

Inhibition of Autophagy and Immune Response: Alpha-fetoprotein Stimulates Initiation of Liver Cancer

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Alpha-fetoprotein (AFP) is a tumorous marker for the diagnosis of hepatocellular carcinoma (HCC), it is synthesized mainly by the embryo yolk sac, fetal liver and the gastrointestinal tract [1]. AFP belongs to the family of protein products of albuminoid genes, which are located in tandem arrangement in chromosome 4 (region 4q11-q13) [2]. AFP is a glycoprotein with molecular weight of about 68-73 kDa and carbohydrate content of 3%-5%. Primary structure of full-length human AFP (HAFP) molecule is represented by 609 amino acid (aa) residues from which a signal peptide of 18 aa residues is removed during processing to produce the mature protein of 591 aa residues [3-5]. AFP has multiple ligand-binding sites and a plasma half-life of approximately 3-5 days [6]. Embryo cells internalize the AFP-ligand complex through the AFP receptor (AFPR)-mediated endocytosis, and cancer cells performed the same to internalize the AFP [7]. The AFPR is mostly absent in normal adult cells, except for a small population of regulatory immune cells, such as myeloid-derived suppressor cells (MDSCs), specific T cells and monocytes. Selective targeting of MDSCs through AFP-binding receptor was able to inhibit the growth of cancer cells [8-10]. Therefore, AFP and AFPR are not only proteins of nutrient transport system and tumor markers, but also involve in regulating the growth and differentiation of cells, and immune response.

Autophagy is closely related with the development and progression of cancer. Autophagy is a type of cellular death that is similar to apoptosis, which in some cases can

suppress cancer by eliminating damaged cells to maintain homeostasis [11]. Autophagy involves multiple signaling pathways, such as PI3K/Akt/mTOR, Bcl-2/beclin1, MAPK/Erk1/2, and AMPK [12]. Wang et al. [13] found that AFP can interact with PTEN, suppress the activity of PTEN to activate the PI3K/AKT/mTOR pathway, and inhibit autophagy in HCC cells that lead to promote malignant behaviors by upregulating the expression of the autophagy-related protein mTOR. These results implicated that AFP inhibits autophagy which is a pivotal factor for the development and progression of liver cancer.

Escape attacking of lymphocytes contribute to HCC initiation and the cancer cells growth *in vivo*. AFP promotes the expression of Fas ligand (FasL) in HCC cells while inhibiting the expression of Fas, Fas has a trait to bind with the specific ligand (FasL), which is highly expressed in HCC cells, and Fas, which is expressed in lymphocytes, these effects can induce apoptosis of lymphocyte through the Fas/FasL pathway and results in HCC cells to escape host lymphocyte immune monitoring [14]. CD8⁺ cytotoxic T lymphocytes (CTLs) play an important role in tumor elimination, while regulatory T cells (Tregs) can suppress the immune attack of CTLs [15]. Some studies found that AFP can lead to immune escape of tumor cells by changing the ratio of CD4⁺ and CD8⁺ T cells. The number of Tregs is high in the peripheral blood of HCC patients, which affects the function of CD8⁺ T lymphocytes and inhibits their immune response to tumor cells, and AFP also has a trait to enhance the number of Tregs [16,17]. Tumor-associated macrophages (TAMs), which are related to the occurrence and development of tumors, are the main cell components of the tumor microenvironment (TME). Activation of the Wnt/ β -catenin signaling pathway can stimulate M2-like

polarization of TAMs and promote tumor growth and metastasis [18]. A study by Laderoute [19] found that AFP, as an immunosuppressive factor, can prevent macrophage-induced cell lysis and promote HCC cells escape from lymphocyte-mediated immune surveillance.

AFP promotes liver cancer cells to evade immune surveillance by inhibiting the maturation and differentiation of dendritic cells (DCs). When cultured DCs were treated with tumor-derived AFP, the DCs differentiation was significantly inhibited, and the immature mononuclear morphology was maintained [20]. Moreover, fatty acid synthesis is required during the maturation and differentiation of DCs, and the expression of sterol regulatory element binding transcription factor 1 (SREBP-1) and downstream target proteins can be inhibited by treatment of DCs with AFP. Downregulation of SREBP-1-mediated fatty acid synthase transcription can reduce the synthesis of fatty acids, thus affecting the maturation and differentiation of DCs [21]. AFP inhibits the maturation of DCs, which not only directly affects DCs uptake, processing and presentation of antigens, but also indirectly affects the roles of other immune cells in inhibiting tumor growth. Pardee et al. [22] found that AFP has the ability to reduce the proliferation of CD4⁺ and CD8⁺ cells when DCs were treated with AFP. In addition to reduce lymphocyte proliferation, the expression of the DCs surface markers HLA-DR, CD40, CD80, CD83 and CD86 also decreased after AFP treatment, and these markers are crucial for the proliferation and differentiation of immune effector cells [23]. Interleukin-12 (IL-12) can stimulate the expression of the activation receptor NKG-2D on natural killer (NK) cells, increase the toxicity of NK cells, and exert an immune response [24], while AFP can indirectly inhibit the activity of NK cell by suppressing the maturation of DCs to reduce the secretion of IL-12 [25,26]. AFP treatment of DCs can also significantly reduce the level of CD1d, which plays a crucial role in the presentation of lipid antigens to NK T lymphocytes [27]. In summary, AFP indirectly blocks the functions of various immune cells and immune factors by inhibiting the maturation of DCs and stimulates immune escape of HCC cells, maintaining continued growth of cancer cell *in vivo*.

The effect of AFP on the malignant transformation of hepatocytes involve the role of multiple factors. Evidences have indicated that infection of Hepatitis B virus (HBV), HBV-x protein (HBx) can block p53-mediated repression of the *AFP* gene promoter, stimulating the expression of AFP [28]. The expression of AFP can promote activation of the PI3K/AKT/mTOR signaling pathway, which play a role in promoting the expression of reprogramming genes such as *KLF4*, *Sox2*, *Oct4*, and *c-myc* [29]. The expression of these genes can induce the reprogramming of HBV-infected liver cells to transform into liver stem cells, which has a potential trait of liver cancer stem cells. AFP can also

promote the expression of *Ras*, *Src* and other oncogenes [30]. Due to the presence of reprogrammed cells and the expression of oncogenes, a condition is established for the malignant transformation of liver stem cells into liver cancer cells. Moreover, studies found that after infection of HBV, HBx molecules stimulate the acetylation of AFP and protect degradation of AFP which is mediated by ubiquitin enzyme [31]. AFP inhibits the activity of PTEN in the cytoplasm, activates the PI3K/AKT/mTOR signaling pathway, promotes expression of the proliferation-related oncogenes *Ras* and *Src* and the metastasis-related genes *CXCR4*, *EpCAM*, and *MMP2/9*, and promotes the proliferation and metastasis of cancer cells. Furthermore, AFP can bind to caspase-3 and inhibit the activity of caspase-3, leading to tumor necrosis-related apoptosis-induced ligand (TRAIL) and other apoptotic molecules failure to induce HCC cells apoptosis *in vivo* [32,33]. Therefore, expression of AFP in HCC cells activates growth signals in the cytoplasm, inhibits the activation of apoptotic enzymes, promotes the malignant transformation of liver cells, the proliferation and metastasis of HCC cells, and inhibits the transduction of apoptotic signals and apoptosis induced by apoptotic factors. Our previous study found that HBV infection can also drive the expression of AFPR [34]. AFPR can bind to AFP, activate Ca²⁺ and cAMP signaling pathways, and cause extracellular AFP to stimulate the proliferation and metastasis of HCC cells. Recent and early studies have found that AFP has a trait to suppress immune response [18,20,35], not only inducing the occurrence of HCC, but also playing important role in protecting these cancer cells from immune attack in the early stage of HCC. In other words, during the early stages of malignant transformation of liver cells, expression of AFP in these cells suppresses attack by immune cells. These effect results in liver stem cells malignant transformation into cancer stem cells, and these liver cancer stem cells survive *in vivo* and secrete more AFP, suppressing cellular and humoral immunity, causing these cancer cells to not only grow *in situ* but also escape immune surveillance during metastasis. Therefore, during malignant transformation of hepatocytes, AFP not only promotes the proliferation and metastasis of cancer cells by activating growth signals, but also inhibits the transduction of apoptotic signals to prevent cancer cell apoptosis. More importantly, AFP can also play an immunosuppressive role, protecting liver cancer stem cells and HCC cells from attack by immune cells and immune factors during the early progression of malignant transformation of HCC cells, and promoting the occurrence and development of the disease.

Recently, evidence indicated that autophagy promotes immune evasion in cancer patients [36], high serum level of AFP in the HCC patients was able to inhibit autophagy of liver cancer cells, which led to microenvironment inflammatory infiltrate to drive growth speed of HCC cells [37]. AFP plays an important role in

inhibiting programmed cell death (including autophagy). Inhibiting programmed cell death leads to a failure to quickly remove potentially dangerous cells which differentiation ability is not compatible *in vivo*, which eventually leads to the malignant transformation of cells and abnormal growth of cancer cells; AFP also inhibits the attack of humoral immunity and immune cells, constructs a favorable environment for the initiation and growth of HCC cells, these effects result in promoting the initiation and development of HCC. AFP is an available biotarget for the therapy of HCC patients.

Financial & Competing Interests Disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes stock ownership or options, consultancies, employment, honoraria, expert testimony, grants or patents received or pending, or royalties.

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