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Cancer/testis antigens and urological malignancies

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Abstract

Cancer/testis antigens (CTAs) are a group of tumour-associated antigens (TAAs) that display normal expression in the adult testis—an immune-privileged organ—but aberrant expression in several types of cancers, particularly in advanced cancers with stem cell-like characteristics. There has been an explosion in CTA-based research since CTAs were first identified in 1991 and MAGE-1 was shown to elicit an autologous cytotoxic T-lymphocyte (CTL) response in a patient with melanoma. The resulting data have not only highlighted a role for CTAs in tumorigenesis, but have also underscored the translational potential of these antigens for detecting and treating many types of cancers. Studies that have investigated the use of CTAs for the clinical management of urological malignancies indicate that these TAAs have potential roles as novel biomarkers, with increased specificity and sensitivity compared to those currently used in the clinic, and therapeutic targets for cancer immunotherapy. Increasing evidence supports the utilization of these promising tools for urological indications.

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Competing interests

P. Kulkarni, T. Shiraishi and R. H. Getzenberg declare associations with the following organization: Johns Hopkins University. See the article online for full details of the relationships.

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Introduction

As a result of the global increase in industrialization, urbanization, and acculturation changes in lifestyles, the worldwide incidence of cancer and the associated health-care costs are rising at an alarming rate. By 2030, the World Health Organization predicts that there will be more than 21 million new cancer cases and 13 million cancer-related deaths per year, generating a substantial cost burden to society.¹ Furthermore, the combination of a rising global population and increased average life expectancy has led to, and continues to lead to, significant increases in the number of elderly patients with cancer, particularly those with urological cancers.² According to a recent report commissioned by the National Institute on Aging (a division of the National Institute of Health in the USA), the number of people on this planet over 65 years of age will double to 1.4 billion (14% of the total population) in the next 30 years,³ underscoring the urgent need for new strategies to combat cancer.

Increased understanding of tumour genetics and pharmacological mechanisms of action, combined with technological advances in high-throughput genomics, have enhanced the development of new biomarkers and therapeutics. ‘Targeted therapies’ have been produced that are mainly, if not exclusively, active against tumours of a particular genotype, identified by a simple diagnostic test.⁴ In this era of ‘personalized’ medicine—which links individual patient biology with cancer risk, diagnosis, prognosis, and treatment—biomarkers play a critical role. Not only have biomarkers contributed greatly to our understanding of the heterogeneous nature of specific cancers, they have also led to significant improvements in treatment outcomes.⁵

Unfortunately, the biomarkers used to detect and treat urological malignancies—such as PSA and urine cytology—present several limitations relating to specificity, sensitivity, and cost-effectiveness. Furthermore, many of the therapeutic treatments currently in use—for example, androgen deprivation therapy (ADT) for prostate cancer^{6–8} and platinum compounds for bladder cancer⁹—are often discontinued as a result of drug resistance, following an initial period of positive response. Thus, biomarkers and therapeutic targets with increased efficacy and specificity—expressed highly in diseased tissue but not in surrounding normal tissue—are urgently needed.

A class of tumour-associated antigens (TAAs) called cancer/testis antigens (CTAs) has demonstrated potential in this context. These TAAs are typically expressed in embryonic stem cells and testicular germ cells, exhibiting little or no expression in most somatic tissues. They are also aberrantly expressed in several types of cancers.^{10–14} Although cancer genes typically harbour mutations associated with their pathological function, mutations in genes encoding the CTAs are, to the best of our knowledge, extremely rare.^{15–17} Nevertheless, CTAs are generally upregulated in response to DNA hypomethylation, which is a common feature of cancer cells.^{18,19} Indeed, treating cells with the DNA methyltransferase inhibitor decitabine (5-aza-2'-deoxycytidine) *in vitro* results in a dramatic upregulation of most, if not all, CTAs.^{20,21} Furthermore, several studies have demonstrated a correlation between CTA expression and promoter methylation status in tissue samples from patients with several different types of cancer.^{22–24}

Taken together, these observations suggest that the underlying cause of CTA-associated pathological function in cancer cells results from aberrant (dose-sensitive) expression of normal CTA genes, rather than the generation of mutated versions of these genes. In support of this theory, a study of fruit flies demonstrated a correlation between aberrant germline gene expression and tumorigenesis.²⁵ Moreover, upregulated CTA expression has been associated with advanced disease and poorer prognosis,²⁶ suggesting a key role of CTAs in tumorigenesis. Given the similarities between embryonic stem cells and cancer stem

cells,^{27–30} it seems likely that CTAs have an important function in disease progression and could, therefore, be useful for diagnosing and treating advanced cancers with stem-cell-like characteristics.

Broadly speaking, the CTAs can be grouped into two classes based on their chromosomal location. The CT-X antigens are located on the X chromosome and non-X CT antigens are located on the autosomes. Most CT-X antigens seem to be unique to primates and constitute several subfamilies of homologous genes, organized in discrete clusters along the X chromosome.³¹ As a result of their restricted expression in an immune-privileged organ, both groups of CTAs represent attractive immunotherapy targets.³² Antigenic CTA-derived peptides that are presented to the immune system with different human leukocyte antigen (HLA) allo-specificities elicit both humoral and cellular immune responses. Indeed, spontaneous humoral and cell-mediated immune responses have been demonstrated against several CTAs and patients with good antibody titres often present with better prognosis.³³

A potential protocol to treat urological malignancies utilizes immunogenic peptides corresponding to CTAs (Figure 1). A tumour sample is first profiled for CTA expression using DNA microarrays and a CTA ‘finger-print’ is generated. Synthetic immunogenic peptides corresponding to the CTAs are then used to pulse *in vitro* immature dendritic cells isolated from the patient. The pulsed cells mature into professional antigen-presenting cells, which are then injected back into the patient to trigger an immune response. This strategy is applicable to most other nonurological cancers, provided specific CTAs are aberrantly expressed. At present, more than 200 CTAs have been described in the literature and two databases—CTpedia³⁴ and ACTAbase³⁵—serve as excellent resources on these antigens. Although emerging evidence has shed light on the functions of a few CT-X antigens in cancer, the majority remain poorly understood. By contrast, the non-X CTAs are fairly well conserved throughout evolution, with established roles in processes such as transformation,³⁶ chromatin remodelling,³² transcriptional regulation, and signalling (Figure 2b).¹¹ Thus, the non-X CT antigens are particularly promising targets for the development of small-molecule therapeutics.

Various CTA-based cancer vaccine trials are already underway (Table 1). Although several CTAs are differentially expressed in patients with urological malignancies (Figure 2), CTA-based therapy has been researched more extensively for other types of cancer—particularly melanoma—than for urological cancers. However, recent reports illustrate the significant progress that has been made with respect to the characterization of CTAs in patients with these malignancies. In this Review, we evaluate the available literature regarding the utility of CTAs as biomarkers and therapeutic tools for patients with prostate, bladder, renal cell, and testicular germ cell tumours (TGCTs).

CTAs and prostate cancer

Worldwide, prostate cancer is the most common urological malignancy. In men, it is the second most frequently diagnosed cancer and the sixth leading cause of cancer death, with 903,500 new cases and 258,400 cancer deaths reported in 2008.² The introduction of serum PSA testing is thought by many to have dramatically improved the prognosis of men diagnosed with prostate cancer. However, two recent reports from Europe and the USA have sparked controversy regarding this issue.^{32,33} Although the European study concluded that routine PSA screening reduced the mortality rate associated with prostate cancer by 20%,³⁷ the US-based population study showed no discernable difference between patients who received PSA screening and control groups.³⁸ Consistent with the latter findings, the US Preventive Services Task Force (USPSTF) has now officially recommended against routine PSA screening for prostate cancer. Although this recommendation remains equivocal, it is

generally accepted that the widespread use of PSA testing results in the over detection and over treatment of potentially indolent disease. On the other hand, the fact that relatively few men present with metastatic disease has been attributed, at least in part, to PSA screening protocols.³⁹

ADT is the gold-standard first-line treatment for metastatic prostate cancer. Although this approach is usually effective to begin with, the response to ADT is often transient and tumours tend to progress to castration-resistant prostate cancer (CRPC). Despite improvements to chemotherapy regimens, CRPC is characteristically life threatening.^{6,7} Thus, there are two major challenges facing the management of prostate cancer today; firstly, to develop new biomarkers that can identify aggressive phenotypes of prostate cancer and, secondly, to develop new treatment options for CRPC.

CTAs as biomarkers

Several studies have investigated the potential role of CTAs as biomarkers in prostate cancer. In early reports, protein expression of cancer/testis antigen 1A (CTAG1A) was detected in 3% of patients with localized prostate cancer and 15% of patients with CRPC.⁴⁰ Subsequently, Hudolin *et al.*⁴¹ reported that, although expression of melanoma antigen family A, 1 (MAGEA1) was observed for just 10.8% of carcinoma samples, antibodies against multiple MAGEA antigens and combinations of CTAG1A and cancer testis antigen 2 (CTAG2) stained 85.9% and 84.8% of samples, respectively. Synovial sarcoma X protein expression has been shown to be largely restricted to metastatic prostate cancers, further supporting a role for CTAs as biomarkers of disease progression.⁴²

In order to elucidate CTA expression patterns in prostate cancer samples, Suyama *et al.*²⁴ produced a customized DNA microarray tiled with DNA probes representing a significant portion of all known CTAs. This study revealed that several CTAs are upregulated in prostate cancer cells, the majority of which are CT-X antigens. Several of the MAGEA and chondrosarcoma-associated gene subfamilies of CT-X antigens were upregulated in CRPCs but not in primary prostate cancers, whereas P antigen family, member 4 (prostate associated; PAGE4) expression was, conversely, upregulated in primary prostate cancers but silent in metastatic disease. These observations suggest that CTAs could potentially be used as biomarkers to distinguish men with aggressive disease (who are likely to require treatment) from men with more indolent disease (for whom a conservative treatment programme, such as active surveillance, might be more appropriate).

To further investigate this potential role, Shiraishi *et al.*⁴³ used a panel of five CTAs—centrosomal protein 55 kDa (CEP55), NUF2, NDC80 kinetochore complex component, homolog (*S. cerevisiae*; NUF2), PAGE4, PDZ binding kinase (PBK) and TTK protein kinase (TTK)—that are differentially expressed in prostate cancer to devise a multiplex real-time PCR assay to predict prostate cancer progression and identify disease recurrence following radical prostatectomy. Expression of all of the CTAs, with the exception of TTK, significantly correlated with prostatectomy Gleason score using Pearson's correlation coefficient (CEP55 $P = 0.0057$; NUF2 $P = 0.0051$; PBK $P = 0.0295$; PAGE4 $P = 0.0236$). However, CTA expression was not shown to correlate with age, preoperative serum PSA level, or tumour stage. Although Gleason score is thought to be one of the strongest predictors of recurrence, it tends to be relatively subjective. The multiplex assay could provide an objective assessment that is quantitative, consistent across institutions, and perhaps even useful as a molecular surrogate for Gleason score. However, it is worth noting that the investigators reported several limitations to the study, including a small sample size and a sample set that was nonreflective of a contemporary series.^{43,41} Additional studies—performed across multiple institutions and involving larger contemporary-matched cohorts

of patients presenting with newly diagnosed prostate cancer—could further validate this CTA-based predictive test.

Using a different approach, Xie *et al.*⁴⁴ developed a multiplex assay that simultaneously measures expression of autoantibodies against CTAG1A, X antigen family, member 1B (XAGE1B), synovial sarcoma, X breakpoint 2 (SSX2), synovial sarcoma, X breakpoint 4 (SSX4), alpha-methylacyl-CoA racemase (AMACR), p90 autoantigen, PC4 and SFRS1 interacting protein 1 (PSIP1; also known as LEDGF) and PSA. This assay demonstrated enhanced sensitivity and specificity over PSA alone with respect to distinguishing prostate cancer from nonmalignant tissue. The presence of a natural immune response against CTAs could, therefore, represent an alternative strategy for exploiting these antigens as diagnostic and prognostic markers for prostate cancer.

Despite these promising preliminary findings, several limitations to the use of CTAs as biomarkers for prostate cancer have already been identified. For example, the frequency of CTA expression is not generally high enough for individual CTAs to serve as single biomarkers; a problem that is exacerbated by the heterogeneity of prostate cancer. Thus, combining the expression analysis of a CTA with that of other CTAs (or non-CTA markers) might be necessary to exploit CTAs as biomarkers for prostate cancer.

CTAs as therapeutic targets

The efficacy of sipuleucel-T—an autologous cellular immunotherapy—for the treatment of men with asymptomatic or minimally symptomatic metastatic CRPC has set a precedence for prostate cancer immunotherapy. This treatment approach is emerging as a promising modality for chemotherapy-resistant prostate cancer and perhaps even early-stage disease. Accumulating evidence suggests that CTAs could be the next generation of immunotherapeutic targets for advanced prostate cancer. CTAG1A and members of the MAGE family have already been extensively studied as therapeutic targets for other types of cancer in several clinical trials^{45–47} and CTAG1A has recently entered a phase I clinical trial for prostate cancer (Table 1).⁴⁸

In other studies, aimed at detecting circulating auto-antibodies, anti-CTAG1A antibodies have been observed more frequently in the serum of patients with advanced disease (18.9%) compared to localized tumours (0.9%).⁴⁰ Similarly, SSX2 antibodies and SSX2-specific T cells have been identified in the peripheral blood of patients with prostate cancer, indicating that patients can have pre-existing immunity to SSX2.^{42,49,50} T-cell-mediated immune responses to PAGE4 have also been reported in patients with prostate cancer.⁵¹ Parmigiani *et al.*⁵² described a novel CTA, ankyrin repeat domain 30B pseudogene 2 (ANKRD30BP2; also known as CTSP-1), which is aberrantly expressed in 58% of prostate tumors and capable of eliciting a humoral immune response (HIR) in about 20% of patients with prostate cancer. The same group also reported that patients with anti-CTSP-1 antibodies have a lower risk of biochemical recurrence and that presence of anti-CTSP-1 antibodies significantly associated with a better prognosis in patients with higher Gleason grade tumours.⁵³

Furthermore, centrosomal protein 290 kDa (CEP290; also known as T21) was identified by the SEREX (Serological Analysis of Recombinant cDNA EXpression libraries) approach, which can identify tumour antigens that can elicit high titre IgG antibody responses by combining serological analysis with antigen cloning techniques.⁵⁴ In this study, which utilized a normal testicular cDNA library and pooled allogeneic sera from patients with prostate cancer, CEP290 was shown to be significantly overexpressed in patients with prostate cancer compared to those with benign prostate epithelium. An HIR against CEP290 was observed in 50% of patients with prostate cancer. Moreover, one research team detected

A kinase (PRKA) anchor protein 4 (AKAP4)— which induces AKAP4-specific and major histocompatibility complex, class I, A (HLA-A)-restricted cytotoxic T lymphocytes (CTLs)—in 65% of prostate cancer tissues (13 of 20).⁵⁵ Taken together, these findings suggest that several CTAs are potential immunotherapeutic candidates that await clinical validation for advanced prostate cancer.

In a phase I clinical trial, 24 patients with metastatic CRPC were treated with ipilimumab—an antibody that blocks CTL-associated antigen 4 (CTLA4)—and granulocyte-macrophage colony-stimulating factor (GM-CSF).⁵⁶ PSA declines of more than 50% were observed in 50% of patients treated with the highest doses ($n = 6$). Interestingly, IgG antibodies against CTAG1A were induced in one of the three clinical responders. These results show that this combination immunotherapy with CTLA4 blockade and GM-CSF can induce the expansion, not only of activated effector CD8⁺ T cells, but also of CTA-specific T cells. Although the physiological functions of most CTAs—especially the CT-X antigens—remain poorly understood,^{11,26} data are beginning to shed new light on immunotherapy approaches for prostate cancer. For example, among the CT-X antigens, MAGEA11 was shown to increase androgen receptor (AR) transcriptional activity via direct interactions with AR and other coactivators.^{57–59} Suyama *et al.*²⁴ demonstrated that MAGEA2 is associated with proliferation and chemosensitivity of prostate cancer cells. Similarly, expression of members of the SSX protein family has been associated with stem cell migration, suggesting a biologically important role that is connected to its metastatic phenotype.⁶⁰ Among the non-X CT antigens, lysine (K)-specific demethylase 5B (KDM5B; also known as JARID1B)—a chromatin remodeller that specifically demethylates histone H3 lysine 4 (H3K4)—is upregulated in prostate cancers (especially metastatic tumours) and potentiates AR transactivation.⁶¹ Collectively, these observations underscore the potential for discovering small molecule therapeutics for prostate cancer by targeting the CTAs.

CTAs and bladder cancer

Bladder cancer, which is reported more frequently in men than in women, is the second most common urological malignancy (after prostate cancer). In 2008, 386,300 new cases of bladder cancer and 150,200 associated deaths were reported worldwide.² Three main types of bladder cancer have been described; transitional cell carcinoma (TCC), squamous cell carcinoma, and adenocarcinoma. TCC, which accounts for about 90% of bladder cancer cases, mainly occurs in the innermost lining of the bladder and can be either superficial (70–80% of cases), muscle invasive, or metastatic. Adenocarcinoma, which accounts for less than 2% of cases, arises from glandular cells.⁶² Although several biomarkers have been identified for bladder cancer, no single biomarker is a reliable predictor of disease recurrence or prognosis, and false-negative rates are generally high. The pathologic diagnosis achieved with cystoscopy still remains the gold standard for detecting and evaluating both early and late stages of bladder cancer. However, although highly specific (90% specificity), this approach is associated with poor sensitivity and is an invasive technique that requires the insertion of a catheter.⁶³

CTAs as biomarkers

Depending on the type and stage of the bladder tumour, different treatment options are administered. Superficial papillary TCC is often treated with transurethral resection of bladder tumour (TURBT).⁶³ Superficial non-papillary TCC—which is generally more aggressive and tends to progress to invasive tumours—is often treated with BCG, a form of immunotherapy.⁶⁴ In some patients, BCG treatment has proven to be helpful in reducing the risk of recurrence and progression after TURBT.⁶⁵ Invasive TCCs are often treated with radical cystectomy in combination with radiation and chemotherapy. Although initial response to chemotherapeutic agents (including platinum compounds) is typically good,

treatment does not generally lead to a cure and tumour recurrence is typical.⁹ Hence, biomarkers with high sensitivity and specificity are urgently needed. It is also important to be able to differentiate between patients with invasive and superficial TCCs (to avoid overtreatment or undertreatment patients), identify patients who are likely to respond to immunotherapy (to reduce unnecessary adverse effects), and to determine the most appropriate timing for radical cystectomy.

Several CTAs, especially CT-X antigens, are highly expressed in bladder cancers (Figure 2).²⁶ One early study reported increased expression of MAGEA1 (21% of cases) and MAGEA3 (35% of cases) in primary TCCs.⁶⁶ Subsequently, increased expression of several MAGE genes has been associated with advanced stages of the disease.⁶⁷ In these immunohistochemical studies, several members of the MAGE gene family were highly expressed in squamous cell carcinoma and TCC compared with normal bladder tissue. Of particular note was MAGEA9, which was highly expressed in the majority of patients (>60%) with higher grades (2 and 3) of TCC and associated lymph node metastases. Increased expression of both MAGEA9 and MAGEA4 was associated with progression to metastatic disease, as well as disease recurrence. Thus, MAGEA9 could be useful, not only as a prognostic and diagnostic marker, but also as an immunotherapeutic target.⁶⁸

Another member of the MAGEA family, MAGEA10, seems to be the most immunogenic of the MAGE antigens and is upregulated in subsets of both noninvasive (19.4%) and invasive (31.3%) bladder cancers compared to normal bladder tissue.⁶⁹ In this study, MAGEA10 expression was used to measure the effectiveness of immunotherapy, underscoring its biomarker potential. Another CT-X antigen that is frequently overexpressed in a variety of tumours is CTAG1A.⁷⁰ This CTA was shown to demonstrate higher expression in TCC than in squamous cell carcinoma using epitope mapping and tissue microarray analysis.⁷¹ Some of the non-X CT antigens have also been shown to be highly expressed in bladder cancers. For example, acrosin binding protein (ACRBP) is overexpressed in 28% of bladder cancers,⁷² and lymphocyte antigen 6 complex, locus K (LY6K), a putative oncogene, is highly upregulated in clinical samples from patients with bladder tumours, as well as in several bladder cancer cell lines.⁷³ JARID1B was shown to be significantly upregulated in bladder cancer specimens compared to normal tissue, with a dose-sensitive effect in several bladder cancer cell lines.⁷⁴

CTAs as therapeutic targets

On the basis that MAGE genes are highly expressed in advanced tumours, Nishiyama *et al.*⁷⁵ used autologous dendritic cells pulsed with MAGEA3 peptide as an immunotherapeutic option. The investigators established that, in 75% of treated patients ($n = 4$), lymph node metastases and liver metastases were significantly reduced, with no severe adverse effects.⁷⁵ As noted earlier, expression levels of CTAs are often upregulated in response to epigenetic modulators. Picard *et al.*⁷⁶ demonstrated that MAGEA9 expression was increased in response to the DNA methyltransferase inhibitor decitabine and histone deacetylase inhibitors MS-275 and 4-phenylbutyrate, highlighting their potential for the targeted treatment of bladder cancer.

CTAs and renal cell carcinoma

Kidney cancer accounts for about 2% of all new cancer cases worldwide,⁷⁷ with 111,100 cases and 43,000 deaths reported in 2008 in the developed world.² Renal cell carcinoma (RCC) accounts for approximately 85% of all kidney cancers; the remaining 15% includes transitional cell cancers of the renal pelvis (approximately 12%), sarcomas, and other soft tissue malignancies (approximately 2%).⁷⁸ At present, surgical resection is the most effective treatment for localized RCC tumours; 5-year survival is 85% for patients with

organ-confined disease who undergo nephrectomy.^{79,80} However, one-third of patients have metastases at diagnosis and, among those with clinically localized disease, 30–40% will develop metastases after surgery.^{76,81} Treatment options in these patients remain limited and 5-year survival is estimated at less than 5%.⁸² Furthermore, RCC is resistant to conventional therapies such as radiation and chemotherapy^{83,84} and prognosis for patients with advanced stages of RCC remains poor, underscoring the urgent need for novel diagnostic and prognostic biomarkers and therapeutic targets.

CTAs as biomarkers

CTA expression profiles vary with cancer type, enabling the classification of cancers based on the frequency of CTA expression. On this basis, RCC is characterized as a cancer type with low expression of relatively few CTAs; thus, the utility of CTAs as biomarkers for RCC seems to be limited.²⁶ Consistent with this prediction, a study involving 57B—a murine pan-specific monoclonal antibody that has been shown to react with MAGEA1, MAGEA3, MAGEA4, MAGEA6, and MAGEA12—demonstrated that none of these CT-X antigens are expressed in RCCs.⁶⁷ Furthermore, the same group also used IHC analysis to show that CTAG1A expression is not upregulated in RCCs.⁸⁵

However, in contrast to these findings, Yamanaka *et al.*⁸⁶ demonstrated that MAGEA1, MAGEA2, MAGEA3, and MAGEA4 genes were expressed in 22%, 16%, 76%, and 30% of RCC samples tested using reverse transcription PCR ($n = 50$) and Garg *et al.*⁸⁷ reported expression of sperm associated antigen 9 (SPAG9) in 88% of patients with RCC using *in situ* RNA hybridization and immunohistochemistry. An HIR against SPAG9 was also detected in the sera of patients with RCC—but not in healthy individuals—by enzyme-linked immunosorbent assay and immunoblotting. Furthermore, recent work from our laboratory has shown that the non-X CT antigen SPAG4 is highly expressed in RCC and regulated by hypoxia inducible factor. Interestingly, low levels of SPAG4 mRNA expression significantly correlate with shorter progression free survival, suggesting that SPAG4 could serve as a promising diagnostic and prognostic biomarker for RCC (P. Kulkarni, unpublished work). These conflicting data can be explained by differences in the sensitivities of the techniques employed, emphasizing the need for additional studies to evaluate the potential of CTAs as biomarkers for RCC.

CTAs as therapeutic targets

Given that RCC represents one of the most immuneresponsive cancers, immunotherapy is a promising strategy for treating patients with RCC.^{88,89} However, as a result of low expression frequencies, the use of CTAs as immunotherapy targets presents additional challenges. Nonetheless, treatment with the decitabine has been shown to induce CTAG1A expression for up to 60 days following treatment, generating a functional protein that is efficiently recognized by HLA-A2-restricted CTAG1A-specific CTLs. This finding supports the use of CTAs as therapeutic targets for RCC.⁹⁰

CTAs and testicular germ cell tumours

Testicular cancer is the most prevalent cancer in men aged 15–44 years, with an average of 4.6 cases and 0.3 deaths per 100,000 men reported in 2008.^{2,91,92} Treatment is successful in virtually 100% of patients with organ-confined disease and 80% of those with metastatic disease.⁹³ However, there are two major challenges that face testicular cancer care; identifying patients that can be treated with orchiectomy alone (without the need for chemotherapy or radiation) and, if disease is metastatic, determining which patients have responded well to nonsurgical treatment (radiation or chemotherapy) and do not require invasive surgery to remove metastases.

Both major forms of TGCTs—nonseminomas and seminomas—are generally diagnosed at a relatively young age (median age of 25 and 35 years, respectively), whereas spermatocytic seminomas—a much less common form of testicular cancer—affects men aged more than 50 years.⁹⁴ Nonseminomas and seminomas often metastasize and are generally treated by orchiectomy and platinum-based therapies. These malignancies are thought to arise from primitive gonocytes and primordial germ cells (PGCs), which are present before birth (Figure 3). This suggests that intratubular germ cell neoplasia unclassified cells, which give rise to seminomas and nonseminomas but not spermatocytic seminomas, actually form during development and are thus termed inaccurately. Spermatocytic seminomas resemble adult spermatogonia and spermatocytes, and probably develop from these cells much later in life than other testicular malignancies. It is, therefore, not surprising that non-seminomas and seminomas are the most aggressive TGCTs, followed by spermatocytic seminomas.⁹⁴ In fact, spermatocytic seminomas most frequently present in older men as painless enlargements of the testis that rarely metastasize and are, as such, generally treated with orchiectomy alone.

CTAs as biomarkers

The use of biomarkers for detecting and monitoring TGCTs has been limited to date. TGCT screening is largely restricted to periodic digital self-examination, as recommended by general practitioners. In order to confirm diagnosis and prognosis after surgery, a blood test for α -fetoprotein and β -human chorionic gonadotropin is commonly performed following treatment (orchiectomy with chemotherapy or radiation). However, these biomarkers are rarely seen in seminomas and are not necessarily expressed in nonseminomas.⁹⁵

Although CTAs are not currently used as markers for TGCT, some studies have produced fairly promising data to support their use—especially for seminomas. As outlined in Supplementary Table 1 online, these tumours normally express cancer/testis antigen family 45 (CT45) members, G antigen (GAGE) family members, MAGEB1, MAGEB2, MAGEC1, MAGEC2, and testis specific protein, Y-linked (TSPY) family members. Although these CTAs seem to be excellent tissue markers, their use as serum-based markers has not yet been investigated. Several laboratories have also demonstrated that CTA expression coincides with the repression of pluripotency genes during sperm development and similarly, during tumorigenesis of their respective cancer lineages.⁹⁴ OCT3/4 and NANOG—transcription factors that trigger the development of induced pluripotent stem (iPS) cells—are upregulated in many TGCTs (including seminomas and nonseminomas, but not spermatocytic seminomas).^{7,96,97} It is not surprising then that spermatocytic seminomas actually express a wider array of CTAs than seminomas and nonseminomas (Supplementary Table 1 online). Increased transition from pluripotency to CTA expression in spermatocytic seminomas could explain the improved prognosis observed in patients who undergo orchiectomy alone compared to seminomas and nonseminomas, which also require adjuvant therapy.

CTAs as therapeutic targets

Given the immune-privileged nature of the testis, CTAs are potential therapeutic targets for non-organ-confined disease. However, as metastatic disease is already highly sensitive to platinum-based therapies and radiation, only a subset of metastatic cancers (about 20%) will require further treatment.⁹⁵ These cases, which are recurrent and generally fatal, might be treatable by CTA-based immunotherapy.

Conclusions

Therapeutic vaccination for cancer treatment depends on T-cell response to TAAs.⁹⁸ Thus, the identification and characterization of immunogenic CTA peptides, together with the identification of respective HLA class I antigen restriction, is a highly viable approach for cancer immunotherapy (Figure 1). Unfortunately, several issues have hampered the success of this approach in a clinical setting. Firstly, low levels of expression, coupled with great intertumoural and intratumoural heterogeneity in CTA expression, mean that these approaches are some-what challenging. Secondly, CTA expression heterogeneity could also impair immunogenicity and immune recognition of cancer cells by the immune system, resulting in decreased vaccination efficacy. An even bigger potential problem is that tumour cell heterogeneity could lead to the emergence of CTA-negative neoplastic clones, capable of escaping treatment-induced and CTA-specific immune surveillance.⁹⁹

A possible solution to these limitations is to boost CTA expression by administering decitabine, a DNA hypomethylating agent used to treat myelodysplastic syndromes.¹⁰⁰ In addition to their effect on CTA expression, such agents are characterized by a more general immunomodulatory response, which leads to the persistent upregulation of molecules involved in tumour antigen presentation.¹⁰¹ Thus, the ability to pharmacologically ‘normalize’ intratumoural CTA distribution and upregulate the expression of HLA antigens, accessory molecules, and costimulatory molecules has fuelled optimism for the use of CTA-based vaccination therapy.⁹⁹ Furthermore, the use of these epigenetic modulators is likely to render cancer cells more immunogenic and less prone to immune surveillance evasion. However, such optimism needs to be balanced with caution. For example, there could be ‘off-target’ effects of epigenetic drugs that could, potentially, result in undesirable consequences. Additionally, some CTAs have putative oncogenic functions when overexpressed, even if only transiently, which could be counterproductive. The benefits and risks of such an approach must, therefore, be carefully contemplated.

Tumour cell heterogeneity is also a major impediment to developing reliable biomarkers. Using a combination of markers is likely to improve the accuracy of prognosis and treatment stratification compared to utilization of a single marker. In fact, there has already been some success in the identification of subtypes of certain urological malignancies, such as prostate cancer, based on genetic signatures.^{102,103} In the future, CTA-based gene signatures could be used to complement clinicopathological factors in the diagnosis of aggressive and indolent cancers, discerning treatment responders from nonresponders, and monitoring therapeutic response. Perhaps more robust mRNA-based platforms designed for high-throughput screening with greater sensitivity than typical DNA microarrays¹⁰⁴—such as the nCounter® Gene Expression Assay (NanoString Technologies, Seattle, WA, USA)—could be utilized to achieve highly reproducible results across multiple institutions. The efficacy of such platforms could be tested directly using total blood samples^{105,106} or circulating tumour cells,¹⁰⁷ providing critical information regarding diagnosis and prognosis in a minimally invasive manner.

The lack of knowledge regarding the functions of most CTAs, especially in relation to the development of cancer, has limited progress in targeting them therapeutically. However, work from our laboratory has identified some interesting characteristics of CTAs that shed new light on their functions. For example, more than 90% of CTAs are predicted to be intrinsically disordered proteins (IDPs).¹⁰⁸ IDPs are proteins that lack a rigid structure, at least *in vitro*, and are frequently overexpressed in several pathological conditions. The inherent ability of IDPs to engage in promiscuous interactions with other proteins when present at high concentrations is thought to explain their pathological effects.¹⁰⁹ Taken together, these observations provide a novel perspective on CTAs, implicating them in the

dose-sensitive processing and transducing of information in cells with altered physiological states. These findings should enhance our understanding of the functions of CTAs and facilitate the development of novel cancer therapeutics.

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Key points

- Restricted expression of cancer/testis antigens (CTAs) in an immune-privileged organ (the adult testis) and aberrant expression in malignant tissue make CTAs ideal biomarkers and immunotherapeutic targets for managing urological cancers
- Unlike many cancer-associated genes, which frequently harbour mutations associated with their pathological function, mutations in genes encoding the CTAs are extremely rare
- The majority of CTAs are thought to be intrinsically disordered proteins that often engage in promiscuous interactions with other proteins when overexpressed
- A key problem for CTA-based therapies is tumour cell heterogeneity, which results in differential expression of CTAs; use of the FDA-approved DNA methylation inhibitor decitabine can circumvent this issue
- Use of a combination of markers is likely to improve the accuracy of prognostication and treatment stratification compared to utilization of a single marker
- The success of clinical trials underscores the immunotherapeutic potential of a CTA-based approach and indicates that CTAs could be used as clinical tools for urological malignancies in the near future

Review criteria

We conducted detailed searches of the medical literature published in PubMed up until March 31st 2012. The search terms we used were “cancer/testis antigens”, “urological malignancies”, “prostate cancer”, “bladder cancer”, “testicular cancer”, “renal cell carcinoma”, “kidney cancer”, “biomarker”, and “therapeutic target”. Only English-language articles were included.

Figure 1.

Schematic diagram of an immunotherapeutic approach to treating urological malignancies that utilizes immunogenic peptides corresponding to CTAs. A tumour sample is profiled for CTA expression using DNA microarrays. A CTA ‘fingerprint’ is generated and immunogenic peptides corresponding to the CTAs are generated that are used to pulse *in vitro* immature dendritic cells isolated from the patient. The pulsed cells mature into professional antigen presenting cells, which are then injected back into the patient to trigger an immune response. Abbreviations: CTA, cancer/testis antigen; Cy3, cyanine 3; Cy5, cyanine 5.

Figure 2.

Normalized expression heat maps. **a** | CT-X genes and **b** | non-X CT genes based on expression profiles from prostate cancer, bladder cancer, renal cell carcinoma, and testicular germ cell tumour samples ($n = 397$) obtained from the Gene Expression Omnibus (GEO) database.¹¹⁰ The complete list of expression profiles, together with the respective GEO dataset (GDS) records, is provided in the Supplementary Information online. The raw data files were converted into expression profiles via the GeneChip Robust Multi-Array Analysis method. Data analyses were performed using R packages,¹¹¹ Bioconductor,¹¹² and gplots.¹¹³ For each gene, the expression values from all samples were averaged for each type of urological malignancy. Mean expression levels for all genes were then normalized to range from 0 to 1. Some genes appear multiple times in this list as a result of different probes present on the chip.*Multiple genes involved.

Figure 3.

Schematic diagram of mammalian spermatogenesis that depicts the stages of testicular germ cell tumour development. Primordial germ cells enter the testis during embryogenesis and develop into gonocytes. These cells, which are only present before birth, are thought to be the precursors of seminomas and nonseminomas. In humans, the process of spermatogenesis begins at puberty, from precursor cells known as spermatogonia, and continues throughout the adult life. Spermatocytic seminoma is thought to develop from spermatogonia or spermatocytes.

Table 1

Selected trials of cancer/testis antigen vaccines for various types of cancer

Vaccine	Adjuvant	Tumour histotype	Phase	Sponsor	Trial identifier
GSK2132231A (MAGEA3 ASCI)	Dacarbazine	Melanoma	I	GSK	NCT00849875
GSK1572932A (MAGEA3 ASCI)	Cisplatin, vinorelbine and radiotherapy	Non-small-cell lung cancer	I	GSK	NCT00455572
CTAG1A-derived peptide or CTAG1A protein	PF-3512676 and montanide ISA 720 VG (with or without cyclophosphamide)	CTAG1A-expressing tumours	I	University of Pittsburgh	NCT00819806
CTAG1A and CTAG2 peptides	None	Prostate cancer	I	Baylor College of Medicine	NCT00711334
CTAG1A, gp100, and MART-1 peptides	Oncovirus poly ICLC and montanide ISA 51 VG (with or without CP 870-893 antibody)	Melanoma	I	H.3Lee Mof3tt Cancer Center and Research Institute	NCT01008527
Recombinant MAGEA3 protein	AS15 (with or without BCG)	Bladder cancer	I	Patrice Jichlinski, University of Lausanne Hospitals	NCT01498172
IMF-001 (recombinant CTAG1A protein and cholesterol hydrophobized pullulan)	None	CTAG1A-positive solid tumours or melanoma	I	ImmunoFrontier, Inc.	NCT01234012
CTAG1A genetically engineered T cells	None	Synovial carcinoma	I	University of Pennsylvania	NCT01343043
Recombinant MAGEA3 protein	AS15	Multiple myeloma	I	Ludwig Institute for Cancer Research	NCT01380145
Mature dendritic cells pulsed with peptide mixes derived from full-length CTAG1A, MAGEA1, and MAGEA3	Decitabine (5-aza-2'-deoxycytidine) and imiquimod	Neuroblastoma, Ewings sarcoma, osteogenic sarcoma, rhabdomyosarcoma, and synovial sarcoma	I	Penn State University	NCT01241162
GSK2241658A (CTAG1A ASCI)	None	Melanoma	I	GSK	NCT01213472
GSK2302032A (PRAME ASCI)	None	Non-small-cell lung cancer	I	GSK	NCT01159964
Recombinant MAGEA3, MART1, and survivin	Therapeutic autologous dendritic cells (with or without keyhole limpet haemocyanin)	Melanoma	I/II	Dermatologische Klinik MIT Poliklinik, Universitaetsklinikum Erlangen	NCT00074230
CTAG1A protein	Poly-ICLC and montanide	Melanoma	I/II	New York University Langone Medical Center	NCT01079741
Autologous T3cells transduced with MAGEA3(A3A) and CTAG1A(C259)	Cytoreductive chemotherapy	Melanoma	I/II	University of Pennsylvania	NCT01350401
PRS pan-DR, MAGEA3, MAGEA1, CTAG1A, and MART1	Cyclophosphamide	Non-small-cell lung cancer	II	Institut Gustave Roussy	NCT01159288
Recombinant MAGEA3 protein	AS15	Urinary bladder neoplasms	II	European Association of Urology Research Foundation	NCT01435356
GSK1203486A (MAGEA3 ASCI)	None	Melanoma	II	GSK	NCT00706238
MAGEA3 and gp100 peptides	Resiquimod (R848)	Melanoma	II	M3D.3Anderson Cancer Center	NCT00960752

Vaccine	Adjuvant	Tumour histotype	Phase	Sponsor	Trial identifier
MAGEA3 and CTAG1A peptides	DT-PACE chemotherapy and autologous transplantation	Multiple myeloma	II/III	University of Arkansas	NCT00090493
GSK1572932A (MAGEA3 ASCI)	None	Non-small-cell lung cancer	III	GSK	NCT00480025
GSK2132231A (MAGEA3 ASCI)	None	Melanoma	III	GSK	NCT00796445

Abbreviations: ASCI, antigen-specific cancer immunotherapeutic; CTAG1A, cancer/testis antigen 1A; CTAG2, cancer/testis antigen 2; DT-PACE, dexamethasone, thalidomide, cisplatin, doxorubicin, cyclophosphamide, and etoposide; gp100, glycoprotein 100; GSK, Glaxo Smith Kline; MAGE, melanoma antigen family proteins; MART1, melanoma antigen recognized by T cells 1; PRAME, preferentially expressed antigen in melanoma; PRS pan-DR, proline-rich sequence pan-DR.