Expression of uPA, uPAR, PAI-1 and PAI-2 in Hepatocellular carcinoma

Ito, Hiroshi

(Citation)
神戸大学医学部神緑会学術誌, 14:7-11

(Issue Date)
1998-08

(Resource Type)
departmental bulletin paper

(Version)
Version of Record

(JaLCDOI)
https://doi.org/10.24546/81007544

(URL)
https://hdl.handle.net/20.500.14094/81007544

社団法人神緑会事業報告　(1)
慢性肝疾患に関するウイルス種及び発癌機序の地域民族差に関する日中疫学調査（第２報）

神戸大学医学部第一病理学教室　伊　東　　宏（38年卒）

Expression of uPA, uPAR, PAI-1 and PAI-2 in Hepatocellular carcinoma

Li Zhou[1], Jing-An Rui[1], Ma Zheng Zhong[2], Yu-Tang Fan[3], Mi Fu-Shun[3], Li Baorong[3]
Weiping Wang[4], Takashi Itoh[4], Yoshitake Hayashi[4], Hiroshi Itoh[4]

[1] Liver Cancer Institute, Post and Telecommunications General Hospital, Beijing, China
[2] Division of Pathology, Beijin Hospital, Beijing Medical University, Beijing, China
[3] Chinese Cancer Research Foundation, Beijing, China
[4] First Division of Pathology, Kobe University School of Medicine, Kobe, Japan

Abstract

It has been more and more clear that the plasminogen activation system (mainly including uPA, uPAR, PAI-1 and PAI-2) plays a very important role aggressiveness of cancer in recent decade. Using immunohistochemistry and ELISA, we analyzed the expression of these four components of the system in cancer and no-cancer tissues of 19 cases for hepatocellular carcinoma. The positive rates of uPA, uPAR, PAI-1 and PAI-2 for immunohistochemical staining in cancer tissues are 78.9%, 68.4%, 57.9% and 31.6%, respectively. Positive staining mainly locates in cytoplasm of cancer and stromal cells and appears granular pattern. Moreover, the strong staining is chiefly in invasive front. No specific staining was detected in non-cancer tissues. In ELISA, we found that there are significant differences between cancer and non-cancer tissues in concentration of uPA, uPAR and PAI-1 ($P = 0.0003$, 0.0041 and 0.0042, respectively), but there is not similar significant difference in PAI-2 ($P = 0.9234$). These results suggest that uPA, uPAR and PAI-1 are related to invasion of HCC. But PAI-2 is different, it may be a real inhibitor of plasminogen activation.

Key words：uPA, uPAR, PAI-1, PAI-2, hepatocellular carcinoma

INTRODUCTION

The main models of cancer progress are invasion and metastasis. In the progress, plasminogen activation system plays a important role. It has already been described in some kinds of cancer, such as breast, colon and lung cancer[1)4)]. Urokinase-type plasminogen activator (uPA) is a serine protease produced and released by cancer and stromal cells[5)6)]. It can transform the zymogen plasminogen into the active proteinase plasmin to accumulate the breaking of basement membrane and degradation of extracellular matrix (ECM)[7)8)]. So it contributes to invasion and metastasis of cancer. The specific receptor of uPA (uPAR) is a important protein on cell membrane. It can bind and anchore

uPA on cell surface and enhance plasmin production[9)10)]. And it has already been reported to take part in the process of cancer invasion to ECM[11)]. There are two inhibitors to plasminogen activation, e.g. PAI-1 and PAI-2, mainly. PAI-1 is regarded as a physiological inhibitor of the two forms of plasminogen activation (uPA and tPA)[12)]. The possible mechanism is that PA-1 protect extracellular matrix (ECM) from degradation[13)]. But there are still arguments about function of PAI-1. Someone reported that high expression of PAI-1 in cancer tissue relate to poor prognosis of the patients and suggested that PAI-1 is not a real inhibitor[1)14)]. PAI-2 is seen as an inhibitor of cellular uPA, generally[15)16)]. It is reported that PAI-2 can inhibit uPA activity truly in malignant cell line and is associated with good prognosis in some cancer patients[17)-19)]. All these indicate that PA system has close relation to cancer, especially in invasion and metastasis.

In our study, we evaluated the expression of uPA, uPAR, PAI-1 and PAI-2 in hepatocellular carcinoma. We aimed to research the characteristic of plasminogen activation system in HCC.

MATERIALS AND METHODS

Samples

There are 19 samples of cancer tissue and 14 samples of non-cancer tissue of hepatocellular carcinoma in our study at all, including 17 cases underwent partial hepatectomy and 2 cases for autopsy. Among them 18 cases are Chinese and the rest is Japanese. All samples are stored in liquid nitrogen and then in −80℃ freezer till use.

Immunohistochemistry

The monoclonal antibodies against uPA, uPAR, PAI-1 and PAI-2 are gotten from American Diagnostica Inc. (Greenwich CT, U.S.A.). The product numbers of these antibodies are shown in Table 1. DAKO-LSAB Kit (Dako Co. CA, U.S.A.) is used in immunohistochemical staining according to the instructions of manufacturer. The frozen slides are put into acetone for 10 minutes. After drying and washing with Tris-buffer, incubating with blocking solution and primary monoclonal antibodies for 30 and 60 minutes in room temperature, respectively. The primary antibodies are diluted 50 folds in diluting solution in advance. Then wash with Tris-buffer and incubate with link-antibody and streptoavidin in room temperature, also. Visualization are performed with aminoethylcarbazole chromogen plus hydrogen peroxide. The sections are counter-stained with haematoxyline and mounted. Microscopic examinations are performed by Three observers.

ELISA

Using Enzyme-Linked immunosorbent Assay (ELISA), we checked protein level of uPA, uPAR, PAI-1 and PAI-2 in cancer and non-cancer tissues of hepatocellular carcinoma. ELISA Kit for uPA, PAI-1 and PAI-2 are obtained from Biopool inc. (product numbers are # 111120, # 210221 and # 220220). To check uPAR expression, we use IMUBIND Total uPAR ELISA Kit (product # 983, American Diagnostica Inc. Greenwich CT, U.S.A.). Firstly, protein samples are prepared and content amounts are measured on 280nm. According to the manufacturer's instructions, diluted samples, antibodies and enzyme conjugates are put into the wells of boards sequencely and incubate in room temperature. Within these steps, wash the wells with wash buffer more than 10 times. Then use substrate solution to appear the color. The last step is to read the absorbance at proper wavelength timely.

Statistics

To evaluate the results of ELISA, t-test was used and P-value was calculated. P-value less than 0.05 was considered significant differences.

Table1. Product numbers of antibodies

uPA	uPAR	PAI-1	PAI-2
#3689	#3937	#3785	#3750

Results

Immunohistochemistry

All samples are studied immunohistochemically. Positive rates of uPA, uPAR, PAI-1 and PAI-2 in cancer tissues are 78.9%, 68.4%, 57.9% and 31.6%, respectively. (Table 2.) There are obvious differences between positive rates of uPA, uPAR and PAI-1 and that of PAI-2. No specific staining is observed in non-cancer tissues. Positive staining locates in cytoplasm of cancer and stromal cells predominantly and appears granular patten. And strong staining focuses on the front of invasion and infiltration of neoplasm, especially in uPA, uPAR and PAI-1. The positive staining of PAI-2 is much weak.

Table 2. Positive rates of immunohistochemistry (n=19)

uPA	uPAR	PAI-1	PAI-2
78.9% (15/19)	68.4% (13/19)	57.9% (11/19)	31.6% (6/19)

ELISA

The levels of protein of uPA, uPAR, PAI-1 and PAI-2 in cancer and non-cancer tissues are measured by ELISA. There are significant differences between cancer and non-cancer tissues in uPA, uPAR uPAR and PAI-1 (P = 0.0003, 0.0041 and 0.0042, respectively). But to be contrast, no such difference appears in PAI-2 (P = 0.9234).

Discussion

In the process of cancer development, direct invasion is one of the important manner (the others including distant metastasis and adjacent lymph node metastasis). This process can make the mass larger to cause many severe complications, for example, dysfunction of organs and rupture of tumor. So its mechanism must be understood. Recently, many papers were published about destruction of basement membrane and degradation of extracellular matrix (ECM) mediated by plasminogen activation system in many kinds of cancer[7)-20)]. But there are less data about this data in hepatocellular carcinoma. In our study, we research the expression and role of urokinase plasminogen activator, its specific receptor and inhibitors (PAI-1 and PAI-2) in HCC initially. Our immunohistochemical data appears high positive rates in uPA, uPAR and PAI-1. And the positive staining of them are strong in cancer and stromal cells, especially in invasion fronts. These findings suggest that uPA, uPAR and PAI-1 play important role in invasion of hepatocellular carcinoma. The uPA and uPAR expressions have been demonstrated to closely relate to cancer invasion in many published paper[7)-11)]. Our results confirm the conclusion. But about PAI-1, there are some agruments. Some reports find that PAI-1 is a poor prognostic marker in some kinds of cancer[1) 14) 21)]. About the mechanism of that, there is a hypothesis. Because PAI-1 is expressed by stromal cells in cancer tissue, so it can inhibit proteolytic activity of uPA inside mass to protect tumor itself from self-proteolysis[22)-23)]. That is why PAI-1 contributes to cancer invasion and cause poor prognosis. Our data supports this hypothesis. In our study, the staining of PAI-1 is strong in cancer and stromal cells

and its positive regions are almost same as those in uPA and uPAR. Such distribution of PAI-1 makes its inhibition against internal uPA activity more effectively. The other confirmation is that someone reported that co-expression of uPA, uPAR and PAI-1 is necessary for optimum invasiveness of cultured lung cancer cells[24]. There is obvious difference between PAI-2 and above three components of plasminogen activation system. Its positive rate in cancer tissue is low in our study and even positive, staining is weak (Fig. 1D). This data appears low expression of PAI-2 in HCC tissue, contrasting with high expression of uPA, uPAR and PAI-1. This finding indicates that PAI-2 is a real inhibitor of uPA activity. The possible mechanism is that lack of specific inhibitor in cancer tissue cause the increase of uPA activity and promote invasion of tumor. It has already been verified by many papers which reported that PAI-2 can inhibit uPA activity in cell lines and its high expression concern with favorable prognosis of cancer patient [17)-19), 25)-27)]. The mechanism of inhibition may be decrease of degradation of extracellular matrix (ECM) caused by uPA[27]. But it is not recognized generally.

Using ELISA, we investigate the protein levels of these components of plasminogen activation system. There are significant differences between cancer and non-cancer tissues in uPA, uPAR and PAI-1,. But in PAI-2, there is not such difference existed. These results indicate high expression of uPA, uPAR and PAI-1 and low expression of PAI-2 in hepatocellular carcinoma. They can confirm our data of immunostaining wholely.

In conclusion, uPA, uPAR and PAI-1 appear high expression in HCC tissues and contribute to its invasion. To be reverse, PAI-2 exhibit low expression and be a real inhibitor of plasminogen activation and then invasion of HCC.

REFERENCES

1) Foekens JA, Schmitt MS, can Putten WLJ et al. Prognostic value of urokinase-type plasminogen activator in 671 primary breast cancer patients. Cancer Res. 1992; 52: 6101-6105.

2) De Bruin PAF, Griffioen G, Verspaget HW et al. Plasminogen activator profiles in neoplastic tissues of human colon. Cancer Res. 1988; 48: 4520-4524.

3) Rochefort H, Capony F, Carcia M. A protease involved in breast cancer metastases. Cancer Metastases Rev. 1: 277-297.

4) Oka T, Ishida T, Nishino T et al. Immunohistochemical evidence of urokinase-type plasminogen activator in primary and metastatic tumors of pulmonary adenocarcinoma. Cancer Res. 1991; 51: 3522-3525.

5) Jankun J, Merrick HW, Goldblatt PJ. Expression and localization of elements of the plasminogen activation system in benign breast disease and breast cancers. Cell Biochem. 1993; 53: 135-144.

6) Kirchheimer JC, Pfluger H, Ritschl P et al. Plasminogen activator activity in bone metastases of prostatic carcinomas as compared to primary tumors. Invasion Metastases 5: 344-355.

7) Dano K, Andreasen PA, Grondaihi-Hansen J et al. Plasminogen activators, tissue degradation and cancer. Adv Cancer Res. 1985; 44: 139-226.

8) Nakajima M, Chop AM. Tumor invasion and extracellular matrix degradative enzyme: regulation of activity by organ factors. Semin Cancer Biol. 1991; 2: 115-127.

9) Ellis V, Behrendt N, Dano K. Plasminogen activation by receptor-bound urokinase.. J Biol. Chem. 1991; 266: 5177-5181.

10) Roldan AL, Cubellis M, Masucci MT et al. Cloning and expression of the receptor for human urokinase plasminogen activator, a

central molecule in cell surface, plasmin dependent proteolysis. The EMBO Journal 1990; 9: 467-474.

11) Schlechte W, Brattain M, Boyd D. Invasion of extracellular matrix by cultured colon cancer cells: dependence on urokinase receptor display. Cancer Commun. 1990; 2: 173-179.

12) Hekman CM, Loskutoff DJ. Kinetic analysis of the interactions between plasminogen activator inhibitor 1 and both urokinase and tissue plasminogen activator. Arch. Biochem. Biophys. 1987; 262: 199-210.

13) Olsen D, Pollanen J, Hoyer-Hansen G et al. Internalization of the urokinase plasminogen activator inhibitor type-1 complex is mediated by the urokinase receptor. J Biol. Chem. 1992; 267: 9129-9133.

14) Grondahl-Hansen J, Christensen IJ, Rosenquist C et al. High levels of urokinase-type plasminogen in extracts of breast carcinomas are associated with poor prognosis. Cancer Res. 1993; 53:2513-2521.

15) Hekman CM, Loskutoff DJ. Fibrinolytic pathways and the endothelium. Semin. Thromb. Hemostasis 1987; 13: 514-527.

16) Andreasen PA. George B. Lund LR et al. Plasminogen activator inhibitors: hormonally regulated serpins. Mol. Cell Endocrinol. 1990; 68: 1-19.

17) Laug WE. Cao XR, Yu YB et al. Inhibition of invasion of HT1080 sarcoma cells expressing recombinant plasminogen activator inhibitor 2. Cancer Res. 1993; 53: 6051-6057.

18) Foekens JA, Buessecker F, Peters HA et al. Plasminogen activator inhibitor 2: prognostic relevance in 1012 patients with primary breast cancer. Cancer Res. 1995; 55: 1423-1427.

19) Umeda T, Eguchi Y, Okino K et al. Cellular localization of urokinase-type plasminogen activator, its inhibitors and their mRNAs in breast cancer tissues. J pathol. 1997; 183: 388-397.

20) Mignatti P, Rifkin DB. Biology and biochemistry of proteinase on tumor invasion. Physiol Rev. 1993; 73:161-195.

21) Bashar-H, Urano-T, Fukuta-k et al. Plasminogen activators and plasminogen activator inhibitor 1 in urinary tract cancer. Urol-int. 1994; 52(1): 4-8.

22) Bianchi-E, Cohen-RL, Dai-L et al. Immunohistochemical localization of the plasminogen activator inhibitor-1 in breast cancer. Int-J-Cancer 1995; 60(5): 597-603.

23) Pappot-H, Gardsvoll-H, Romer-J. Plasminogen activator inhibitor type 1 in cancer therapeutic and prognostic implications. Biol-Chem-Hoppe-Seyler 1995; 376(5): 259-267.

24) Liu-G, Shuman-MA, Cohen-RL. Co-expression of urokinase, urokinase receptor and PAI-1 is necessary for optimum invasiveness of cultured lung cancer cells. Int-J-Cancer 1995; 60(4): 501-506.

25) Chambers-SK, Ivins CM, Carcangiu ML. Expression of plasminogen activator inhibitor-2 in epithelial ovarian cancer. Int-J-Cancer 1997; 74(6): 571-575.

26) Duggan C. Kennedy S, Krammer MD et al. Plasminogen activator inhibitor type 2 in breast cancer. Br-J-Cancer 1997; 76(5): 622-627.

27) Baker MS, Bleakley P, Woodrow GC, Doe WF. Inhibition of cancer cell urokinase plasminogen activator by its specific inhibitor PAI-2 and subsequent effects on extracellular matrix degradation. Cancer Res. 1990; 50: 4647-4684.