PEER REVIEWED ARTICLE

TAP: NOVEL TARGETS FOR CANCER VACCINE DEVELOPMENT

Reduced expression of TAP (transporters associated with antigen presentation) correlates with poor immune responses in cancer. The restoration of this pathway through expression or up-regulation of TAP in TAP-deficient cancer cells can enhance the functioning of the major histocompatibility complex (MHC) Class 1 antigen-presenting pathway and increase the production of tumour-infiltrating cytotoxic T-cells. Accordingly it provides a promising approach for the development of cancer vaccines.

The immune system can target tumour cells for destruction by recognizing tumour-associated or tumour-specific antigens displayed on their surface. In principle, immunosurveillance is a fundamental defense mechanism that can recognize and destroy malignant cells before they develop into tumours. Advances in our understanding of tumour-associated antigens have stimulated the clinical development of immunotherapies for the treatment of cancer. The clinical appeal of immunotherapy is the potential to control disseminated metastatic disease with a minimum of toxic side effects because of the immune system's exquisite specificity. Many of the therapies involve vaccination with protein/peptide antigens, plasmids, or recombinant virus-encoding genes for antigens, or whole cell vaccines that consist of autologous or allogeneic tumour cells, or autologous dendritic cells with a variety of modifications.[1] These diverse approaches aim to stimulate a T cell-mediated antitumour immune response. Although conceptually appealing, the success of cancer vaccines and immunotherapies in humans is variable. In most cases, the vaccines are very well tolerated and specific immune responses to particular antigens can be achieved, but the response rate of the disease to the therapy is low. The reasons for the low response rates are thought to be because of several factors, which include low immunogenicity and tolerance to tumour-associated antigens, immunosuppressive microenvironments and defects in the cellular machinery for antigen processing and recognition.[2] If the cellular machinery for antigen processing is defective or fails, cancer cells can escape immunosurveillance. The correlation between increased tumourigenicity and a decrease in major histocompatibility complex (MHC) Class I expression has been well established. A decrease in cell surface expression of MHC Class I can be the result of a defect in the MHC Class I biosynthetic pathway.[3–10] One central component of this pathway is a group proteins called transporters associated with antigen processing (TAP). Reduced expression or loss of TAP represents a central mechanism correlating with poor immune responses in cancer. The restoration of this pathway by expression (or up-regulation) of TAP genes provides a promising approach for the development of new cancer vaccines.

Figure 1

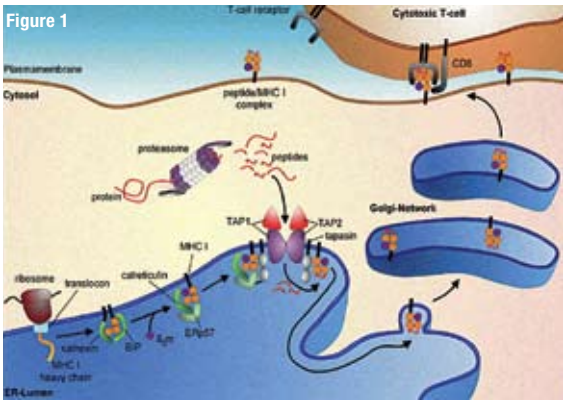

Figure 1: Role of TAP (TAP1/TAP2) and associated proteins in loading peptides to MHC Class I molecules and presentation to cytotoxic T-cells.

Figure 2: AdhTAP1 treatment leads to fewer and smaller tumours in mice bearing B16F10 tumours.

TAP

TAP plays a central role in immunosurveillance as it functions to shuttle peptides from inside the cell (proteosome) to MHC Class I, which are transported to the cell surface where they can be recognized as foreign by cytotoxic T lymphocytes. Activated cytotoxic T cells can infiltrate tumours and destroy tumour cells. TAP is a member of the ATP-binding-cassette (ABC) transporter family. It delivers cytosolic peptides into the endoplasmic reticulum where they bind to newly synthesized MHC Class I molecules.[11]

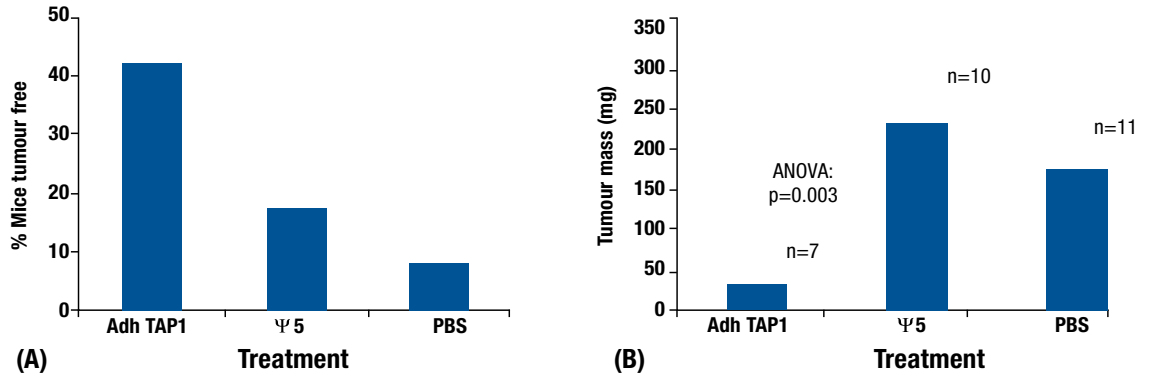

Figure 2

The TAP structure is formed of two proteins: TAP1 and TAP2, which assemble into a heterodimer. TAP is found in the lumen of the endoplasmic reticulum associated with the peptide-loading complex. This complex of β2 microglobulin, calreticulin, ERp57, TAP, tapasin and MHC Class I functions to hold MHC molecules until they have been fully loaded with peptides.[12] The intracellular components of this pathway and the presentation of MHC Class I antigens to T-cells are shown diagrammatically in Figure 1.

There is abundant evidence that a variety of solid tumours (for example, melanoma, ovarian, breast, lung) have deficiencies in TAP levels. In a study evaluating human melanoma tissues levels of TAP1 and TAP2 were greatly reduced in metastatic lesions and the reduction in TAP levels in primary lesions correlated with lesion thickness, disease stage, faster disease progression and lower survival rates.[13] Loss of the TAP complex is highly correlated with loss of human leucocyte antigen (HLA) expression in cervical carcinoma.[14] In addition, a higher frequency of down-regulation of this complex has been observed for metastatic lesions than for primary lesions.[5] The TAP complex has been particularly strongly implicated in tumourigenicity of several cancers such as melanomas, cervical carcinomas and renal cell carcinomas.[5,15] Thus, these findings suggest that TAP down-regulation may represent an important and widespread mechanism for immune escape of malignant cells in a variety of tumours, and raised the question whether genetic transfer of TAP genes could restore immune recognition of tumours and provide the basis for a new approach to the development of therapeutic cancer vaccines.

Restoration of TAP as a Strategy for Cancer Vaccine Development

Whereas the correlation between the down-regulation of TAP and increased tumourigenesis had been widely established the research of Dr Wilfred Jeffries and colleagues at the University of British Columbia, Vancouver BC provided the first critical demonstration that TAP1 gene transfer into tumour cells and cancer bearing animals could significantly improve the immune recognition of tumour-associated antigens. Details of these studies were published in a series of landmark publications, which also demonstrated that TAP1 and TAP2 gene transfer could also improve the potency of vaccines for the treatment of viral diseases.[16–20]

In a study evaluating TAP levels in tissues from nine cases of human small cell lung cancer and 10 cases of non-small cell lung cancer, 59% of tumour lesions were negative for TAP expression as determined by immunoperoxidase staining of the tissues for this protein. Using this methodology only one of 19 tumours tested strongly positive for TAP.[18] The researchers demonstrated that a nonreplicating adenovirus encoding the gene for TAP1 (AdhTAP1) could restore TAP1 expression in the mouse lung carcinoma cell line CMT.64, and increased tumour-specific immune responses. This cell line was derived from an aggressive metastatic small cell lung cancer and is defective in TAP. The ability of TAP to restore an immune response has also been demonstrated in a series of animal studies. In a mouse model of small cell lung cancer animals receiving the TAP1 gene administered via a vaccinia virus vector demonstrated improved immunogenicity and increased survival. Up to 60% of cancerous mice that had restored expression of TAP1 were still alive after 100 days and metastasis was reduced. In contrast, 50% of untreated mice died of multiple tumours after 40 days.[16] In a separate series of studies, AdhTAP1 produced effective immune responses in a mouse model of melanoma.[20] This model, which uses the B16F10 cell line, is widely used to evaluate T-cell-based vaccine strategies, as it is a highly metastatic and poorly immunogenic cell line and is defective in levels of TAP1 and TAP2. These studies determined that melanoma-bearing mice that were administered AdhTAP1 were less likely to develop tumours, had a 10-fold slower tumour growth rate and improved survival time. In contrast to 100% of animals that died by week 3, more than 40% of the AdhTAP1-treated animals were tumour-free at this time (Figure 2). In addition, the treated animals also showed

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increased levels of tumour-infiltrating lymphocytes and memory cells further validating restoration of immune function. It is particularly significant that the B16F10 model is deficient in many antigen processing components (including tapasin, LMP-2, LMP-7, LMP10, and PA28α/β) yet expression of TAP1 function alone could lead to significant restoration of MHC Class I surface expression.

Collectively, these studies show that TAP1 gene transfer and expression of small amounts of TAP results in several critical effects:

- It restores the MHC Class 1 antigen-presenting pathway.
- It increases the number of tumour-infiltrating cytotoxic T-cells and dendritic cells.
- It enhances memory T-cell subpopulations.
- It improves animal survival.

As these immune effects are central to the development of a successful cancer vaccine the potential importance of using TAP expression in the immunotherapy of cancer was recognized. Moreover, studies on TAP expression also demonstrated a number of potential advantages for the development of a therapeutic product:

- It allows the immune system to recognize all tumour antigens presented on tumour cells.
- Only a small proportion of tumour cells need to be treated.
- It is independent of genetic variability of MHC Class 1 proteins.
- It has application to many solid tumours.
- It is relevant to immuno-compromised individuals.
- It can be administered by simple injection.

Collectively these animal studies have provided the basis for evaluating the up-regulation of TAP in human clinical studies.

As a prerequisite for entry into the clinic the reproducible manufacturing and safety of TAP1 constructs needs to be established. Particular focus is on the use of a commercial cell-based manufacturing system for adenoviral-based vaccines that greatly reduces or eliminates the production of live virus through recombination events.[21] In addition, safety studies in animals and Phase I/II studies in man will need to establish an immunostimulatory dose of TAP1 without the induction of widespread autoimmunity to self-antigens. Clinical development strategies include the up-regulaton of TAP expression alone or in conjunction with other tumour-associated antigens as a therapeutic cancer vaccine or in combination with other immunotherapies. Initial clinical trials, starting this year, will target the treatment of HER2/neu breast cancer. This approach will evaluate the use of AdhTAP1 in concert with a set of novel HER2/neu antigens.[22] The overall strategy of this approach is to target both MHC Class I (TAP-dependent stimulation of cytotoxic T-cells) and MHC Class II pathways (TAP-independent stimulation of T-helper cells) to achieve activation of different T-cell populations for a robust and prolonged immune response. Patients selected for these trials will be those who have low to moderate expression of the HER2/neu antigen and are not prime candidates for monoclonal antibody therapy using Herceptin (trastuzumab). The ability to measure TAP levels in tumour tissue biopsies will provide an additional selection tool for selecting patients that could best benefit from the combined treatment. This will be the first clinical trial to test the combined effect of a tumour-associated antigen and restoration of TAP expression. The wide variety of solid tumours that have reduced levels of TAP suggest that this approach will have widespread application in the treatment of cancer.

ALTHOUGH A NUMBER OF VACCINE AND CELLULAR IMMUNOTHERAPIES HAVE PROGRESSED TO LATE-STAGE CLINICAL TRIALS, IMMUNOTHERAPY FOR THE TREATMENT OF CANCER IS STILL IN ITS INFANCY.

Although a number of vaccine and cellular immunotherapies have progressed to late-stage clinical trials, immunotherapy for the treatment of cancer is still in its infancy. The US approval, in 2010, of Provenge (sipucel-T), the first marketed autologous cellular immunotherapy for the treatment of late-stage prostate cancer, has drawn attention to the emergence of this field and the opportunities for development of improved, simpler and more cost effective therapeutic vaccine products. Currently, Gardasil, used for the prevention of cervical cancer caused by the human papilloma virus, remains the only preventative cancer vaccine on the market. While the results of TAP1 expression in experimental animals look extremely promising the results of the clinical studies will determine if this approach can provide a safe and effective platform for the development of a range of novel cancer therapeutics that are HLA-specific and tumour-specific. **Pharma**


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