Review

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INDUCED DIFFERENTIATION OF HEPATOCELLULAR CARCINOMA BY NATURAL PRODUCTS

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Abstract

Hepatocellular carcinoma is one of the most common malignant tumors worldwide. For the difficulty of the giving sufficient dose because of the poor liver function and the low sensitivity of hepatoma cells for the chemotherapeutic agents, chemotherapy adds little to overall survival of hepatocellular carcinoma patients. The induction of terminal differentiation in tumor cells represents a possible therapeutic strategy with less toxicity. Gekko sulfated polysaccharides, isoverbascoside, Ginsenoside-Rh2, Camptothecin, 9-nitro-camptothecin, tachyplesin, Matrine, tylophorine, 7-OH-4-CH (3)- coumarin and arsenic trioxide are known to have a differentiation-inducing capability on hepatocellular carcinoma in vitro and/or in vivo. Although the therapeutic effect of the differentiation-inducing agents may not be potent when compared with that of conventional chemotherapeutic agents, they have multiple therapeutic targets, low toxicity and less probability of drug resistance. More data are required on the molecular mechanisms of therapeutic effects, dose response and potential toxicities.

Keywords: Differentiation; hepatocellular carcinoma; herb

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide. Despite the high mortality and frequency of this cancer, surgical resection is an option for only a small proportion of patients because of the high ratio of metastases, recurrence and second HCC. Chemotherapy adds little to overall survival of HCC patients because of the low dose of drug and the low sensitivity of HCC cells: Malignant transformation of hepatocytes may occur in the context of chronic liver injury, regeneration and cirrhosis. Thus it is difficulty to give sufficient dose for HCC patient because of the poor liver function. Multidrug resistance protein P-glycoprotein is physiologically expressed at the bile canalicular membrane, where it participates in the biliary excretion of various lipophilic drugs and xenobiotics (Roelofsen et al., 1997). P-glycoprotein is up-regulated in liver regeneration and hepatocarcinogeneses (Daoudaki et al., 2003; Minemura et al., 1999).

Since prognosis and survival of patients with HCC is still very poor, novel strategies and agents, which are more selective for HCC but lower toxicity for normal liver cells, are expected of the development. The development of malignancies can be considered as the result of change of the normal process of cell differentiation. Thus, induction of terminal differentiation of HCC represents a possible therapeutic strategy with less toxicity. However, development of differentiation-inducing agents to treat malignant tumors, especially for solid tumors, has been limited to date (Kawamata et al., 2006). Analysis of induction of differentiation by natural products might be helpful for comprehending the cellular and molecular mechanisms of herbal medicine.

Polysaccharides and glycoside

A wide variety of anti-tumor activities of sulfated polysaccharides, such as anti-metastasis, proliferation inhibition and immune regulation, have been observed (Wu, 2006a, b). Gekko swinhonis Gūenther (Chinese name: Bihu) has been used as the anti-cancer drug in traditional Chinese medicine more than hundreds of years. Our group has reported that WRCP, which is composed of aqueous extracts of Gekko swinhonis Guenther and other Chinese herbs, could suppress the proliferation and induce the differentiation of HCC cell line Bel-7402 (Yan et al., 2007). Furthermore, we found that Gekko sulfated polysaccharides suppress the proliferation and induce the differentiation of HCC cells, but did not suppress the proliferation of normal liver cell line L-02. The cytotoxicity of Gekko sulfated polysaccharides is negligible for L-02 cells. Moreover, Gekko sulfated polysaccharides did not induce apoptosis and had little cytotoxicity for HCC cells (Wu et al., 2006c).

All-trans retinoic acid (ATRA) induces the differentiation of HCC and has been used for preventing HCC. Dramatically, there is a significant difference of differentiation induction between Gekko sulfated polysaccharides and ATRA. ATRA blocks tumor cells in S phase, whereas Gekko sulfated polysaccharides block Bel-7402 cells in G_2/M phase. Bel-7402 cells changed to polygon shape after treatment with ATRA, but they presented spindle shape after exposure to Gekko sulfated polysaccharides. ATRA induces the apoptosis of HCC cells, whereas Gekko sulfated polysaccharides do not induce the apoptosis of HCC cells (Wu et al., 2006c).

Malignant transformation of hepatocytes may occur in the context of chronic liver injury, regeneration and cirrhosis. Proliferation of hepatocyte and production of extracellular matrix (ECM) are response for the injury. When the injury is limited in time, the result of the repair is restoration of normal liver structure. When the injury is persistent, however, there is net accumulation of ECM, resulting in cirrhosis. Transforming growth factor-beta (TGF-beta) is the most important cytokine suppressing the inflammation and stimulating ECM production in the liver. TGFbeta 1 was overexpressed in HCC, especially in patients with small HCC and well-differentiated HCC (Matsumoto et al., 2003; Idobe et al., 2003; Ito et al., 1990; Kira et al., 1997; Song et al., 2002; Tsai et al., 1997). Moreover, TGF-beta1 induces growth inhibition and differentiation of HuH7 cells (Damdinsuren et al., 2006). TGF-beta1 induced epithelial-to-mesenchymal transformation in SMMC-7721 cells. SMMC-7721 cells clearly switched to the spindle shape morphology after TGF-beta1 treatment (Xu et al., 2003). Our research showed that Bel-7402 and SMMC-7721 cells changed to spindle shape and the expression of TGF-beta1 was up-regulated after exposure to Gekko sulfated polysaccharides (Wu et al., 2006; unpublished data).

Isoverbascoside, a phenylethanoid glycoside which are isolated from Chinese folk medicine herb *Pedicularis striata* Pall (Chinese name:Maxianhao), induced the differentiation and G0/G1 and arrested HCC cell line SMMC-7721 (Rui-Chuan et al., 2002).

Ginsenosides

Ginsenosides are thought to be the major effective ingredients in ginseng (Chinese name: Renshen).

Ginsenoside-Rh2 (G-Rh2) inhibits the proliferation and induces the apoptosis and differentiation of SMMC-7721 cells (Park et al., 1997; Park et al., 1998; Zeng and Tu, 2004a). G-Rh2 increases the proportion of SMMC-7721 cells in G1 phase, and decreases those in S and G2/M phases. Moreover, G-Rh2 down-regulates the expression of positive-regulating factors (Cyclin D1, Cyclin E) and up-regulates the expression of negative-regulating factors (P16 protein, p21 gene) in SMMC- 7721 cells. G-Rh2 effectively reduces telomerase activity through affecting transcription level of hTERT, and arresting cell cycle progression. The down- regulation of telomerase activity in SMMC-7721 cells is closely related to G-Rh2-induced differentiation (Zeng and Tu, 2004b).

Ginsenoside-Rg5 (G-Rg5) blocks cell cycle of SK-HEP-1 cells at the Gl/S transition phase by down-regulating cyclin E-dependent kinase activity. The down-regulation of cyclin E-dependent kinase activity is caused mainly by inducing CDK2 inhibitor and p21, and decreasing the expression of cyclin E (Lee et al., 1997). However, Ginsenoside-Rg1 (G-Rg1) stimulates cell-growth of SK-HEP-1 cells by inducing the intracellular levels of cyclin E/cdk2 complex, which in turn up-regulates cyclin E-dependent kinase activity (Lee and Lee, 1996). Thus Ginsenoside have many ingredients which could promote or inhibit the progression of HCC. Further data on the long-term safety of ginseng for HCC patients are therefore required.

Alkaloids

Camptothecin is a plant-derived alkaloid from *Camptotheca acuminata* (Chinese name: Xishu) that has a strong toxicity for tumor cells. Hydroxycamptothecin can induce Hep G2 cells to differentiation (Zhang et al., 2000a). Hydroxycamptothecin increases the proportion of Hep G2 cells in G2 phase, and up-regulates the expression of wide P53 (Zhang et al., 2000b). Treatment of HepG2 cells with 9-nitro-camptothecin results in a dose-dependent inhibition of cell proliferation and DNA synthesis. HepG2 cells treated by 9-nitro-camptothecin are arrested in the G2 phase of the cell cycle. Dramatically, 9-nitro-camptothecin at low concentrations induces differentiation of HCC (Khaoustov et al., 1995). Moreover, high dose of 7-OH-4-CH(3)-coumarin (Chinese name: Xiangdousu), which could be found in many Chinese herbs, suppresses the proliferation of the SMMC-7721 cells in a dose-dependent manner, while low concentrations could trigger differentiation (Pan et al., 2004). Thus dose is a key factor that makes some drugs exhibit cytotoxicity or differentiation inductor for HCC.

Tylophorine, which could be found in *Tylophora crebriflora* S.T.Blalse (Chinese name: Wuerteng), and its analogs are phenanthroindolizidine alkaloids. Several Tylophorine analogs have been isolated from the *Tylophora* genus of plants. Tylophorine analogs could inhibit the proliferation and induce the differentiation of HepG2 cells. Tylophorine analogs have inhibitory effects on cyclic AMP response elements, activator protein-1 sites and nuclear factor-kappa b binding site-mediated transcriptions (Gao et al., 2004).

Matrine, which could be extracted from a Chinese herb *Radix Sophorae flavescentis* (Chinese name: Kushen), has great effects as anti-inflammation, anti-arrhythmia and anti-fibrosis of liver cell (Zhang and Huang., 2004; Long et al., 2004). Recent evidences indicate that matrine also has great effects as anti-tumor agent, such as inhibiting proliferation, inducing apoptosis, modulating immune response, reducing invasion and metastasis of HCC cells (Ma et al., 2005; Chui et al., 2005; Wang et al., 2003). Moreover, matrine induces the differentiation of SMMC-7721 cells (Wang et al., 2003). Tissue homeostasis requires a balance among cell division, differentiation and death. The decision of proliferation, differentiation and apoptosis are linked by cell cycle regulators. Tumor is a kind of cell cycle disease that has the abnormal interface of division, differentiation and death (Wu 2006d). As a cell biological modifier, matrine could reverse the abnormal biologic behaviour of tumor cells and recover the balance among cell division, differentiation and death.

Tachyplesin

Overexpression of c-myc has been frequently observed in HCC. Moreover, c-myc/ TGF-alpha transgenic mice are prone to liver cancer. Thus, c-myc plays a pivotal role in hepatocarcinogenesis (Calvisi and Thorgeirsson 2005; Cavin et al 2005; Cadoret et al., 2005). MYC inactivation induced HCC cells differentiating into hepatocytes and biliary cells and forming bile duct structures. It is associated with rapid loss of the expression of the tumor marker alpha-fetoprotein, the increase of the expression of liver cell markers cytokeratin 8 and carcinoembryonic antigen (Shachaf et al., 2004). Tachyplesin, which is isolated from acid extracts of Chinese horseshoe crab (*Tachypleus tridentatus*, Chinese name: Pangxie) hemocytes, effectively inhibits the proliferation and induces the differentiation of hepatocarcinoma cells. The expression of p21 protein is up regulated and that of c-myc protein is down regulated (Ouyang et al., 2002). After tachyplesin treatment, the cell cycle is arrested at G0/G1 phase, the protein levels of mutant p53, cyclin D1 and CDK4 and the mRNA level of c-myc gene are decreased, whereas the levels of p16 protein and p21 mRNA are increased (Li et al., 2003).

Although tumor cells remain dormant as long as MYC remains inactivated, MYC reactivation immediately restores their neoplastic features (Shachaf et al., 2004). Thus induction of differentiation through MYC inactivation may have the high potential of recrudescence. Further data on the long-term therapeutic effects of tachyplesin for HCC are therefore required.

Arsenic trioxide

Arsenic trioxide (As_2O_3, Chinese name: Pishuang) has inhibitory effect on growth of experimental HCC. The mechanisms may involve decrease of cell division, direct cytotoxicity, apoptosis of tumor cells, and inhibitory effect on angiogenesis through blocking VEGF (Tan et al., 2005; Luo et al., 2006). Zheng et al. reported that As_2O_3 induces differentiation of HCC by virtue of producing H_2O_2, while the combination of As_2O_3 and sodium selenite may induce the differentiation of hepatoma cells by virtue of enhancing the activities of antioxidative enzymes and reducing the formation of H_2O_2 (Kang et al., 2000; Zheng and Zheng, 2002; Zheng et al., 2002).

The therapeutic efficacy of As_2O_3 against HCC is not satisfactory even at high dosage. Other therapeutic strategies are required to enhance the efficacy of As_2O_3 against HCC. Retinoic acid induces the differentiation of HCC with the alteration of the levels of RAR/RXR heterodimer, RXR/RXR homodimer, or RAR/RAR homodimers (Wan et al., 1998; Feng et al., 2001; Tsang and Crowe et al., 2001). Retinoic acid have been used for treating patients with HCC, but the therapeutic effect of the differentiation-inducing agents is not potent when compared with that of conventional chemotherapeutic agents. Attractively, all-trans-retinoic acid (ATRA) can strongly potentate As_2O_3-induced growth-inhibition and apoptosis, and ARTA and low dose of As_2O_3 can produce a significant synergic effect (Lin et al., 2005). Whether the combination of ARTA and the low dose of As_2O_3 is a possible therapeutic strategy for HCC is unknown and must be identified.

As_2O_3 down-regulates the expression of VEGF, inhibits angiogenesis induced by HCC, and enhances the apoptosis of tumor cells at doses greater than 1 mg/kg, but a mouse lost weight and failed to thrive at dose of 4 mg/kg and greater (Liu et al., 2006; Tan et al., 2005). In contrast, low dose (<1 mg/kg) of As_2O_3 promotes tumor growth, up-regulates the expression of VEGF and tumor angiogenesis, and has no effect on the apoptosis of tumor cells (Liu et al., 2006). Thus, different dose of As_2O_3 have opposing effects on tumor growth and angiogenesis. Clearly, appropriate dosage of As_2O_3 is required to treat human patients to avoid toxicity and undesirable side effects. The results demonstrate that As_2O_3 has a narrow window of therapeutic opportunity with respect to dosage, and that low dose of the drug as used in metronomic therapy should be used with extreme caution.

Conclusion

HCC is one of the most common malignant tumors worldwide. For the difficulty of the giving therapeutic dose because of the poor liver function and the low sensitivity for the chemotherapeutic agents, chemotherapy adds little to overall survival of HCC patients. The induction of terminal differentiation in tumor cells represents a possible therapeutic strategy with less toxicity. Gekko sulfated polysaccharides, isoverbascoside, Ginsenoside, Camptothecin, 9-nitro-camptothecin, Tachyplesin, Matrine, Tylophorine, 7-OH-4-CH (3)- coumarin and arsenic trioxide are known to have a differentiation-inducing capability on HCC. More data are required on the molecular mechanisms of therapeutic effects, dose response and potential toxicities. Although the therapeutic effect of the differentiation-inducing agents may not be potent when compared with that of conventional chemotherapeutic agents, they have multiple therapeutic targets, low toxicity and less probability of drug resistance. Combination of differentiation-inducing agents with chemotherapy therapy might be a potential way of treating HCC patients. New designs for trials to demonstrate activity in human subjects are required. Quality assurance of appropriate extracts is essential prior to embarking on clinical trials.

References

1.Cadoret, A., Desbois-Mouthon, C., Wendum, D., Leneuve, P., Perret, C., Tronche, F., Housset, C and Holzenberger, M. (2005). c-myc-induced hepatocarcinogenesis in the absence of IGF-I receptor. Int. J. Cancer., **114(4):**668-672.

2.Calvisi, D.F. and Thorgeirsson S.S.(2005). Molecular mechanisms of hepatocarcinogenesis in transgenic mouse models of liver cancer. Toxicol. Pathol., **33(1):**181-184.

3.Cavin, L.G., Wang, F., Factor, V.M., Kaur, S., Venkatraman, M., Thorgeirsson, S.S. and Arsura, M. (2005). Transforming growth factor-alpha inhibits the intrinsic pathway of c-Myc-induced apoptosis through activation of nuclear factor-kappaB in murine hepatocellular carcinomas. Mol. Cancer. Res., **3(7):**403-412.

4.Chui, C.H., Lau, F.Y., Tang, J.C., Kan, K.L., Cheng, G.Y., Wong, R.S., Kok, S.H., Lai, P.B., Ho, R., Gambari, R. and Chan, A.S. (2005). Activities of fresh juice of Scutellaria barbata and warmed water extract of Radix Sophorae Tonkinensis on anti-proliferation and apoptosis of human cancer cell lines. Int. J. Mol. Med.,**16(2)**:337-341.

5.Damdinsuren, B., Nagano, H., Kondo, M., Natsag, J., Hanada, H., Nakamura, M., Wada, H., Kato, H., Marubashi, S., Miyamoto, A., Takeda, Y., Umeshita, K., Dono, K. and Monden, M. (2006). TGF-beta1-induced cell growth arrest and partial differentiation is related to the suppression of Id1 in human hepatoma cells. Oncol. Rep.,**15(2):**401-408.

6.Daoudaki, M., Fouzas, I., Stapf, V., Ekmekcioglu, C., Imvrios, G., Andoniadis, A., Demetriadou, A. and Thalhammer, T. (2003). Cyclosporine a augments P-glycoprotein expression in the regenerating rat liver. Biol. Pharm. Bull., **26(3):**303-307.

7.Feng, Y., Wang, L.Y., Cai, T., Jin, J.W., Zhou, G.F., Cao, L.H. and Zha, X.L. (2001). All-trans-retinoic acid increased the expression of integrin alpha5beta1 and induced "anoikis" in SMMC-7721 hepatocarcinoma cell. J. Exptl. Clin. Cancer. Res., **20(3):**429-438.

8.Gao, W., Lam, W., Zhong, S., Kaczmarek, C., Baker, D.C. and Cheng, Y.C. (2004). Novel mode of action of tylophorine analogs as antitumor compounds. Cancer. Res., **64(2):**678-688.

9.Idobe, Y., Murawaki, Y., Kitamura, Y. and Kawasaki, H. (2003). Expression of transforming growth factor-beta 1 in hepatocellular carcinoma in comparison with the non-tumor tissue. Hepatogastroenterol., **50(49):**54-59.

10. Ito, N., Kawata, S., Tamura, S., Takaishi, K., Yabuuchi, I. and Matsuda, Y. (1990). Expression of transforming growth factor-beta 1 mRNA in human hepatocellular carcinoma. Japan. J. Cancer. Res., **81(12):**1202- 1205.

11. Kang, J.H., Shi, Y.M. and Zheng, R.L. (2000). Effects of ascorbic acid and DL-alpha-tocopherol on human hepatoma

cell proliferation and redifferentiation. Acta. Pharmacol. Sin., **21(4):**348-52.

12. Kawamata, H., Tachibana, M., Fujimori, T. and Imai, Y. (2006). Differentiation-inducing therapy for solid tumors. Curr Pharm Des., **12(3):**379-385.
13. Khaoustov, V.I., Ozer, A., Smith, J.R., Noda, A., Mearns, M., Krishnan, B., Slagle, B.L. and Yoffe, B. (1995). Induction of senescent cell-derived inhibitor of DNA synthesis gene, SDI1, in hepatoblastoma (HepG2) cells arrested in the G2-phase of the cell cycle by 9-nitrocamptothecin. Lab. Invest.,**73(1):**118-127.
14. Kira, S., Nakanishi, T., Suemori, S., Kitamoto, M., Watanabe, Y. and Kajiyama, G. (1997). Expression of transforming growth factor alpha and epidermal growth factor receptor in human hepatocellular carcinoma. Liver, **17(4):**177-182.
15. Lee, K.Y., and Lee, S.K. (1996). Ginsenoside-Rg1 positively regulates cyclin E-dependent kinase activity in human hepatoma SK-HEP-1 cells. Biochem. Mol. Biol. Int., **39(3):**539-546.
16. Lee, K.Y., Lee, Y.H., Kim, S.I., Park, J.H. and Lee, S.K. (1997). Ginsenoside-Rg5 suppresses cyclin E-dependent protein kinase activity via up-regulating p21Cip/WAF1 and down-regulating cyclin E in SK-HEP-1 cells. Anticancer. Res., **17(2A):**1067-1072.
17. Li, Q.F., Ou-Yang, G.L., Peng, X.X. and Hong, S.G. (2003). Effects of tachyplesin on the regulation of cell cycle in human hepatocarcinoma SMMC-7721 cells. World. J. Gastroenterol., **9(3):**454-458
18. Lin, L.M., Li, B.X., Xiao, J.B., Lin, D.H. and Yang, B.F. (2005). Synergistic effect of all-trans-retinoic acid and arsenic trioxide on growth inhibition and apoptosis in human hepatoma, breast cancer, and lung cancer cells in vitro. World. J. Gastroenterol.,**11(36):**5633-5637.
19. Liu, B., Pan, S., Dong, X., Qiao, H., Jiang, H., Krissansen, G.W. and Sun, X. (2006). Opposing effects of arsenic trioxide on hepatocellular carcinomas in mice. Cancer. Sci., **97(7):**675-681.
20. Long, Y., Lin, X.T., Zeng, K.L. and Zhang, L. (2004). Efficacy of intramuscular matrine in the treatment of chronic hepatitis B. Hepatobiliary. Pancreat. Dis. Int., **3(1):**69-72.
21. Luo, L., Qiao, H., Meng, F., Dong, X., Zhou, B., Jiang, H., Kanwar, J.R., Krissansen, GW. and Sun, X. (2006). Arsenic trioxide synergizes with B7H3-mediated immunotherapy to eradicate hepatocellular carcinomas. Int. J. Cancer.,**118(7):**1823-1830.
22. Ma, L.D., Zhang, Y., Wen, S.H., He, Y.J., Liu, X.S., Kang, G.F. and Jiang, J.K. (2005). Inhibition of tumor growth in tumor-bearing mice treated with matrine. Zhonghua. Zhong. Liu. Za. Zhi., **27(6):**339-341.
23. Matsumoto, H., Nagao, M., Ogawa, S., Kanehiro, H., Hisanaga, M., Ko, S., Ikeda, N., Fujii, H., Koyama, F., Mukogawa, T. and Nakajima, Y. (2003). Prognostic significance of death-associated protein-kinase expression in hepatocellular carcinomas. Anticancer. Res., **23(2B):**1333-1341.
24. Minemura, M., Tanimura, H. and Tabor, E. (1999). Overexpression of multidrug resistance genes MDR1 and cMOAT in human hepatocellular carcinoma and hepatoblastoma cell lines. Int. J. Oncol., **15(3):**559-563.
25. Ouyang, G.L., Li, Q.F., Peng, X.X., Liu, Q.R. and Hong, S.G. (2002). Effects of tachyplesin on proliferation and differentiation of human hepatocellular carcinoma SMMC-7721 cells. World. J. Gastroenterol., **8(6):**1053-1058.
26. Pan, J., Zhang, Q., Zhao, C.Y. and Zheng, R.L. (2004). Redifferentiation of human hepatoma cells induced by synthesized coumarin. Cell Biol Int. **28(5):**329-333.
27. Park, J.A., Lee, K.Y., Oh, Y.J., Kim, K.W. and Lee, S.K. (1997). Activation of caspase-3 protease via a Bcl-2-insensitive pathway during the process of ginsenoside Rh2-induced apoptosis. Cancer. Lett.,**121(1):**73-81.
28. Park, J.A., Kim, K.W., Kim, S.I. and Lee, S.K. (1998). Caspase 3 specifically cleaves p21WAF1/CIP1 in the earlier stage of apoptosis in SK-HEP-1 human hepatoma cells. Eur. J. Biochem., **257(1):** 242-248.
29. Roelofsen, H., Vos, T.A., Schippers, I.J., Kuipers, F., Koning, H., Moshage, H., Jansen, P.L. and Müller, M. (1997). Increased levels of the multidrug resistance protein in lateral membranes of proliferating hepatocyte-derived cells. Gastroenterology, **112(2):**511-521.
30. Rui-Chuan, C., Jin-Hua, S., Gao-Liang, O., Ke-Xia, C., Jin-Quan, L. and Xiao-Guang, X. (2002). Induction of

differentiation in human hepatocarcinoma cells by isoverbascoside. Planta. Med., **68(4):**370-372.

31. Shachaf, C.M., Kopelman, A.M., Arvanitis, C., Karlsson, A., Beer, S., Mandl, S., Bachmann, M.H., Borowsky, A.D., Ruebner, B., Cardiff, R.D., Yang, Q., Bishop, J.M., Contag, C.H. and Felsher, D.W. (2004). MYC inactivation uncovers pluripotent differentiation and tumour dormancy in hepatocellular cancer. Nature, **431(7012):**1112-1127.
32. Song, B.C., Chung, Y.H., Kim, J.A., Choi, W.B., Suh, D.D., Pyo, S.I., Shin, J.W., Lee, H.C., Lee, Y.S. and Suh, D.J. (2002). Transforming growth factor-beta1 as a useful serologic marker of small hepatocellular carcinoma. Cancer, **94(1):**175-180.
33. Tan, B., Huang, J.F., Wei, Q., Zhang, H., Ni, R.Z. (2005). Anti-hepatoma effect of arsenic trioxide on experimental liver cancer induced by 2-acetamidofluorene in rats. World. J. Gastroenterol.,**11(38):**5938-5943.
34. Tsai, J.F., Jeng, J.E., Chuang, L.Y., Chang, W.Y., Hsieh, M.Y., Lin, Z.Y. and Tsai, J.H. (1997). Urinary transforming growth factor-beta 1 in relation to serum alpha-fetoprotein in hepatocellular carcinoma. Scand. J. Gastroenterol., **32(3):**254-260.
35. Tsang, K.J. and Crowe, D.L. (2001). Retinoic acid and extracellular matrix inhibition of matrix metalloproteinase 9 expression is mediated by the mitogen activated protein kinase pathway. Intern. J. Oncol., **18(2):**369-374.
36. Wan, Y.J., Cai, Y. and Magee, T.R. (1998). Retinoic acid differentially regulates retinoic acid receptor-mediated pathways in the Hep3B cell line. Exptl. Cell. Res., **238(1):**241-247.
37. Wang, Y., Peng, C., Zhang, G., Liu, Y., Li, H. and Shan, J. (2003). Study on invasion and metastasis related factors in differentiation of SMMC-7721 cells induced by matrine. Zhong. Yao. Cai., **26(8):**566-569.
38. Wu, X.Z. (2006a). Sulfated oligosaccharides and tumor: promoter or inhibitor? Panminrva. Med., **48(1):**27-31.
39. Wu, X.Z. (2006b). Effects of sulfated polysaccharides on tumor biology. West. Indian. Med. J., **55(4):** 270-273.
40. Wu, X.Z., Chen, D. and Xie, G.R. (2006c). Effects of Gekko sulfated polysaccharide on the proliferation and differentiation of Hepatic Cancer Cell Line. Cell. Biol. Int., **30(8):** 659-64.
41. Wu, X.Z. (2006d). A new classification system of anticancer drugs – based on cell biological mechanisms. Med. Hypotheses., **66(5):**883-887.
42. Xu, Z., Shen, M.X., Ma, D.Z., Wang, L.Y. and Zha, X.L. (2003). TGF-beta1-promoted epithelial-to-mesenchymal transformation and cell adhesion contribute to TGF-beta1-enhanced cell migration in SMMC-7721 cells. Cell. Res., **13(5):**343-350.
43. Yan, Z.C., Chen, D., Wu, X.Z., Xie, G.R., Ba, Y. and Yan, Z. (2007). Effects of aqueous extracts of Aconitum carmichaeli, Rhizoma bolbostemmatis, Phytolacca acinosa, Panax notoginseng and Gekko swinhonis Guenther on Bel-7402 cells. World. J. Gastroenterol., **13(19):**2743-2746.
44. Zeng, X.L., and Tu Z.G. (2004a). Induction of differentiation by ginsenoside Rh2 in hepatocarcinoma cell SMMC-7721. Ai. Zheng., **23(8):**879-884.
45. Zeng, X.L., and Tu Z.G. (2004b). Effect of telomerase on ginsenoside Rh2-induced differentiation of hepatocarcinoma cell line SMMC-7721. Ai. Zheng., **23(12):**1655-1659.
46. Zhang, M.J. and Huang, J. (2004). Recent research progress of anti-tumor mechnism matrine. Zhongguo. Zhong. Yao. Za. Zhi., **29(2):**115-118.
47. Zhang, X., Zhou, Y. and Xu, B. (2000a). Differentiation of human hepatoma Hep G2 cells induced by 10-hydroxycamptothecin. Chin. Med. J. (Engl)., **113(8):**712-713.
48. Zhang, X.W., Jiang, J.F. and Xu, B. (2000b). Differentiation-inducing action of 10-hydroxycamtothecin on human heptoma hepG2 ce11s. Acta. Pharmacol. Sin., **21(4):**364-368.
49. Zheng, Q.S. and Zheng, R.L. (2002). Effects of ascorbic acid and sodium selenite on growth and redifferentiation in human hepatoma cells and its mechanisms. Pharmazie, **57(4):**265-269.
50. Zheng, Q.S., Zhang, Y.T. and Zheng, R.L. (2002). Ascorbic acid induces redifferentiation and growth inhibition in human hepatoma cells by increasing endogenous hydrogen peroxide. Pharmazie, **57(11):**753-757.