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REVIEW ARTICLE OPEN ACCESS

Role of Vascular Endothelial Growth Factor and Transforming Growth Factor-β in Hepatocellular Carcinoma

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Abstract

Hepatocellular carcinoma is one of the most common malignant tumor and leading cause of death worldwide. Tumor genes, signals from tumor stroma, recruited inflammatory cells and hypoxia has been reported to switch on angiogenesis. Vascular endothelial growth factor is a pleiotropic factor that regulates cell proliferation, vascular permeability and plays an important role in tumor angiogenesis through its different angiogenic receptors. Multifunctional regulatory cytokine transforming growth factor-β also controls cell proliferation, differentiation, extracellular matrix production and survival/or apoptosis of many types cells. By raising level of epithelial–mesenchymal transition, transforming growth factor-β creates a favourable microenvironment for tumor cell growth. Depending upon the stage of cancer progression it can either initiate tumor development, progression and metastasis or can act as a tumor suppressor. In the present review, an attempt has been made to comprehensively define the role of vascular endothelial growth factor and transforming growth factor-β in the development and progression of hepatocellular carcinoma.

Keywords: Hepatocellular carcinoma; VEGF; TGF-β; angiogenesis; cancer

Introduction

Hepatocellular carcinoma (HCC) has been reported as the seventh most common cancer worldwide and the third leading cause of cancer-related deaths (1). High mortality rate has been observed in patients with HCC due to lack of effective treatment options, especially at the advanced stages. Although surgery and percutaneous ablation can achieve long-term control in patients with early HCC, recurrence rates are high (approximately 50% at 3 years)(2). Moreover, because of asymptomatic nature of early HCC, lack of awareness and poorly defined screening strategies, most patients (approximately 80%) present with advanced or unresectable disease (3).

Etiological factors associated with HCC include chronic liver diseases, hepatitis virus

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(HBV & HCV) and alcoholism. Irrespective of the cause liver cirrhosis has emerged as one of the major risk factor for tumor development and it has been observed that almost 80% of patients with HCC pass through this stage before developing tumor (4). Further, aflatoxin has been reported to have a synergistic effect on Hepatitis C virus (HCV) induced liver cancer (5). From epidemiological studies, it is evident that contamination of food with AFB1 is the major risk factor for human liver cancer (6,7).

HCC is considered an extreme type of vascular tumor growth and is dependent upon angiogenesis, which is growth of new blood vessels. During the pathogenesis and progression of cancer from the preinvasive and dormant form to the invasive and metastatic phase, angiogenesis has been observed to play a critical role by yielding angiogenic factors necessary for tumor growth, invasion and metastasis. Multiple studies have demonstrated that the degree of tumor vascularity correlates positively with disease stage, the likelihood of metastases, and cancer recurrence (8).

Vascular endothelial growth factor (VEGF) is a well known, potent angiogenic factor that has been reported to enhance vascular permeability, promotes neovascularization and endothelial cell proliferation in HCC due to its endothelial cell mitogen property (9). In addition to VEGF, further during the development of liver diseases, transforming growth factor-b (TGF-b) has been reported to play an important role in fibrosis of the lesions (10).

Although in normal liver TGF-β inhibits hepotocyte cell growth but has been observed to get activated in those with injured livers and promote the development of hepatocellular carcinoma (11). During cancer progression, activation of TGF-β signalling leads to the adherence of TGF-β ligand to its receptor on the cell membrane (12).

VEGF as Angiogenesis Biomarkers of Hepatocellular Carcinoma

Described for the first time in 1989 (13), VEGF protein family currently comprises several members: VEGF (or VEGF-A), VEGF-B, VEGF-C and VEGF-D, VEGF-F, placental growth factor (PlGF), and their receptors VEGFR-1, VEGFR-2 and VEGFR-3 (14). VEGF is a ligand of the VEGF tyrosine kinase receptor superfamily and includes VEGF-A, -B, -C and -D, with splice variants of VEGF-A resulting in several different isoforms (15,16).

The VEGF family ligands bind to tyrosine kinase receptors VEGFR1, VEGFR2 and VEGFR3 (Figure-1); each receptor contains an extracellular domain of approximately 750 amino acid residues, arranged within seven immunoglobulin like folds (17). Additionally, heparin sulphate proteo-glycans (HSPGs) as well as neuropilins (NRP-1 and NRP-2) can act as coreceptors for VEGF and promote VEGFR activation (18,19). VEGF/VEGFR pathway is influenced and regulated by many factors like local environmental hypoxia, various hormones, growth factors and cytokines. Hormones such as androgens upregulate stromal cell and malignant cell VEGF production and angiogenesis, enhancing prostate cancer growth (20,21).

VEGF has been described as the main angiogenic growth factor and the level of its expression has been used as a biomarker in the identification of angiogenic diseases. Expression level of VEGF has been reported to increase with the progression and pathogenesis of HCC and has been reported to be very high at advanced stages of cancer. In solid tumors due to neovascularization, VEGF facilitates tumor growth and metastasis by supplying continuous nutrient flow and its

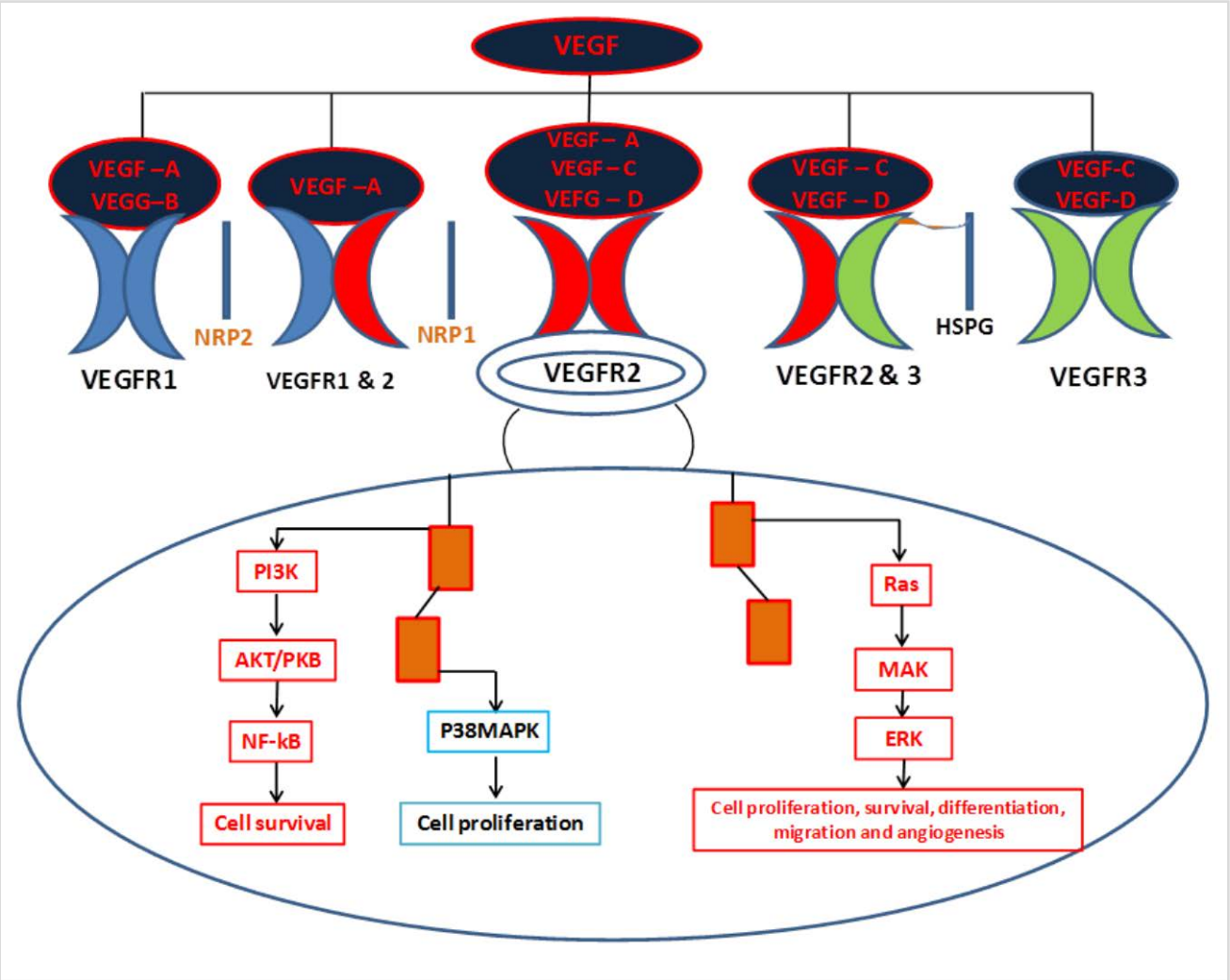

Figure-1. VEGF receptor binding; The five mammalian vascular endothelial growth factors (VEGF-A-D) bind to the receptor tyrosine kinases, VEGF receptor (VEGFR1-3) and co-receptors (HSPG, NRP-1 and NRP-2). VEGFR-binding leads to the formation of homodimers and/or heterodimers. Proteolytic cleavage enables VEGF-C and -D to bind VEGFR2 forming a homodimer. The binding and activation of VEGFR2 lead to downstream signalling of the PI3K, MAPK, and Ras pathways; which promote cell survival, proliferation, differentiation, migration and angiogenesis.

expression correlates with tumour progression (22). It has been identified as one of the important angiogenic factor in HCC. Its expression has been reported to increase gradually from low-grade dysplastic nodules to high-grade dysplastic nodules to early HCC (23). During the early stages of HCC, higher status of neo-angiogenesis and cell proliferation activity has been observed as compared to advanced form of HCC (23). The degree of VEGF expression during development of HCC has been observed to correlate with micro vascular density, unpaired arteries (i.e., arteries not accompanied by bile ducts, indicative of angiogenesis) and with CD34 staining as a marker of sinusoidal capillarization in HCC (23). VEGF-C gene polymorphism resulting into its over expression has been observed to occur frequently in HCC and has been suggested to be a predictive factor for advanced stage of HCC (24).

VEGF has been accounted as the chief angiogenic factor responsible for the neoangiogenesis, development and/or progression/ spread of HCC tumour (25) and HCC patients with over expression of VEGF in serum and tissue has been associated with poor survival rate (26). VEGF induced formation of new microvessels, is critical for the growth and progression of various human solid tumors as it enables delivery of oxygen and nutrients to the rapidly growing tumors (27). Some of these rapidly growing tumors concomitantly also secrete angiopoietins (28).

Tumor Suppressive and Oncogenic Functions of TGF-β in HCC

TGF-β is a cytokine with multiple functions like regulation of cell proliferation, differentiation and extracellular matrix production (29). Deregulation of TGF-β expression or signalling has been implicated in the pathogenesis of a variety of diseases, including cancer and fibrosis. Expression of TGF-β has been reported to be very low that increases dramatically during the later stages of cancer development and is thus actively secreted by tumor cells and thus it does not act merely as a bystander

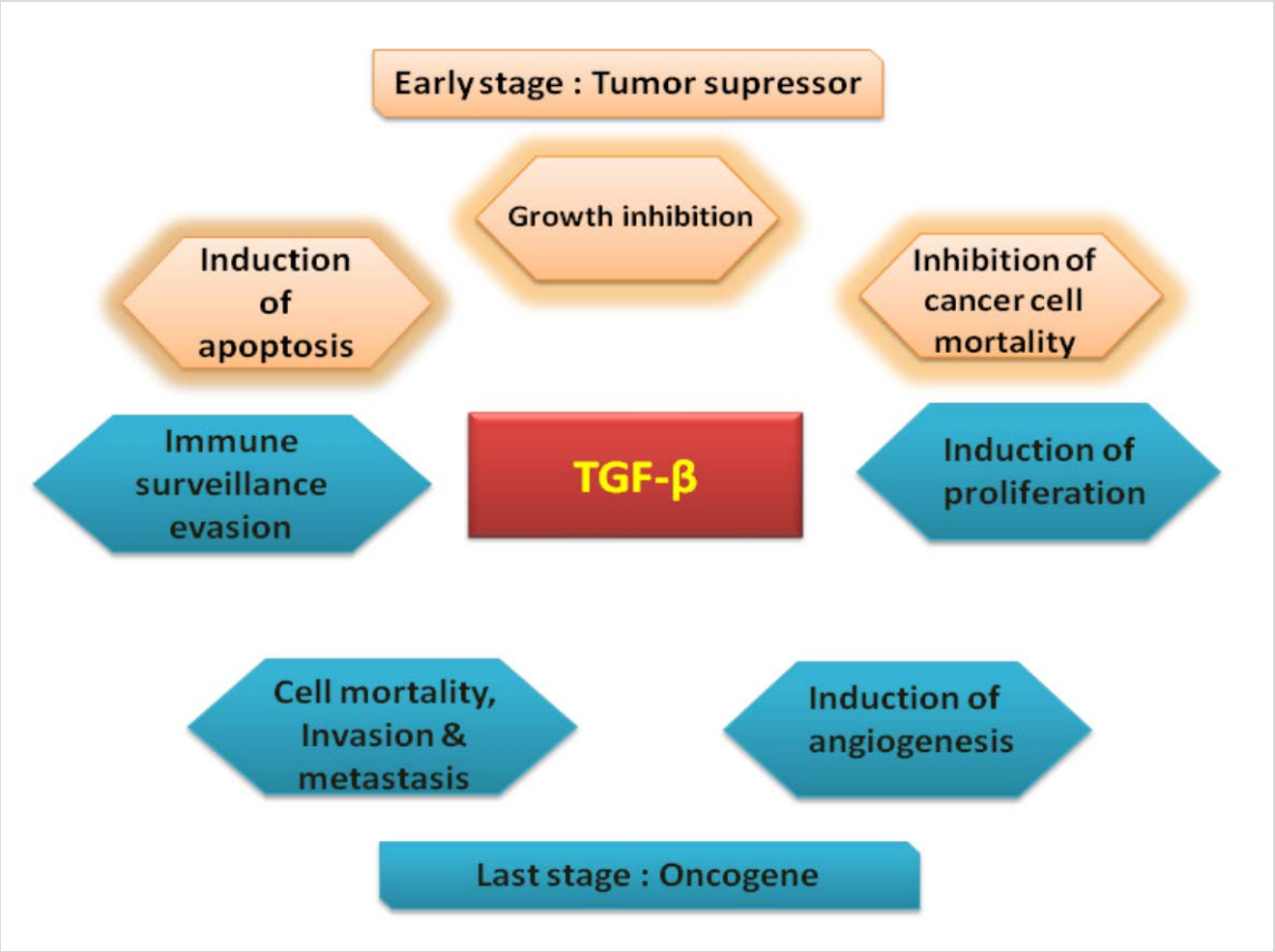

Figure-2. Major areas where TGF-β affects tumorigenesis

but rather contributes to cell growth, invasion, metastasis and decreases host-tumor immune response during advanced stages (30). TGF-β is the prototypic member of a large family of secreted proteins, which can be further divided into two subfamilies, depending on the signal pathways they activate - the TGF-β/Activin/ Nodal subfamily and the Bone Morphogenetic Protein (BMP) subfamily (31).

TGF-βs are able to regulate tumor initiation, progression, and metastatic development. It is widely accepted that TGF-βs act as both tumor suppressors and tumor promoters, depending on the cancer type and tumor development timing (32,33). During the early stage of tumor development, TGF-β1 has been reported to acts as a tumor suppressor mainly due to its ability to inhibit the proliferation of epithelial cells. Further it can also cause G1 cell cycle arrest by declining expression of oncogenes such as c-Myc and ID, and/or by inducing the expression of CDK inhibitors such as p21Cip1 and p15Ink4b (34,35). TGF-β has been reported to play a dual role of tumor suppressor as well as tumor promoter (Figure 2), however till date the mechanism is not very clear. In HCC expression of TGF-β has been reported to increase concomitantly with collagen deposition and the reduction in proteolytic degradation (36).

Tumor suppressor function of TGF-β is evident by the frequent mutational defects of Smad4, TβRI or TβRII (TGF-β type II receptor) observed in many malignancies such as cancers of the stomach, colon, prostate, breast, lung, liver and pancreas (37,38). Dual role of TGF-β has been observed in epithelial cell of liver during the development and progression of HCC. In the initial phase it exerts a tumour-suppressive, which is quite evident by the enhanced susceptibility to HCC because of reduced availability of TGF-β type II receptor (TβRII) due to the ectopic expression of soluble TβRII in hepatocytes (39) or reduced TβRII expression in heterozygous mice has confirmed the tumor suppressive function of TGF-β signalling pathway (40).

In most of cancers that arise from epithelial cells, TGF-β suppressed malignant cell growth by inducing cell cycle arrest through the up-regulation of cyclin-dependent protein kinase (CDK) inhibitors such as p15 and p21, activating apoptosis through induction of pro-apoptotic proteins like Death associated protein kinase (DAP) (41). Contrary to this expression in case of prostate and colorectal cancers, expression of TGF-β1 in cancer cells was found to strongly correlate with progression and metastasis (42-44). Most commonly mutated TGF-β pathway genes in cancer being TGFBR2, TGFBR1, SMAD4 and SMAD2.

Conclusion

HCC exhibits numerous molecular abnormalities, which may be involved in the process of cancer development and progression. Thus, it is important to identify key players which may be involved in the pathogenesis and progression of cancer, so that accurate prognosis can be made and a reasonable selection criterion can be applied to patients with HCC, particularly during the early stages. It has been observed that in various tumors including HCC, whenever there is local invasion of cancer cells or metastasis to distal parts take place, there is elevated levels of VEGF in blood through angiogenesis. Further tumor suppressive function of TGF-β plays an important role. Thus, both VEGF and TGF-β regulates angiogenesis, tumor progression and production of the extracellular matrix and immune suppression in HCC via different mechanism. In such scenario, better under-

standing of VEGF and TGF-β pathway will lead to the development of modalities that can target cancer cells in HCC. Till date the exact mechanisms involved in the malignant transformation of TGF-β is not clear and subject to future research with therapeutic point of view.

Conflict of Interests

The authors declare that there is no conflict of interests.

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Abbreviations

- BMP Bone Morphogenetic Protein
- CTGF Connective tissue growth factor
- ERK Extracellular signal-regulated kinase
- HCC Hepatocellular carcinoma
- HSPGs Heparin sulphate proteoglycans
- MAPK Mitogen-activated protein kinase
- NRP Neuropilins
- TGF-β Transforming growth factor-beta
- VEGF-A Vascular endothelial growth factor-A

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