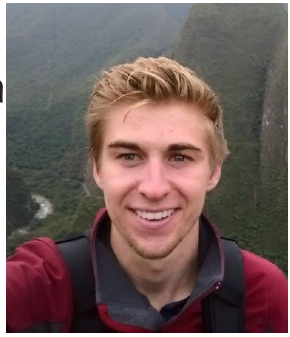
MAGEA Gene Family: Its Role in Cancer Metabolism and Capacity as an Immunotherapeutic Target



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Writer's Comment: This formal scientific literature review was written in response to the culminating assignment of Dr. Amy Clarke's UWP104F (Writing in the Health Sciences) class. I chose to write on cancer/testis (CT) antigens because of their loose relation to my own research experience at UC Davis and because of my interest in translational research. Intended for a specialized audience, this review summarizes some of the enormous amount of recent research on CT antigens conducted in the past five years. It attempts to report recently elucidated mechanistic features of these proteins, establish an idea of the "state-of-the-art" of clinical applications of CT antigens to cancer diagnosis and treatment, and provide suggestions for the direction of future research. I would like to offer my sincere thanks to Professor Clarke for providing the opportunity to write this piece and for her expert guidance in approaching and styling my review.

Instructor's Comment: Logan stood out from the very beginning of UWP 104F (Writing in the Health Professions) as someone already easily conversant in the discourse community of his field. Indeed, if he hadn't been my student, the proficiency of the literature review published here might make me wonder if Logan were an undergraduate. Its strength stems from a number of things: Logan's keen intelligence, his work in the lab investigating this topic, his skill as a writer. But the beating heart of this piece is Logan's humanity. He is working on a real world problem and he feels the responsibility intensely. It was an honor and a pleasure to work with Logan.

– Amy Clarke, University Writing Program

Abstract

Cancer-testis (CT) antigens are valuable immunotherapeutic targets in the treatment of cancer. The Melanoma-Antigen-A (MAGE-A) gene family is the best-studied group of CT antigens. MAGE-A is derepressed in cancer cells due to unspecific global demethylation. Once active, MAGE-A proteins interfere with normal AMP-activated protein kinase (AMPK), mammalian target of rapamycin (mTor), and p53 pathways, allowing cancer cells to adopt germline-like metabolic qualities. MAGE-A-targeted immunotherapies provide significant clinical benefit when an immune response is induced. However, only a small percentage of patients develop immune responses to vaccines alone. We present evidence that combinatorial treatments including the immunostimulant AS15 and targeting multiple CT antigens increase the proportion of patients who develop an immune response to the vaccine. MAGE-A-targeted immunotherapies offer a safe and valuable supplement to existing cancer treatments. However, further refinement of combination immunotherapies is necessary.

Keywords: *cancer/testis (CT) antigens; melanoma-antigen-A (MAGE-A); MAGE-A gene family; cancer immunotherapy; AS15; cancer metabolism; DNA hypomethylation.*

Introduction

There were an estimated 1.7 million new cases of cancer and 600,000 deaths due to cancer in the United States in 2015 (1). Despite increasing survival rates, much work remains to improve the efficacy of cancer therapy modalities. Since van der Burgeen et al.'s 1991 discovery of the first cancer-testis (CT) antigens, known as the Melanoma Antigen-A (MAGE-A) gene family, CT antigens have been viewed as potential immunotherapeutic targets in the treatment of many cancers (2, 3). CT antigens are proteins normally expressed solely in germ cells; however, they are also expressed in a variety of cancer cells (2). Additionally, these antigens are expressed as major histocompatibility (MHC) type I complexes in cancerous cells, but not in germ cells (2). This abnormal expression pattern and the lack of MHC type I complexes in normal tissue allow CT antigens to act as cancer biomarkers and possible

targets for antibody- or vaccine-based immunotherapy (2). The twelve-member MAGE-A gene family (MAGE-A1-12) is the best-studied group of CT antigens (4). These genes are expressed in many cancers, but why they are expressed and how they enhance carcinogenicity has until recently remained unclear (4). Applied clinically, MAGE-A3-targeted cancer vaccines have shown limited success in past clinical trials (2).

This review describes recently elucidated carcinogenic mechanisms of the MAGE-A family of genes and conveys the current clinical state of MAGE-A-targeted immunotherapy. We focus on the MAGE-A family of genes as these are the best-studied CT antigens. Understanding the mechanistic role of MAGE-A antigens may help with the design of future immunotherapeutic treatments. Current MAGE-A-targeted immunotherapy treatments provide a valuable supplement to existing cancer therapies and can create measurable clinical benefits for patients.

Mechanism of Derepression of CT Antigen Genes

MAGE-A and related CT antigen gene expression is repressed in non-germline tissues via DNA methylation of promoter regions (5). Recently, using a systematic analysis of many genome-wide methylation profiles, Kim *et al*. concluded that derepression of CT antigen genes is likely explained by unspecific global hypomethylation in cancerous cells (6). In cancerous cells, large genomic regions are hypomethylated in lamina-associated domains (LAD) involved in DNA replication (6). CT antigen gene promoters follow a prototypical methylation pattern and are associated with LAD regions (6). As LAD regions are hypomethylated in cancerous cells, it follows that CT antigen gene promoters are hypomethylated and CT antigens are therefore expressed in these cells (6). Hypomethylated CT antigen promoter genes occurred in LAD regions regardless of the presence of CpG islands where DNA methylation frequently occurs; this suggests that an alternate, possibly specific, mechanism of CT antigen derepression exists but has yet to be elucidated (6). Additional support for this hypothesis comes from the treatment of human esophageal cancer cell line Eca109 with decitabine (7). Treatment with demethylating agent decitabine induced MAGE-A4 and MAGE-A8 expression in Eca109 cells (7). Accumulating evidence indicates that genomic derepression of CT antigen genes in cancerous

cells is likely largely explained by global unspecific hypomethylation patterns common to cancer.

Role of MAGE-A Proteins in Cancer Metabolism

MAGE-A proteins activate germline-like processes in tumor cells. The activation of these pathways grants cancer cells aberrant metabolic capabilities by affecting the control of well-known cellular pathways. Recently, MAGE-A3 and MAGE-A6 have been shown to act as oncogenes by suppressing the growth-restrictive AMP-activated protein kinase (AMPK) pathway (8). MAGE-A3/6 recruits the TRIM28 E3 ubiquitin ligase; the MAGE-A3/6-TRIM28 complex inhibits AMPK through ubiquitination and subsequent proteasomal degradation (8, 9). The inhibition of AMPK impacts both cellular glucose metabolism and mammalian target of rapamycin (mTOR)-dependent protein anabolism (8). Researchers concluded that MAGE-A3/6- TRIM28 contributes to abnormal mTOR hyperactivity, and MAGE-A3/6 knockouts produced decreased mTOR activity (8, 9). The MAGE-A3/6-TRIM28 complex also inhibits autophagic flux, allowing for abnormal cellular accumulation of both proteins and organelles (8). The presence of MAGE-A proteins allows cells to evade normal growth-suppressive pathways.

It is well documented that MAGE proteins activate RING finger E3 ubiquitin ligases (10). However, recent research indicates that MAGE-A CT antigens can also act as inhibitors to E3 ubiquitin ligases (10). MAGE-A proteins bind to the p53-targeted E3 ubiquitin ligase MDM2, competitively interfering with necessary MDM2/MDM4 dimerization (10). As a result, cellular MDM4 levels increase, inhibiting p53's ability to promote transcription (10). Given these observations, it appears that MAGE-A proteins aid in the well-documented coordinated inhibition of p53 within cancer cells (9, 10). MAGE-A proteins contribute to cellular carcinogenicity through multiple recently elucidated mechanistic pathways. They allow the cell to evade normal control mechanisms in well-studied pathways, and they give cancerous cells access to germline-like metabolic processes.

Diagnostic use of the MAGE-A Family

CT antigens and their mRNA transcripts have traditionally been measured in tissue samples (2). However, recent research indicates that MAGE-A mRNA transcripts can also be found in the blood of patients with cancer (11, 12). Blood serum levels of MAGE-A mRNAs were significantly increased in breast cancer patients compared to women without malignant carcinomas (11). Additionally, MAGE-A mRNA transcripts were observed at significantly higher levels in ovarian cancer patients compared to healthy women (12). Furthermore, in ovarian cancer patients the presence of one or more mRNA species in the blood indicated a poor prognosis (12). This conclusion mirrors the established prognostic trend of CT antigens found in tissue samples: the expression of one or more CT antigens suggests a poor prognosis (2). As blood samples may be acquired more easily than tumor tissue samples, researchers should investigate whether MAGE-A mRNA is present in the blood of patients with different types of cancer. This discovery could aid in the early detection of cancer and facilitate tracking the progression of MAGE-A-targeted immunotherapy treatments in patients during clinical trials (11). Further research should focus on developing a clinical diagnostic test for use in the early detection of cancer.

MAGE-A-based Immunotherapy Clinical Trials

Phase II clinical trials studying vaccines targeting the MAGE-A family of proteins have recently been completed. In 2012, Russo *et al*. in coordination with MolMed SpA tested the clinical efficacy of treatment of late-stage melanoma patients with biweekly injections of autologous lymphocytes genetically engineered to express a MAGE-A3 tumor antigen (13). Compared to pre-infusion samples, an approximately 44-fold increase of anti-MAGE-A3 T-cells was observed in 6 of 22 patients (27%) (13). No abnormal lymphocyte count was observed, and only one adverse event (grade 1 toxicity—nausea) was related to treatment (13). A significant correlation (P=0.0038) was observed between the increase of anti-MAGE-A3 T-cells and the disease control rate: patients with the anti-MAGE-A3 immune response survived significantly longer than those without the immune response (13). The significant relationship between immune response and clinical benefit has also been observed in

patients with advanced esophageal, stomach, and lung cancer in response to a MAGE-A4-targeted vaccine (14). The ability of patients to produce an immune response did not appear to be related to their general immune system health. Instead, the ability to mount an anti-MAGE-A3 immune response likely relies on unknown factors (13). This clinical trial shows the potential benefit of autologous lymphocytes expressing MAGE-A3 in the treatment of late-stage cancer. However, phase III studies are required to validate the conclusions of Russo *et al*. This treatment provided a discernible clinical benefit to patients who developed an immune response to the vaccine, but only 27% of patients developed an immune response. The cause of vaccine non-response must be investigated to increase the potency of MAGE-A-targeted cancer vaccines in late-stage cancer immunotherapy.

In 2013, Kruit *et al*. and the European Organisation for Research and Treatment of Cancer, in collaboration with GlaxoSmithKline Biologicals, tested the efficacy of an immunotherapeutic treatment targeting MAGE-A3 tumor antigens combined with the immunostimulants AS02B and AS15 (15). The study was performed on 72 stage III/IV melanoma patients with MAGE-A3-expressing tumors (15). Patients in the AS15 treatment group had higher CD4 T-cell response rate to MAGE-A3 (76%) than patients in the AS02B treatment group (21%). In both groups, there was a frequent association between CD4+ T-cell response and clinical benefit (15). Additionally, one patient in the AS15 treatment group produced a CD8+ T-cell response, while no patients in the AS02B group showed this response (15). Both treatments had few adverse effects, and almost all treatment-related adverse effects were grade 1/2-toxicity events (15). Kruit *et al*. concluded that phase III clinical trials and further development of the MAGE-A3/AS15 treatment should move forward (15). While treatment including the immunostimulant AS15 did drastically increase the CD4 T-cell response compared to earlier clinical trials, 24% of patients still did not develop an immune response to the vaccine. The mechanisms preventing immune response to the MAGE-A3-targeted vaccine must be studied to offer clinical benefit to all patients.

Vaccines targeting multiple CT antigens have also been tested to maximize the proportion of patients developing an immune response. Krishnadas *et al*. tested the effectiveness of an autologous dendritic cell vaccine targeting MAGE-A1, MAGE-A3, and NY-ESO-1 in children

with relapsed neuroblastoma and sarcoma (16). After treatment with the combination vaccine, 6 of 9 patients developed an immune response to at least one of the antigens (16). Consistent with previous clinical trials, researchers found that the vaccine therapy had few adverse effects (16). The group plans to continue future trials to improve the proportion and duration of clinical responses (16). Despite the small number of patients involved in the study, multiple CT antigen-targeted vaccines appear to be valuable options in maximizing the number of patients who develop an immune response to treatment. Future studies should investigate the clinical value of treatment combining multiple CT antigen-targeted vaccines and the immunostimulant AS15. Combination of these two treatments could increase the number of patients who develop an immune response to the vaccine and thus could lead to significant clinical benefit for a higher proportion of patients.

Safety of MAGE-A-targeted Immunotherapy Treatments

CT antigen-based treatments could offer safe alternatives to chemotherapy treatment. Clinical trials applying MAGE-A-targeted vaccines largely support this belief (13, 15, 16). Additionally, a recent study exclusively analyzing the clinical safety of the MAGE-A3/AS15 treatment concluded that this combinatorial treatment is safe and warrants further study (17). Like all CT antigens, MAGE-A proteins are thought to only express MHC class I antigens in cancerous cells (2). As expected, immunotherapeutic treatment with MAGE-A3 vaccines has been shown to have no effect on either male or female fertility in a rat model (18). However, some researchers have questioned if CT antigens are only expressed in germline cells (19, 20). CT antigens PIWIL2 and PEPP2—thought to follow the normal CT antigen expression pattern—have also been found in normal leukocytes (19). MAGE-A12 has been found to be naturally expressed in the human brain, and MAGE-A1/8/9 are likely expressed naturally in the human brain (20). Neuronal death has been observed after treatment with MAGE-A3-targeted vaccines in some patients; this is possibly a treatment-related effect (20). Because all members of the MAGE-A family are highly structurally similar, vaccines targeting MAGE-A3 could cause an immune response against multiple members of the MAGE-A family (20). Therefore, clinicians should be

cautious in using "highly active" immunotherapies targeting MAGE-A family members (20). Additional study of normal MAGE-A protein expression patterns is required.

Conclusion

The MAGE-A gene family becomes derepressed in cancerous cells due to unspecific DNA demethylation. Once active, MAGE-A proteins interfere with the AMPK, mTor, and p53 pathways, allowing cancer cells to hijack germline metabolic qualities. MAGE-A proteins and mRNA transcripts can be detected in cancerous tissue, and mRNA transcripts can also be detected in the blood of some cancer patients. In the future, the detection of MAGE-A mRNA in blood could be applied clinically in the early diagnosis of cancer. Current clinical trials show that MAGE-A3-targeted vaccines provide significant clinical benefit to patients who develop immune responses to the vaccine. Unfortunately, a small percentage of patients develop immune responses to the vaccine alone. Further research is needed to elucidate the mechanisms of vaccine nonresponse. Combination therapies including the immunostimulant AS15 or multiple CT antigen targets appear to cause immune responses in a greater proportion of patients. However, clinicians must not create immune responses that are too highly active, as these responses could affect off-target sites. Further refinement of MAGE-A-targeted combination immunotherapies will likely provide a more widely applicable and efficacious cancer treatment in the near future.

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