunda TGFβR-1 boyanmasındaki yoğunluk ile fibrozis skorundaki artışın korelasyon gösterdiği saptandı ($p< 0.05$). İnaktif sirozun izlendiği vakalarda TGFβR-1 boyanması görülmedi. HSK grubunda saptanan en önemli bulgu ise; tümör çapı ile TGFβR-1 boyanmaları arasındaki ters korelasyon oldu. TGFβ ve reseptörü olan TGFβR-1 karaciğerdeki yara iyileşmesi sürecinde görevli olan en önemli moleküllerdendir. Ancak etkileri hepatokarsinogenezin erken dönemlerinde belirgindir. Komplet siroz ve 3 cm ve daha büyük çaplardaki HSK'larda anti-TGFβR stratejilerinin monoterapi şeklinde kullanılması faydalı olmayacaktır.

Anahtar Kelimeler: *Siroz, hepatoselüler karsinoma, TGFβ, TGFβR-1.*

INTRODUCTION

Liver fibrosis is the common response to hepatotoxicity and its most unfavorable result is hepatocellular carcinoma. Hepatocellular carcinoma (HCC) constitutes approximately 5.4% of cancers and in some populations is the most common cancer (1). More than 85% of cases of HCC occur in countries with high rates of chronic hepatitis B virus (HBV) infection. The other most prominent factors associated with HCC include chronic hepatitis C virus (HCV) infection, chronic alcohol consumption, aflatoxin-B1 contaminated food and virtually all cirrhosis-inducing conditions. The lethality of HCC stems in part from its resistance to existing anticancer agents, a lack of biomarkers that can detect surgically resectable incipient disease, and underlying liver disease that limits the use of chemotherapeutic drugs (2).

Hepatocyte apoptosis, which is the first cellular response to many toxic events, and accompanies viral hepatitis, correlates with disease severity and hepatic fibrosis. In particular, hepatic stellate cells (HSC), the key fibrogenic cell type in liver, contribute to apoptosis and inflammation. Phagocytosis of apoptotic bodies by quiescent HSC facilitates the phenotypic transformation to myofibroblasts. Transforming growth factor-β (TGFβ) is generated when cells phagocytose apoptotic bodies, especially by HSC. Although TGFβ is a potential inhibitor of T-cell function, it is also a strong fibrogenic signal in liver (3). Liver fibrosis represents the common response of the liver to toxic, infectious, or metabolic agents and is characterized by excessive accumulation of extracellular matrix (4). Increased collagen deposition leads to reduced oxygen levels in the surrounding tissue and consequently upregulates TGFβ (5). Among the cytokines involved in wound healing, such as hepatitis, TGFβ is particularly prominent (6).

TGFβ is synthesized as a prohormone. Once activated, TGFβ signals through a complex of 2 related but structurally and functionally distinct serine-threonine kinase receptors, called type 1 and 2. Binding of the homodimeric TGFβ to transforming growth factor-β receptor-2 (TGFβR-2) enables the formation and stabilization of type 1/type 2 receptor complexes, most likely heterotetramers. The TGFβR-2 kinase then phosphorylates TGFβR-1. This is the critical event in TGFβ signaling and serve as the initiation point for downstream events (7).

Many different strategies of molecular therapy have focused on the inhibition of TGFβ effects by blocking its synthesis, using TGFβ binding proteins, soluble receptors, or targeting its downstream signal transduction pathways. Although these progresses in treatment of liver injury and fibrogenesis are encouraging, one issue that requires additional attention remains that targeting these molecular therapeutics to spesific cell types (hepatocytes, Kupffer cells or HSC) is critical in avoiding undesired effects on other organs or cell types (8). Pathophysiological relationship between hepatic fibrosis and cancer progression critically relies on TGFβ which might be of particular relevance for the therapy of liver carcinoma (9).

There are many researches in the English literature that show TGFβ analogues and TGFβR antagonists have an effect on HCC. Most of them prove that these agents decrease tumor load and diameter. There are also researches about the effect of these agents on cirrhosis and preneoplastic liver lesions (10,11). But according to our knowledge, there is no research about TGFβR-1 expressions through hepatitis-cirrhosis-HCC process, in human tissues with wide serials.

Here in this study we planned to see the expressions of this molecule in hepatitis, cirrhosis and HCC groups.

MATERIALS and METHODS

Formalin fixed-paraffin embedded sections from fine needle, incisional, wedge or excisional biopsies of 65 viral hepatitis, 57 cirrhosis and 21 HCC cases were immunostained with monoclonal antibody for TGFβR-1 (TGFβR-1, labvision, corporation, neomarkers, rabbit polyclonal antibody, 1/50) using avidin-biotin complex immunohistochemical method. The intensity of immunoreactivity in any kind of cell were evaluated as slight, moderate or intense. The distribution of the immunoreactivity was evaluated according to the stained amounts (1/3, 2/3 or 3/3 of the tissue) on that slide. All the results were correlated with the hematoxylen and eosine and reticulin stained slides.

A control group composed of 10 normal liver tissue (fetal, childhood or adult livers from autopsy materials or resection materials which were done because of non-liver pathologies) was also immunostained with the same antibody.

Biostatistical analysis, using SPSS 13 data base, were done to see, if there are any meaningful results between age, sex, etiology, hepatic activity index, fibrosis scores; tumor diameter, vascular invasion, mul-

tifocality, presence of cirrhosis and TGFβR-1 expressions.

RESULTS

There were no positive stainings in our control group which is composed of normal livers of different ages.

We saw positive stainings with TGFβR-1 in central vein, sinusoid and portal vein endothelial cells, portal and sinusoidal macrophages, portal fibroblasts, few bile duct epithelial cells and lymphocytes in the research group.

In the viral hepatitis group; mean age was 34 (0-67), 43 of them were male (66%). Etiology of 48 cases (73%) were HBV, 16 cases were (25%) HCV and 1 case was HBV + HCV. There were no significant relationship between TGFβR-1 expression and age, sex, virus type, piecemeal necrosis, lobuler degeneration and portal inflammation scores. There was no correlation with histological activity index (HAI) either. The only positive result in the viral hepatitis group was the one between fibrosis score and the intensity of TGFβR-1 staining. The intensity was positively correlated with the increasing fibrosis score ($p< 0.05$) (Table 1).

In the cirrhosis group; mean age was 30 (0-66), 39 of them were male (63%). Etiology of the cases were mostly composed of viral and cryptogenic cirrhosis, but we also examined biliary, autoimmune, alcoholic and metabolic (e.g., diabetes mellitus, storage diseases) cirrhosis (Table 2). TGFβR-1 expression was mostly seen in periportal areas where inflammation was still active. But in the cases with inactive cirrhosis and the space of Disse was fulfilled with collagen, there were no TGFβR-1 expressions. So we especially analysed the cirrhosis group with viral etiology and could not find any correlation between the expression of TGFβR-1 and the HAI. There were no significant results with TGFβR-1 stain in cirrhosis group.

Table 2. Etiologic distribution of cirrhosis group.

Etiology	n	%
Hepatitis B virus	5	9
Hepatitis C virus	14	25
Biliary	11	19
Autoimmune	3	5
Alcoholic	3	5
Metabolic	8	14
Cryptogenic	13	23

In HCC group mean age was 50 (13-77), 16 of them were male (69%). Etiology of 8 cases (38%) were HBV, 2 of them were (10%) HCV and 11 of them were unknown (52%). There were no correlation between TGFβR-1 expression and the age, sex, etiology, presence of cirrhosis, differentiation of tumor cells, vascular invasion or multifocality. The only significant result in HCC group was the inverse correlation between tumor diameter and TGFβR-1 expression both in terms of distribution and intensity. The expression was significant in the smaller tumors (< 3 cm), whereas it was less noticable in the greater ones (> 3 cm). But it was stable over 3 cm, even if the diameter gets bigger (Table 3).

CONCLUSION

In the English literature, most of the researches were done with HCC cell cultures derived from animal tumors and concluded that there are significant differences between human and animal tumors. These results prove that, researches using the human tissues are more meaningful. As mentioned above, TGFβRs are the most important an d popular signaling molecules in liver fibrogenesis, and TGFβR-1 is the key receptor.

There were no positive stainings with TGFβR-1 in our control group, composed of normal livers of diffe-

Table 1. p values of the variables in viral hepatitis group (Kendall's tau_b correlations).

p< 0.05	Age	Sex	Virus type	Piecemeal necrosis	Lobuler degeneration	Portal inflammation	Histological activitiy index (HAI)	Fibrosis
Distribution of TGFβR-1	0.304	0.517	0.948	0.752	0.480	0.942	0.853	0.368
Intensity of TGFβR-1	0.157	0.612	0.917	0.201	0.499	0.794	0.486	0.044

TGFβR-1: Transforming growth factor-β receptor-1.

Table 3. p values of the variables in hepatocellular carcinoma group (Kendall's tau_b correlations).

p< 0.05	Age	Sex	Etiology	Presence of cirrhosis	Tumor size	Tumor differantiation	Multifocality	Vascular invasion
Distribution of TGFβR-1	0.792	0.868	0.428	0.578	0.010	0.106	0.324	0.324
Intensity of TGFβR-1	0.792	0.868	0.428	0.578	0.010	0.106	0.324	0.324

TGFβR-1: Transforming growth factor-β receptor-1.

rent ages. We interpreted this result as; this receptor is not expressed on the cell surface unless there is a primary liver damage.

We saw positive stainings with TGFβR-1 in central vein, sinusoid and portal vein endothelial cells, portal and sinusoidal macrophages, portal fibroblasts, few bile duct epithelial cells and lymphocytes in the research group, showing the role of the other cells, except HSC, in liver fibrosis.

In the viral hepatitis group; there were no significant relationship between TGFβR-1 expression and age, sex, virus type, piecemeal necrosis, lobuler degeneration and portal inflammation scores. There was no correlation with HAI either. This result got along with the results of Spee B. et al. concluding the severity of the inflammation does not change the mRNA levels of TGFβR-1 (12). The only positive result in the viral hepatitis group was the one between fibrosis score and the intensity of TGFβR-1 staining. The intensity was positively correlated with the increasing fibrosis score ($p< 0.05$). This intensity was mostly located at the endothelial cells of periportal sinusoids and portal collateral veins, compared with the similar study (10). In this study, only the vascular smooth muscle wall was stained with TGFβR-1. Whether the endothelial cells were stained or not, it is an important result that the vascular network, as a therapy target, has the same receptor. Paik SY et al. Studied only dysplastic nodules and HCC, and also found out positivity in non-neoplastic hepatocyte cytoplasms (10). We found cytoplasmic positivity in hepatocytes in all groups. The well-differentiated peripheral cells of the HCC were also stained, while the poorly-differentiated central cells did not.

There were no significant results with TGFβR-1 stain in cirrhosis group. In the cirrhosis group, fibrosis score was not correlated with TGFβR-1 expressions as it had been in the viral hepatitis. So we needed to confirm with reticulin stain that, in active cirrhosis (31 cases), caused by chronic hepatitis, the intensity and the distribution of TGFβR-1 expressions were more significant than in inactive cirrhosis (26 cases). This result supported the idea that fibrogenesis is a dynamic process and when the space of Disse is fulfilled with mature collagen (complete, inactive cirrhosis), TGFβR-1 downregulates.

In HCC group there were no correlatin between TGFβR-1 expression and the age, sex, etiology, presence of cirrhosis, differentiation of tumor cells, vascular invasion or multifocality. The only significant finding in HCC group was the inverse correlation between tumor diameter and TGFβR-1 expression. The expression was significant in the smaller tumors (< 3 cm), whereas it was less noticeable in the greater ones (> 3 cm). But it was stable over 3 cm, even if the diameter gets bigger.

When these results were interpreted altogether we found that the intensity of TGFβR-1 expression was increased in correlation with the fibrosis scores in hepatitis; sustained in active but decreased in inactive cirrhosis and almost disappeared in HCC. Ueno T. et al. showed the similar expressions for TGFβR-2, in hepatitis-cirrhosis and HCC groups (11). These results can be interpreted as; TGFβR-1 expression increases in inflammation in correlation with fibrosis, decreases during carcinogenesis, and almost disappears in HCC greater than 3 cm. These results support the idea that receptor down-regulation or mutation occur in the late stages of hepatocarcinogenesis.

According to our results, TGFβ and TGFβRs can be useful agents in antifibrogenic therapy in chronic hepatitis and active cirrhosis but will have a limited effect in inactive cirrhosis and HCC, and will not have a beneficial effect to the tumors greater than 3 cm. We are still studying larger series to prove our hypothesis.

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