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The Expressions of Nerve Growth Factor and Its Receptor p75^{NGFR} in Hepatocellular Carcinoma: Their Relation with the Clinicopathologic Factors

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Background : Nerve growth factor (NGF) has been suggested to participate in tumor progression and it can interact with its receptor p75^{NGFR}. In the present study, we investigated the expressions of NGF and p75^{NGFR} in hepatocellular carcinoma (HCC). **Methods :** We performed immunohistochemistry for NGF, p75^{NGFR} and PCNA in 45 cases of HCCs, and examined the relationships between the clinicopathologic factors and the immunohistochemical results. **Results :** NGF was detected in 84.4% (38/45) of the tumor cells and in 64.4% (29/45) of the non-tumorous hepatocytes. Furthermore, a NGF expression was present in 28.9% (13/45) of the endothelial cells in the HCCs, but in 80% (36/45) of the endothelial cells in the non-tumor liver tissue. The tumor cells were negative for p75^{NGFR} in all the HCCs. Although a p75^{NGFR} expression was present in all the nerve fibers in the non-tumor liver tissues, it was markedly reduced (42.2%; 19/45) in the HCCs and a p75^{NGFR} expression was observed at the sinusoids or around the large vessels. The HCCs expressing NGF, either in the tumor cells or the endothelial cells, showed a larger size than those HCCs that didn't express NGF. The NGF positive tumors showed a tendency toward a higher PCNA-labeling index than did the negative tumors. **Conclusions :** The changed localization of the NGF expression and the decreased expression of p75^{NGFR} are associated with hepatic carcinogenesis. We suggest that a NGF expression may contribute to the progression of HCC.

Key Words : Nerve growth factor; p75^{NGFR} (Receptor, Nerve growth factor); Carcinoma, Hepatocellular

The liver is innervated by both sympathetic and parasympathetic fibers, and most of these fibers enter the liver in close association with the hepatic artery, portal vein or bile duct.[1] Furthermore, several studies have demonstrated that the autonomic nervous system participates in the regulation of the hepatic blood circulation and metabolism.[2,3] There are 2 known types of hepatic innervation: 1) the innervation of parenchymal cells related to hepatic metabolism and regeneration; and 2) catecholamine-containing nerve fibers that are in close contact with hepatocytes and the vasculatures.

Nerve growth factor (NGF) is a prototypic member of the neurotrophin family, and NGF is essential for the survival, differentiation and maintenance of neuronal cells in both central and peripheral nervous systems.[4] NGF is composed of α and β subunits in most tissues.[4] Moreover, the biological activity of NGF is confined to its β subunit, whereas both subunits are believed to be required for protection from proteolytic degradation. NGF can interact with two types of membrane binding sites: TrkA and p75^{NGFR}.[5] p75^{NGFR} is a low affinity NGF receptor that structurally resembles members of the p55 tumor necrosis factor receptor family and it activates Jun N-terminal kinase and ceramide to promote apoptosis.[6-8] On the other hand, TrkA is a high affinity receptor with tyrosine kinase activity, and binding of TrkA with NGF results in intracellular signaling through the mitogen-activated protein kinase cascade and the phosphatidylinositol-3 kinase cascade, which both lead to the differentiation and survival of neuronal cells.[6-8] Several recent reports have indicated that NGF is involved in the repair of lung and skin tissue, in addition to its known involvements in allergic inflammation and fibrosis[9,10] and these reports have suggested that NGF is in-

volved in tumor growth, invasion, and metastasis.[11-14] NGF and its two receptors are expressed in various cancers, including lung, breast and prostatic cancers, which suggests that NGF participates in tumorigenesis via the autocrine or paracrine pathways.[11,13,14] However, the role of NGF in cancer progression remains controversial.

Human hepatocellular carcinoma (HCC) is the fifth most common type of malignancy and it is the third leading cause of cancer death worldwide.[15] Despite the recent advances in diagnostic and therapeutic advances in the treatment of HCC, its prognosis is still poor. Without treatment, HCC that is diagnosed at an advanced stage usually leads to death within months, and any long-term survival is rare.[16]

The previous investigations that focused on the expressions of NGF and its receptors in HCC tissues have yielded contradictory results. Some researchers have found that hepatocytes expressed NGF and TrkA only in the walls of the arteries that are associated with tumors[17,18] whereas other researchers found that the hepatocytes in fibrotic rat livers expressed TrkA mRNA, but not NGF mRNA.[19] Thus, it is unclear which liver tissue cell types are involved in the cross-talk mediated by NGF during the progression of HCC.

In this study, we investigated the expressions and localizations of NGF and its receptor $p75^{NGFR}$ in both HCC and the non-tumorous liver tissues. In addition, we examined the relationships between the clinicopathologic factors and the expressions of NGF and $p75^{NGFR}$ in HCC and the relationships between the proliferating cell nuclear antigen-labeling index (PCNA-LI) and the expressions of NGF and $p75^{NGFR}$.

MATERIALS AND METHODS

Patients and specimens

This study was approved by the Human Ethics Committee of Chonbuk National University Hospital, Korea. We retrospectively studied the HCC specimens and the non-tumor specimens obtained from 45 patients who underwent surgical resection between 1991 and 2001 at the Chonbuk National University Hospital. None of the patients had received any therapy prior to surgery. Of the 45 patients with HCC, 37 were men and 8 were women. The mean patient age was 55.5 years (range: 12-79 years). The grading system developed by Edmondson and Steiner was used for histological grading the HCC.[20] The clinicopathologic findings of these specimens are summarized in Table 1.

Immunohistochemistry

An immunoperoxidase method with employing streptavidin-biotinylated horseradish peroxidase complex (DAKO, Carpinteria, CA, USA) was used. Tissue blocks that contained non-tumor liver tissues and HCC tissues were selected and then 4 μm thick sections were cut from the formalin-fixed, paraffin-embedded tissue blocks. After deparaffinization, the sections were subjected to a microwave antigen retrieval procedure in 0.01 M sodium citrate buffer for 10 min. The sections were then incubated in methanol that contained 0.3% hydrogen peroxide at room temperature for 20 min to block the endogenous peroxidase activity. After blocking the endogenous biotin, the sections were incubated with Protein Block Serum-Free (DAKO, Carpinteria) at room temperature for 10 min to block any nonspecific staining, and then the sections were incubated for 2 h at room temperature with anti-NGF (Santa Cruz Biotechnology, Santa Cruz, CA, USA), $p75^{NGFR}$ (DAKO, Carpinteria) and PCNA (DAKO, Carpinteria) antibodies. After washing, the sections were incubated with a biotin-conjugated secondary antibody at room temperature for 30 min and they were finally incubated with peroxidase conjugated streptavidin at room temperature for 30 min. The peroxidase activity was detected with the enzyme substrate

Table 1. Clinicopathologic findings in the 45 HCC cases

Clinicopathologic factors	Number of cases
Age (year) (55.5±12.9) (mean±SD)	
<60 (49.0±11.6)	28
≥60 (66.2±6.1)	17
Sex	
Male	37
Female	8
Number of masses	
1	30
>1	15
Tumor size (cm) (3.8±2.1) (mean±SD)	
<2 (1.41±0.29)	10
≥2 (4.43±1.91)	35
Histologic grade by Edmondson and Steiner	
1 & 2	26
3 & 4	19
Vessel invasion	
Present	20
Absent	25
Cirrhosis	
Present	25
Absent	20
HBs Ag (n=37)	
Positive	25
Negative	12

SD, standard deviation.

3 amino-9-ethyl carbazole. For negative controls, sections were treated in the same manner except they were treated with Tris-buffered saline instead of the primary antibody.

Evaluation of the immunohistochemical results

To semi-quantitatively determine the expressions of NGF and $p75^{NGFR}$, a scoring system was developed by multiplying the degree of the staining intensities and the percentage of the positively stained areas. The intensities of cell staining were graded according to the following scale: 0, no staining; 1+, mild staining; 2+, moderate staining; 3+, marked staining, and the areas of staining were evaluated by using the following scale: 0, <10% of the cells stained positively; 1+, 10-30% of the cells stained positively; 2+, 30-70% of the cells stained positively; and 3+, >70% of the cells stained positively. Accordingly, the maximum possible score was 9 and the minimum possible score was zero. Specimens with scores >2 were considered positive for a NGF expression or a $p75^{NGFR}$ expression. PCNA-LI was defined as the percentage of tumor cell nuclei that showed positive PCNA staining.

Statistical analysis

The expressions of NGF and $p75^{NGFR}$ in the tumor and non-tumor tissues were compared using Fisher's exact test. The possible relations between the clinicopathologic factors and the expressions of NGF or $p75^{NGFR}$ in the tumor tissues were assessed by using Fisher's exact test. The relations between the NGF and $p75^{NGFR}$ expressions in the tumor tissues and the PCNA-LI values were assessed by using Student's t-test. p-values <0.05 were considered statistically significant.

RESULTS

The expression of NGF and its relation with the clinicopathologic factors

In the non-tumor liver tissues, a NGF expression was observed in the sinusoidal endothelial cells (Fig. 1A) and the hepatocytes. In addition, a NGF expression was also observed in the epithelial cells of the bile ductules, in the nerve bundle cells and in the stromal cells. In the HCC tissues, NGF was found to be highly expressed in the tumor cells, which showed an intense cytoplasmic expression and in the sinusoidal endothelial cells (Fig. 1B, C). Furthermore, NGF was detected in the tumor cells in 84.4% (38/45 cases) of the cases, and in the non-tumor hepatocytes in 64.4% (29/45 cases) of the cases (Table 2), and the NGF expression had a tendency to exchange its localization between the tumor hepatocytes and the non-tumor hepatocytes (p=0.052). Moreover, while NGF was detected in the tumor endothelial cells in only 28.9% (13/45 cases) of the cases, it was detected in the non-tumor endothelial cells in 80.0% (36/45 cases) of the cases, and the difference was significant (p<0.001). As shown in Table 3, a NGF expression in the tumor cells was associated with a larger tumor size (p=0.034). In addition, a NGF expression in the

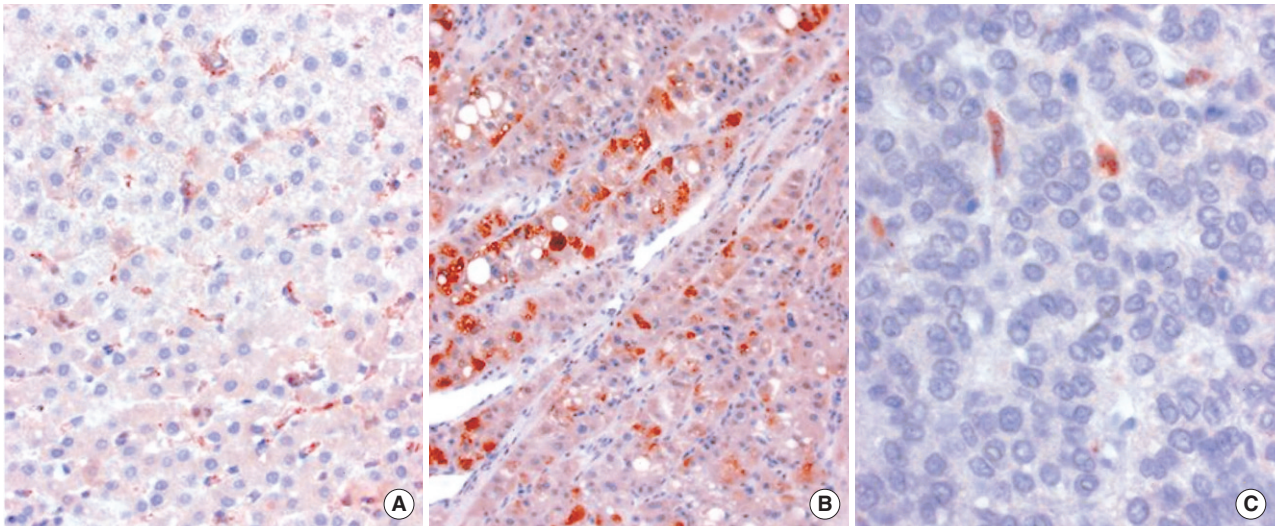

Fig. 1. In non-tumor liver tissues, NGF was present in sinusoidal endothelial cells (A). In HCC tissues, NGF was highly expressed in tumor cells (B) and in sinusoidal endothelial cells (C).

Table 2. Immunohistochemical staining results for NGF and p75^{NGFR}

NGF	Total Number			Hepatocyte			Endothelium			p75^{NGFR}	Tumor	Non-tumor	p-value
	T	NT	p-value	T	NT	p-value	T	NT	p-value				
Positive	39	43	0.266	38	29	0.052	13	36	<0.001		19	45	<0.001
Negative	6	2		7	16		32	9			26	0	

Fisher's exact test.
NGF, nerve growth factor; T, tumor; NT, non-tumor.

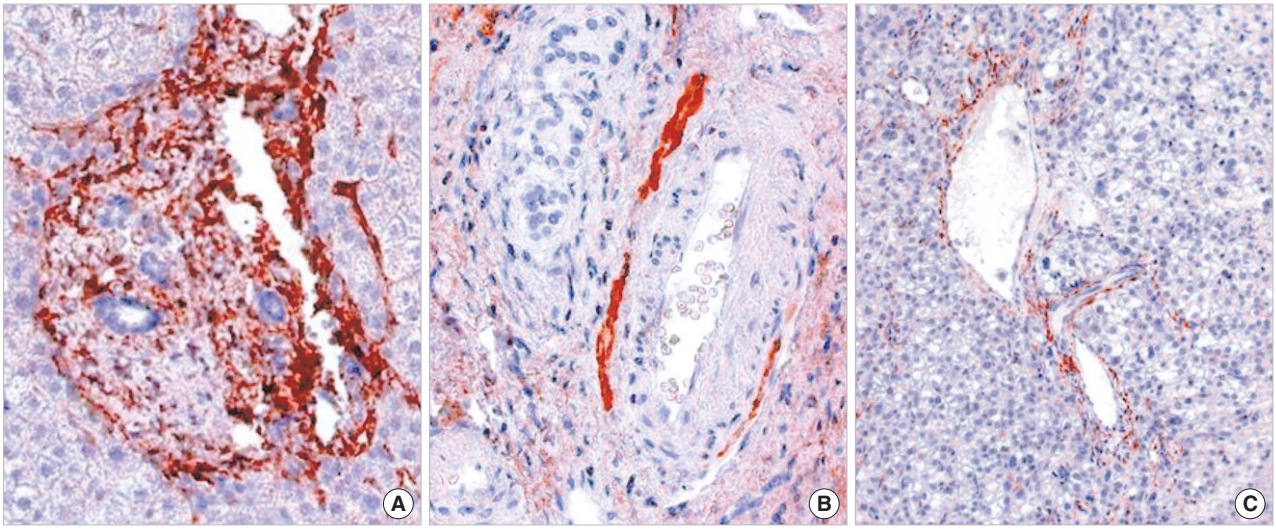

Fig. 2. In non-tumor liver tissues, p75^{NGFR} was expressed in nerve fibers in portal areas (A), especially around blood vessels (B). In HCC tissues, p75^{NGFR} was not expressed in tumor cells but was expressed in the nerve fibers, especially around large tumor vessels (C).

tumor endothelial cells was associated an advanced age (p=0.047), the female gender (p=0.034) and a larger tumor size (p=0.042). No other clinicopathologic factors were found to be correlated with the expression of NGF in HCC.

The expression of p75^{NGFR} and its relation with the clinicopathologic factors

In the non-tumor liver tissue, p75^{NGFR} was expressed in the nerve fibers in the portal areas (Fig. 2A), and especially around the blood vessels (Fig. 2B) and the bile ducts in all cases (45/45 cases, Table 2). However, in the tumor tissues, p75^{NGFR} showed positivity in 42.2% (19/45 cases) of the cases in the nerve fibers, and especially in the nerve fibers around the large tumor vessels (Fig. 2C). No tumor cells expressed p75^{NGFR}. The expression of p75^{NGFR} in the tumor tissues was significantly lower than that in the non-tumor liver tissues (p<0.001). As shown in Table 4, none of the clinicopathologic factors were found to be correlated with a p75^{NGFR} expression.

Correlations between the expressions of NGF or p75^{NGFR} and the PCNA-LI values

The PCNA-LI values in the tumor tissues ranged from 12% to 79% (average: 40.6%). As shown in Table 5, the tumors with cells expressing NGF tended to have higher PCNA-LI values than did those tumors that did not express NGF (p=0.054). There was no significant correlation between the NGF expression in the tumor endothelial cells and the PCNI-LI or between the p75^{NGFR} expression in tumor and the PCNI-LI was found.

DISCUSSION

This study demonstrates the following: 1) the expression of NGF was significantly lower in the tumor endothelial cells than that in the non-tumor endothelial cells, but there was a tendency for a higher NGF expression in the tumor cells than that in the non-tumor hepatocyes; 2) the expression of p75^{NGFR} was significantly lower in tumor tissues than that in the non-tumor tissues;

Table 3. Relations between NGF expression and clinicopathologic factors in HCCs

Clinicopathologic factors	TC (+)	TC (–)	p-value	TEC (+)	TEC (–)	p-value
Age (year)						
<60	22	6	0.227	3	19	0.047
≥60	16	1		10	13	
Sex						
Male	32	5	0.590	8	29	0.034
Female	6	2		5	3	
No. of tumor						
1	24	6	0.395	9	21	1.000
>1	14	1		4	11	
Tumor size (cm)						
<2	6	4	0.034	0	10	0.042
≥2	32	3		13	22	
Histologic grade						
1 & 2	21	5	0.681	10	3	0.182
3 & 4	17	2		16	16	
Vessel invasion						
Present	17	3	1.000	5	15	0.745
Absent	21	4		8	17	
Cirrhosis						
Present	22	3	0.682	10	15	0.100
Absent	16	4		3	17	
HBs Ag (n=37)						
Positive	20	5	1.000	6	18	1.000
Negative	10	2		3	9	

Fisher's exact test.
NGF, nerve growth factor; HCC, hepatocellular careinoma; TC, tumor cell; TEC, tumor endothelial cell; Ag, antigen.

Table 5. Relations between PCNA-LI values and the expressions of NGF and $p75^{NGFR}$

	PCNA-LI (mean±SD)	p-value
NGF		
Positive in TC	42.50±14.71	0.054
Negative in TC	30.28±16.54	
Positive in TEC	43.15±15.30	0.487
Negative in TEC	39.56±15.66	
$p75^{NGFR}$		
Positive in tumor	39.58±13.73	0.377
Negative in tumor	41.35±16.85	

Student's t-test.
NGF, nerve growth factor; TC, tumor cell; TEC, tumor endothelial cell; LI, labeling index; SD, standard deviation.

3) the expression of NGF in either the tumor cells or the tumor endothelial cells was associated with a larger tumor size; 4) the HCCs with NGF-expressing tumor cells tended to have higher PCNA-LI values than did those HCCs with tumor cells that did not express NGF. These findings suggest that both the changed localization of the NGF expression and the decreased expression of $p75^{NGFR}$ are associated with hepatic carcinogenesis. Furthermore, these results suggest that a NGF expression may contribute to the progression of HCC in association with the increased proliferation of tumor cells.

Table 4. Relations between $p75^{NGFR}$ expression and clinicopathologic factors in HCCs

Clinicopathologic factors	Total no.	Positive	Negative	p-value
Age (year)				
<60	28	13	15	0.543
≥60	17	6	11	
Sex				
Male	37	18	19	0.113
Female	8	1	7	
No. of tumor				
1	30	15	15	0.203
>1	15	4	11	
Tumor size (cm)				
<2	10	5	5	0.720
≥2	35	14	21	
Histologic grade				
1 & 2	26	11	15	1.000
3 & 4	19	8	11	
Vessel invasion				
Present	20	7	13	0.545
Absent	25	12	13	
Cirrhosis				
Present	25	13	12	0.224
Absent	20	6	14	
HBs Ag (n=37)				
Positive	25	9	16	1.000
Negative	12	6	6	

Fisher's exact test.
HCC, hepatocellular careinoma.

The liver is innervated by two separate but intercommunicating plexuses that are around the hepatic artery and the portal vein.[21] Besides their presence around vascular structures in the portal tracts, nerve fibers, and mostly sympathetic nerve fibers, are present in the parenchyma along the sinusoids. Furthermore, the release of neurotransmitters from the intrasinusoidal fibers modulates the functions of the hepatocytes and perisinusoidal cells and this partly controls carbohydrate and lipid metabolism, and it induces the contractions of perisinusoidal cells, thereby regulating the intrasinusoidal blood flow.[22]

NGF can interact with two types of cell membrane receptors, i.e., TrkA and $p75^{NGFR}$.[5] It has been demonstrated that NGF induces cell proliferation in breast cancer cell lines via TrkA and the activation of its downstream mitogen-activated protein kinase cascade.[14] In addition, NGF's effect on cell survival in breast cancer cell lines has been shown to be dependent on the activation of $p75^{NGFR}$ and its downstream NFκB signal cascade.[23] Both types of NGF receptor may participate in tumor progression by promoting cell proliferation and preventing apoptosis. Recent evi-

dence has indicated that both TrkA and $p75^{NGFR}$ can dimerize in response to NGF binding, and TrkA homodimers and TrkA-$p75^{NGFR}$ heterodimers promote cell survival, whereas conversely, $p75^{NGFR}$ homodimers mediate cell death.[24]

In recent years, many studies have concluded that NGF is involved in tumor biology, such as in growth, invasion and metastasis, and the expressions of NGF and/or of its receptors have been reported in many types of carcinomas.[14,25-27] In particular, Kishibe *et al.*[17] showed that NGF is expressed in mouse HCC tissues and mouse HCC cell lines at much higher levels than that in a normal liver, and immunostaining showed that NGF is expressed not only in HCCs, but also in early preneoplastic lesions, which indicates that its expression represents an early change during murine hepatic carcinogenesis. In addition, Rasi *et al.*[28] reported that NGF is expressed in human tissues with cirrhosis and HCC, but not in normal liver tissues or in the liver tissues with cirrhosis and without HCC. Further, these researchers showed that NGF was mainly localized on tumor hepatocytes, and to a lesser extent on the endothelial cells and Kupffer cells in HCC tissues. However, NGF was localized on the biliary epithelial cells and hepatic stellate cells in the cirrhotic tissues. The location that expressed NGF was similar to our results except that NGF was not expressed on the non-tumor hepatocytes. This difference of results might be partly due to the different sources of antibody. In the present study, NGF was found to be expressed at higher levels in the tumor cells than that in the non-tumor hepatocytes, which suggests that NGF participates in the tumorigenesis of HCC. Additionally, the HCCs with NGF expressing tumor cells tended to have higher proliferating activities. Even though $p75^{NGFR}$ was remarkably expressed in the walls of the large vessels in HCC, its expression was not observed in tumor cells or in the non-tumor hepatocytes, which suggests that HCC tumor cells and normal hepatocytes are not the major targets of NGF. These results concur with those of a previous murine study, in which a NGF expression was observed in the neoplastic hepatocytes, but the expressions of TrkA and $p75^{NGFR}$ were not observed in the tumor cells.[17] Furthermore, TrkA was observed in the walls of the hepatic arteries, and this might have contributed to the development of tumor-associated arteries.[17] The TrkA expression in the arterial walls suggests that NGF may enhance hepatic innervation and further promote blood vessel development, and that the NGF produced by HCC cells has a paracrine action toward the non-tumor cell components in the HCC tissues.

In agreement with our results, Yuanlong *et al.*[29] reported that the expression of p75NTR was significantly decreased in HCC tissues as compared with their adjacent noncancerous counterparts, and the up-regulation of $p75^{NGFR}$ inhibited the growth of HCC cell lines by inducing cell cycle arrest. These results suggested that $p75^{NGFR}$ might be a potential tumor suppressor.

To sum it up, our findings suggest that the location showing a NGF expression is changed in HCC, and the NGF produced by the HCC tumor cells is a paracrine factor that participates in the progression of HCC. A deeper understanding of the role of neurotrophins in hepatic carcinogenesis might provide valuable clues for creating novel strategies to treat HCC.

REFERENCES

1. Tiniakos DG, Lee JA, Burt AD. Innervation of the liver: morphology and function. Liver 1996; 16: 151-60.
2. Kyosola K, Penttila O, Ihamaki T, Varis K, Salaspuro M. Adrenergic innervation of the human liver: a fluorescence histochemical analysis of clinical liver biopsy specimens. Scand J Gastroenterol 1985; 20: 254-6.
3. Hartmann H, Beckh K, Jungermann K. Direct control of glycogen metabolism in the perfused rat liver by the sympathetic innervation. Eur J Biochem 1982; 123: 521-6.
4. Fahnestock M. Structure and biosynthesis of nerve growth factor. Curr Top Microbiol Immunol 1991; 165: 1-26.
5. Maness LM, Kastin AJ, Weber JT, Banks WA, Beckman BS, Zadina JE. The neurotrophins and their receptors: structure, function, and neuropathology. Neurosci Biobehav Rev 1994; 18: 143-59.
6. Chapman BS. A region of the 75 kDa neurotrophin receptor homologous to the death domains of TNFR-I and Fas. FEBS Lett 1995; 374: 216-20.
7. Dobrowsky RT, Werner MH, Castellino AM, Chao MV, Hannun YA. Activation of the sphingomyelin cycle through the low-affinity neurotrophin receptor. Science 1994; 265: 1596-9.
8. Casaccia-Bonnefil P, Carter BD, Dobrowsky RT, Chao MV. Death of oligodendrocytes mediated by the interaction of nerve growth factor with its receptor p75. Nature 1996; 383: 716-9.
9. Micera A, Vigneti E, Pickholtz D, *et al.* Nerve growth factor displays stimulatory effects on human skin and lung fibroblasts, demonstrating a direct role for this factor in tissue repair. Proc Natl Acad Sci U S A 2001; 98: 6162-7.
10. Micera A, Puxeddu I, Aloe L, Levi-Schaffer F. New insights on the involvement of Nerve Growth Factor in allergic inflammation and fibrosis. Cytokine Growth Factor Rev 2003; 14: 369-74.
11. Oelmann E, Sreter L, Schuller I, *et al.* Nerve growth factor stimulates clonal growth of human lung cancer cell lines and a human glioblastoma cell line expressing high-affinity nerve growth factor binding

sites involving tyrosine kinase signaling. Cancer Res 1995; 55: 2212-9.
12. McGregor LM, McCune BK, Graff JR, *et al.* Roles of trk family neurotrophin receptors in medullary thyroid carcinoma development and progression. Proc Natl Acad Sci U S A 1999; 96: 4540-5.
13. Pflug BR, Onoda M, Lynch JH, Djakiew D. Reduced expression of the low affinity nerve growth factor receptor in benign and malignant human prostate tissue and loss of expression in four human metastatic prostate tumor cell lines. Cancer Res 1992; 52: 5403-6.
14. Descamps S, Lebourhis X, Delehedde M, Boilly B, Hondermarck H. Nerve growth factor is mitogenic for cancerous but not normal human breast epithelial cells. J Biol Chem 1998; 273: 16659-62.
15. Bosch FX, Ribes J, Cleries R, Diaz M. Epidemiology of hepatocellular carcinoma. Clin Liver Dis 2005; 9: 191-211.
16. Tang ZY. Hepatocellular carcinoma: cause, treatment and metastasis. World J Gastroenterol 2001; 7: 445-54.
17. Kishibe K, Yamada Y, Ogawa K. Production of nerve growth factor by mouse hepatocellular carcinoma cells and expression of TrkA in tumor-associated arteries in mice. Gastroenterology 2002; 122: 1978-86.
18. Oakley F, Trim N, Constandinou CM, *et al.* Hepatocytes express nerve growth factor during liver injury: evidence for paracrine regulation of hepatic stellate cell apoptosis. Am J Pathol 2003; 163: 1849-58.
19. Cassiman D, Denef C, Desmet VJ, Roskams T. Human and rat hepatic stellate cells express neurotrophins and neurotrophin receptors. Hepatology 2001; 33: 148-58.
20. Edmondson H, Steiner P. Primary carcinoma of the liver: a study of 100 cases among 48,900 necropsies. Cancer 1954; 7: 462-503.
21. MacSween RN, Scothorns RJ. Developmental anatomy and normal structure. In: MacSween RN, Anthony PP, Scheuer PJ, Burt AD, Portmann BC, eds. Pathology of the liver. London: Churchill Livingstone, 1994; 1-49.
22. Dhillon AP, Sankey EA, Wang JH, *et al.* Immunohistochemical studies on the innervation of human transplanted liver. J Pathol 1992; 167: 211-6.
23. Descamps S, Toillon RA, Adriaenssens E, *et al.* Nerve growth factor stimulates proliferation and survival of human breast cancer cells through two distinct signaling pathways. J Biol Chem 2001; 276: 17864-70.
24. Niederhauser O, Mangold M, Schubenel R, Kusznir EA, Schmidt D, Hertel C. NGF ligand alters NGF signaling via p75(NTR) and trkA. J Neurosci Res 2000; 61: 263-72.
25. Dalal R, Djakiew D. Molecular characterization of neurotrophin expression and the corresponding tropomyosin receptor kinases (trks) in epithelial and stromal cells of the human prostate. Mol Cell Endocrinol 1997; 134: 15-22.
26. Zhu ZW, Friess H, Wang L, *et al.* Nerve growth factor exerts differential effects on the growth of human pancreatic cancer cells. Clin Cancer Res 2001; 7: 105-12.
27. Missale C, Codignola A, Sigala S, *et al.* Nerve growth factor abrogates the tumorigenicity of human small cell lung cancer cell lines. Proc Natl Acad Sci U S A 1998; 95: 5366-71.
28. Rasi G, Serafino A, Bellis L, *et al.* Nerve growth factor involvement in liver cirrhosis and hepatocellular carcinoma. World J Gastroenterol 2007; 13: 4986-95.
29. Yuanlong H, Haifeng J, Xiaoyin Z, *et al.* The inhibitory effect of p75 neurotrophin receptor on growth of human hepatocellular carcinoma cells. Cancer Lett 2008; 268: 110-9.