5T4 Oncotrophoblast Glycoprotein: Janus Molecule in Life and a Novel Potential Target against Tumors

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5T4 oncotrophoblast glycoprotein is a transmembrane protein expressed on the embryonic tissue and various malignant tumor cell surfaces. It plays a vital role in the multiple biological and pathological processes including massive cellular migration during the embryogenesis, cell invasion associated with implantation, and neoplastic metastasis in the progression of tumorigenesis. Its restricted profile of expression stratifies criteria of tumor-associated antigen and makes it a new promising candidate for immunotherapy for cancer. Hence, illustrating this molecular function is necessary for discovering the principle of the tumor diffusion and aggravation and is helpful for developing novel and effective strategies of cancer therapy.

Key Words: 5T4 oncofetal antigen, tumor-associated antigen, immunotherapy, tumorigenesis

Introduction

5T4 oncofetal antigen is a kind of heavily glycosylated transmembrane protein defined by a monoclonal antibody raised against wheat germ agglutinin-glycoprotein from human syncytiotrophoblast microvillus plasma membrane (StMPM) (1). This protein has been chemically characterized as a 72 kDa product encoded by the TPBG gene (trophoblast glycoprotein gene) (2, 3). A wealth of researching have shown that 5T4 oncotrophoblast antigen is associated with diverse physiologically and pathologically cellular events, such as the cellular migration, the increase of motility, the change of the morphology and the integrity of the membrane (4, 5). It is indicated that 5T4 glycoprotein plays a bifunctional role in different biological processes. Through the immunohistochemical analysis, this trophoblast cell surface antigen is found to be expressed on a variety of carcinomas and weakly on the specialized epithelia but with a restricted pattern of expression in normal adult tissues. This profile of the expression, undoubtedly, makes it qualified as a tumor-associated antigen, a target for anti-cancer therapy.

A positive role in biological development

5T4 oncofetal antigen was first defined on the embryonic cell surface with a heavily glycosylated modification before two decades, and the biological and physiological functions of this protein in vivo had been elusive for many years. Nowadays, however, more and more studies in this field have given copious insight into this attractive aspect in the range from its molecular structure to its distribution, furthermore, to its potential functions.

5T4 protein with a high level expression is a type I membrane protein whose extracellular domain contains seven leucine-rich repeat regions (LRRs) flanked by cysteine-rich region (6, 7), which are believed to mediate specific protein-protein interactions (8, 9), and includes a cytoplasmic region that contains a PDZ domain-binding motif (10). Hence, there is a hypothesis that 5T4 oncofetal antigen is involved in the process of the embryogenesis. In murine embryonic experiments, the results indicate that 5T4 antigen is first expressed following hatching of blastocyst from zona pellucida, an event that occurs immediately prior to embryo implantation. Furthermore, during murine post-implantation development, 5T4 exhibits restricted expression to different epithelial cell types derived from all three germ layers, and in some regions of the brain (11). The process of implantation includes the invasion of trophoblast cells into the maternal host tissue to establish the embryo within the uterus. So, it is suggested that 5T4 antigen is involved into this event, and that it also participates into the developing epithelia and neural system (6).

Another study shows that there is a relationship between the early differentiation of mouse embryonic stem cells and the expression of 5T4 antigen coincident with the cellular processes.
The direct evidence on the involvement into pathological events, such as metastasis of cancer cells is from the immunohistochemical analysis to the plentiful specimens of different carcinomas (1). The result demonstrates that 5T4 protein is expressed at high level on the surface of the tumor cells including the carcinoma of ovary (14), colon (15), stomach (15, 16), lung (17) and cervix (18), but most normal or non-neoplastic tissues is negative except some specialized epithelial (19). Furthermore, the data from clinics show that the 5T4 expression is strongly associated with metastasis in patients with diverse cancer (20-22). Besides these, more facts from studies on the function of 5T4 gene ex vivo support this hypothesis that 5T4 oncofetal antigen promotes the invasion and diffusion of malignant tumors.

The group of Peter Stern shows that overexpression of 5T4 cDNA in the transfected murine cells leads to the change of cellular morphology and cellular motility (5). In the mice epithelial cells stably expressing full-length 5T4 cDNA, cell-cell contact is altered and 5T4 expression is associated with dendritic morphology, accompanied by abrogation of actin/cadherin-containing contacts and increased motility (4). The experiment also displays a “polkadot” pattern of 5T4 antigen expression: heterogeneous in intensity between cells, but distributed over the entire cell surface. Through the cellular assay, it is also shown that 5T4 is concentrated at microvillus projections of the plasma membrane accordant to the observation in various carcinoma cell lines. These projections function in cell adhesion and invasion by expressing an array of surface molecules.

Cellular movement, such as migration, invasion, and the reduction of adhesion is a complex and integrated process, which is associated with diverse molecules assembly, multiple signaling pathways cross-talking and various interactions between cells and cells and/or environment. As a sensor, 5T4 cell surface antigen can shuttle different signals between cytoplasm and outer surrounding through both extracellular and intracellular domains (4). The former includes LRR short sequence motif and heavily glycosylated N-linked sequence, which are involved into cell attachment to influence the motility (23); the latter contains PDZ binding domain (10), which is capable of interacting with PDZ domain protein that plays a central role in organizing diverse cell signaling assemblies, and could direct remodeling of cytoskeleton, alter the integrity of cellular membrane and then influence the cellular contact or/and adhesion (24).

Hence, 5T4 antigen is a bifunctional molecule as janus which plays opposite roles in life. Recently, however, this molecule has become attractive in anti-cancer immunotherapy as a new promising candidate (2) because of its restricted expression pattern on normal adult tissues but high expression on tumor cells and the significant relationship with the poor prognosis in various cancers including colorectal carcinoma, gastric carcinoma, cervical cancer, ovarian carcinoma and so on (14, 16, 20-22, 25).

**A negative role in pathological events**

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**A novel promising target to immunotherapy for cancers**

Historical data show that immune system clearly plays a role in cancer progression. For example immunosuppression is associated with cancer development (26). And it is suggested that tumors are immunogenic based on the fact that some tumor-specific antibodies or/and tumor-specific immune effector cells, which could recognize some components, so-called tumor antigen, encoded by cancer-causing genes can be detected in patients who suffer different cancers. This makes it possible to stimulate the immune system to attack cancers (27).

As above mentioned, 5T4 oncofetal antigen is expressed at a high level on the surface of trophoblast membrane and a variety of malignant tumor tissues but with a restricted pattern of expression on the normal adult tissues expect weakly positive in some specialized epithelia. It therefore satisfies the criteria for a tumor-associated antigen (TAA) and is an ideal target for the immunotherapy of cancers (2, 28). Furthermore, 5T4 cellular surface antigen is able to promote the metastasis and the invasion of malignant cells in the progression of cancers. Hence, developing the therapeutic strategies targeting to 5T4 is an effective method not only to lead the death of cancerous cells depending on the relative immune mechanisms including antibody-dependent cell-
mediated cytotoxicity (ADCC) (29) and complement-dependent cytotoxicity (CDC) (30) but also to inhibit the diffusion of neoplastic cells which is difficult to control in most of advanced cancers and is associated with a poor clinical outcome.

The immunotherapy targeting to 5T4 molecule could be classified into two categories: passive immunotherapy and active immunotherapy. Essentially, the former strategy supplies the immune response through the antibodies, the infusion of antigen-specific T cells and cytokines rather than activating the immune system directly. This approach is rapid albeit short-lived since the immune system is not engaged; the attack may not be full-fledged.

The use of monoclonal antibody (McAb) is the most successful approach in the passive immunotherapy, notably in breast cancer (31). This strategy targeting to 5T4 antigen, predominantly, is the use of the single chain antibody such as single chain antibody Fv (scFv) and the conjugated engineered antibody. It has been reported that high affinity scFv was isolated from a monoclonal antigen recognizing the 5T4 antigen, which could overcome several drawbacks in traditional monoclonal therapy, including a strong human anti-mouse immune (HAMI) response, and limited tumor penetration due to size of the molecules (32). Furthermore, a research group has shown that they constructed a fusion protein from a Fab of a monoclonal antibody against 5T4 antigen, and an engineered superantigen of Staphylococcal enterotoxin A (SEA) (33), which could induce a strong, local cytotoxic T-cell attack resulting in direct killing tumor cells and leading to inflammation and local accumulation of tumouricidal cytokines (34-37). The recombinant product, named ABR-214936, has been utilized in the therapy of human non-small-cell lung carcinoma (17) and in a phase II study of the therapy in patients with advanced renal cell carcinoma (33). The result from the study shows this drug is effective to prolong the survival of patients with mild and easily managed side effects but well tolerance.

Other effective strategy for passive immunotherapy is the infusion of tumor antigen-specific T cells because of its key role in a host response to a tumor. Although cancer cells can escape T-cell killing (38), this will be restored via the genetical modification for T cell ex vivo to possess McAb specificity for a protein epitope by retroviral transduction with a chimeric T-cell receptor (39). Such, the engineered T cells are capable of recognizing one single antigen, then killing the cells presenting it. Griffiths RW, et al. reported that they isolated and expanded T cells from renal cell carcinoma patients, and then genetically modified them through introduction of a chimeric-signalling protein which consists of a single chain antibody fragment capable of
binding 5T4 antigen directly at the cell surface and activating the T cell by virtue of a CD3ζ-signalling domain (40). It is observed that 5T4, CD3ζ-transduced lymphocytes significantly enhanced killing of the renal cell lines and were able to generate higher amounts of interferon-γ on contact with 5T4 expressing cell lines. This perhaps is caused by the possessing of the extracellular spacer of antigen-dependent CD3ζ chimeric immune receptor (41).

Active immunotherapy for cancer, in fact, is vaccine-based therapies because they generate an intrinsic immune response by introducing into the immune system activators. The advantage of this therapy includes high specificity, long-term efficacy, and giving a body a protection from tumorigenesis. Vaccines for this approach targeting 5T4 antigen contain: viral vector vaccines (42, 43) and dendritic cell (DC) vaccines (44). Figure 1 displays a therapeutic strategy targeting 5T4 antigen for destroying tumor cells through a viral vector encoding a fusion protein (45).

MVA is an efficacious potential vaccine vector in tumor therapy models, and it attenuated by extensive passage in chick embryo fibroblast cells (46). The most significant attribute is that it is able to break immune tolerance to specific TAAs in murine models (47) and human clinical trials (48). So, many therapeutic viral vectors are constructed based on this virus, such as TroVax, a highly attenuated strain of vaccinia virus, modified vaccinia Aankara (MVA strain), encoding the h5T4 protein (49, 50) (Figure 2). Other viral vaccine vectors are based either on a replication-defective adenovirus, like Adh5T4 (42) or on a lentiviral vectors, like the equine infectious anaemia virus vector (ELAV) (51). DC vaccines are derived from immortalized dendritic cell lines, such as DC2.4 encoding human or mouse 5T4 antigen by retroviral transduction (52).

Many reports have shown that immunizing mouse model with viral vaccine vectors alone or recombinant with the treatment with engineered T cells could delay or suppress the challenge of B16h5T4 melanoma and enhance antigen-specific cellular immune response (42, 50). Harrop and colleagues evaluated the therapeutic efficacy in a phase I and phase II trial of patients with colorectal cancer (49). The result shows that this vector is well tolerated in all patients with no serious adverse events attributing to vaccination, and 5T4-specific immune responses are boosted in the presence of MVA neutralizing antibodies. This concludes that vaccination with TroVax is safe and well tolerated and that immune response to 5T4 can be induced without any evidence of autoimmune toxicity.

Active therapeutic strategies for malignant tumor are more promising approaches compared with the passive ones, albeit there are more obstacles to conquer. The reason is that the aim of therapy is to obtain an essential improvement of the immunity against tumor in a body, which involves the whole immune system defensive and activated mechanism such as breaking the immune tolerance to tumor antigens and interacting between different immune effectors cells. As 5T4 antigen, this molecule is shared by both embryonic tissue and various carcinoma tissues but not in the normal adult tissues, whereas embryo is not an autologous fraction in the maternal host and 5T4 protein is immunogenic in normal status. So, it is suggested that 5T4 protein is involved into a mechanism that could make embryo more self relative to maternal host. Further, this mechanism also participates into the process which renders the tumor cells less immunogenic. Although it has been reported that there is a repertoire of CD8+ T cell recognizing 5T4 in normal human and they are potential to function as anti-tumor effectors in the immune defense, their function is suppressed in the patients with cancers (53, 54).

The recent evidence suggests that CD4+CD25+ regulatory T cells (Treg) (55), which are capable of controlling self-antigen specific response in the periphery, may play a role in controlling anti-tumor immune response (56). The vaccination using DC vaccine will lead to increasing Treg and its depletion could facilitate the generation of 5T4 specific CD8+ T cells (44). The precise mechanism through which this occurs is unclear, but it is possible that the cytokine milieu within the tumor promotes the generation of Tregs recognizing tumor-associated antigen, such as TGF-β (57, 58). So, the use of active immunotherapeutic strategy against 5T4 must consider more elements within immune system and microenvironment in vivo for its better efficacy.

**Conclusions and perspectives**

5T4 oncofetal antigen plays double roles in both biological development and pathological progression. Its restricted profile of expression, the biological functions and the distribution among the tissues make it a new promising candidate in the immunotherapy for cancer. And many successes have been met. Whereas, more information about this protein in the complex events of impanation and metastasis, interaction with immune effectors causing the reduction of tumor immunogenicity and regulation on its gene expression is difficult to obtain via the direct study in human tissues. However, the comparison between mouse and human 5T4 protein sequences shows a high homology, 81% identity as a whole alignment and the coding regions of the human and mouse 5T4 genes have been cloned, respectively (7, 60). This opens the possibility of more holistic in vivo
approaches to study the function of 5T4 oncofetal antigen by transgenesis or using knockout or and knock-down methodology which should provide new insight into its role in normal and malignant status, and then give more new inspiration for developing therapeutic strategies targeting this protein in cancer or another diseases.

References


