Review

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

Yu Zhao[1] and Yuxia Wang[1, 2]

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. *Cellular & Molecular Immunology*. 2007;4(2): 99-104.

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 processes

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 luecine-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

[1]Beijing Institute of Pharmacology and Toxicology, Beijing 100850, China;

[2]Corresponding to: Dr. Yuxia Wang, Beijing Institute of Pharmacology and Toxicology, Beijing 100850, China. Tel: +86-10-6687-4604, Fax: +86-10-6821-1656, E-mail: wangyuxia1962@hotmail.com

Received February 24, 2007. Accepted March 29, 2007.

changes in motility and morphology (12). The same event is observed during early human embryonic stem cell differentiation. It implies that 5T4 is a useful marker of early embryonic stem cell differentiation in humans and mice (13).

The intriguing information from comparative neurology discloses a novel function of 5T4 in the process of the formation dendrodendritic synapses between GCs and mitral/tufted cells (6). Many LRR-containing membrane proteins in the central nervous system are known to be involved in the formation of neuronal circuits. The present study on the mechanism in the stratification of neurons in the murine olfactory bulb (OB) shows that 5T4 is expressed by a subset of superficial granule cells (GCs), a major population of local interneurons of the OB. These 5T4 positive GCs project their peripheral dendrites preferentially to the superficial stratum of the external plexiform layer (EPL). Additionally, 5T4 expression parallels in time the formation of dendrodendritic synapses between GCs and mitral/tufted cells. Thus, 5T4 might be involved in the superficial-stratum preferred dendritic arborization of the 5T4 positive GCs or superficial-sratum specific formation of dendrodendritic synapses between tufted cell dendrites and spines of 5T4-positive GCs.

As above described, 5T4 oncofetal antigen is a vital molecule whose major effect is to promote the regular migration of different cells. Cellular migration is an important circumstance not only in the physiological development but also in the various pathological progressions, especially in the tumorigenesis and metastasis of malignant cells.

A negative role in pathological events

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 complicate 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 immuno-therapy 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 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-

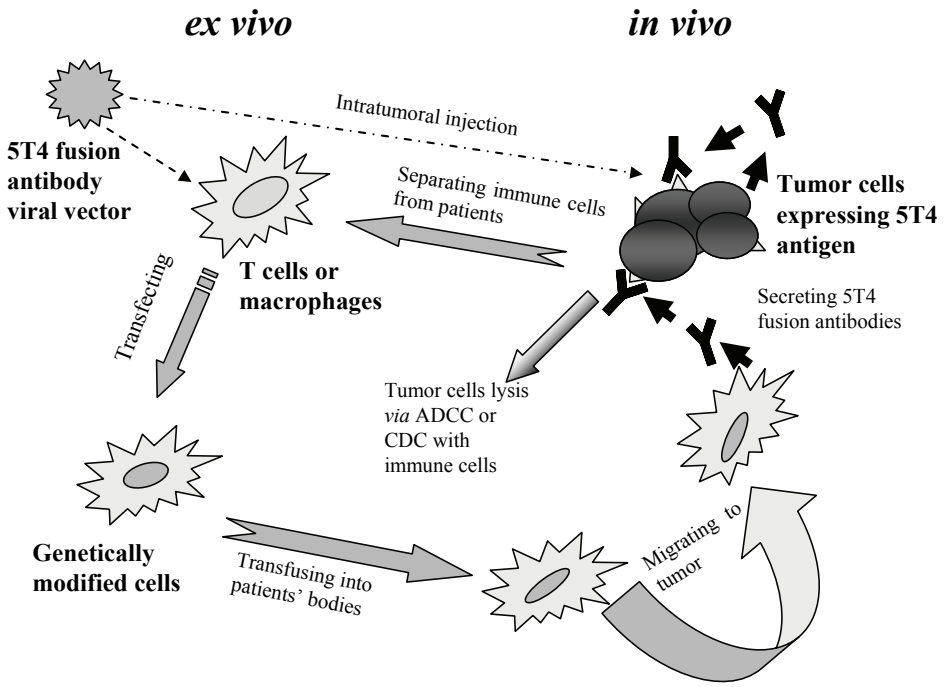

Figure 1. Genetic delivery of 5T4 fusion antibody constructs. Therapeutic strategy that recruit both humoral and cellular arms of the immune response through contrasting engineered retroviral vectors that produce fusion proteins is able to interact simultaneously with 5T4-positive cells and with the receptor/ligands of the immune effector moieties. Tumor cells transduced by vector particles containing the 5T4 scFv-Hg1 constructs will produce fusion protein, which will then be secreted from the cell. The immune conjugate will then bind back to the 5T4 antigen at the cell surface, directing lysis of both transduced and neighbouring untransduced cells, through monocytes, macrophages, and NK cells and complement fixation. Alternatively, macrophages can be transduced *ex vivo*, and then introduced back into the patient. The tumor tropic properties of these cells will take them to sites of the cancer where secretion of the fusion protein will direct cell lysis.

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

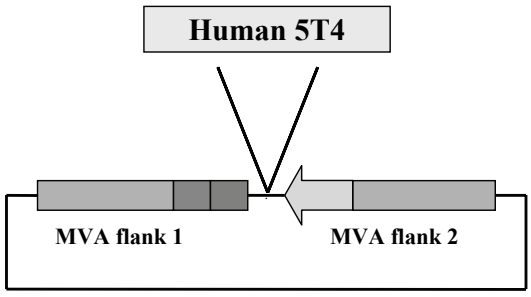

Figure 2. Structure of MVA h5T4 viral vector. Pox viral transfer plasmids encoding human 5T4 were constructed through 5T4 cDNA ligated into monoclonal sites of the engineered pox transfer plasmids.

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 dentritic 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 (EIAV) (51). DC vaccines are derived from immortalized dentritic cell lines, such as DC2.4 encoding human or mouse 5T4 antigen by retroviral transduction (52).

Many reports have shown that immunizating 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 effecters 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 effetors 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

1. Southall PJ, Boxer GM, Bagshame KD, Hole N, Bromley M, Stern PL. Immunohistological distribution of 5T4 antigen in normal and malignant tissues. Br J Cancer. 1990;61:89-95.
2. Hole N, Stern PL. Isolation and characterization of 5T4, A tumor-associated antigen. Int J Cancer. 1990;45:179-184.
3. Hole N, Stern PL. A 72kD trophoblast glycoprotein defined by a monoclonal antibody. Br J Cancer. 1998;57:239-246.
4. Carsberg CJ, Myers KA, Stern PL. Metastasis-associated 5T4 antigen disrupts cell-cell contacts and induces cellular motility in epithelial cells. Int J Cnacer. 1996;68:84-92.
5. Carsberg CJ, Myers KA, Evans GS, Allen TD, Stern PL. Metastasis-associated 5T4 oncofoetal antigen is concentrated at microvillus projections of the plasma membrane. J Cell Sci. 1995;108:2905-2916.
6. Imamura F, Nagao H, Naritsuka H, Murata Y, Taniguchi H, Mori K. A leucine-rich repeat membrane protein, 5T4, is expressed bu a subtype of granule cells with dendritic arbors in specific strata of the mouse olfactory bulb. J Comp Neurol. 2006;495:754-768.
7. Shaw DM, Woods AM, Myers KA, et al. Glycosylation and epitope mapping of the 5T4 glycoprotein oncofoetal antigen. Biochem J. 2002;363:137-145.
8. Kobe B, Deisenhofer J. The luecine-rich repeat: a versatile binding motif. Trends Biochem Sci. 1994;19:415-421.
9. Kobe B, Deisenhofer J. Proteins with luecine-rich repeat. J Struct Bio. 1995;5:409-416.
10. Awan A, Lucic MR, Shaw DM, et al. 5T4 interacts with TIP-2/GIPC, a PDZ protein, with implications for metastasis. Biochem Biophy Res Commun. 2002;290:1030-1036.
11. Barrow KM, Ward CM, Rutter J, Ali S, Stern PL. Embryonic expression of murine 5T4 oncofoetal antigen is associated with morphogenetic events at implantation and in developing epithelia. Dev Dyn. 2005;233:1535-1545.
12. Ward CM, Barrow K, Woods AM, Stern PL. The 5T4 oncofoetal antigen is an early differentiation marker of mouse ES cells and its absence is a useful means to assess pluriptency. J Cell Sci. 2003;116:4533-4542.
13. Ward CM, Eastham AM, Stern PL. Cell surface 5T4 antigen is transiently upregulated during early human embryonic stem cell differentiation: effect of 5T4 phenotype on neural lineage formation. Exp Cell Res. 2006;312:1713-1726.
14. Wrigley E, McGown AT, Rennison J, et al. 5T4 oncofetal antigen expression in ovarian carcinoma. Int J Gynecol Cancer. 1995;5:269-274.
15. Starzynska T, Marsh PJ, Schofield PF, Roberts SA, Myers KA, Stern PL. Prognostic significance of 5T4 oncofetal antigen expression in colorectal carcinoma. Br J Cancer. 1994;69:899-902.
16. Starzynska T, Rahi V, Stern PL. The expression of 5T4 antigen in colorectal and gastric carcinoma. Br J Cancer. 1992;66:867-869.
17. Forsberg G, Ohlsson L, Brodin T, et al. Therapy of human non-small-cell lung carcinoma using antibody targeting of modified superantigen. Br J Cancer. 2001;85:129-136.
18. Jones H, Roberts G, Hole N, McDicken IW, Stern P. Investigation of expression of 5T4 antigen in cervical cancer. Br J Cancer. 1990;6:69-100.
19. Ali A, Langdon J, Stern PL, Partidge M. The pattern of expression of 5T4 oncofoetal antigen on normal, dysplastic and malignant oral mucosa. Oral Oncol. 2001;37:57-64.
20. Mulder WM, Stern PL, Stukart MJ, et al. Low intercellular adhesion molecule 1 and high 5T4 expression on tumor cells correlate with reduced disease-free survival in colorectal carcinoma patients. Clin Cancer. 1997;3:1923-1930.
21. Starzynska T, Wiechowska-Kozlowska A, Marlicz K, et al. 5T4 oncofetal antigen in gastric carcinoma and its clinical significance. Eur J Gastroenterol Hepatol. 1998;10:479-484.
22. Naganuma H, Kono K, Mori Y, et al. Oncofetal antigen 5T4 expression as a prognostic factor in patients with gastric cancer. Anticancer Res. 2002;22:1033-1038.
23. Myers KA, Rahi-Saund V, Davison MD, Young JA, Cheater AJ, Stern PL. Isolation of a cDNA encoding 5T4 oncofetal trophoblast glycoprotein. An antigen associated with metastasis cantains leucine-rich repeats. J Biol Chem. 1994;269:9319-9324.
24. Baruch ZH, Wendell AL. Mechanism and role of PDZ domains in signaling complex assembly. J Cell Sci. 2001;114:3219-3231.
25. Conner ME, Davidson SE, Stern PL, Arrand JR, West CM. Evaluation of multiple biologic parameters in cervical carcinoma: high macrophage infiltration in HPV-associated tumors. Int J Gynecol Cancer. 1993;3:103-109.
26. Old LJ. Immunotherapy for cancer. Sci Am. 1996;275:136-143.
27. Berkow R, Beer MH. Cancer and the immune system. In: The Merck Manual of Medical Information. White House Station, NL; Merck Research Laboratories. 1997:792-794.
28. Woods AM, Wang WW, Shaw DM, et al. Characterization of the murine 5T4 oncofoetal antigen: a target for immunotherapy in cancer. Biochem J. 2002;366:353-365.
29. Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc recptors modulate *in vivo* cytoxicity against tumor targets. Nat Med. 2000;6:443-446.
30. Le X, McWaters A, Wiener J, Wu J, Mills GB. Anti-HER2 antibody and heregulin suppress growth of HER2-over-expressing human breast cancer cells through different mechanisms. Clin Cancer Res. 2000;6:260-270.
31. Cobleigh MA, Vogel CL, Tripathy D, et al. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J Clin Oncol. 1999;17:2639-2648.
32. Shaw DM, Embleton MJ, Westwater C, et al. Isolation of a high affinity scFv from a monoclonal antibody recognizing the oncofoetal antigen 5T4. Biochim Biophys Acta. 2000;1524: 238-246.
33. Shaw DM, Connolly NB, Patel PM, et al. A phase II study of a 5T4 oncofoetal antigen tumor-targeted superantigen (ABR-214936) therapy in patients with advanced renal cell carcinoma. Br J Cancer. 2007;69:567-574.
34. Dohsten M, Abrahmsen L, Bjork P, et al. Monoclonal antibody-superantigen fusion proteins: tumor-specific agents for T-cell-based tumor therapy. Proc Natl Acad Sci U S A. 1994;91: 8945-8949.
35. Dohsten M, Hansson J, Ohlsson L, et al. Antibody-targeted superantigens are potent inducers of tumor-infiltrating T lymphocytes *in vivo*. Proc Natl Acad Sci U S A. 1995;92:9791-9795.
36. Dohsten M, Hedlund G, Akerblom E, et al. Monoclonal

antibody-targeted superantigens: a different class of anti-tumor agents. Proc Natl Acad Sci U S A. 1991;88:9287-9291.
37. Litton MJ, Dohlsten M, Hansson J, et al. Tumor therapy with an antibody-targeted superantigen generates a dichotomy between local and systemic immune responses. Am J Pathol. 1997;150: 1607-1618.
38. Garrido F, Ruiz-Cabello F, Cabrera T, et al. Implication for immunosurveillance of altered HLA class I phenotypes in human tumours. Immunol Today. 1997;18:89-95.
39. Thistlethwaite F, Mansoor W, Gilham DE, Hawkina RE. Engineering T-cell with antibody-based chimeric receptors for effective cancer therapy. Curr Opin Mol Ther. 2005;7:48-55.
40. Griffiths RW, Gillham DE, Dangoor A, et al. Expression of 5T4 oncofoetal antigen in renal cell carcinoma: a potential target for T-cell-based immunotherapy. Br J Cancer. 2005;93:670-677.
41. Guest RD, Hawkins RE, Kirillova N, et al. The role of extracellular spacer regions in the optimal design of chimeric immune receptors: evaluation of four different scFv and antigens. J Immunother. 2005;28:203-211.
42. Jiang HR, Gilham DE, Mulryan K, Kirillova N, Hawkins RE, Stern PL. Combination of vaccination and chimeric receptor expressing T cells provides improved active therapy of tumors. J Immunol. 2006;177:4288-4298.
43. Mulryan K, Ryan MG, Myers KA, et al. Attenuated recombinant vaccinia virus expressing oncofetal antigen (tumor-associated antigen) 5T4 induces active therapy of established tumors. Mol Cancer Ther. 2002;1:1129-1137.
44. Ali S, Mulryan K, Taher T, Stern PL. Immunotherapy success in prophylaxis cannot predict therapy: prime-boost vaccination against the 5T4 oncofoetal antigen. Cancer Immunol. Immunother. 2007;56:165-180.
45. Myers KA, Ryan MG, Stern PL, et al. Targeing immune effector molecules to human tumor cells through genetic delivery of 5T4-specific scFv funsion protein. Cancer Gene Ther. 2002;9: 884-896.
46. Meyer H, Sutter G, Mayr A. Mapping of deletions in the genome of the highly attenuated vaccinia virus MVA and their influence on virulence. J Gen Virol. 1991;72:1031-1038.
47. Overwijk WW, Lee DS, Surman DR, et al. Vaccination with a recombinant vaccinia virus encoding a "self" antigen induces antoimmune vitiligo and tumor cell destruction in mice: requirement for $CD4^+$ T lymphocytes. Prco Natl Acad Sci U S A. 1999;96:2982-2987.
48. Carroll MW, Restifo NP. Poxviruses as vectors for cancer immunotherapy. In: Cancer vaccines and immunotherapy. Cambridge University Press; 2000:47-61.
49. Harrop R, Connolly N, Redchemko I, et al. Vaccination of colorectal cancer patients with modified vaccinia Ankara delivering the tumor antigen 5T4 (TroVax) induces immune responses which correlate with disease control: a phase I/II trial. Clin Cancer Res. 2006;12:3416-3424.
50. Harrop R, Ryan MG, Myers KA, Redchenko I, Kingsman SM, Carrol MW. Active treatment of murine tumors with a highly attenuated vaccinia virus expressing the tumor associated antigen 5T4 (TroVax) is $CD4^+$ T cell dependent and antibody mediated. Cancer Immunol Immunother. 2005;55:1081-1090.
51. Lamikanra A, Myers KA, Ferris N, Mitrophanous KA, Carroll MW. *In vivo* evaluation of an EIAV vector for the systemic genetic delivery of therapeutic antibodies. Gene Ther. 2005;12: 988-998.
52. Mendoza L, Bubenik J, Simova J, et al. Tumour-inhibitory effects of dendritic cells administered at the site of HPV 16-induced neoplasms. Folia Biol (Praha). 2002;48:114-119.
53. Smyth LJ, Elkord E, Taher TE, et al. CD8 T-cell recognition of human 5T4 oncofetal antigen. Int J Cancer. 2006;119:1638-1647.
54. Redchenko I, Harrop R, Ryan MG, Hawkins RE, Carroll MW. Identification of a major histocompatibility complex class I-restricted T-cell epitope in the tumor-associated antigen, 5T4. Immunology. 2006;118:50-57.
55. Clarke SL, Betts GJ, Plant A, et al. $CD4^+CD25^+FOXP3^+$ regulatory T cells suppress anti-tumor immune responses in patients with colorectal cancer. PLoS ONE. 2006;1:e129.
56. Curiel TJ, Coukos G, Zou LH, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med. 2004;10:942-949.
57. Schramn C, Huber S, Protschka M, et al. TGF-β regulates the $CD4^+CD25^+$ T-cell pool and the expression of Foxp3 *in vivo*. Int Immunol. 2004;16:1241-1249.
58. Huber S, Schramm C, Lehr HA, et al. Cutting edge: TGF-β signaling is required for the *in vivo* expansion and immunosuppressive capacity of regulatory $CD4^+CD25^+$ T cells. J Immunol. 2004;173:6526-6531.
59. King WK, Sheppard CF, Westwater C, Stern PL, Myers AK. Organisation of the mouse and human 5T4 oncofoetal leucine-rich glycoprotein genes and expression in foetal and adult murine tissues. Biochim Biophy Acta. 1999;1445:257-270.