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Review Article

Alpha-fetoprotein: A molecular bootstrap for hepatocellular carcinoma

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ABSTRACT

Hepatocellular carcinoma (HCC) is well known for its aggressive nature and high recurrence rates. Alpha-fetoprotein (AFP) secreting tumors are more common in HCC. However, a few proportion of HCC do not produce AFP more than the basal level. AFP secreting tumors are more aggressive in nature since the ability of AFP to promote effective progression, growth, and metastasis of tumor. AFP also intervenes the immune system to evade the immune responses against cancer cells. AFP-producing tumors contain poorly differentiated cells similar to embryonic stem cells of liver mimicking rapid replication, proliferation, and AFP secretion by fetal liver. In this review, we highlight the crucial roles of AFP in immune evasion, aggressiveness, progression, and tumor biology of HCC.

Keywords: Alpha-fetoprotein, Hepatocellular carcinoma, Differentiation, Recurrence, Stemness

INTRODUCTION

Hepatocellular carcinoma (HCC) remains a major contributor for cancer-related mortality and morbidity as well and ranks the second most leading cause for global cancer mortalities.[1] HCC is one of the few types of cancers which secretes alpha-fetoprotein (AFP). Among HCC tumors, nearly more than 50% of the HCC are AFP-secreting tumors.[2] AFP is an oncofetal protein and was incorporated in the international guidelines for HCC surveillance and is the most commonly used serological biomarker in the diagnosis of HCC.[3,4] Recently, AFP was described as a marker of aggressiveness in HCC and used to predict the survival of HCC patients.[5] Human AFP is a glycoprotein containing 4% of carbohydrate content and a total molecular weight of 69,000 Daltons.[6] AFP has several functions in HCC such as proliferation, angiogenesis, and enhancement of antiapoptotic ability of HCC cells. It is also well known that AFP supports HCC progression by suppressing the immune system, leading to immune escape of HCC cells.[7] These properties make the AFP-secreting tumors more aggressive in nature.

ORIGIN AND POTENTIAL SOURCES OF AFP IN HCC

AFP is secreted in large quantities by fetal liver and yolk sac during the embryonal development in pregnancy.[8] After birth, liver loses the ability to synthesize AFP and thus contributes to the low circulating levels of AFP in adults. Very low levels of AFP synthesis are characteristic of mature hepatocytes of adult liver. After oncogenic transformation, the adult liver cells regain the ability to synthesize and secrete high levels of AFP such as the fetal liver tissue which is the basis of AFP

secretion by HCC tumors.[7] The ability to synthesize AFP is more dependent on the status of cellular differentiation, such that the undifferentiated HCC cells contribute to high levels of AFP secretion, while differentiated HCC cells secrete very low quantities of AFP. Highly differentiated tumors in HCC patients were characterized by serum negativity of AFP or weak positivity.[7]

Different animal models for liver injury and hepatocarcinogenesis showed that AFP levels upsurge during hepatocyte regeneration. Hepatocyte injury by carbon tetrachloride and partial hepatectomy models demonstrated that adult hepatocytes become more regenerative and prolific after injury. In this case, AFP rise is a mere consequence of this injury mediated hepatocyte proliferation.[9] In severe hepatocyte injury mediated by high doses of d-galactosamine and N-nitrosomorpholine, hepatocyte regeneration by adult hepatocytes fails. In such conditions, a subset of biliary epithelial cells comes into play to reconstruct the damaged liver, which is known as oval cells that have the ability to give rise to bile duct epithelia as well as functionally mature hepatocytes. The proliferation of these biliary epithelial cells reactivates fetal genes and AFP synthesis suggesting that these cells have the stemness potential. Hence, HCC tumors originating from both proliferating oval cell lineage and dedifferentiating mature hepatocyte lineage are AFP secreting tumors constituted by dedifferentiating cells.[9]

AFP ARBITRATE IMMUNE ESCAPE OF HCC

Tumor immune surveillance is mainly preserved by three key immune cells, namely, dendritic cells (DCs), T lymphocytes (T cells), and natural killer cells (NK cells). These cells constitute the antigen-presenting cells. AFP is shown to modulate the immunological properties and the survival of these immune cells. AFP increased the expression of pro-apoptotic genes Bid, Bad, Bax, and ApaF and pro-apoptotic proteins such as Caspase-3, FasL, TRAIL, and Fas in peripheral lymphocytes.[10,11] It is also reported that elevation in serum AFP levels is associated with increased circulating premature DCs and decreased mature DCs in the peripheral venous blood of patients with HCC. This observation was further supported by the *in vitro* experiment on the effect of tumor-derived AFP on DCs, which shows that AFP inhibits the maturation and differentiation of DCs. Tumor-derived AFP also shown to downregulate the production of chemokines and inflammatory cytokines in DCs.[12] Moreover, the AFP is also involved in the inhibition of proliferation of T cells and NK cells. Tumor necrosis factor-alpha and interleukin-12 production by DCs were significantly lowered when treated with AFP.[13,14] These potential mechanisms of AFP significantly hamper the tumor immune responses and facilitate the tumor growth and progression by these immune evasion strategies in HCC.

AFP PROMOTES HCC PROGRESSION AND METASTASIS

AFP is often regarded as a marker for poor differentiation of HCC. In line with this, multiple levels of evidence, including meta-analyses, show that elevated AFP levels are associated with poor tumor differentiation, aggressiveness of the tumor, microvascular invasion, and prognosis in HCC.[15-18] In addition to this, AFP is a proven risk factor associated with pathological grading and poor tumor cell differentiation.[2] Fujiki *et al*. showed that HCC with an AFP level >800 ng/mL was associated with poor tumor cell differentiation when compared to AFP level <200 ng/mL.[19] Another multicentric study showed that AFP levels more than 1000 ng/mL are an independent risk factor and associated with poor differentiation of tumor cells than AFP levels between 100 and 1000 ng/mL in patients with HCC.[20] A systematic review on the role of AFP on the prognosis of HCC following liver transplantation (LT) reveals that the rate and degree of poorly tumor differentiation are higher in HCC with elevated pre-LT AFP levels.[21] In other types of cancers such as colon cancer, AFP is involved in poorly differentiated tumors. A case of adenocarcinoma of ascending colon with poor differentiation which is of neuroendocrine origin was reported to secrete AFP and it was found to be elevated.[22]

The expression and clinical significance of cancer stem cell (CSC) marker CD133 or prominin were analyzed by a study using immunohistochemical staining, in which it is observed that CD133 is highly expressed in HCC tissues. Interestingly, the high expression of CD133 was associated with the elevated AFP levels, indicating that CD133-positive HCC stem cells could be the probable reason behind AFP secretion in HCC.[23] It is also reported that epithelial cell adhesion molecule (EpCAM) and AFP coexpressing HCC cells exhibit the features of CSCs and hepatocyte maturation lineages and also display stem cell-like gene expression.[24] The previous studies also observed that a subset of HCC cells positive for EpCAM and AFP is characteristic of stem cell-like features and shows self-renewal capabilities. It is also suggested that these cells are tumor-initiating cells (TIL) by the *in vivo* transplantation experiments on mice, in which EpCAM and AFP-positive HCC cells were isolated from clinical samples of HCC tumors and inoculated to mice which later developed HCC tumors.[25] Recent studies investigated the role of association and role of AFP in HCC metastasis, which reveal that AFP levels are significantly elevated in patients having intrahepatic and lung metastasis when compared to patients having no metastasis showing a high correlation between AFP and metastasis.[26] Furthermore, the levels of AFP are significantly higher in patients having distant organ (lung) metastasis than intrahepatic metastasis and no metastasis. Interestingly, proteomic analysis of HCC tissue samples shows that the CSC marker EpCAM is coexpressed with the

proteins involved in metastasis such as K19, MMP2, MMP9, and CXCR4. *In vitro* experiments with HCC cell lines also demonstrate that AFP promotes migration and invasion of HCC cells.[26] Therefore, AFP-secreting cells in HCC tumor may represent poorly differentiated cells, including CSCs and undifferentiated HCC progenitor cells, which fail the normal hepatocyte function and promote the growth, progression, and metastasis of HCC.

AFP-NEGATIVE HCC CELLS SUBSIDIZE LIVER FUNCTION

Almost one-third of HCC comprise tumors that do not produce AFP which has more favorable prognosis, superior survival, and low recurrence than AFP-producing HCCs. It is suggested that AFP is a remarkable biomarker to discriminate the biological behavior of HCC tumors with respect to aggressiveness, differentiation, and invasive characters.[27] A retrospective study analyzed a total of 1173 HCC patient data by categorizing into groups based on low and high AFP levels. The results show that the functions of liver, such as albumin synthesis, were significantly higher in low AFP group than patients having high AFP levels. Moreover, the bilirubin levels were significantly lower in low AFP group when compare to HCC with high AFP levels. Considering the AFP as a marker poor differentiation, the high AFP-producing HCC is poorly differentiated and is more aggressive and invasive with less hepatocyte function. On the other hand, non-AFP-producing tumors are typically composed of well-differentiated cells with normal hepatocyte function and are less aggressive and invasive than poorly differentiated cells.[28]

Differentiated HCC cells retain most of the normal functions of hepatocytes, such as synthetic activity, even after their transformation. The tumor component of the liver is capable of serving those retained functions which contribute to the whole organ function. A study reported the antithrombin III synthetic activity of liver in pre- and post-operative periods, which concludes that antithrombin III activity in serum was significantly decreased postoperatively when compared to pre-operative measurements.[29] Another study clarifies on the notion that removal of tumor lowers the synthetic functions of liver by eliminating the tumor cells capable of normal synthetic activity. Measurements of thrombopoietin levels showed that post-operative increase in their levels is low in HCC when compared to cirrhotic patients after resection. In addition, the platelet count was decreased in HCC after surgery when compared to pre-operative platelet count.[30] These observations create an impression, despite the fact that HCC tumors endure a threat to the physiological system, these tumors are not absolutely useless for the reason that their ability to serve partially to the whole organ function of liver is accountable. Especially, the non-AFP-producing tumors contain cells of normocytic phenotypes and may serve the liver function as well.

CONCLUSION

AFP is considered as a hallmark of HCC biomarkers worldwide. However, one-third of HCC are AFP negative or having normal levels of AFP. Hence, AFP fails to serve as an appropriate tool for screening HCC in general but is a crucial biomarker to diagnose advanced stage HCC and poorly differentiated tumors suggesting that AFP is more specific toward aggressive and dedifferentiated cancer cells. HCC cells become dedifferentiated cells by acquisition of biological characters of embryonic stem cells and hepatic progenitor cells such as rapid replication, self-renewal, and AFP synthesis. AFP secreted from dedifferentiated HCC cells hijacks the immune system by targeting key immune cells involved in tumor immune response to support the progression and growth of the tumor. This tumor-derived AFP is critical for maintain the biological behavior of the tumor, such as aggressiveness, invasiveness, progression, metastasis, and recurrence, which makes AFP as the master controller of HCC.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Conflicts of interest

There are no conflicts of interest.

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