4. Potential of cancer-testis antigens as targets for cancer immunotherapy

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Abstract. Cancer-testis antigens are tumour antigens with limited expression in male germ cells in the testis, ovary and trophoblasts. Recently, their expression has been seen in different types of tumours. Due to the existence of the blood-testis barrier, testis is considered an immune-privileged site; and testis-specific genes, if expressed in cancers, can be immunogenic. For this reason, cancer-testis antigens are promising candidates for cancer immunotherapy and have become a major focus for the development of vaccine-based clinical trials in recent years. These antigens may be used as biomarkers for early detection of cancers. Expression of these genes in cancer reflects gene reprogramming and may have a role in neoplastic features such as metastasis and immune evasion.

Introduction

Tumourigenesis and germ cell development have many common characteristics. There is a great variety of evidence for this theory. First of all, many kinds of epithelial tumours secrete Chorionic Gonadotropin and other...
trophoblastic hormones. In addition, a new category of tumour antigens called Cancer-Testis Antigens (CTAs) has been found with limited expression in normal tissues rather than germ cells and trophoblast [1]. Immature germ cells of fetal ovary (oogonia and primary oocytes) express CTAs but their expression has not been seen in oocytes in the resting primordial follicles. Cytotrophoblast and syncytiotrophoblast of the placenta express some CTAs. Expression of these antigens in the placenta has a special pattern; some of them are not expressed in the placenta but some are highly expressed, and their expression is not completely paralleled with their presence in the fetal germ cells. Some characteristics of malignant tissues, such as invasiveness, destructiveness, and metastatic features, are shared with trophoblastic cells, and thus gene expression profile in the placenta can be similar to cancer. Some CTAs are expressed in nongametogenic tissues such as the pancreas, liver, and spleen; but at levels much less than germ cells [2]. This expression in non-testicular tissues are usually at less than 1% of their expression levels in testis and has not been confirmed at protein level by immunohistochemical analysis. Another group of genes has been described with limited expression in testis and brain [3]. These two tissues share another feature of having blood barriers.

It was recently reported that some CTAs such as N-RAGE, NY-ESO, MAGE, and SSX are expressed in both adult and fetal human mesenchymal stem cells of the bone marrow but after differentiation of osteocytes and adipocytes, their expression is down-regulated. It has been suggested that expression of CTA, in addition to being a special characteristic of gametogenesis, can be a stem cell marker. This restricted expression of these antigens in undifferentiated somatic and germ cells is suggestive of their essential role in embryonic development. In addition, expression of CTAs in cancer stem cells may provide special targets for treatment of cancer recurrences and metastasis. Maintenance of undifferentiated phenotype in cancer stem cells is needed for expression of CTAs; so cancer cells, in which CTAs are expressed, may have lost their differentiation ability. Supposing that cancer stem cells are sources of metastasis and recurrence, drugs targeting CTAs may be efficient in cancer treatment [4].

To prevent autoimmunity, a proper tumour antigen for immunotherapy must have no or highly restricted expression in normal tissues. Because of their highly tissue restricted expression, CTAs are considered as promising target molecules for cancer vaccines. Spontaneous humoral and cell-mediated immune responses have been found for at least some of them.

Until now at least 70 families of cancer-testis genes with 140 members have been placed in this group and their expression has been studied in different types of tumours. Some of them have been originally identified
Table 1. CTAs of X chromosomes expressed in less than 5 normal tissues [2].

<table>
<thead>
<tr>
<th>CT antigen</th>
<th>Expression in normal tissues rather than testis</th>
<th>Expression in cancer according to digital differential display</th>
<th>Expression in cancer tissues</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOM-TES-85(LUZP4) Xq23</td>
<td>None</td>
<td>None</td>
<td>Hepatocellular carcinoma, Lung cancer, Ovarian cancer, Melanoma, Glioma, Bladder cancer, Breast cancer</td>
<td>Lucine Zipper Protein(Possibly transcriptionsal regulatory protein)</td>
</tr>
<tr>
<td>NY-ESO-1 (CTAG1B) Xq28 Placenta, Bone</td>
<td>Chondrosarcoma</td>
<td>Brain tumours, Melanoma, Ovarian cancer, Non small cell lung carcinoma, Breast cancer, Hepatocellular carcinoma, Esophageal carcinoma</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>FATE1 Xq28 Adrenal gland, Placenta, Brain</td>
<td>Adrenal tumour, Germ cell tumour</td>
<td>Hepatocellular carcinoma</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>MAGEB1 Xp21 Salivary gland, skin, Brain, Spinal cord</td>
<td>Skin tumour</td>
<td>Hepatocellular carcinoma, Esophageal squamous cell carcinoma</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>SAGF1 Xq26 Bone marrow, Brain</td>
<td>Germ cell tumour, Leukemia</td>
<td>Head and neck squamous cell carcinoma, Sarcoma</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>SPANXA1 Xq27 Bone marrow, Liver</td>
<td>Germ cell tumour, Leukemia</td>
<td>Melanoma</td>
<td>Association with nuclear envelope of human spermatids and spermatozoa</td>
<td></td>
</tr>
<tr>
<td>SPANXB2 Xq27 Connective tissue</td>
<td>Soft tissue/muscle tissue tumour</td>
<td>Melanoma</td>
<td>Association with nuclear envelope of human spermatids and spermatozoa</td>
<td></td>
</tr>
<tr>
<td>SPANXC Xq27 None</td>
<td>None</td>
<td>Melanoma</td>
<td>Association with nuclear envelope of human spermatids and spermatozoa</td>
<td></td>
</tr>
<tr>
<td>SPANXD Xq27 Connective tissue, Skin, Liver</td>
<td>Soft tissue/muscle tissue tumour</td>
<td>Melanoma</td>
<td>Association with nuclear envelope of human spermatids and spermatozoa</td>
<td></td>
</tr>
<tr>
<td>TAF7L Xq22 Eye, Lung, Germ cell</td>
<td>Head and neck</td>
<td>Transcription</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
because of their immunogenicity in cancer patients and their recognition by CD8+ T cells and antibodies [5]. Classifying genes in this gene group is based on some characteristics: 1) mRNA expression in normal tissues is almost limited to testis, fetal ovary, and placenta, and 2) mRNA expression has been seen in different cancers. In cancers immunohistochemical analysis also has shown their expression at protein level.

Contrary to other differentiation antigens, CTAs expression in cancers is predominately heterogeneous and usually small subsets of cancer cells express CTAs. One reason for this heterogeneous expression could be the fact that CTA expression marks cancer stem cells, and this expression disappears after the differentiation of cancer cells [5]. It is possible to assess expression of CTAs in normal tissues by means of digital differential display to find those with more limited expression in normal tissues which are more appropriate for cancer immunotherapy (Tables 1 and 2).

CTAs can be targets for siRNA and if expressed at cell surface, they can serve as targets for monoclonal antibodies. It is worthy to mention that for each type of cancer, CTA expression is usually related to worse outcome, higher grade and metastasis; hence it shows a relationship between their expression and the level of dedifferentiation.

### Classification of CTAs

CTAs are classified according to their gene locations on chromosomes to cancer-testis-X CT-X or non X-CT. About 50% of cancer-testis genes, including
including those which have been used in cancer immunotherapy, are located on the X chromosome. These CT-X genes usually form gene families connected to inverted DNA repeats. Study of the sequence of the human X chromosome has shown that about 10% of all genes on the X chromosome belong to the cancer-testis gene family. In normal testis, the CT-X genes are generally expressed in the spermatogonia, which are proliferating germ cells. The biological function of some CT-X genes has been characterized. For instance, MAGE genes can have a role in signal transduction and transcription modulation and a member of GAGE family can repress apoptosis [6]. Most of CT-X genes are clustered in two regions: Xp11 and Xq26-q28. High level of multigenicity of many CTAs can be attributed to gene duplication and subsequent divergence [7].

Expression of CT-X antigens is different in different types of tumours. Their highest expression frequency has been seen in bladder cancer,lung

<table>
<thead>
<tr>
<th>CT antigen</th>
<th>Chromosome location</th>
<th>Expression in normal tissues rather than testis</th>
<th>Expression in cancer according to digital differential display</th>
<th>Expression in cancer tissues</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRAMEF2</td>
<td>1p36</td>
<td>Brain</td>
<td>Primitive neuroectodermal tumour</td>
<td>None</td>
<td>Unknown</td>
</tr>
<tr>
<td>BRDT</td>
<td>1p22</td>
<td>Brain, Mouth, Muscle, Prostate</td>
<td>Germ cell tumour, Head and neck tumour</td>
<td>Lung cancer</td>
<td>Possibe transcriptional regulatory protein</td>
</tr>
<tr>
<td>SPO11</td>
<td>20q13</td>
<td>Brain, connective tissue</td>
<td>Soft tissue/ muscle tissue tumour</td>
<td>Formation of double-strand breaks in paired chromosome homologues</td>
<td></td>
</tr>
<tr>
<td>SYCP1</td>
<td>1p13</td>
<td>Connective tissue</td>
<td>Germ cell tumour, Soft tissue/ muscle tissue tumour</td>
<td>Brain tumour</td>
<td>Major component of synaptonemal complexes</td>
</tr>
<tr>
<td>TPTE</td>
<td>21p11</td>
<td>None</td>
<td>Germ cell tumour, Adrenal tumour</td>
<td>Hepatocellular carcinoma</td>
<td>PTEN-related tyrosine phosphatase</td>
</tr>
<tr>
<td>ADAM2</td>
<td>8p11</td>
<td>Brain, Connective tissue, Prostate</td>
<td>Soft tissue/ muscle tissue tumour, Prostate cancer</td>
<td>Multiple myeloma</td>
<td>Membrane-anchored protein structurally related to snake venom disintegrins, cell-cell and cell-matrix interactions</td>
</tr>
</tbody>
</table>

Table 2. CTAs of non-X chromosomes expressed in less than 5 normal tissues [2].
cancer, ovarian cancer, hepatocellular carcinoma, and melanoma. CT-X genes are usually expressed in parallel, and tumours that express them tend to express several CT-X antigens. For example, in a study, it was revealed that 40% of breast tumours and 65% of melanomas expressed three or more CT-X [8]. CT-X genes have more restricted expression in normal tissues compared with non X-CTs and hence more likely to be used in immunotherapy approaches.

In contrast to CT-X genes, the genes for non-X cancer testis genes are distributed throughout the genome and do not generally form gene families and are not located within genomic repeats. In the testis, they are expressed more predominantly in later stages of germ cell differentiation, such as in spermatocytes. It seems that many have a role in meiosis; so their aberrant expression in cancer may contribute to abnormal chromosome segregation and aneuploidy [1].

These two groups of CTAs seem to have different functions because of their expression in different stages of spermatogenesis. RT-PCR and digital differential display analysis show that CT-X genes are under more stringent transcriptional restriction in somatic tissues than non X-CT genes which have more expression in somatic tissues.

Identification of CTAs

Several strategies have been used to identify CTAs. Many CTAs have been identified as a result of their recognition by CD8+ T cells and antibodies. Strategies such as T cell epitope cloning and SEREX rely on the ability of CTAs to elicit humoral or cellular immune response. So, gene products identified by such tools are true antigens, whereas genes identified by other strategies may not be immunogenic.

T cell epitope cloning

Many antigens recognized by CD8+ T cells have been discovered by cDNA libraries constructed from tumour cells transduced into target cells, which express the suitable HLA molecule. Subsequently, antitumour T cells were isolated from tumour infiltrates to detect the antigen epitopes presented by HLA on the surface target cells. This approach was first used by Bruggen et al., and the first cloned antigen by this technique was the melanoma antigen MAGE-1. This antigen was shown to be a target antigen for one of the cytotoxic T cell clones and the first recognized immunogenic tumour antigen eliciting T cell responses in a cancer patient [9]. Other new tumour
antigens including B melanoma antigen (BAGE) and G antigen (GAGE) gene family were identified by this strategy [10, 11].

**Serological analysis of cDNA expression libraries (SEREX)**

This method was first developed by Sahin and his colleagues who used antibody repertoire of cancer patients to identify antigens. Using this method, antibody response can be detected. In this approach, a cDNA expression library is constructed from a fresh tumour specimen and cloned into phage expression vectors. Then, E.coli cells are transduced by these recombinant phages. Recombinant proteins expressed by bacteria are incubated with serum from the autologous patient.

Clones reactive with high-titer antibodies are distinguished and nucleotide sequence of the cDNA insert will be identified. This technique was applied to identify CTAs NY-ESO-1, CT7/MAGE-C1, SCP-1, OY-TES-1, HOM-TES-85, CAGE, cTAGE, and PASD1 [3]. But the clinical significance of these anti-tumour antibodies is unknown; so, the antigens recognized by these antibodies should be screened for T cell recognition by reverse T-cell immunology. To accomplish this, antigen presenting cells should be either loaded with selected major histocompatibility complex (MHC) class I binding peptides or transduced by cDNA of the antigen [2].

**Differential gene expression analysis**

Differential display is a powerful tool for the comparison of gene expression between two or more mRNA populations. This technique consists of PCR and denaturing polyacrylamide gel electrophoresis steps to provide DNA fingerprints of tissues. RNAs extracted from the sources to be compared are reverse transcribed with one of a possible set of four degenerate oligonucleotide primers (dT)12VC, (dT)12VA, (dT)12VG, or (dT)12VT where V is C, A, or G. First-strand cDNA is used as a template in the PCR with oligo(dT) primer mixture and a decamer sequence that has been randomly generated. The complex mixtures of cDNAs are then separated by electrophoresis by a denaturing polyacrylamide gel. Cancer-testis genes can be efficiently identified through comparison of testis and cancer tissue libraries [2].

**DNA and tissue microarrays**

Microarrays are miniature devices having thousands of DNA sequences as gene-specific probes, immobilized on solid support (nylon, glass, silicon).
cDNA targets labeled with a radioactive, fluorescent, or chemiluminescent tag are hybridized with sequences on array, and the intensity of the signal generated by each bound probe indicates the relative abundance of that transcript in the sample. Using this technology, gene pool of tumour samples are compared with DNA sequences derived from testis-specific genes. This has been applied to identify a new CTA named STK31 in colorectal cancer.

Tissue microarray technology is a powerful tool for simultaneous analysis of hundreds of tissue specimens in a single experiment. A tissue microarray is constructed by taking core biopsies of paraffin-embedded tissues and re-embedding them on a single arrayed ‘master block’. Tissue microarrays are dependent on a variety of techniques such as immunohistochemistry for protein expression and fluorescence in situ hybridization (FISH) to detect DNA alterations. Tissue microarrays have the advantage of examining a single gene product per experiment in a large number of samples. So, it is possible to assess expression of a single testis gene in various tumour samples [2].

Massively parallel signature sequencing (MPSS)

In this approach millions of short sequence tags associated to transcript from different RNA preparations are generated and MPSS data of normal testis and different cancer tissues are compared. Using this approach a new CT gene called CT45 was found which is frequently expressed in lung cancer.

Serial analysis of gene expression (SAGE)

Serial analysis of gene expression (SAGE) is a method that has the ability to quantitate and compare large numbers of transcripts. Only a portion of the cDNA transcript, which is known as a SAGE tag, is needed to analyze the expression profile of each particular tissue. As the first concatemers (DNA segments composed of repeated sequences linked end to end) of SAGE tags are made; then, up to 30 tags will be sequenced at once. The frequency of each tag in the concatenated sequence shows the abundance of the corresponding transcripts in that cell. So, expression levels of a sequence can be compared between two populations. SAGE libraries can be used to analyze the differences in gene expression between cells or tissues [2].

A more comprehensive approach to identify CT genes is a combination of four platforms: MPSS, Expressed sequence tags (ESTs), Cap-analysis of Gene Expression (CAGE) and RT-PCR. This comprehensive approach
resulted in categorization of 153 CT genes according to their expression in normal tissues rather than testis [12].

A CT database has been recently established by the Ludwig Institute for Cancer Research (http://www.cta.lncc.br/) which contains results of standardized RT-PCR analysis of each CTA in a panel of 22 normal tissues and 32 cancer cell lines.

**Function of CTAs**

Biological functions of CTAs are not completely understood, but the function of non X-CTs is better clarified than CT-X antigens. The fact that expression of CTAs is much higher in high grade tumours and metastases provides clues that CTA expression has a role in tumourigenesis rather than being unrelated by-products of this process. It was found that cell lines expressing at least one of the three MAGE genes were more resistant to TNF-mediated cytotoxicity. Transfection of cells with MAGEA2 or MAGEA6 genes also gives them a proliferative advantage [2]; and Mage-A2 protein strongly down-regulates p53 trans-activation [12]. MAGE-A11 has a role in the regulation of androgen receptor function and MAGE-A4 binds to oncoprotein gankirin [12]. Multiple MAGE proteins can form complexes with Kap-1, a co-receptor of p53; and suppression of these gene products by siRNA results in p53 expression and apoptosis. Similar anti-apoptotic activity was found for CAGE.

A newly identified CT gene, Spermatogenesis Associated 19 (SPATA19) has been proposed to participate in cell differentiation, multicellular organismal development and spermatogenesis. It was shown that SPATA19 is expressed in basal cell carcinoma [13] and prostate adenocarcinoma [14]. SPATA19 contains a mitochondria-targeting signal and works as an adhesive molecule between the adjacent mitochondria of the sperm sheath, so it has a role in the maintenance of the normal mitochondrial sheath. Activation of the mitochondrial pathway can reverse cellular energy metabolism to non-malignant phenotype and also promotes reactive oxygen species (ROS) production by mitochondria and increase the susceptibility of tumour cells to apoptosis. Consequently, targeting of the mitochondria is a promising strategy for induction of apoptosis in tumour cells. SPATA19 has the special characteristic of having a mitochondria-targeting signal, so it can be a potential target in this field [14].

TSGA10 is a new cancer-testis gene whose function is partly identified. Mouse homologue of TSGA10 mRNA has mitotic arrest deficient domain and was first detected in the postmeiotic phase of spermatogenesis. It is then
processed to a major fibrous sheath protein of the sperm tail. Mitotic arrest deficient is a mitotic checkpoint protein. The mitotic spindle checkpoint monitors proper attachment of the bipolar spindle to the kinetochores of aligned sister chromatids. Recently, a protein–protein interaction between hypoxia inducible factor 1 (HIF-1), a transcriptional regulator of genes involved in oxygen homeostasis, and the TSGA10 was identified by yeast two- hybrid screening. Recent models suggest that TSGA10, after processing, can also serve as a scaffold for protein complexes involved in regulating signal transduction and cell division processes [15-17]. TSGA10 has been reported to be expressed in different types of tumours including 84.6% of acute lymphoblastic leukemia samples [18, 19].

The outer dense fiber (ODF) proteins have preferential expression during mammalian spermiogenesis. These proteins co-assemble along the axoneme during the development of the sperm tail and play a role in maintaining the passive elastic structures and elastic recoil of the sperm tail. It has been shown that ODF1 and ODF2 are expressed in basal cell carcinoma and prostate cancer [13, 14].

In another experiment, it was suggested that SSX has a functional role in cell migration and a potentially similar function in cancer cell metastasis. It has been revealed that when SSX is down-regulated in a melanoma cell line expressing SSX, the migration of cells will decrease [2].

As many of the important characteristics of cancer cells such as migration, invasion, immune subversion, apoptosis resistance, and induction of angiogenesis are also seen in gametogenesis or placentation processes, it is possible that CTA products controlling gametogenesis processes give similar characteristics to cancer cells.

Briefly, CTA functions can be categorized as follows:

- Structural components of spermatozoa such as TSGA10.
- Possible role in transcription regulation such as MAGE-A, SSX, HOM-TES-85, E2Flke/ HCA661, TAF7L, BRDT, PLU-1, BORIS, NXF2.
- Possible role in signal transduction such as LIP1, SGY1, MAGE.
- Helicase-like features such as CAGE, HAGE.
- Cell to cell binding such as SPA17, TPX1, ADAM2.
- Enzymatic actions such as ADAM2, LIP1, TSP50, LDHC, TPTE.
- Probable role in inhibition of apoptosis such as CAGE.
- Components of synaptonemal complex such as SCP1, SPO11 [2].
It has been suggested that expression of these antigens in tumour tissues is restricted to cells that maintain stem cell properties. CTAs may be true hallmarks of cancer stem cells and can be considered as targets for interference in recurrence and metastatic processes.

**Regulation of CTA expression**

An important question regarding their expression is about the mechanism of their transcriptional silencing in normal tissues except testis and their derepression in malignancies. Activation of CTAs in cancer may be the result of induction of a gametogenic program in cancer and different CTA expression profile observed in cancer may be a reflection of CTA expression profile in different stages of gametogenesis or placentation [12]. Especially for CT-X genes, expression can be induced by DNA methyl-transferase 1 inhibitors, 5-aza-2'-deoxycytidine (5DC), and by histone deacetylase (HDAC) inhibitors. It has been proven that DNA methylation is the primary silencing mechanism for these genes and demethylation is necessary and sufficient to produce their expression. It was also shown that heavy methylation represses gene expression in cells despite the presence of transcription factors required for expression. In another study, it was shown that the site specific hypomethylation of MAGE-A1 in tumour cells depends on demethylation and then persistent local inhibition of remethylation [2].

Multiple sequence alignment results and comparison of the 5' flanking regions of the mouse and human TSGA10 genes indicate that the homologue of the first exon of the mouse gene is located at 8.3 kb upstream of human exon 1. This result may indicate TSGA10 genes use different exon 1 sequences and different promoters; so, different mechanisms may act in different animals. The presence of an alternative promoter in human and pig TSGA10 genes compared with mouse and rat genes still needs to be investigated [16].

The mechanism of epigenetic regulation is somehow clear for some genes. For instance, recent data indicate that reciprocal binding of CCCTC-binding factor (zinc finger protein, CTCF) and CCCTC-binding factor like (BORIS) to the NY-ESO-1 promoter mediates epigenetic regulation of this CTA in lung cancer cells, and suggest that induction of BORIS may be a novel strategy to enhance immunogenicity of pulmonary carcinomas. It has also been shown that intratumoural heterogeneity of expression of CTAs in melanoma is regulated by methylation and using the demethylation agent 5-aza-2'-deoxycytidine they could induce expression of several CTAs [2].
CTAs as targets for cancer immunotherapy

Blood-testis barrier

Pathophysiologic data suggest that a blood-testis barrier exists in testis. As spermatogenesis begins at puberty, new cell surface antigens are expressed when the immune system has refined the ability to distinguish self from nonself. So, sperms in the testis do not stimulate immune responses. In addition, although antigen-presenting cells are commonly seen in the interstitial spaces of the testis, these cells are scarcely seen within the seminiferous tubules. So, testis is considered as an immune-privileged site. The mechanical barrier is made by tight junctions between Sertoli cells along the basolateral aspect and between capillary endothelial cells. The apparent lack of human leukocyte antigen (HLA) class I expression on the surface of germ cells is also important in making the testis an immune privileged site.

Figure 1. The Blood-Testis Barrier is a result of tight junctions between Sertoli. cells along the basolateral aspect and between capillary endothelial cells. 1: Basal lamina, 2: Sertoli cell, 3: Tight junction Modified from: http://en.wikipedia.org/wiki/Blood-testis_barrier.
Immunogenicity of CTAs

The characteristics of CTAs (expression in different types of cancers but not in normal tissues rather than testis) make them promising candidates for immunotherapy. To use CTAs in immunotherapy approaches, the first step is to prove their immunogenicity. Ability of CTAs to elicit cellular and humoral responses has led directly to the development of antigen-specific cancer vaccines. Humoral responses to CTAs have been seen in several tumours usually by means of ELISA testing against recombinant CT protein. For instance, antibodies against SCP-1 in pancreatic cancer, antibodies against NY-ESO-1, SCP-1, and SSX-2 in breast cancer, antibodies against CTSP-1 in prostate, thyroid, and breast tumours, antibodies against TSGA10 in hepatocellular carcinoma and malignant melanoma, and antibodies against MAGEA3, SSX2, and NY-ESO-1 in multiple myeloma, have been detected. Among these CTAs, NY-ESO-1 is the most attractive for cancer immunotherapy. The prevalence of anti-NY-ESO-1 antibodies in patients with advanced NY-ESO-1 positive tumours is estimated at 25-50% and the antibody titer increases with progression of the cancer and decreases after removal of the tumour [12].

CTAs are also immunogenic to cytotoxic T lymphocytes. For instance, Sp17 specific HLA-A1 and B27 restricted cytotoxic T lymphocytes generated from peripheral blood of a healthy donor were able to kill HLA-matched myeloma cells [2]. HLA-restricted T-cell epitopes have been identified for several CTAs including MAGE-A, NY-ESO-1 and SSX. Concurrent humoral and cellular responses have been detected for NY-ESO-1, MAGE-A and SSX antigens [12].

Another important point is to investigate the composite expression of CTAs in tumours. This is crucial for developing polyvalent vaccines because it is necessary to know what proportion of patients with a certain type of cancer can benefit from a polyvalent vaccine and which antigens are more suitable to be used in a polyvalent cancer vaccine for a certain type of cancer.

Cancer vaccine trials using CTAs

Vaccination with antigens specifically expressed by tumours can trigger the immune system and produce specific anti-tumour response. There are a growing number of human tumour specific antigens and their encoded MHC epitopes; and among them many epitopes are encoded by CTAs.

Multiple clinical trials have been conducted using MAGE-A3 or NY-ESO-1. Both have resulted in tumour regression in melanoma patients.
Immunological responses were also detected in both but more frequently in NY-ESO-1 than MAGE-A3 vaccinated patients [20, 21].

Over 34 trials with different NY-ESO-1 vaccine formulations have been performed. NY-ESO-1 peptide, protein, and pox-NY-ESO-1 vaccines can all induce strong NY-ESO-1 humoral and cellular responses in patients with no pre-existing NYESO-1 immunity. The NY-ESO-1 Protein/ISCOMATRIX® trial conducted by Jonathan Cebon had some hopeful results and a Phase II randomized trial is now ongoing. The salmonella/NY-ESO-1 vaccine, which has had considerable therapeutic effects in mice, is now being prepared for the clinic and NY-ESO-1 adenovirus constructs for vaccination will be developed in the near future.

Although the field of antigen-specific cancer vaccine is still in its early steps, it is anticipated that CTAs will be at the center of attention for immunotherapy in the future.

**Future directions**

As mentioned above, expression of CTAs often shows marked specificity for tumour cells. These markers can be used for early detection of cancer cells and specific gene immunotherapy of cancer. Active immunotherapy is still in preclinical and clinical trial phases of development but it will become available in the clinics in the near future. The growing knowledge in CTAs and their ability to elicit cellular and humoral responses will provide new tools for active immunotherapy of patients. Recent studies have shown that CTAs can be useful in adaptive T-cell transfer approaches [12].

Recent experiments postulate a relation between germline stem cells and cancer stem cells (CSC) with some evidence for CTA expression in both stem cells. This hypothesis needs to be investigated in different tumours and tissues. The embryonic stem (ES) cell and germline stem cell genes are subverted in precancerous stem cells. For example, the germline stem cell protein piwil2 may play an important role during the development of CSC, and these kinds of proteins may be used as common biomarker for early detection, prevention, and treatment of cancers [22].

Finally, many changes in tumour cells are caused by post-translational modifications which are not detected by DNA/RNA analyses; so proteomics-based studies of different tumour types are now underway. As a result, modifications of CTAs at protein level in tumoural cells may be detected and compared with normal cells with the aim to find new biomarkers for cancers [2].
Conclusion

CTAs are tumour antigens with limited expression in male germ cells in the testis and different types of tumours. Due to the existence of the blood-testis barrier, if testis specific proteins are expressed in other sites of body, they can elicit immune response. CTAs are promising candidates for cancer immunotherapy and have become a major focus for the development of vaccine-based clinical trials in recent years. In addition, these antigens may be used as biomarkers for early detection of cancers in the future.

References


