Heparanase and hepatocellular carcinoma: Promoter or inhibitor?

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Heparan sulphate proteoglycans (HSPGs) consist of a core protein and several heparan sulphate (HS) side chains covalently linked. HS also binds a great deal of growth factors, chemokines, cytokines and enzymes to the extracellular matrix and cell surface. HSPGs can thus influence a growth inhibiting sequences are contained within the tumor cell surface heparan sulfate. Degrading different HSPGs by heparanase may play different roles in HCC. Systemic studies examining the processing, expression, localization and function of heparanase should shed a light on the role of heparanase in HCC.

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Key words: Apoptosis; Heparanase; Heparan sulphate; Hepatocellular carcinoma; Infection; Metastasis

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide. Extracellular matrix (ECM) remodeling plays an important role in the development of HCC[1].

Heparan sulphate proteoglycans (HSPGs), one of the main components of ECM, are abundant macromolecules associated with the cell surface and ECM of a wide range of cells of vertebrate and invertebrate tissues. The basic HSPG structure consists of a core protein and several heparan sulphate (HS) side chains covalently linked. Extracellular HSPGs can maintain the ECM self assembly and integrity with other macromolecules, while cell surface HSPGs may act as co-receptor for several signal pathway molecules. In fact, HS chains also bind a great deal of growth factors, chemokines, cytokines and enzymes to the ECM and cell surface. HSPGs can thus influence a
number of normal and pathological processes, among which are tissue repair, inflammation, tumor growth and metastasis, and angiogenesis[3].

Recent discoveries indicated that HSPGs localized within the tumor microenvironment can be attacked by enzymes that alter proteoglycan structure resulting in dramatic effects on tumor growth and metastasis[4,5]. Heparanase, an endoglycosidase, can specially cleave HS side chains from HSPGs and release a multitude of bioactive molecules. Then, the generated HS fragments and released bioactive mediators could facilitate tumor metastasis cooperatively. In addition, heparanase also exhibits non-enzymatic activities, including cell adhesion and survival, upregulation of vascular endothelial growth factor (VEGF) and tissue factor, induction of signal transduction, and enhancement of certain HSPG shedding from the tumor cell surface[6-10].

A large body of evidence suggest that the expression of heparanase in the tumor closely relates with the potential for tumor invasion, angiogenesis and metastasis in most tumors examined[7-10]. However, there are a lot of conflicting reports about the relationship between heparanase and HCC. It is timely to review the literature to evaluate the arguments for and against the possible roles of heparanase in HCC.

HEPARANASE AND HEPATITIS B VIRUS (HBV) AND HEPATITIS C VIRUS (HCV) INFECTION

Cell surface heparan sulfate mediates entry and initiation of infection of HBV and HCV, the most important pathogenic factors for HCC. Proper structure and sulfation levels of heparan sulfate are prerequisite for this mediation[17-23]. Heparanase might inhibit HS-mediated HCV and HBV entry and the initiation of infection[24]. Degradation of cell surface heparan sulfate by pretreatment with heparanases resulted in a marked reduction of HCV envelope glycoprotein E2 binding to HepG2 cells[18]. Treatment of Namalwa B cells and human erythroleukemia K562 cells with heparanase I also reduced the cellular binding of HBV nucleocapsids[21]. However, HCV E2 bound to target cells via putative receptors in a noncompetitive manner. Incomplete inhibition of heparan sulfate might lead to a partial E2 blockade and evasion of the host immune response[20]. El-Assal et al[24] reported that heparanase expression was significantly higher in HCV-related HCC compared with that in HCV-negative patients. It is possible to assume that HCV enhances heparanase expression that may be involved in the HCV-related pathological and malignant changes.

HEPARANASE EXPRESSION IN LIVER DISEASES

A biphasic pattern of heparanase expression is also significantly observed in rat liver following partial hepatectomy, peaking at 12 h and 96-168 h and decreasing at 360 h post-surgery[25]. Elevated heparanase levels are noted in the early stages of thioacetamide induced rat liver fibrosis, with no further increase evident in rats exhibiting higher fibrotic grades[25]. Reduction or no significant difference in heparanase expression levels are found in liver fibrosis or cirrhosis samples resected from human patients[26,28-31].

There are conflicting reports about the expression level of heparanase in HCC. Examining HCC patients’ specimens by reverse transcriptase-polymerase chain reaction (RT-PCR) or Real-Time Quantitative RT-PCR, in situ hybridization, Western blotting, immunohistochemistry and tissue microarrays (TMAs), five out of the seven studies reported that heparanase was over-expressed in HCC[24,28-31]. However, two studies indicated that the expression level of heparanase was lower than that in adjacent noncancerous tissue[26,27] (Table 1).

HEPARANASE AND HCC

Heparanase and metastasis of HCC

Metastasis is a sequential process including breaking off from the primary tumor, traveling through the bloodstream and stopping at a distant site. Heparanase enhances HCC metastasis by degrading ECM and releasing ECM-resident growth factors and angiogenic factors. Furthermore, non-enzymatic activities of heparanase, such as promoting cell adhesion, might also play a role in HCC metastasis[6-10].

Hepatoma heparanase was first purified from a human hepatoma cell line Sk-hep-1 in 1998[22]. El-Assal et al[24] reported that expression of heparanase mRNA was significantly correlated with larger tumor size, potential for tumor invasion and tumor microvessel density. Many research studies also support the concept that heparanase expression closely relates with metastasis and recurrence of HCC, tumor differentiation and tumor stage[22,23]. More recently, some researchers reported that down-regulating heparanase expression either by antisense oligodeoxynucleotide or by RNA interference could significantly inhibit the invasiveness, metastasis, and angiogenesis of human HCC SMMC7721 cells both in vitro and in vivo[28]. Yang et al[34] reported that two polypeptide antibodies, anti-MAP1 (multiple antigenic peptides)- and anti-MAP2-antibody, can effectively inhibit the heparanase activity of HCCLM6 human hepatocellular carcinoma cells in vitro and influence their invasive ability. Recently, PI-88, an heparanase inhibitor, showed preliminary efficacy as an adjunct therapy for post-operative HCC[38]. Glycosaminoglycan mimetics may also compete with cellular heparan sulfate chains for the binding to CXC-chemokine Stromal cell-Derived Factor-1 (SDF-1) / CXCL12 and may affect heparanase expression, leading to inhibition of SDF-1/CXCL12-mediated migration and invasion of the HuH7 human hepatoma cells[36].

However, Ikeguchi et al[36] reported that heparanase mRNA in HCC was significantly lower than that of noncancerous liver tissue and heparanase expression did not correlate with tumor differentiation, tumor stage, or patient prognosis. In another study conducted by Ikeguchi’s group, the expression level of heparanase was low in HCC...
and a high expression level of heparanase was associated with better disease-free 5-year survival rate.\(^5\) Ogawa et al.\(^5\) established rat HCC cell lines with a high metastatic potential and found that one cell line, showing high levels of lung metastasis when injected subcutaneously in nude mice, exhibited decreased heparanase mRNA expression compared with other cell lines.

In a study of fibroblasts transfected with various oncogenes, one cell line exhibiting a metastatic phenotype was not found to have a significant increase in heparanase activities, though another one having the highest metastatic potential was shown to contain the greatest heparanase activity\(^5\). The hypothesis is that high heterogeneity of HCC might contribute to such discrepancy. Growth promoting as well as growth inhibiting sequences are contained within the tumor cell surface heparan sulfate.\(^5\) Degrading different HSPGs by heparanase may play different roles in the complex process of metastasis.

**Heparanase and apoptosis of HCC**

The HS side chains of HSPGs could bind a multitude of growth factors, chemokines, cytokines and enzymes in ECM and cell surface, such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor and hepatocyte growth factor. The cleaving of HSPGs by heparanase could release HS-bound growth factors and exhibit complicated effects\(^7-10\). bFGF might enhance endothelial cell and tumor cell proliferation, contributing to HCC progression\(^40-41\). El-Assal et al.\(^42\) reported that bFGF and heparanase co-expressed in HCC patients' specimen and this co-expression was associated with higher tumor microvessel density than that in specimens with expression of either factor alone.

Heparanase is involved in the activation of several signal pathways including bFGF-induced signal transduction\(^42-47\) (Figure 1). In an in vitro study of melanoma cells, heparanase seemed necessary for phosphorylation of extracellular signal-related kinase (ERK) or focal adhesion kinase (FAK) in response to bFGF\(^40\). Kato et al.\(^47\) reported that in postmastectomy wound fluids, syndecan-1 was converted from an inhibitor to an activator of bFGF by the degrading activities of heparanase. The release of bFGF and HS degrading fragments by heparanase might promote bFGF-receptor binding and activation\(^42-47\).

Heparanase expression closely related with apoptosis of several tumor cells, including HCC cells.\(^27,48,49\) Ikekuchi et al.\(^51\) found a significant positive correlation between heparanase mRNA expression levels and the percentages of apoptotic hepatocytes in liver tissues. In addition to mitogenic effects, bFGF also could enhance some tumor progression by protecting tumor cells from apoptosis\(^50-56\). Targeting bFGF by neutralizing antibody or antisense oligonucleotides could result in apoptosis of some tumor cells.\(^57-59\) Cell surface HSPGs could not only act as co-receptors for formation of bFGF high-affinity receptor complexes, but could also function directly as receptors for bFGF-induced signal transduction, depending on core protein or HS specific manner\(^60,62\). One possibility is that the alteration of cell surface HSPGs resulting from heparanase might down-regulate HSPG-mediated bFGF-induced signal pathways, resulting in apoptosis of tumor cells.

![Figure 1: Heparan sulphate proteoglycan (HSPG) and fibroblast growth factor (FGF)-induced signal transduction.](image-url)

**Table 1: Studies examining the pro-metastatic role of heparanase in HCC**

| Studies          | No.  | Methods       | Positive rate of HCC tissue | Correlation between heparanase expression and HCC progression |
|------------------|------|---------------|-----------------------------|-------------------------------------------------------------|
| El-Assal et al\(^39\), 2001 | 55\(^4\) | RT-PCR        | 47%                         | Significant positive correlation                             |
| Ikekuchi et al\(^30\), 2002 | 50\(^4\) | QRT-PCR       | < adjacent noncancerous tissue | No significant correlation                                    |
| Ikekuchi et al\(^30\), 2003 | 48\(^4\) | QRT-PCR       | < adjacent noncancerous tissue | Significant negative correlation                             |
| Xiao et al\(^31\), 2003 | 11\(^3\) | QRT-PCR, WB, ISH, IHC | > normal and cirrhosis tissue | Significant positive correlation                             |
| Chen et al\(^32\), 2004 | 33\(^4\) | RT-PCR       | 48.5%, > adjacent cirrhosis tissue | Significant positive correlation                             |
| Liu et al\(^33\), 2005 | 33\(^4\) | RT-PCR       | 48.5%, > adjacent normal tissue | Significant positive correlation                             |
| Chen et al\(^34\), 2008 | 120\(^5\) | IHC in TMAs  | 45.83%, > adjacent tumor tissue, cirrhosis, and normal liver tissue | Significant positive correlation                             |

\(^{35}\) HCC tissue samples; \(^{36}\) Both HCC tissue samples and non-cancerous liver samples were obtained from the same patients; \(^{37}\) 16 normal liver tissue samples, 14 liver cirrhosis tissue samples and 11 HCC tissue samples; \(^{38}\) HCC tissue samples and paracancerous tissue samples were obtained from 33 HCC patients; paracancerous tissues of 9 cases of benign liver tumor were used as normal controls; \(^{39}\) 48 cases of adjacent HCC liver, 62 cases of cirrhosis, and 23 cases of normal liver tissues. HCC: Hepatocellular carcinoma; RT-PCR: Reverse transcriptase-polymerase chain reaction; QRT-PCR: Real-time quantitative RT-PCR; WB: Western blotting; ISH: In situ hybridization; IHC: Immunohistochemistry; TMAs: Tissue microarrays.

**Figure 1:** Heparan sulphate proteoglycan (HSPG) and fibroblast growth factor (FGF)-induced signal transduction. Basic FGF (bFGF) enhances tumor progression by protecting tumor cells from apoptosis. Cell surface HSPGs could act as a co-receptor for formation of a bFGF high-affinity receptor complex. The alteration of cell surface HSPGs resulting from heparanase might down-regulate HSPG-mediated bFGF-induced signal pathway, resulting in apoptosis of tumor cells.
Location of heparanase in HCC

Human heparanase is synthesized as a 65 kDa inactive precursor within late endosomes and lysosomes. Then heparanase undergoes proteolytic cleavage, yielding 8 and 50 kDa protein subunits that heterodimerize to form an active enzyme in lysosome. Active heparanase translocates to the nucleus, cell surface or ECM[6,8]. Different locations of heparanase may exert different activities. Cell surface expression and secretion of heparanase in EB mouse lymphoma cells markedly promotes tumor angiogenesis and metastasis compared with intracellular enzyme[6,8]. However, nuclear heparanase induces differentiation of some tumor cells, such as esophageal cancer cells, mammary cancer cells and leukemic cells. Furthermore, a nuclear location of heparanase represents a better prognosis in tumor patients than its cytoplasmic location[65-68].

During liver regeneration, the location of heparanase exhibits a dramatic alteration from cytoplasm to cell surface in a time-dependent manner[69]. Xiao et al[28] and Chen et al[29] reported that high heparanase expression in HCC was localized within the cytoplasm of tumor cells and there was a significant correlation between the expression level of heparanase mRNA and tumor stage. Does the role of heparanase in HCC depend on its location?

CONCLUSION

There are a lot of conflicting reports about the role of heparanase in HCC. Several questions are intriguing and shouldn’t be ignored. (1) In a human glioma cell xenograft tumor model, moderate heparanase expression levels significantly enhanced tumor development, whereas high heparanase expression levels inhibited tumor growth[70]. Another study also showed that extensive heparanase inhibited bFGF binding in human metastatic melanoma 70W cells, while treatment of 70W cells with low heparanase concentrations enhanced bFGF binding[71]. Does the effect of heparanase depend on its expression level in HCC? (2) During the course of colon adenoma-carcinoma progression, active heparanase increases in the early stage, while latent heparanase predominantly increases in the late stage. The possibility was that enzymatic activities and non-enzymatic activities of heparanase have different roles in the early and late stages of colon cancer development[72]. Do enzymatic activities and non-enzymatic activities of heparanase play different roles during HCC development? (3) HSPGs may have promoting or inhibiting activities depending on the core protein and localization[73]. For example, Glypican-3 and syndecan-1 might act as promoter and inhibitor during the development of HCC, respectively[74,75]. Degrading different HSPGs by heparanase may play different roles in HCC; and (4) Researchers have already observed that heparan sulfates could occur in hepatic nucleus and hypothesized that alteration of heparan sulfates detected in HCC might be involved in HS-related gene expression[6,8,76]. For example, DNA topoisomerase I activity is modulated by heparan sulfates present in normal liver cells but is markedly reduced or absent in their transformed counterparts[80]. Interestingly, active heparanase also could translocate to the nucleus and degrade nuclear HS[65-70,81]. Is heparanase the criminal for the lack of biologically active HS in HCC? Do the effects of heparanase depend on its location in HCC? Systemic studies examining the processing, expression, localization and function of heparanase should shed a light on the role of heparanase in HCC.

REFERENCES

1 Wu XZ, Chen D, Xie GR. Extracellular matrix remodeling in hepatocellular carcinoma: effects of soil on seed? Med Hypotheses 2006; 66: 1115-1120
2 Bishop JR, Schuksz M, Esko JD. Heparan sulphate proteoglycans fine-tune mammalian physiology. Nature 2007; 446: 1030-1037
3 Sasissekharan R, Shriver Z, Venkataraman G, Narayanasami U. Roles of heparan sulphate glycosaminoglycans in cancer. Nat Rev Cancer 2002; 2: 521-528
4 Fjeldstad K, Kolset SO. Decreasing the metastatic potential in cancers—targeting the heparan sulfate proteoglycans. Curr Drug Targets 2005; 6: 665-682
5 Sanderson RD, Yang Y, Kelly T, MacLeod V, Dai Y, Theus A. Enzymatic remodeling of heparan sulfate proteoglycans within the tumor microenvironment: growth regulation and the prospect of new cancer therapies. J Cell Biochem 2005; 96: 897-905
6 Nakajima M, Irimura T, Di Ferrante D, Di Ferrante N, Nicolson GL. Heparan sulfate degradation: relation to tumor invasive and metastatic properties of mouse B16 melanoma sublines. Science 1983; 220: 611-613
7 Parish CR, Freeman C, Hulett MD. Heparanase: a key enzyme involved in cell invasion. Biochim Biophys Acta 2001; 1471: M99-M108
8 Vlodavsky I, Friedmann Y. Molecular properties and involvement of heparanase in cancer metastasis and angiogenesis. J Clin Invest 2001; 108: 341-347
9 Ilan N, Elkin M, Vlodavsky I. Regulation, function and clinical significance of heparanase in cancer metastasis and angiogenesis. Int J Biochem Cell Biol 2006; 38: 2018-2039
10 Vlodavsky I, Elkin M, Abboud-Jarrous G, Levi-Adam F, Fuks L, Shafat I, Ilan N. Heparanase: one molecule with multiple functions in cancer progression. Connect Tissue Res 2008; 49: 207-210
11 Zeharia E, Jia J, Zhang X, Baraz L, Lindahl U, Perez-T, Vlodavsky I, Li JP. Newly generated heparanase knock-out mice unravel co-regulation of heparanase and matrix metalloproteinases. PLoS One 2009; 4: e5181
12 Nasser NJ, Avivi A, Shafat I, Edovitsky E, Zeharia E, Ilan N, Vlodavsky I, Nevo E. Alternatively spliced Spalax heparanase inhibits extracellular matrix degradation, tumor growth, and metastasis. Proc Natl Acad Sci USA 2009; 106: 2253-2258
13 Nadir Y, Brenner B. Heparanase coagulation and cancer progression. Best Pract Res Clin Haematol 2009; 22: 85-92
14 Yang Y, Macleod V, Miao HQ, Theus A, Zhan F, Shaughnessy JD Jr, Sawyer J, Li JP, Zeharia E, Vlodavsky I, Sanderson RD. Heparanase enhances syndecan-1 shedding: a novel mechanism for stimulation of tumor growth and metastasis. J Biol Chem 2007; 282: 13326-13335
15 Mahtouk K, Hose D, Raynaud P, Hundemer M, Jourdan M, Jourdan E, Pantose V, Baudard M, De Vos J, Larroque M, Moehler T, Rossi JF, Réme T, Goldschmidt H, Klein B. Heparanase influences expression and shedding of syndecan-1, and its expression by the bone marrow environment is a bad prognostic factor in multiple myeloma. Blood 2007; 109: 4914-4923
16 Levy-Adam F, Feld S, Suss-Toby E, Vlodavsky I, Ilan N. Heparanase facilitates cell adhesion and spreading by
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clustering of cell surface heparan sulfate proteoglycans. PLoS One 2008; 3: e2219

17 Hilgard P, Stockert R. Heparan sulfate proteoglycans initiate dengue virus infection of hepatocytes. Hepatology 2000; 32: 1041-1047

18 Barth H, Schafer C, Adah MI, Zhang F, Linhardt RJ, Toyoda H, Kinoshita-Toyoda A, Toida T, Van Kuppevelt TH, Depla E, Von Weizsacker F, Blum HE, Baumert TF. Cellular binding of hepatitis C virus envelope glycoprotein E2 requires cell surface heparan sulfate. J Biol Chem 2003; 278: 41003-41012

19 Olena L.V, Kuzmina T, Sobolev BN, Kuraeva TE, Kolesanova EF, Archakov AI. Identification of glycosaminoglycan-binding sites within hepatitis C virus envelope glycoprotein E2*. J Viral Hepat 2005; 12: 584-593.

20 Barth H, Schnober EK, Zhang F, Linhardt RJ, Depla E, Boson B, Cosset FL, Patel AH, Blum HE, Baumert TF. Viral and cellular determinants of the hepatitis C virus envelope-heparan sulfate interaction. J Viral 2006; 80: 10579-10590

21 Vanlandschoot P, Van Houtte F, Serruys B, Leroux-Roels G. The arginine-rich carboxy-terminal domain of the hepatitis B virus core protein mediates attachment of nucleocapsid to cell-surface-expressed heparan sulfate. J Gen Virol 2005; 86: 75-84

22 Cooper A, Tal G, Lider O, Shaul Y. Cytokine induction by the hepatitis B virus capsid in macrophages is facilitated by membrane heparan sulfate and involves TLR2. J Immunol 2005; 175: 3165-3176

23 Heo TH, Chang JH, Lee JW, Fong SK, Dubuisson J, Kang CY. Incomplete humoral immunity against hepatitis C virus is linked with distinct recognition of putative multiple receptors by E2 envelope glycoprotein. J Immunol 2004; 173: 446-455

24 El-Assal ON, Yamanoi A, Ono T, Kohno H, Nagasue N. The clinicopathological significance of heparanase and basic fibroblast growth factor expressions in hepatocellular carcinoma. Clin Cancer Res 2001; 7: 1299-1305

25 Goldshmidt O, Yeikilis R, Mawasi N, Paizi M, Gan N, Ilan O, Lider O, Shaul Y. Cytokine induction by hepatitis C virus and its correlation with spontaneous apoptosis in hepatocytes. J Pathol 2004; 203: 594-602

26 Ikeguchi M, Ueta T, Yamane Y, Hirooka Y, Kaibara N. Quantitative analysis of heparanase messenger RNA expression in hepatocellular carcinoma. J Surg Oncol 2002; 81: 148-154; discussion 154

27 Ikeguchi M, Hirooka Y, Kaibara N. Heparanase gene expression and its correlation with spontaneous apoptosis in hepatocytes of cirrhotic liver and carcinoma. Eur J Cancer 2003; 39: 86-90

28 Xiao Y, Kleeff J, Shi X, Büchner MW, Friess H. Heparanase expression in hepatocellular carcinoma and the cirrhotic liver. Hepatol Res 2003; 26: 192-198

29 Chen G, Dang YY, Luo DZ, Peng ZB, Tang XL. Expression of heparanase in hepatocellular carcinoma has prognostic significance: a tissue microarray study. Oncol Res 2008; 17: 183-189

30 Chen XP, Liu YB, Rui J, Peng SY, Peng CH, Zhou ZY, Shi LH, Shen HW, Xu B. Heparanase mRNA expression and point mutation in hepatocellular carcinoma. World J Gastroenterol 2004; 10: 2795-2799

31 Liu YB, Gao SL, Chen XP, Peng SY, Fang HQ, Wu YL, Peng CH, Tang Z, Xu B, Wang JW, Deng GL, Li HJ, Feng XD, Qian HR. Expression and significance of heparanase and nm23-H1 in hepatocellular carcinoma. World J Gastroenterol 2005; 11: 1378-1381

32 Pikas DS, Li JP, Vlodavsky I, Lindahl U. Substrate specificity of heparanases from human hematoma and platelets. J Biol Chem 1998; 273: 18770-18777

33 Zhang Y, Li L, Wang Y, Zhang J, Wei G, Sun Y, Shen F. Downregulating the expression of heparanase inhibits the invasion, angiogenesis and metastasis of human hepatocellular carcinoma. Biochem Biophys Res Commun 2007; 358: 124-129

34 Yang JM, Wang HJ, Du L, Han XM, Ye ZY, Fang Y, Tao HQ, Zhao ZS, Zhou YL. Screening and identification of novel B cell epitopes in human heparanase and their anti-invasion property for hepatocellular carcinoma. Cancer Immunol Immunother 2009; 58: 1387-1396

35 Liu CJ, Lee PH, Lin DY, Wu CC, Jeng LB, Lin PW, Mok KT, Lee WC, Yeh HZ, Ho MC, Yang SS, Lee CC, Yu MC, Hu RH, Peng CY, Lai KL, Chang SS, Chen PJ. Heparanase inhibitor PI-88 as adjuvant therapy for hepatocellular carcinoma after curative resection: a randomized phase II trial for safety and optimal dosage. J Hepatol 2009; 50: 958-968

36 Friand V, Haddad O, Papy-Garcia D, Hamzaoui H, Vassy R, El-Assal ON, Kuzmina TI, Sobolev BN, Kuraeva TE, Kolesanova EF, Archakov AI. Identification of glycosaminoglycan-binding sites within hepatitis C virus envelope glycoprotein E2*. J Viral Hepat 2005; 12: 584-593.

37 Schwartz IC, Inoue T, Irimura T, Damen JE, Greenberg AH, Wright JA. Relationships between heparanase activity and increasing metastatic potential of fibroblasts transfected with various oncogenes. Cancer Lett 1990; 51: 187-192

38 Liu D, Shriver Z, Venkataraman G, El Shabrawi Y, Yamanoi A, Ono T, Kohno H, Nagasue N. Downregulating the expression of heparanase inhibits the invasion, angiogenesis and metastasis of human hepatocellular carcinoma. J Biol Chem 2009; 19: 1511-1524

39 Ogawa K, Nakanishi H, Takeshita F, Futakuchi M, Asamoto M, Maimidak T, Tatamatsu M, Shirai T. Establishment of rat hepatocellular carcinoma cell lines with differing metastatic potential in nude mice. Int J Cancer 2001; 91: 797-802

40 Elkin M, Ilan N, Ishai-Michaeli R, Friedmann Y, Papo O, Pecker I, Vlodavsky I. Heparanase as mediator of angiogenesis: mode of action. FASEB J 2001; 15: 1661-1663

41 Cross MJ, Claesson-Welsh L. FGF and VEGF function in angiogenesis: signalling pathways, biological responses and therapeutic inhibition. Trends Pharmacol Sci 2001; 22: 201-207

42 Presta M, Dell’Era P, Mitola S, Moroni E, Ronca R, Rusnati M. Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. Cytokine Growth Factor Rev 2005; 16: 159-178

43 Gungis-Velitski S, Zetser A, Flugelman MY, Vlodavsky I, Ilan N. Heparanase induces endothelial cell migration via protein kinase B/Akt activation. J Biol Chem 2004; 279: 23536-23541

44 Reiland J, Kempf D, Roy M, Denkis Y, Marchetti D. FGF2 binding, signaling, and angiogenesis are modulated by heparanase in metastatic melanoma cells. Neoplasia 2006; 8: 596-606

45 Kato M, Wang H, Kaimulainen V, Fitzgerald ML, Ledbetter S, Ornitz DM, Bernfield M. Physiological degradation converts the soluble syndecan-1 ectodomain from an inhibitor to a potent activator of FGF-2. Nat Med 1998; 4: 691-697

46 Ginath S, Menzcer J, Friedmann Y, Ainghorn H, Aviv A, Tajima K, Dantes A, Klegerman M, Vlodavsky I, Amsterdam A. Expression of heparanase, Mdm2, and erbB2 in hepatocellular carcinoma cell lines. Proc Natl Acad Sci USA 2002; 99: 568-573

47 Yonehara H, Yonehara S. Oncogenic K-Ras and basic fibroblast growth factor prevent Fas-mediated apoptosis in fibroblasts through activation of mitogen-activated protein kinase. J Cell Biol 2000; 148: 357-366

48 Gu Q, Wang D, Wang X, Peng R, Liu J, Deng H, Wang Z, Jiang T. Basic fibroblast growth factor inhibits radiation-
induced apoptosis of HUVECs. II. The RAS/MAPK pathway and phosphorylation of BAD at serine 112. *Radiat Res* 2004; 161: 703-711.

52 Huang J, Wu L, Tashio S, Onodera S, Ikejima T. Fibroblast growth factor-2 suppresses oridonin-induced L929 apoptosis through extracellular signal-regulated kinase-dependent and phosphorylation-dependent 3-kinase-independent pathway. *J Pharmacol Sci* 2006; 102: 305-313.

53 Alavi AS, Acevedo L, Min W, Cherson DA. Chemoeristence of endothelial cells induced by basic fibroblast growth factor depends on Raf-1-mediated inhibition of the proapoptotic kinase, ASK1. *Cancer Res* 2007; 67: 2766-2772.

54 Ballif BA, Blenis J. Molecular mechanisms mediating mammalian mitogen-activated protein kinase (MAPK) kinase (MEK)-MAPK cell survival signals. *Cell Growth Differ* 2001; 12: 397-408.

55 Chang F, Steelman LS, Shelton JG, Lee JT, Navolanic PM, Blalock WL, Franklin R, McCubrey JA. Regulation of cell cycle progression and apoptosis by the Ras/Raf/MEK/ERK pathway (Review). *Int J Oncol* 2003; 22: 469-480.

56 Cox AD, Der CJ. The dark side of Ras: regulation of apoptosis. *Oncogene* 2003; 22: 9999-10036.

57 Fukumoto M, Takahashi JA, Murai N, Ueba T, Kono K, Nakatsu S. Induction of apoptosis in glioma cells: an approach to control tumor growth by blocking basic fibroblast growth factor autocrine loop. *Anticancer Res* 2000; 20: 4059-4065.

58 Inoue K, Ferrotte P, Wood CG, Slaton JW, Sweeney P, Dinney CP. Gene therapy of human bladder cancer with adenovirus-mediated antisense basic fibroblast growth factor. *Cancer Res* 2000; 60: 4422-4431.

59 Valesky M, Spang AJ, Fisher GW, Farkas DL, Becker D. Noninvasive dynamic fluorescence imaging of human melanomas reveals that targeted inhibition of bFGF or FGFR-1 in melanoma cells blocks tumor growth by apoptosis. *Mol Med* 2002; 8: 103-112.

60 Bernfield M, Götze M, Park PW, Reizes O, Fitzgerald ML, Linnebur J, Zako M. Functions of cell surface heparan sulfate proteoglycans. *Annu Rev Biochem* 1999; 68: 729-777.

61 Mundhenke C, Meyer K, Drew S, Friedl A. Heparan sulfate proteoglycans as regulators of fibroblast growth factor-2 receptor binding in breast carcinomas. *Am J Pathol* 2002; 160: 185-194.

62 Chua CC, Rahimi N, Forsten-Williams K, Nugent MA. Heparan sulfate proteoglycans function as receptors for fibroblast growth factor-2 activation of extracellular signal-regulated kinases 1 and 2. *Circ Res* 2004; 94: 316-323.

63 Nadav L, Eldor A, Yakoby-Zeevi O, Zamir E, Pecker I, Ilan N, Geiger B, Vlodavsky I, Katz BZ. Activation, processing and trafficking of extracellular heparanase by primary human fibroblasts. *J Cell Sci* 2002; 115: 2179-2187.

64 Goldshmidt O, Zcharia E, Abramovitch R, Metzger S, Aingorn H, Friedmann Y, Schirrmacher V, Mitrani E, Goldshmidt O. Heparanase regulates adhesion and tumor progression of human glioma cells. *Br J Cancer* 2004; 90: 1357-13580.

65 Schubert SY, Ilan N, Shusky M, Ben-Izhak O, Vlodavsky I, Goldshmidt O. Human heparanase nuclear localization and enzymatic activity. *Lab Invest* 2004; 84: 535-544.

66 Ohkawa T, Naomoto Y, Takaoka M, Nobuhisa T, Noma K, Motoki T, Murata T, Uetsuka H, Kobayashi M, Shirakawa Y, Yamatsuji T, Matsuura N, Matsuoka J, Haisa M, Gunduz M, Tsujigiwa H, Nagatsu H, Hosokawa M, Nakajima M, Tanaka N. Localization of heparanase in esophageal cancer cells: respective roles in prognosis and differentiation. *Lab Invest* 2004; 84: 1289-1304.

67 Nobuhisa T, Naomoto Y, Takaoka M, Tabuchi Y, Ooka K, Kitamoto D, Gunduz E, Gunduz M, Nagatsu H, Haisa M, Matsuoka J, Nakajima M, Tanaka N. Emergence of nuclear heparanase induces differentiation of human mammary cancer cells. *Biochem Biophys Res Commun* 2005; 331: 175-180.

68 Kobayashi M, Naomoto Y, Nobuhisa T, Okawa T, Takaoka M, Shirakawa Y, Yamatsuji T, Matsuoka J, Mizushima T, Matsuura H, Nakajima M, Nakagawa H, Rustgi A, Tanaka N. Heparanase regulates esophageal keratinocyte differentiation through nuclear translocation and heparan sulfate cleavage. *Differentiation* 2006; 74: 235-243.

69 Dowek I, Kaplan-Cohen V, Naroditsky I, Sabo E, Ilan N, Vlodavsky I. Heparanase localization and expression by head and neck cancer: correlation with tumor progression and patient survival. *Neoplasia* 2006; 8: 1055-1061.

70 Nobuhisa T, Naomoto Y, Okawa T, Takaoka M, Gunduz M, Motoki T, Nagatsu H, Tsujigiwa H, Shirakawa Y, Yamatsuji T, Haisa M, Matsuoka J, Kobayashi Y, Yamatsuji T, Haisa M, Matsuoka J, Kurebayashi J, Nakajima M, Tanaka N, Saka J, Dong J, Tanaka N. Translocation of heparanase into nucleus results in cell differentiation. *Cancer Sci* 2007; 98: 535-540.

71 Zetser A, Bashenko Y, Miao HQ, Vlodavsky I, Ilan N. Heparanase affects adhesives and tumorigenic potential of human glioma cells. *Cancer Res* 2003; 63: 7733-7741.

72 Doviner V, Maly B, Kaplan V, Gingis-Velitski S, Ilan N, Vlodavsky I, Sherman Y. Spatial and temporal heparanase expression in colon mucosa throughout the adenoma-carcinoma sequence. *Mod Pathol* 2006; 19: 878-888.

73 Timár J, Lapis K, Dudás J, Sebestyén A, Kopper L, Koválszky I. Proteoglycans and tumor progression: Janus-faced molecules with contradictory functions in cancer. *Semin Cancer Biol* 2002; 12: 173-186.

74 Matsumoto A, Ono M, Fujimoto Y, Gallo RL, Bernfield M, Kohgo Y. Reduced expression of syndecan-1 in human hepatocellular carcinoma with high metastatic potential. *Int J Cancer* 1997; 74: 482-491.

75 Capurro MI, Xiang YY, Lobe C, Filmus J, Glypican-3 promotes the growth of hepatocellular carcinoma by stimulating canonical Wnt signaling. *Cancer Res* 2005; 65: 6245-6254.

76 Fedarko NS, Conrad HE. A unique heparan sulphate in the nuclei of hepatocytes: structural changes with the growth stage of the cells. *J Cell Sci* 1986; 82: 587-599.

77 Ishihara M, Fedarko NS, Conrad HE. Transport of heparan sulphate into the nuclei of hepatocytes. *J Biol Chem* 1986; 261: 13575-13580.

78 Rykova VI, Grigorieva EV. Proteoglycan composition in cell nuclei of mouse hepatoma. *Biochemistry (Moscow)* 1998; 63: 1271-1276.

79 Dudás J, Ramadori G, Knittel T, Neubauer K, Raddatz D, Egedy K, Koválszky I. Effect of heparin and liver heparan sulphate on interaction of HepG2-derived transcription factors and their cis-acting elements: altered potential of hepatocellular carcinoma heparan sulphate. *Biochem J* 2000; 350 Pt 1: 245-251.

80 Koválszky I, Dudás J, Olah-Nagy J, Pogány G, Tóváry J, Timár J, Kopper L, Jeney A, Izzoz RV. Inhibition of DNA topoisomerase I activity by heparan sulphate and modulation by basic fibroblast growth factor. *Mol Cell Biochem* 1998; 183: 11-23.

81 Chen L, Sanderson RD. Heparanase regulates levels of syndecan-1 in the nucleus. *PLoS One* 2009; 4: e4947.

S- Editor Tian L  L- Editor O'Neill M  E- Editor Lin YP