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 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 (Roelofs en 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 Günter (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 Günter 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 G2/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. TGF-beta 1 was overexpressed in HCC, especially in patients with small HCC and well-differentiated HCC (Matsumoto et al., 2003; Idohe 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 G1/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.Blase (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 upregulated 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$_2$O$_3$, 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$_2$O$_3$ induces differentiation of HCC by virtue of producing H$_2$O$_2$, while the combination of As$_2$O$_3$ 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$_2$O$_2$ (Kang et al., 2000; Zheng and Zheng, 2002; Zheng et al., 2002).

The therapeutic efficacy of As$_2$O$_3$ against HCC is not satisfactory even at high dosage. Other therapeutic strategies are required to enhance the efficacy of As$_2$O$_3$ 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$_2$O$_3$-induced growth-inhibition and apoptosis, and ARTA and low dose of As$_2$O$_3$ can produce a significant synergic effect (Lin et al., 2005). Whether the combination of ARTA and the low dose of As$_2$O$_3$ is a possible therapeutic strategy for HCC is unknown and must be identified.

As$_2$O$_3$ 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$_2$O$_3$ 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$_2$O$_3$ have opposing effects on tumor growth and angiogenesis. Clearly, appropriate dosage of As$_2$O$_3$ is required to treat human patients to avoid toxicity and undesirable side effects. The results demonstrate that As$_2$O$_3$ 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, Tylorophrine, 7-OH-4-CH₃-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


