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# UROKINASE-TYPE PLASMINOGEN ACTIVATOR, ITS RECEPTOR AND INHIBITOR EXPRESSION IN HEPATOCELLULAR CARCINOMA RELATION TO CANCER INVASIVENESS AND PROGNOSIS*

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**Objective: To study the relevance of uPA, uPAR and PAI-1 to hepatocellular carcinoma (HCC). Methods: The expression at protein level of uPA, uPAR and PAI-1 was determined in 48 cases of HCC and 12 cases of benign tumors of liver (as control) by immunohistochemistry. Results: When compared to cancer-adjacent liver tissue and the control, positive rate of immune staining for uPA, uPAR and PAI-1 on cell membrane were significantly higher in HCC cells ($P<0.05$). Positive staining of uPA and uPAR was seen in 16 of 22 and 19 of 22 cases of HCC with invasion, respectively ($P<0.01$ and $P<0.001$). In 8 of 8 cases with cancer embolus, and in 6 of 6 cases with lymph node metastasis was the expression of positive uPAR. Compared with 2 of 17 cases without recurrence, uPAR was positive in 15 of 17 recurrent cases ($P<0.01$). In 36 cases who survived, 17 was positive uPAR and 15 positive PAI-1, while in 12 cases who died 2 years after surgery, 12 were positive for uPAR and 9 positive PAI-1, respectively ($P<0.01$ and $P<0.05$). In 15 positive cases for all three parameters, 11 had cancer invasion and 7 died within 2 years, while in negative cases, 2 had invasion and none died within 2 years ($P<0.05$). Conclusion: Expression of uPA, uPAR and PAI-1 is increased in HCC, uPA and uPAR may contribute significantly to HCC invasion and metastasis. uPAR and PAI-1 are associated with poor prognosis of HCC.**

**Key words: Carcinoma, Hepatocellular, Plasminogen Activators, Neoplasm invasiveness, Neoplasm metastasis, Immunohistochemistry**

## INTROUCTION

Urokinase-type plasminogen activitor (uPA), its receptor (uPAR) and plasminogen activator inhibitor type-1 (PAI-1) have been shown to play roles in some tumor invasion and metastasis. However, the action of uPA, uPAR and PAI-1 in the invasiveness and prognosis of hepatocellular carcinoma (HCC) remained unclear. In this article, the relationship

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The abbreviation used was: uPA urokinase-type plasminogen activator; uPAR uPA receptor; PAI-1 plasminogen activator inhibitor type-1; HCC hepatocellular carcinoma.

between biological behavior of HCC and uPA, uPAR and PAI-1 was presented by our immunohistochemical data.

## MATERIALS AND METHODS

### Handing of Tumor Specimens

All specimens in this study were obtained by surgery in our institute from 1993 to 1994. 48 specimens of HCC, including cancer tissue and cancer-adjacent liver tissue (Table 1), and 12 control specimens of liver tissue from benign tumor were collected. All specimens were fixed in 10% Forman solution and embedded in paraffin blocks, these blocks was cut into 4 μ m tick section, mounted on glass slides.

### Immunohistochemistry

Immunohistochemical straining for uPA. uPAR and PAI-1 was performed by ordinary ABC method. The mouse monoclonal antibody to uPA and PAI-1 (1: 400), and rabbit polyclonal antibody to uPAR (1: 250) were used, all of them were supplied by Molecule Genetics Institute in Shanghai Medical University. All sections were first incubated with 0.01 M citrate in microwave, pH 6.0. Related test reagent consist of 0.05 M Tris, 1: 200 rabbit IgG and horse against mouse IgG (Vector Co.), 1: 100 ABC complex and 0.04% DAB (Fluk Co.) Standard of antibody localization was on the basis of immune staining on cell membrane.

### Statistics:

All statistical analyses were carried out with SAS6.11 software, chi-square test was used in comparison of difference between test groups. P values below 0.05 were considered significant.

## RESULTS

The results have shown that positive staining for uPA, uPAR and PAI-1 was higher in cancer tissue of HCC than in cancer-adjacent tissue and the control, $P<0.05$ respectively. Positive staining of uPA and uPAR were seen in 16 of 22 and 19 of 22 in cases with invasiveness (including satellite, portal cancer embolus and metastasis), while in those without invasiveness, positive uPA and uPAR were 7 of 26 and 10 of 26, difference between two groups was significant, $P<0.001$ and $P<0.01$ respectively. Positive uPAR was found in 8 of 8 cases with portal cancer

*Table 1. Total data of 48 patients with HCC*

| Variable | Study patients n=48 | % |
|---|---|---|
| Tumor size | | |
| ≤ 5 cm | 26 | 54. 2 |
| > 5 cm | 22 | 45. 8 |
| Tumor capsule | | |
| with | 14 | 29. 2 |
| without | 34 | 70. 8 |
| Invasion case | 22 | 45.8 |
| Positive lymph nodes | 6 | 12. 5 |
| Cancer embolus | 8 | 16. 7 |
| Satellite cancer | 14 | 29. 2 |
| AFP | | |
| ≤ 20ng/ml | 10 | 20. 8 |
| > 20 ng/ml | 38 | 79. 2 |
| HbsAg | | |
| Positive | 37 | 77. 1 |
| Negative | 11 | 22. 9 |
| Edmondson grade | | |
| I | 3 | 6.3 |
| II-III | 39 | 81. 3 |
| IV | 6 | 12.5 |
| Tumor recurrence | 17 | 33.3 |
| Death | 12 | 25.0 |

Invasion cases: satellite cancer and/or tumor metastasis (including portal embolus and/or lymph metastasis). AFP: α-fetoprotein. HbsAg: hepatitis B surface antigen. Recurrence and death: in 2 years after surgery.

*Table 2. Comparison of results for uPA, uPAR and PAI-1 between groups*

| Variable | n | uPAR(+) | uPA (+) | PAI-1(+) |
|---|---|---|---|---|
| Cancer tissue | 48 | 29* | 23* | 24* |
| Cancer-adjacent tissue | 48 | 3 | 4 | 2 |
| Control | 12 | 0** | 1** | 1** |

Control: normal liver tissue of benign liver tumor. (+): positive. *: cancer compare to cancer-adjacent, $P<0.05$. **: control compare to cancer, $P<0.05$.

*Table 3. Test results of 48 cases with HCC*

| Variable | N=48 | uPAR (+) | uPA (+) | PAI-1 (+) |
|---|---|---|---|---|
| Tumor size | | | | |
| ≤ 5 cm | 26 | 16 | 13 | 12 |
| > 5 cm | 22 | 13 | 9 | 12 |
| Tumor capsule | | | | |
| with | 14 | 7 | 5 | 5 |
| without | 34 | 22 | 18 | 17 |
| Invasion case | 22 | 19 | 16 | 14 |
| Positive lymph nodes | 6 | 6 | 4 | 5 |
| Cancer embolus | 8 | 8 | 5 | 5 |
| Satellite cancer | 14 | 9 | 9 | 7 |
| AFP | | | | |
| ≤ 20 ng/ml | 10 | 4 | 5 | 3 |
| > 20 ng/ml | 38 | 25 | 18 | 21 |
| HBsAg | | | | |
| Positive | 37 | 25 | 20 | 22 |
| Negative | 11 | 4 | 3 | 2 |
| Edmondson grade | | | | |
| I | 3 | 2 | 1 | 2 |
| II-III | 39 | 22 | 19 | 18 |
| IV | 6 | 5 | 3 | 4 |
| Tumor recurrence | 17 | 15 | 11 | 10 |
| Death | 12 | 12 | 8 | 9 |

Invasion cases: satellite cancer and/or tumor metastasis (including portal embolus and/or lymph metastasis). AFP: α–fetoprotein. HbsAg: hepatitis B surface antigen. Recurrence and death: in 2 years after surgery.

embolus, and in 6 of 6 cases with metastatic lymph node. When cases with tumor recurrence compared to those without recurrence, positive uPAR were 15 of 17 to 14 of 31, $P<0.01$. When death cases in 2 years after surgery compared to survival, positive uPAR were 12 of 12 to 17 of 26, $P<0.01$. While positive PAI-1 was 9 of 12 to 15 of 36, $P<0.05$. Expression of uPA, uPAR and PAI-1 were increased in cases without tumor capsule and in those with poor cell differentiation. Results also showed that in 15 patients whose three parameters, including uPA, uPAR and PAI-1, were all positive, 11 had cancer invasiveness, 8 had tumor recurrence, and 6 of 8 recurred in 1 year after surgery, earliest one in 3 months after tumor removing, 6 of 8 died in postoperative 1 year, while in 8 patients whose all three parameter were negative, 2 had cancer invasiveness, 3 recurrence, 1 recurred in 8 years after tumor removing, none died in 2 years after surgery, difference of invasiveness and mortality were significant between tow groups, $P<0.05$ respectively.

## DISCUSSION

Degradation of the extracellular matrix during cancer invasion results from a combined action of several proteolytic enzyme systems, including collagenases, metalloproteinases and serine proteases such as urokinase-type plasminogen activator (uPA). uPA have been shown to play roles in tumor invasion and metastasis.[1,2] uPA is a highly specific serine protease that is synthesized by tumor cell and various tissue cell, it is secreted as an single-chain inactive proenzyme (pro-uPA), pro-uPA can bind to uPA receptor (uPAR) localizing at tumor cell surface where it is converted into a active two-chain form (tcuPA), active uPA efficiently convert plasminogen to plasmin, a broad-spectrum protease that degrades extracellular matrix components such as fibrin, fibronectin, proteoglycans and laminin.[3]

Our data showed that uPAR had higher expression in HCC, in cases with invasiveness, positive uPAR was 86.3 per cent, in those with portal cancer embolus and lymph nodes metastasis, positive uPAR was 100 per cent respectively, while in those with recurrence and death in 2 years after surgery, positive uPAR were 88.2 and 100 per cent respectively. Results suggested that uPAR may contribute to invasiveness and poor prognosis in HCC. uPAR might be involved in efficient activation of uPA, confinement of uPA activity and progress of proteolysis action mediated by uPA. The interaction between uPA and uPAR is specific, as well as species-specific. uPAR in tumor cell surface could constitute an optimum environment and location for proteolysis during tumor cell invasion and metastasis.[4]

Higher expression of uPA and PAI-1 had also been found in HCC. This suggested a possible role of coordination and regulation between uPA, uPAR and PAI-1. Some studies showed that PAI-1 binds to the receptor-bound tcuPA and neutralizes uPA activity at cell surface, subsequently, the uPA-uPAR-PAI-1 complex is internalized, while the complex is degraded in the lysosomes, the internalized uPAR is

recycled and relocated closed to a different adhesive cell surface, this may cause changes in uPAR location at cell surface and uPA activity, resulting in enchanced cell surface proteolysis activity and tumor invasiveness.[5-7] Results also showed that while three parameters (including uPA, uPAR and PAI-1 were all positive in HCC, there was a strong trend of tumor invasion and metastasis, as well as earlier tumor recurrence and higher death rate. Compared with those that three parameters were all negative, the cases positive had a poor prognosis. This suggested that the interaction between uPA, uPAR and PAI-1 may related to the invasiveness and metastasis of HCC. In addition, death cases after surgery was associated with higher PAI-1 expression, this suggested that PAI-1 has a valuation of prognosis for HCC, the result is similar to some reports on PAI-1.[8] Our recent experiments performed by Northern blot and metastatic model of HCC in nude mouse also suggested that PAI-1 may contribute significantly to HCC invasion and metastasis.

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