MINI-REVIEW

Expression and Role of ICAM-1 in the Occurrence and Development of Hepatocellular Carcinoma

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Abstract

Intercellular adhesion molecule-1 (ICAM-1) is a member of the immunoglobulin superfamily, its main function being to participate in recognition and adhesion between cells. ICAM-1 is considered closely related to occurrence, development, metastasis and invasion process of hepatocellular carcinoma (HCC). A variety of inflammatory cytokines and stimulus affect its expression through the nuclear factor-kappa B (NF-ϰB) signal transduction pathway. In the initial stage of inflammation, hepatocirrhosis and tumor development, ICAM-1 is expressed differently, and has varied effects on different cells to promote occurrence of malignancy and metastasis. ICAM-1 has diagnostic significance for AFP-negative or suspected HCC, and may be a prognositic significance. It is thus widely used in studies as a biomarker which reflects cancer cells metastasis as well as curative effect of drugs. Many new treatments of HCC may be based on the effects of ICAM-1 on different levels of function.

Keywords: ICAM-1 - NF-ϰB signal transduction pathways - hepatocellular carcinoma - inflammation

Introduction

Intercellular adhesion molecule-1(ICAM-1) is a member of the immunoglobulin superfamily, which belongs to Cell adhesion molecule (CAM). The interaction, mediated by ICAM-1, between cell-cell and between cells and extracellular matrix, is a form of communication between cells, which plays an important role in cells differentiation, movement, and immunity. ICAM-1, one of the most important molecule in the tumor-associated mechanism, often be used for an index of tumors arises, metastasis, recurrence and prognosis. In this review, we have described the expression and the role of ICAM-1 in the occurrence and development of liver cancer.

Basic Characteristics of ICAM-1

Features of ICAM-1

ICAM-1 is a highly glycosylated single-chain glycoprotein with a 55KD polypeptide core. The main function of ICAM-1 is to participate in recognition and adhesion between cells. The receptors of ICAM-1 are lymphocyte function associatedantigen-1 (LFA-1) and macrophage surface antigen - 1 (Mac-1), and the type of combine is heterogeneous affinity. ICAM-1 is the essential molecule in the interaction between LFA-I-dependent T-cells and fibroblasts, as well as the agglutination between lymphocytes or lymphocyte and endothelial cell under the stimulation by phorbol ester. The effects mentioned above could be restrained by their own monoclonal antibodies.

Distribution of ICAM-1

ICAM-1 is a large class of glycoprotein molecules which located on the surface of cells. They mainly distribute on lymphocytes in the bone marrow, whereas, there is almost none on the peripheral blood cells. In normal tissues, ICAM-1 is expressed mostly in the spleen. Because the normal lymphocytes express low levels of ICAM-1, what's more, lymphocytes provide a good environment for ICAM-1 expression, all that had been described in Sarah H. Hayes and Gail M. Siegel's experiments (Hayes et al., 2009). In addition, in vascular endotheliocytes, mucous epitheliums of the tonsils, the thymus epitheliums, hepatic sinusoids lining cells, as well as some fibroblasts in some tissues have low levels of ICAM-1's expression (Dustin et al., 2011).

ICAM-1's expression relatively increased in metastatic tumors, what is also the highest in lymphoid tissues (Hayes et al., 2009). But glands, and some connective tissue/skin have the lowest expression, the reason of the latter is not clear. In a variety of tumors, besides the tumor cells expressing different levels of ICAM-1, almost in all of them can find lymphocytes with ICAM-1. Lymphocyte with ICAM-1 can't be found in brain because of the blood-brain barrier. The tumor in lymphoid tissue is easier to invade than non-lymphoid tissues, which may be related to the function of immune cells in tumor metastasis (Dustin et al., 2011).

Expression of ICAM-1

Human ICAM-1 genes are located on chromosome

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19p13.3 ~ 13.2 areas, 15.5kb long. Various cytokines and stimulation effect the expression of ICAM-1 will not directly by acting on ICAM-1's DNA, but through activating nuclear factor-kappa B (NF-κB) signaling pathway, stimulating its transcription and translation.

NF-κB signaling pathway is basically composed of receptor and signaling adapter protein on proximal of receptor, IκB kinase complex, IκB proteins and NF-κB dimers. It is the key point in the mechanism of hepatocarcinoma. Under normal circumstances, the two subunits of NF-κB, P50 and P65 sequestered in the cytoplasm by its inhibitor proteins IκB, so they are in a non-active state (Moon et al., 2011).

NF-κB's activity is controlled at several levels, including nuclear translocation, post-translational modifications, such as phosphorylation and acetylation, and DNA binding. The mechanism accepted now is as follows: TNF-α, IL-1β and other inflammatory factors, can activate I-κB kinase (IKK), leading IκB phosphorylation, ubiquitination, and then IκB protein is degraded, NF-κB dimers are released. NF-κB dimers are further activated through a variety of translation and modification, which made their nuclear localization signals exposed, and are rapidly induced into the nucleus, combining with specific binding locus. In the initiating-process of transcription, they participate in mRNA synthesis, and achieve the regulation of genes' expression. High level of expression of ICAM-1 is related to the content of mRNA in the tissue; and the latter content level is based on the degree of cells malignant transformation in liver tissue. Otherwise, the expression of ICAM-1 mRNA is related to the degree of cirrhosis and metastasis in the liver. ICAM-1 is a downstream factor of NF-κB pathway, and its expression is mainly affected by NF-κB pathway. Therefore, down-regulation of ICAM-1 expression through suppressing NF-κB pathway may restrain cancer's adhesion and invasion, what might become a potential treatment of hepatocellular carcinoma (HCC) (Prasad et al., 2009; Connelly et al., 2011).

ICAM-1 Expression and Significance in the Evolution of HCC

ICAM-1 expression and significance in various liver diseases

In normal liver tissue, hepatocytes don't express ICAM-1, liver sinusoidal endothelial cells (LSEC) and vascular endothelial cells have a faint expression of ICAM-1. In various kinds of hepatitis, the expression of ICAM-1 is enhanced in LSECs and vascular endothelial cells, and it will be positive on liver cells, bile duct epithelial cells, lymphocytes, and fibroblasts. ICAM-1 levels in the serum of patients with liver disease will be significantly higher than normal, which help the lymphocytes adhere to the vascular endothelium, infiltrating to the liver tissue, what is an extremely important step in immune responses. In many injuries, ICAM-1 up-regulation more often occurred before the immune response to injuries.

Liver is an important site for T cells differentiation. T cells in the liver express more LFA-1 antigen than in peripheral, and LFA-1 is the basis of ICAM-1 adhesion.

LFA-1/ICAM-1 interaction mediating cells adhesion and migration is conducive to the differentiation of T cells in the liver (Shetty et al., 2011). The process of T lymphocytes' recognizing and killing target cells is restricted by major histocompatibility complex (MHC), and adhesion molecules are also indispensable. ICAM-1 is a kind of molecule which plays a central role in the process of cell-cell contacting. Cells over-expressing ICAM-1 may become targets for T cells infiltration. ICAM-1 can enhance immune cells adhesion and migration though binding with lymphocyte function-associated antigen -1 (LFA-1), and increase the immune cells susceptibility of cytotoxic damage, thus, mediate immune-mediated liver injury. With chronic inflammatory stimulation, TOLL-like receptors (TLRs) can induce tunmorigenesis and immune escape, but the mechanisms remain controversial. NF-κB pathway has been researched mostly, TLRs could activate the NF-κB pathway though MyD88, and then raise ICAM-1 expression. TLR3 seem to be an indispensable factor in ICAM-1's up-regulation, they regulate the interaction between cells' function and white blood cells' function though adjusting the expression of ICAM-1, but experimental evidence is also deficiency (Zhang et al., 2011).

NF-κB pathway activated by the above process, increases the expression of ICAM-1, and effects on creating of inflammatory cytokines, and many other aspects such as cells' against apoptosis, cells' survival and proliferation, angiogenesis, tumor's progression and metastasis (Porta et al., 2011). One study showed that CD45 marked tumor inflammation was significantly related to ICAM-1 expression (Prasad et al., 2009). ICAM-1 expressed in vascular endothelial cells can interact with the leukocyte integrin CD11/CD18, make inflammatory cells solidly combined in the vascular endothelium, and induce inflammatory cells transude to the outside of vascellum, so as to promote inflammation. On the other hand, ICAM-1 secreted by tumor cells themselves can promote lymphocyte and microglia cells accumulation; thereby contribute to inflammatory reaction.

HBV and HCV infection is closely related to the incidence of HCC. When patients are infected with HBV, HBV-specific cytotoxic T lymphocyte (CTL) secrete IFN-γ and TNF-a, which stimulate the hepatocytes to produce ICAM-1 mRNA and express ICAM-1. ICAM-1 lead T cell to destruct HBV infected hepatocytes and clear HBV. What's more, ICAM-1 can improve CTL's ability to identify and kill HBV infected hepatocytes, but also can make serum ALT, AST and TB increase at the same time (Shi et al., 2010). That let to speculion that a rise of SICAM-I in serum may be due to hepatocytes' secretion or ecclasis, and also related with lymphocyte's infiltration and hepatocytes weak inactivation of SCIAM-1 after damaged. So, ICAM-I, at a certain degree, can reflect the extent of the necrosis and dysfunction of hepatocytes.

ICAM-1 expression and significance in liver cirrhosis

About 50% to 90% patients of HCC are associated with cirrhosis. Cancer-associated fibroblasts (CAFs) are the major cell types in the solid tumor or the neoplasm. They play important roles in cancer invasion, angiogenesis and

metastasis. Normal fibroblasts can promote tumor cells differentiation, and inhibit the growth of tumor cells, but CAFs can stimulate tumor cells' growth and infiltrating. Micro-environment composed by interleukin-interleukin (IL) -6 and activated ICAM-1 expressed by CAFs, may support the metastasis of cancer cells. Lars Mueller's study found that TNF-a clearly induced the expression of ICAM-1 mRNA by CAFs and LFs (Mueller et al., 2010). Their results also raised the possibility that CAFs activated by inflammatory chemicals provide a significant cellular source of ICAM-1 (Mueller et al., 2010). The up-regulation of ICAM-1 is controlled by TNF-a on two levels: transcription and protein expression.

ICAM-1 expression and significance in HCC

Normal human's hepatocytes do not express ICAM-1, sICAM-1 only express weakly on Sinusoidal endothelial cells and vascular endothelial cells on the portal system. The level of ICAM -1 in HCC patients is significantly higher than the normal. The investigation made by Shunichi Matsuoka et al on ICAM-1's expression in diethylnitrosamine-induced liver has shown that: even in the same liver, in normal liver tissue area, the area of atypical hyperplasia and the cancer region, ICAM-1's expression is different (Matsuoka et al., 2009). ICAM-1's expression in endothelial cells and mesenchymal cell is inverse to the degree of atypical hyperplasia, but in the hepatocytes, that is on the contrary, the density of ICAM-1 on cytomembrane and cytoplasm of hepatocytes is significantly higher than non-cancerous cells. In addition, ICAM-1 found in the endoplasmic reticulum of dysplasia liver cells shows that, besides secreted by immune cells which stimulated by tumor antigens, ICAM-1 is also released by tumor cells themselves, what may be one mechanism of tumor cells evading the body's immunity. ICAM-1 promote cell's atypical hyperplasia, but the exact mechanisms are not yet clear, however, the atypical hyperplasia tissue and the normal tissue can be distinguished by it (Matsuoka et al., 2009).

The level of ICAM-1 in stage III, IV HCC is obviously higher than it in stage I HCC, and is significantly increased in metastatic HCC. SICAM-1's level is markedly decreased after surgery removing the tumor. While in the tissues of HCC, the expression of ICAM-1mRNA shows the same pattern. Visibly, ICAM-1 in tissues and serum may suggest the stage of liver cancer, imply the potential of invasion and metastasis, and play a role in the process of metastasis

Oval cells (OVCs) is a kind of adult stem cells located in liver tissue, which have the potential of self-replication and multi-differentiation. They derived from bone marrow hematopoietic stem cells (HSCs), and play an important role in physiological and pathological physiological processes of hepatocytes' development, growth, proliferation and cancerization. It can differentiate into liver cells in some specific state or micro-environment. When the hepatocytes are damaged severely or their proliferation is inhibited, OVCs are activated and express ICAM-1. ICAM-1 can mediate directional migration of OVCs, make these precancerous cells migrate in the liver tissue and differentiate into hepatocarcinoma cells. This process can be enhanced by inflammatory mediators in the early stage and be affected by NF-ϰB. TNF-α and TGF-β control the expression of ICAM-1 by OVCs, through promoting or inhibiting NF-ϰB pathway, and then have an affect on HCC.

ICAM-1 and HCC Metastasis

ICAM-1 is highly expressed on the membrane of hepatocarcinoma cells. Its expression related to tumor's size and whether the capsule is complete. While the size, capsule and the quantity of the tumor decided whether the tumor has invasiveness. It indicated that ICAM-1 is closely related to the metastasis of HCC. The mechanism may be act like that, ICAM-1 combine with its natural ligand LFA-1, leading cancer cells' ecclasis, and then these cells can walk with the lymphocytes to the circulation, transferring to other place. In the small hepatocellular carcinoma with a capsule, the adhesion intercellular is closely under the capsule; ICAM-1's expression is not increased. When the capsule is ruptured by the tumor, the tumor cells would adhere to the extracellular matrix, and with the help of positive movement of tumor cells, the matrix is degradation and the cancer cells invade the vascular and surrounding tissue.

Other studies suggested that, ICAM-1 which combine can trigger the calcium concussion, activate protein, and take a part in disint egration of the cells' local adhesion, contraction of cells and the reconstruction of cytoskeleton, so as to promote the transfer of cancer cells (Bernier et al., 2011). Studies on "high fat diet" mouse found that the level of cytokines like sICAM-1, IL-16 had risen in the serum of "high fat diets" mouse, and these cytokines could help to change the expression of metastasis associated protein, and promote the transfer of tumor (Kim et al., 2011).

What's more, the high expression of ICAM-1 helps cancer cells to separate from each other and transfer. Sarah H. Hayes's studies have shown that cancer cells' adjustment on the expression of ICAM-1, on one hand, can make it easier to metastasize and invade, and not to be recognized by immune cells, on the other hand, can effectively prevent it from be dissolved and killed by the immune cells, and benefit to escaping the destruction of specific cell-mediated immunity.

At the same time, ICAM-1 plays a key role in tumor angiogenesis. The current study shows that, NF-kB is strongly activated in the early stage of artery damage, and together with increasing expression of ICAM-1, MCP-1, TNF-a, IL-1b, IL-6 and so on. ICAM-1 and VCAM -1 strongly raise various inflammatory cytokines into the area, promoting the formation of vascular intima (Song et al., 2011). Studies on isolated endothelial cells show that ,isolated tumor endothelial cells (TEC) and normal endothelial cells (NEC), together, expressed CD31, CD105, VE-cadherin, vascular cellular adhesion molecule-1 (VCAM-1), ICAM-1, and E-selectin, and formed capillaries in basal membrane (Naschberger et al., 2011). Calreticulin (CRT), which located in the endoplasmic reticulum, recently, has been report that in addition to inhibiting angiogenesis, it can also enhance the expression of ICAM-1 on tumor endothelial cells to

improve the antitumor effects (Wang et al., 2012).

Significance of ICAM-1 in HCC Diagnosis and Prognosis

HCC is a kind of malignancy which is difficult to predict. It usually early presents the invasion to vascellum and metastasis, so to detect HCC in an early is extremely important for patients to get the surgery opportunity. In some patients with serum AFP-negative, space-occupying-lesion in liver were found in imaging studies 1-4 months after sICAM - 1 had prompted the recurrence of tumor (sICAM-1> 1 000 µg/L) (Mueller et al., 2010). Clinical researches showed that the level of sICAM-1 was significantly higher in HCC group than that in the benign tumor group and normal control group, and was positively correlated with the degree of cirrhosis (Jung et al., 2012). The same conclusion was detected at gene level (Rizk et al., 2012). Thus, sICAM-1 has a value of auxiliary diagnosis in AFP negative or suspicious positive patients. It not only can be used as a biological index for early diagnosis, but also has an important clinical significance for assessing HCC patient's condition, curative effect judgment, recurrence and prognosis assessment.

More interesting is that, ICAM-1may become a breakthrough for some non-invasive technique to detect organ-specific inflammation. The superparamagnetic iron oxide (SPIO)-based nanomicelle termed leukocyte-mimetic nanoparticle (LMN) can preferentially localize to cells with inflammation-induced overexpression of ICAM-1.While the novel MRI quantitative susceptibility mapping (QSM) technique can map LMN to describe the level of inflammation in organs (Wong et al., 2012).

Development of Therapeutic and Preventive Approahces for HCC using ICAM-1

Currently, people are actively studying the new methods in treating or preventing against HCC. Many drugs are proven, on the molecular level, effective at anti-tumor through ICAM-1. Such as: cinobufotalin and silymarin, which can reduce ICAM-1's expression by TNF-α working on NF-κB pathway, so as to inhibit tumor metastasis (Li et al., 2010; Yin et al., 2013). Dexamethasone and Tan could antagonize ICAM-1 and play a role in suppressing tumor metastasis. The monoclonal anti-body which anti-ICAM-1 can significantly reduce the adhesion between leukocytes and SLE after ischemic, and obviously reduce the injury of liver tissue. Some ICAM-1-related cytokines and even genes are also being considered: FK506 and cyclosporine inhibit the sICAM-1 in HLE and inhibit cancer's growth and invasion. KAI1 gene inhibits invasion and metastasis of hepatocellular carcinoma MHCC97 - H cells in vitro and in animal models.

Summary

In short, ICAM-1 is closely related to all about occurrence, development and metastasis of HCC. A variety

of inflammatory cytokines and stimulus have effect on the expression of ICAM-1 through NF-κB signaling pathway. In the initial stage of inflammation, ICAM-1's expression increased mainly in the sinus endothelial cells and vascular endothelial cells. Its significance is to mediate lymphocytes adhesion to vascular endothelial cells, infiltrate the liver tissue and destruct HBV infected liver cells by T cell-mediated and cleare HBV. In cirrhosis of the liver stage, ICAM-1 and (IL) -6 etc compose the micro-environment, which may support the transfer of cancer cells. In the high tumor stage, cancer cells' ICAM-1 expression not only helps to separate and metastasis between cancer cells, but also helps them to evade immune destruction of specific cells. What's more, it mediated OVCs' directional migration, differentiation and precancerous cells' intrahepatic metastasis. Diagnostic significance of ICAM-1 is to detect HCC with AFP-negative or suspected-positive earlier, and prognosticate their metastasis and prognosis. As a biomarker, ICAM-1 can reflect cancer cells' metastasis and prognosis. Now it is widely used in various studies, and can well assess the curative effect of drugs, genes, factors. Many new treatments of HCC are based on the effects on ICAM-1 in different levels, producing antitumor function, but the mechanisms are still not fullyknown.

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