Expression of HIF-2α/EPAS1 in hepatocellular carcinoma

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METHODS: Expression of HIF-2α/EPAS1 was investigated immunohistochemically on paraffin-embedded sections from 97 patients with HCC. To further confirm that HIF-2α/EPAS1 in HCC tissues also correlated with angiogenesis, a parallel immunohistochemistry study of vascular endothelial growth factor (VEGF) was performed on these 97 cases.

RESULTS: HIF-2α/EPAS1 could be detected in 50 of 97 cases (51.6%), including 19 weakly positive (19.8%), and 31 strongly positive (31.1%), the other 47 cases were negative (48.4%). The expression of HIF-2α/EPAS1 was significantly correlated with tumor size, capsule infiltration, portal vein invasion, and necrosis. A parallel immunohistochemical analysis of VEGF demonstrated its positive correlation with capsule infiltration, portal vein invasion, and HIF-2α/EPAS1 overexpression, which supported the correlation of HIF-2α/EPAS1 up-regulation with tumor angiogenesis. No apparent correlation was observed between HIF-2α/EPAS1 and capsular formation, presence of cirrhosis, and histological grade.

CONCLUSION: HIF-2α/EPAS1 is expressed in most of HCC with capsular infiltration and portal vein invasion, which indicates a possible role of HIF-2α/EPAS1 in HCC metastasis.
rehydration. Evaluation of staining was semiquantitatively graded based on score determination by intensity distribution as strong ++ (dark brown), weak + (brown), or negative + (no staining). The score was determined independently by at least three of four observers.

**Statistical analysis**

A computer software SPSS 10.0 program was used for statistical analysis. Spearman’s correlation coefficient test was used to assess the relationship between HIF-2α/EPAS1, VEGF expression versus histological grade and tumor size. \( \chi^2 \) test was used to assess the correlation between HIF-2α/EPAS1, VEGF, versus existence of necrosis, cirrhosis, capsular formation, capsular infiltration and portal vein invasion. \( P<0.05 \) was considered to be statistically significant.

**Table 1** Correlation between HIF-2α/ EPAS1 and clinicopathological features of HCC patients

| Pathological grade | HIF-2α/EPAS1 expression (No of cases) | Significance |
|--------------------|------------------------------------|--------------|
|                     | -  +  ++                          | No of cases 97 | |
| Grade I            | 9  4  3                          | 16            | NS |
| Grade II           | 23 8 7                           | 38            | |
| Grade III-IV       | 15 7 22                          | 43            | |
| Tumor size         |                                   |               | |
| ≤2                 | 22 3 8                           | 33            | |
| 2-5                | 16 14 13                         | 43            | 0.001* |
| >5                 | 9 2 10                           | 21            | |
| Cirrhosis          |                                   |               | |
| With               | 27 9 17                          | 53            | NS |
| Without            | 20 10 14                         | 44            | |
| Capsule formation  |                                   |               | |
| With               | 8 1 9                            | 18            | NS |
| Without            | 39 18 22                         | 79            | |
| Capsule infiltration|                                 |               | |
| With               | 3 3 10                           | 16            | 0.011* |
| Without            | 44 16 21                         | 81            | |
| Portal vein invasion|                                |               | |
| With               | 15 15 18                         | 48            | 0.001* |
| Without            | 32 4 13                          | 49            | |
| Necrosis           |                                   |               | |
| With               | 19 12 23                         | 54            | 0.010* |
| Without            | 28 7 8                           | 43            | |

\* \( P<0.05 \) vs the expression of HIF-2α/EPAS1 was significant in HCC tissues with capsule infiltration, portal vein invasion, necrosis, and tumor size. NS, not significant.

**RESULTS**

In the 97 cases studied, positive staining of HIF-2α/EPAS1 protein was localized mainly in the cytoplasm, and occasionally and faintly in the membrane of HCC cells (Figures 1A and 1B). In general, most positive stains were observed in the perinecrotic regions near the tumor foci (Figure 1C). There was expression of HIF-2α/EPAS1 in cancer cells, whereas no positive staining was seen in the stroma cells of HCC (Figure 1D). Immunoreactivity of HIF-2α/EPAS1 was also observed in macrophages, as previously reported[10]. In addition, we also found positive staining in the intraluminal surfaces of hepatic vessels. All the positive immunoreactivity in macrophages was in the cytoplasm. The immunoreactivity was even stronger in macrophages than that in tumor cells (Figure 1E). All the tumor cluster infiltrating to the tissue showed moderate to strong positive staining of HIF-2α/EPAS1 (Figure 1F). Fifty of 97 cases (51.5%) were positive, including 31 strongly positive (31.9%), 19 weakly positive (19.8%). In the 50 HIF-2α/EPAS1-positive cases, 35 cases (64.8%) had necrosis, and 15 cases (16.27%) had no necrosis. Positive staining was seen in 33.3% of the small-sized HCC (<2 cm), 62.7% of the medium-sized (2-5 cm), and 57.1% of the HCCs larger than 5 cm. HCC larger than 5 cm in diameter though not significantly different between the two groups, had a relatively lower HIF-2α/EPAS1 expression compared to medium-sized tumors. HIF-2α/EPAS1 staining was seen in 81.2% of HCC patients with capsule infiltration, and 45.6% in those without them. Positive staining was also seen in 68.7% in HCC patients with portal vein invasion, and 34.6% in those without them (Table 1). HIF-2α/ EPAS1 positivity was significantly correlated with tumor size, capsule infiltration, portal vein invasion, and existence of necrosis (\( P<0.05 \) respectively). To further confirm whether HIF-2α/EPAS1 expression in HCC tissues also correlated with angiogenesis, a parallel immunohistochemical study of VEGF was performed on these 97 cases, in which VEGF expression was assessed as a major marker for angiogenesis (Table 2). Positive staining was found mostly in the cytoplasm of tumor cells (Figures 2A and 2B), and only weaker staining in stroma areas was detectable. VEGF expression in tumors had strong correlation with capsule infiltration, portal vein invasion, and necrosis (\( P<0.05 \) respectively). The overexpression of VEGF in capsule infiltration and portal vein invasion was found to correlate positively with HIF-2α/EPAS1 expression (\( P<0.05 \), Table 3), supporting the correlation of HIF-2α/EPAS1 up-regulation with tumor metastasis and angiogenesis in HCC.

**Table 2** Correlation between VEGF protein and clinicopathological features of HCC patients

| Variants           | No of cases 97 | VEGF expression | Significance |
|--------------------|----------------|-----------------|-------------|
| Pathological grade |                |                 |             |
| Grade I            | 6 6 0 0        | 0               | NS          |
| Grade II           | 76 28 22 26    | 3               | 4           |
| Grade III-IV       | 15 8 3 4       | 0               |             |
| Tumor size         |                |                 |             |
| ≤2                 | 13 11 0 2      | 0               | NS          |
| 2-5                | 49 16 14 19    | 0               |             |
| >5                 | 35 15 11 9     | 0               |             |
| Cirrhosis          |                |                 |             |
| With               | 53 20 10 23    | 0               | NS          |
| Without            | 44 22 15 7     | 0               |             |
| Capsule formation  |                |                 |             |
| With               | 18 7 5 6       | 0               | NS          |
| Without            | 79 35 20 24    | 0               |             |
| Capsule infiltration|               |                 |             |
| With               | 16 4 2 10      | 0.011*          |             |
| Without            | 81 38 23 20    | 0.011*          |             |
| Portal vein infiltration|         |                 |             |
| With               | 48 14 16 18    | 0.020*          |             |
| Without            | 49 20 9 12     | 0.011*          |             |

\* \( P<0.05 \) vs the expression of VEGF was significant in HCC with capsule infiltration, portal vein invasion, and with necrosis. NS, not significant.

**Table 3** Correlation between HIF-2α/ EPAS1 and VEGF protein expression in HCC tissues

| Variants           | No of cases | Staining score | Significance |
|--------------------|-------------|----------------|--------------|
| HIF-2α/EPAS1       | 97          | 47 19 31       | 0.017        |
| VEGF               | 97          | 42 25 30       |              |
DISCUSSION

Angiogenesis appeared to be one of the crucial steps in tumor’s transition from small, harmless cluster of mutated cells to a large, malignant growth, capable of spreading to other organs throughout the body\textsuperscript{[12]}. HCC is a typical hypervascular tumor of the digestive organs. It seems likely that the formation of tumor vessels precedes tumor growth and is indispensable in maintaining tumor viability, because hepatic arterial embolization frequently causes necrosis and induces a marked reduction in tumor size. In the present study, we investigated the expression of HIF-2α/EPAS1 in HCC tissues. To further confirm whether HIF-2α/EPAS1 in HCC tissues also correlated

Figure 1. Characterization of HIF-2α/EPAS1 expression in human hepatocellular carcinoma tissues by IHC technique. A: Weak expression of HIF-2α/EPAS1 in membranes and cytoplasms of HCC cells (×400). B: Strong cytoplasmic immunoreactivity of HIF-2α/EPAS1 in HCC cells (×400). C: HIF-2α/EPAS1 positive staining (arrows) in perinecrotic region near tumor in a HCC sample (with capsular infiltration and portal vein invasion) (×400). D: Strong HIF-2α/EPAS1 expression in HCC tissues whereas no staining in adjacent stroma cells (×400). E: Strong staining in the cytoplasm of macrophages compared with cancer cells, showing weak staining for HIF-2α/EPAS1 (×400). F: Moderate to strong positive staining of HIF-2α/EPAS1 in tumor clusters infiltrating to the tissue (×400).

Figure 2. Parallel study of VEGF protein expression in HCC samples. A mAb against VEGF was used for immunostaining of slides from HCC patients. A: HCC with capsular infiltration and portal vein invasion showing strong staining (++) in the cytoplasm of tumor cells. B: HCC without capsular infiltration and portal vein invasion showing weak staining (+) (×200).
with angiogenesis, we performed an immunohistochemistry study of VEGF protein. We also examined the correlation between HIF-2α/EPAS1, VEGF protein expression and clinicopathological features.

Our data showed that tumor size was correlated with HIF-2α/EPAS1. Small HCC had significantly lower HIF-2α/EPAS1 expression compared with medium-sized tumors. What was contrary to previous finding[13,14] was that tumors with large sizes had higher expression than smaller and moderate sizes, even these tumors were relatively less vascular compared with the large-sized ones. However, it has been reported that the intercapillary distance increased as the tumor size or weight increased, possibly because of different rates of endothelial cells and neocellular turnover[15,16]. The turnover time of endothelial cells was 50 to 60 hours while that of the neoplastic cells was 22 hours, and significantly shorter[17]. On the other hand, the characteristics of tumor microcirculation could offer another explanation for the reduction of HIF-2α/EPAS1 expression as the tumor became smaller or larger. Generally, blood flow, oxygen pressure, and pH values were less in tumors than in the counterpart normal tissues[18], and because of the absence of lymphatic vessels, the interstitial pressure was often high in tumors, leading further transport problems[19]. As a result, hypoxia and necrosis were a general phenomenon of tumors, especially large ones[20]. It was assumed that rapid cell proliferation at the center of tumor could lead to increased interstitial pressure, which may lead to compression closure of capillaries and consequent tumor necrosis[21]. The current results showed that HCCs of 2 to 5 cm in diameter had the highest HIF-2α/EPAS1 expression compared with smaller and larger tumors. This observation could be considered important for regional chemotherapy, because intuitively, tumor, tumor metastasis, and tumor death should be closely correlated with tumor-induced hypoxia and necrosis. Cells in hypoxic regions have been thought to be more resistant to the effects of radiotherapy and many conventional chemotherapeutic agents than their normoxic counterparts[22,23].

We also found strong immunoreactivity in macrophages. The significance of HIF-2α/EPAS1 in these cells warrants further study. As they have been shown to be one of the terminally differentiated cells that can produce a number of potent angiogenic cytokines such as VEGF[24,25], their chemotaxis, infiltration, degranulation may promote tumor angiogenesis and progression. A parallel IHC study of angiogenesis marker demonstrated that in HCC tissues, overexpression of both VEGF and HIF-2α/EPAS1 was coincidentally found, supporting the notion that HIF-2α/EPAS1 expression is correlated with angiogenesis in HCC.

In the current work, significantly more HIF-2α/EPAS1 protein expression was present in perinecrotic regions. This, when taken with the fact that macrophages appeared to be more pro-angiogenic at these sites[26] may help to explain our observation. As HIF-2α/EPAS1 has been shown to be accumulated by hypoxic macrophages in human tumors[27,28], our finding may indicate that HIF-2α/EPAS1 protein may be released by macrophages and is part of the mechanism by which this protein is most expressed in perinecrotic regions. On the other hand, direct support for microenvironmental mechanisms of HIF-alpha activation in diverse types of human tumor could offer an alternative explanation.

Results from our analysis of HIF-2α/EPAS1 expression in perinecrotic areas were consistent with a number of reports from clinical studies on breast[28-30], ovarian[31], and lung[32,33] cancers and in hemangioiomas[10]. These reports all demonstrated that macrophage hotspots were remotely located from the vascular hotspots of tumors, suggesting that macrophages may preferentially migrate toward areas of relative hypoxia[20]. This in turn might attract macrophages into tumor, which then contribute to angiogenic process, giving rise to association between high levels of angiogenesis and extensive necrosis[29]. Macrophages might be attracted to necrotic tumors by chemotactic factors, such as VEGF[26,34,35].

As a potent pro-angiogenic cytokine, VEGF has been reported to be overexpressed in both malignant tumors[30,36] and stroma cells[30,34] and macrophages[26,34,37]. Expression of VEGF was up-regulated in poorly vascularized areas of breast carcinomas[28,29,38]. VEGF positive macrophages were restricted to areas of VEGF production[26,28]. Evidence is accumulating that VEGF might be activated in stroma cells, especially in macrophages, with the process mediated by the VEGF receptor fit-1[15]. Thus, the subcellular mechanisms mediating hypoxia on VEGF gene by macrophages are not known at present. This most likely involved one or more of the pathways activated by hypoxia in transformed cells[37,40], including the activation of such transcription factors as hypoxia-inducible factors (HIFs) 1, 2 (otherwise known as EPAS1). In this study, we observed the overexpression of VEGF protein in tumor and VEGF expression positively correlated with HIF-2α/EPAS1 expression. We found that the highest VEGF expression was detectable mostly in tumor areas and only weaker staining in necrotic and stroma areas was detectable.

It is widely accepted that angiogenesis necessitates the degradation of extracellular matrix, this process requires protease activation and release. Plasmin was thought to be one of the key proteases involved in this process[39]. Angiogenesis also appear to be involved in the invasion of tumors into surrounding tissues, because this invasion requires concomitant neovascularization through the sprouting of endothelial cells in the tumor stroma. It has been reported that VEGF induced both urokinase-type plasminogen activator (PA) and tissue type PA in endothelial cells[40], and hypoxia might promote cellular invasion by stimulating the expression of urokinase type plasminogen activator (uPAR)[41]. Therefore, enhanced expression of these angiogenic factors would likely indicate the ability of tumors to invade the tumor stroma as well as the ability to promote the development of new blood vessels. Based on these considerations, we examined both portal involvement and capsule infiltration. These two clinicopathological features have been thought to be the most important clinical factors in assessing liver tumor, and HCC in particular, as they were strongly correlated with the metastasis of HCC[42,44]. Our results were in agreement with this concept. We found that the portal vein involvement and capsule infiltration were correlated with the expression of both HIF-2α/EPAS1 and VEGF proteins. HCC patients with capsular infiltration and portal vein invasion had more HIF-2α/EPAS1 and VEGF expression than those without them, indicating that HIF-2α/EPAS1 and VEGF expression may be associated with a poor prognosis of patients with HCCs.

The clinical significance of HIF-2α/EPAS1 expression in tumors remains largely unexplored as monoclonal antibodies available for immunohistochemistry have been recently developed. Talks et al recently reported the expression of HIF-alpha in a panel of normal human tissues and benign or malignant tumors and first showed the expression of the molecule in a good percentage of human carcinomas[11]. However, studies on the HIF-2α/EPAS1 expression with angiogenic factors and receptors, with microvessel density or with other molecular markers or with prognosis of human carcinomas are few. Investigations regarding these angiogenic factors which have been partially done for endothelial carcinoma[13,33,45], and regarding the status of signal transduction via HIF-2α/EPAS1 when the receptors do and do not bind to this protein and when dimerization with aryl hydrocarbon receptor nuclear translocator occurs between HIF-2α/EPAS1 and other HIF-α protein family, should help clarify the significance.
of HIF-2α/EPAS1 in human cancers, including HCC.

In this study, we found cytoplasmic immunoreactivity of HIF-2α/EPAS1, but equivocal staining was sometimes observed in the nuclei which we did not regard as positive. Although it was assumed that nuclear HIF was the active form, clearly it was synthesized and also degraded in the cytoplasm[45,46]. These findings, at least in part, could explain the cytoplasmic location of HIF-2α/EPAS1, which was a tumor specific finding and could better reflect the HIF up-regulation pathways in paraffin embedded material. This suggestion was in accordance with the scoring system proposed by Zhong et al[46].

In conclusion, HIF-2α/EPAS1 expression in HCC and its clinical association with necrosis seem to be a good predictive tool and possibly a target therapy for metastasis of liver cancer, especially HCC. The finding that medium-sized HCCs had the highest expression of HIF-2α/EPAS1 compared with smaller and larger HCCs can be used, after further evaluation, as a therapeutic guide during the selection of cases for chemotherapy.

REFERENCES

1. Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH, Gassen M, Gearhart JD, Lawler AM, Yu AY, Semenza GL. Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. Genes Dev 1998; 12: 149-162
2. Peng J, Zhang L, Drysdale L, Fong GH. The transcription factor EPAS1/ hypoxia-inducible factor 2 alpha plays an important role in vascular remodeling. Proc Natl Acad Sci U S A 2000; 97: 8386-8391
3. Tian H, Mcknight SL, Russell DW. Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. Genes Dev 1997; 11: 72-82
4. Semenza GL. HIF-1 and human disease: one highly involved gene. Genes Dev 2000; 14: 1983-1991
5. Morrow CS, Cowan KH. Antineoplastic drug resistance and breast cancer. Ann NY Acad Sci 1993; 698: 289-312
6. Wiesener MS, Turler H, Allen WE, William C, Eckardt KU, Talks KL, Wood SM, Gatter KC, Harris AL, Pugh CW, Ratcliffe PJ, Maxwell PH. Induction of endothelial PAS domain protein-1 by hypoxia: characterization and comparison with hypoxia-inducible factor-1 alpha. Blood 1998; 92: 2260-2268
7. Jain S, Maltepe E, Lu MM, Simon C, Bradfield CA. Expression of ARNT, ARNT2, HIF1α, HIF2α and Ah receptor mRNAs in the developing mouse. M eCh Ev 1998; 73: 117-123
8. Ema M, Taya S, Yokotani N, Sogawa K, Matsuda Y, Fujii-Kuriyama Y. A novel BHLH-PAS factor with dose-response similarity to hypoxia-inducible factor 1α regulates the VEGF expression and is potentially involved in lung and vascular development. Proc Nati Acad Sci U S A 1997; 94: 4273-4278
9. Flammei, Frob orth T, von Reuten M, Kappel A, Damert A, Risau W. HRF, a putative basic helix-loop-helix-PAS-domain transcription factor is closely related to hypoxia-inducible factor-1 alpha and developmentally expressed in blood vessels. M eCh Ev 1997; 63: 51-60
10. Flammei, J, Krieg M, plate KH. Up-regulation of vascular endothelial growth factor in stromal cells of hemangioblastomas is correlated with up-regulation of the transcription factor HRF/ HIF-2 α. A m J Pathol 1998; 15: 25-29
11. Talks KL, Turley H, Gatter KC, Maxwell PH, Pugh CW, Ratcliffe PJ, Harris AL. The expression and distribution of the hypoxia-inducible factors HIF-1 alpha and HIF-2 alpha in normal human tissues, cancers, and tumor-associated macrophages. A m J Pathol 2000; 157: 411-421
12. Folkman J. Fighting cancer by attacking its blood supply. S c i A m 1996; 275: 150-154
13. Favier J, Plouin P, Corvol P, Gasc JM. Angiogenesis and vascular architecture in phaeochromocytomas: distinctive traits in malignant tumors. A m J Pathol 2002; 161: 1235-1246
14. Cayer A, Rossignal F, Clottes E, Penault LF. aHIF but not HIF-1α/p4 β transcription a poor prognostic marker in human breast cancer. In S E R M U484, Clermont-Ferrand, French. Breast Cancer
15. Vapoul P. Hypoxia in neoplastic tissue. M icrovasc Res 1977; 13: 399-408
16. Tannock IF, Steel GG. Quantitative techniques for the study of the anatomy and function of small blood vessels in tumors. J Natl Cancer Inst 1969; 42: 771-782
17. Tannock IF, Hayashi S. The proliferation of capillary endothelial cells. Cancer Res 1972; 32: 77-82
18. Vapoul P, Kallioni F, Okuni FF, Blood oxygen, nutrient supply, and metabolic microenvironment of human tumors: a review. Cancer Res 1989; 49: 6449-6465
19. Jain RK. Transport of molecules, particles, and cells in solid tumors. Annu R e V i o m e d Eng 1999; 1: 241-263
20. Lyng H, Skre ttin A, Rofstad EK. Blood flow in six human melanoma xenograft lines with different growth characteristics. Cancer Res 1992; 52: 584-592
21. Jain RK. Determinants of tumor blood flow: a review. Cancer Res 1988; 48: 2641-2658
22. Blancher C, Harris AL. The molecular basis of hypoxia response pathways: tumour hypoxia as a therapy target. Cancer Metastasis Rev 1998; 17: 187-194
23. Richard DE, Berra E, PoussySegur J. Angiogenesis: how a tumor adapts to hypoxia. Biochem Biophys Res Commun 1999; 266: 718-722
24. Harney HJ, Dimitriadis E, Kay E, Redmond HP, Boucher-Hayes D. Regulation of macrophage production of vascular endothelial growth factor (VEGF) by hypoxia and transforming growth factor-beta. An n S urg Oncol 1998; 5: 271-278
25. Gaudry M, Bregerie O, Andreieu V, El Benja J, Piccialdo MA, Hakim J. Intracellular pool of vascular endothelial growth factor in human neutrophils. Blood 1997; 90: 4153-4161
26. Lewis JS, Lee JA, Underwood JC, Harris AL, Lewis CE. Macrophage responses to hypoxia: relevance to disease mechanisms. J Leukoc Biol 1999; 66: 889-900
27. Griffiths F, Binley K, Iqbal S, Kan O, Maxwell P, Ratcliffe P, Lewis C, Harris AL, Kingsman S, Naylor S. The macrophage - a novel system to deliver gene therapy to pathological hypoxia. Gen E r 2000; 7: 255-262
28. Leek RD, Hunt NC, Landers RJ, Lewis CE, RoydsJA, Harris AL. Macrophage infiltration is associated with VEGF and EGFR expression in breast cancer. J Pathol 2000; 190: 430-436
29. Leek RD, Landers RJ, Harris AL, Lewis CE. Necrosis correlates with high vascular density and focal macrophage infiltration in invasive carcinoma of the breast. B r J Cancer 1999; 79: 991-995
30. Hlatky L, Tisonou C, Hahnfeldt P, Coleman CN. Mammary fibroblasts may influence breast tumor angiogenesis via hypoxia-induced vascular endothelial growth factor up-regulation and protein expression. Cancer Res 1994; 54: 6083-6086
31. Negus RP, Stamp GW, Hadley J, Balkriff FR. Quantitative assessment of the leukocyte infiltrate in ovarian cancer and its relationship to the expression of C-C chemokines. A m J Pathol 1997; 150: 1723-1734
32. Shoji M, Hancock WW, Abe K, Micko C, Casper KA, Baine RM, Wilcox JN, Danave I, Dillechay DL, Matthews E, Contrino J, Morrissey JH, Gordon S, Edgington TS, Kreutzer DL, Kudryk B, Kreutzer D, Richles FR. A diviation of coagulation and angiogenesis in cancer: immunohistochemical localization in situ of clotting proteins and vascular endothelial growth factor in human cancer. A m J Pathol 1998; 152: 399-411
33. Girommanolaki A, Koukurakis MI, Sirividi E, Turley H, Talks KL, Pezzella F, Gatter KC, Harris AL. Relation of hypoxia inducible factor 1α and 2 α in operable non-small cell lung cancer to angiogenesis/ molecular profile of tumors and survival. B r J Cancer 2001; 85: 881-890
34. Polverini PJ, Lebiovich SJ. Induction of neoangiogenesis in vivo and endothelial proliferation in vitro by tumor-associated macrophages. L ab I n v e s t 1984; 51: 635-642
35. Barleon B, Sozzani S, Zhou D, Weich HA, Mantovani A, Mare M. Migration of human monocres in response to vascular endothelial growth factor in human cancer. B r J Cancer 1998; 78: 171-177
36. Huang GW, Yang LY, Sheng LX, Li HL, Qing JQ, Yang ZL. The relationship between VEGF and HIF-1α protein in hepatocellular carcinoma. Z h o n g h u a X i a o h u a Z a z h i 2000; 10: 627-628
37 Xiong M, Elson G, Legarda D, Leibovich SJ. Production of vascular endothelial growth factor by murine macrophages: regulation by hypoxia, lactate, and the inducible nitric oxide synthase pathway. Am J Pathol 1998; 153: 587-598

38 Lewis JS, Landers RJ, Underwood JC, Harris AL, Lewis CE. Expression of vascular endothelial growth factor by macrophages is up-regulated in poorly vascularized areas of breast carcinomas. Pathol 2000; 192: 150-158

39 Pepper MS, Montesano R. Proteolytic balance and capillary morphogenesis. Cell Differ Dev 1990; 32: 319-327

40 Pepper MS, Vassalli JD, Orci L, Montesano R. Proteolytic balance and capillary morphogenesis in vitro. EXS 1992; 61: 137-145

41 Graham CH, Fitzpatrick TE, McCrae KR. Hypoxia stimulates urokinase receptor expression through a heme protein-dependent pathway. Blood 1998; 91: 3300-3307

42 Los M, Zaemari S, Foekens JA, Gabbink MF, Voest EE. Regulation of the urokinase-type plasminogen activator system by the von Hippel-Lindau tumor suppressor gene. Cancer Res 1999; 59: 4440-4445

43 Arii S, Tanaka J, Yamazoe Y, Minematsu S, Morino T, Fujita K, Maetani S, Tobe T. Predictive factors for intrahepatic recurrence of hepatocellular carcinoma after partial hepatectomy. Cancer 1992; 69: 913-919

44 Primary liver cancer in Japan. Clinicopathologic features and results of surgical treatment. Liver Cancer Study Group of Japan. Ann Surg 1990; 211: 277-287

45 Hui EP, Chan AT, Pezzella F, Turler H, To KF, Poon TC, Zee B, Mo F, Teo PM, Huang DP, Gatter KC, Johnson PJ, Harris AL. Coexpression of hypoxia-inducible factor 1 alpha and 2 alpha, carbonic anhydrase IX, and vascular endothelial growth factor in nasopharyngeal carcinoma and relationship to survival. Clin Cancer Res 2002; 8: 2595-2604

46 Zhong H, De Marzo AM, Laughner E, Lim M, Hilton DA, Zagzag D, Buechler P, Isaacs WB, Semenza GL, Simons JW. Overexpression of hypoxia-inducible factor 1 alpha in common human cancers and their metastases. Cancer Res 1999; 59: 5830-5835

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