Research advance on role of Coxsackie and adenovirus receptor (CAR) in tumor progression

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Coxsackie and adenovirus receptor (CAR) is originally identified as the cellular receptor of 2- and 5-type adenoviruses. Many researches have suggested that CAR can affect the growth, adhesive ability and cytoskeleton of tumor cells, and has complicated functions in metastasis and invasion of tumors. Moreover, the expression of CAR has close relationship with tumor progression and cytoreduction mediated by adenoviruses. CAR has become a new hotspot in the research on mechanism of tumor progression and gene therapy. Our review focuses on the structure and function of CAR and its role in mediating occurrence and progression of tumor.

Biological behaviors of multiple cells are involved in the development and progression of malignant tumors. A lot of molecules can cause malignant transformation of normal cells and changes of microenvironment via interactions between tumor cells and host normal cells. They also are involved in many important biological processes, such as signaling transduction, invasion, metastasis and anti-apoptosis of tumor cells, and so on. However, studies on roles of such molecules in tumor progression, invasion and metastasis are still very limited. Understanding the roles and interactions of these molecules would greatly benefit the diagnosis and treatment of cancers. Coxsackie and adenovirus receptor (CAR) is initially discovered and identified as a cellular receptor for type II and type V adenoviruses. In recent years CAR has attracted much attention due to rising of adenovirus vector-based gene therapy. Complicated biological functions of CAR have been found since then. Subsequently, further studies on CAR have revealed that CAR not only has physiological functions in alloantigen adhesion, leukocyte extravasation, protein transport, growth regulation of cells, and so on, but also plays important roles in tumor progression, invasion and metastasis. Due to the imminence of tumor progression and gene therapy, studies on CAR mainly focus on these two aspects. Research advances on the roles of CAR in tumor progression are reviewed in this article.

Cloning of CAR and Relationship Between Its Structure and Basic Functions

Structure and location of CAR. CAR, with a molecular weight of 46 ku, is a type I transmembrane glycoprotein and belongs to immunoglobulin (Ig) superfamily. It consists of extracellular, transmembrane and intracellular domains. Its open reading frame encodes 365 amino acids, including one fragment of leader sequence of 19 amino acids, one extracellular domain of 216 amino acids, one transmembrane domain of 25 amino acids and one intracellular domain of 105 amino acids. At present, it is thought that the extracellular domain of CAR is mainly associated with virus infection and homotypic adhesion, while the transmembrane and intracellular domains are related to cell growth, adhesion, and neoplasm formation and metastasis of tumor cells. Structural analysis of CAR protein suggests that its intracellular domain contains many protein modification and binding site sequences, which are related to its complicated functions. For example, initial two amino acid Cys-Cys residues in the transmembrane and intracellular domains are the sites for lipid modification after transcription, and C-terminal GSIV motif is a binding site of PDZ protein. Intracellular site deficiency mutations affect biological functions of CAR. For example, Cys-Cys residues are required for cellular expression and locating of CAR, while some other deficiency mutations would lead to ectopic expression of CAR in different cellular regions.

CAR locates on cell membrane, while CAR has its sublocation in tissues. Raschperger et al. used immunofluorescent methods to stain different tissues in mice, and found that CAR is mainly expressed in closely-connected tissues, such as mesothelial cells in the endocardium, cells in nerve-muscle joint of the bones, hepatocytes, and so on, but is not expressed in the artery, vein, lymph ducts and nodes. Sublocation of CAR indicates that CAR-based gene therapy via adenovirus mediation should be tissue-specific. Selective distribution of CAR in tight junctions suggests its roles in stabilizing homeostasis and osmosis.

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CAR glycosylation and its significance. Protein glycosylation plays vital roles in protein folding, configuration, location and stability as well as connection between cells. Furthermore, glycosylation affects many physiological processes, such as cell adhesion, translocation, recognition and binding of a ligand to its receptor, and so on. Therefore, glycosylation is involved in the development of many diseases. CAR is the cellular receptor of most adenoviruses and Coxsackie B viruses. It is well known that CAR is also identified as a glycoprotein because it contains two extracellular Ig-like regions: N106 in D1 region and N201 in D2 region. Excoffon et al. transfected glycosylated CAR and glycosylation-mutated regions into CAR-negative cell line CHO-K1, and found that glycosylation did not affect intracellular location of CAR, but it was required for cell adhesion; when deglycosylation of D2 region in CAR was defect, the binding rate of adenovirus was reduced (Theil coefficient = 0.93) accordingly, while glycosylation of D1 region did not affect the rate (Theil coefficient = 1.06). Many aspects still remain unclear, such as, whether there are other potential unfound glycosylation sites in CAR and their roles, whether adenovirus binding depends on the presence or certain level of glycosylation, as well as the roles of CAR glycosylation in its various biological behaviors.

Roles of CAR in Tumor Development and Progression

Relationship between CAR expression and tumor growth. Zhang et al. transfected CAR into CAR-negative bladder cancer cell line T24 via retrovirus vectors and used transfected and untransfected cells to conduct animal tumor formation experiments, and found that the volumes of tumors formed from CAR-transfected cells were obviously smaller than those of tumors from untransfected cells [(217 ± 30) mg vs. (651 ± 84) mg, p < 0.05]. However, Qin et al. used both CAR-silenced and -unsilenced non-small cell lung cancer NCI-H1703 cells to form subcutaneous tumors in immunodeficiency mice, and found that tumor volumes in CAR-silenced group were significantly smaller than those in control group (p < 0.001) after 40 days, the tumors continuously grew only in 20% of the mice in CAR-silenced group three months later while the tumors in remaining mice disappeared, re-expression of wild type CAR was detected in tumor tissues in the 20% mice of CAR-silenced group by flow cytometry, indicating CAR plays an important role in promoting tumor formation. The two contrary conclusions suggest differences in the biological behaviors of CAR in different kinds of tumors. Therefore, to unveil underlying molecular mechanisms of these phenomena and to find commonness in the differences come to be more important.

Effect of CAR on skeleton of tumor cells. Fok et al. transfected recombinant plasmids containing full length of CAR into human glioma cell line U87-MG, and found that compared with the cells transfected with blank plasmids, CAR-transfected cells were weaker in invasion capability (p < 0.01); furthermore, indirect fluorescent staining indicated that CAR affected cell invasion through direct interaction with microtubules. While Huang et al. investigated neuronal growth cone particles with high expression of CAR, and found that CAR directly bind to F-actin with overlapped locations. The above findings suggest that CAR plays an important role in affecting metastasis of tumor cells through dynamically regulating cellular skeleton structure.

Effect of CAR adhesion function on tumor progression. In the development and progression of malignant tumors, the adhesions between cells or between cells and matrix play decisive roles in the malignant phenotype of cells. It is known that adhesion molecules, such as Integrin and Cadherins, are involved in many biological processes, including signaling transduction, invasion and metastasis, during tumorigenesis via interactions between tumor cells and host normal cells. Among the novel adhesion molecules, CAR is one of them drawing the most attention. It is involved in cellular adhesion and cellular motility, and its extracellular domain mediates homotypic adhesion. CAR has a similar expression pattern to E-Cadherin in clinical tumor tissues: its down-regulated expression in primary lesions leads to metastasis of tumor cells; while its re-expression in metastatic lesions causes cell adhesion to metastatic tissues. Moreover, studies have proven that CAR can regulate expression levels of other adhesion molecules. Wang et al. proved that exogenous expression of CAR promoted homotypic adhesion between tumor cells and reduced malignant invasion phenotype of tumor cells by in vitro experiments. Yamashita et al. introduced recombinant plasmids containing full length of CAR into mouse melanoma cell line B16, and found that the migration rate of transfected cells was obviously lower than that of untransfected ones (p < 0.001); when injected B16 cells via the tail vein in mice, the aggregation rate of CAR-transfected B16 cells in the lungs was evidently lower than that of untransfected B16 cells (15% vs. 85% at 2 h; 21% vs. 79% at 24 h; 9% vs. 91% at 48 h); the mRNA level of integrin was reduced after CAR transfection. All these data suggest that CAR inhibits tumor metastasis which might via down-regulating integrin expression.

Cytoreduction Function of Adenoviruses Mediated by CAR

Adenoviruses infect local tumor cells, diffuse into neighboring cells via bystander effect and oncolysis and exert cell-killing effect to reduce tumor load. The bystander effect refers to that exogenous killing genes (such as Adv-TK) diffuse from virus-infected cells to uninfected cells via intercellular passages. Oncolysis is a process in which released viruses infect adjacent cells after virus replication and lysis of infected tumor cells. Many studies have proven that adenoviruses stick to cells via CAR and enter into target cells via cellular endocytosis mediated by integrin. Adenovirus-mediated Cytoreduction is well known, but its transduction efficiency is still nonideal. Based on pivotal roles of CAR as a receptor of adenovirus-targeted tumor cells, a lot of studies focus on the effects of CAR distribution and expression on its efficiency. Many studies show that the expression level of CAR is negatively correlated to the malignant extent of tumors, such as bladder cancer, head and neck squamous cell carcinoma, and endometrial carcinoma. While some other studies show that the expression level of CAR is positively correlated to the malignant extent of tumors, such as prostate carcinoma and breast cancer. All these indicate that the distribution of CAR is tissue-selective and its expression...
level decides, to a large extent, the transduction efficiency and cytoreduction of adenoviruses. Qin et al. used adenovirus vectors to transfect genes into a series of non-small cell lung cancer cell lines (NCI-H226, NCI-H1703, NCI-H1437, and NCI-H2122) with different expression levels of CAR and detected the mRNA and protein levels of the carried genes, and found that the expression level of CAR is positively correlated to the transduction efficiency of adenoviruses and elevating the expression level of CAR by transfection enhances the infection rate of adenoviruses (p < 0.05).

Based on these findings, many researchers start looking for approaches to enhance CAR expression and expect to increase cytoreduction of adenoviruses. Chen et al. used HDAC inhibitor TSA to treat ovarian carcinoma cell line A2780, and found that with the up-regulation of CAR expression, the intratumor killing effect mediated by ADV/TK on A2780 cells treated by 5 and 100 nmol/L TSA was 4-10 times of that on untreated cells. Anders et al. inhibited pivotal genes MEK and Raf-1 in Raf-MEK-ERK pathway, and found that the expression level of CAR was up-regulated and the oncolysis of adenoviruses was enhanced by 28 folds of control. This indicates that after CAR expression is increased, the killing effect of adenoviruses on tumor cells is really enhanced and CAR plays an important role in cytoreduction mediated by adenoviruses.

**Relationship Between CAR and Tumor Prognosis**

According to above findings, it is shown that CAR affects tumor progression and cytoreduction mediated by adenoviruses. Therefore, the relationship between CAR and tumor prognosis attracts more and more attention. Giaginis et al. examined CAR expression in 41 cases of endometrial adenocarcinoma, and found that the expression level of CAR was negatively correlated to differentiation status (p = 0.016) while positively correlated to diffusion extent of tumors (p = 0.057), and the patients with high expression level of CAR had long survival (median follow-up: 64 months). Martin et al. studied 114 cases of breast cancer, and discovered that the expression level of CAR was higher in the patients with tumor diffusion and metastasis than in control group (p < 0.05) and high expression of CAR was correlated to long-term survival ( > six years) of breast cancer patients (p < 0.01). Similar studies are done in ovarian carcinoma. Reimer et al. proved that CAR was associated with the malignant biological behaviors of ovarian carcinoma and high expression of CAR was an independent factor affecting prognosis. Above studies prove that CAR affects the prognosis of tumors, however, due to the tissue-specific and distribution-specific features of CAR, its effects on different tumors and the mechanisms need to be further studied.

**Perspectives**

Tumor development and progression is a process which involves multiple factors, multiple steps and multiple genes. So far, the mechanisms of tumor development and progression still remain unclear and approaches of gene therapy have to be improved. CAR is a cellular receptor for coxsackie-adenoviruses and attracts attentions due to gene therapy mediated by adenoviruses. It has been proven that both distribution and expression level of CAR affect the efficacy of gene therapy mediated by adenoviruses, but its mechanisms still need to be investigated. Moreover, CAR has many biological effects during tumor development and progression. Possibly due to different experimental methods and designs, the conclusions from different researchers are not unanimous, and these discrepancy need to be corrected or analyzed further. Fully understanding mechanisms of the functions of CAR in tumor progression and gene therapy mediated by adenoviruses would greatly benefit the recognition of biological nature of malignant tumors, also provide a new and more effective platform for therapy of malignant tumors.

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