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Review Article

Harnessing the Power of MAGE Proteins in Cancer Immunotherapy for Multiple Myeloma

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Abstract

This document is a comprehensive review that focuses on the role of melanoma-associated antigen genes (MAGE) family proteins in cancer, with a specific emphasis on their involvement in multiple myeloma (MM). The expression patterns of MAGE proteins in different tissues and their association with critical cellular processes such as cell cycle progression, apoptosis, and gene expression regulation were discussed. The document also highlighted the potential utility of MAGE proteins in cancer immunotherapy, including their use in prognosis and the development of MAGE-based cancer vaccines. In cancer vaccine therapy antigen selection is a crucial step, so by focusing on the vast potency of MAGE, we tried to mention it as a potent antigen for therapy of MM by reviewing the current studies. However, we acknowledged the need for further research and extensive clinical trials to evaluate the effectiveness, safety, and potency of MAGE antigens.

1. INTRODUCTION

The prevalence of cancer is alarming, with multiple myeloma (MM) being a significant contributor to mortality rates worldwide. According to the International Agency for Research on Cancer/GLOBOCAN (1), there were 117,077 deaths and 176,404 new cases of MM in 2020 alone, indicating a significant burden on patients and the healthcare system. Unfortunately, there is no definite cure for MM, and most patients require regular treatments throughout their lives (2). MM management may be necessary for many patients due to the lack of a definitive remedy for the disease. The increasing number of cases and the chronic nature of MM highlight the urgent need for new and effective treatments to improve patients' quality of life. Efforts to discover a suitable drug for treating MM began in the 1950s when alkylating agents were introduced in combination with corticosteroids. Autologous stem cell transplantation followed and was put into use in the 1980s. Before 2000, other drugs like thalidomide, lenalidomide, and pomalidomide were also prescribed to MM patients. However, due to the presence of proteasome inhibitors, histone deacetylase inhibitors, and nuclear export inhibitors, the approach to treatment has become more optimistic. In 2015, the use of immunotherapies such as monoclonal

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antibodies and chimeric antibody receptor (CAR) T-cell products shed new light on better management and resulted in higher survival rates for MM patients (3). This highlights the progress made in finding more effective treatments for MM and the potential of immunotherapies to revolutionize cancer treatment.

Although the use of conventional treatments has helped in achieving partial success in the fight against MM, their various side effects may hinder their efficacy (4-6), leading physicians and care providers to consider alternative agents such as cancer vaccines to improve treatment outcomes with fewer undesirable side effects. Cancer vaccines can be preventive or therapeutic, with the latter using cancer antigens to activate the immune system to invade and destroy cancer cells. Also, depending on the source of providing immune cells we have two groups of autologous and heterologous cancer vaccines which is explained more explicitly in **Figure 1**. The selection of the right antigen is crucial to trigger a strong response in complex diseases such as MM.

The melanoma-associated antigen genes (MAGE) protein family, containing a common MAGE homology domain (MHD) with 180 amino acids, has been identified as a potential target for immune therapies given its correlation with poor prognosis and chemotherapy resistance in many cancers (7, 8). Studies have shown that the MAGE family can enhance cancer cell survival and tumor metastasis through its relationship with the p53 tumor suppressor and adjustment of the function of RING-type E3 ubiquitin ligases (9, 10). The study aims to explore the potential role of MAGE-based cancer vaccines in treating MM and investigate their safety and effectiveness. By highlighting the potential of MAGE-based cancer vaccines, the study offers hope for MM patients who are seeking more effective and less toxic alternatives to current treatments.

2. MAGE GENES

2.1. Overview of MAGE

The MAGE family's first member was discovered in 1991 during experiments on melanoma cells, marking a significant breakthrough in understanding the role of these proteins in cancer (11). MAGE genes are highly conserved across eukaryotes, with a conservation rate of 46%, and mammalian copies of the gene continue to expand. Over forty human proteins are considered cancer testis antigens (CTAs), primarily expressed in the testis and silent in other tissues. While MAGE proteins serve an important role during normal germ cell development in testis or placenta, they are abnormally highly reactivated during oncogenic transformations (7). Cytolytic T-lymphocytes can recognize the tumor-associated antigens expression patterns of MAGE-related antigens, making them promising targets in cancer immune- and target-therapies (12).

The members of the MAGE family can be divided into two categories: Type I and Type II. Type I, which includes MAGE-A, MAGE-B, and MAGE-C on the X chromosome, are regarded as CTAs and play an important role in cancer cell survival and tumorigenesis (13). As such, they are rarely expressed in healthy adult tissues but are highly expressed in various malignancies, including hepatocellular carcinoma, brain cancer, breast cancer, prostate cancer, melanoma, lung adenocarcinoma, gastric cancer, esophageal squamous cell carcinoma, bladder cancer, and ovarian cancer (12). MAGE proteins are typically expressed intracellularly and should be degraded with proteasome into short peptides, which are then conveyed into the endoplasmic reticulum and loaded onto the MHC-I. This process enables MAGE proteins to be used as targets for immunotherapy when they are complexed with MHC-I. However, the precise physiological roles and underlying mechanisms that regulate the expression of the MAGE family in cancer are not yet fully comprehended (14).

2.2. Function of MAGE protein in normal cells

The MAGE protein family has been shown to play a role in various cellular processes, including cell cycle progression, apoptosis, and neurogenetic disease. In a normal body, their primary function is to regulate substrate specificity, enhance ligase activity, and mediate the recruitment of substrates (15). Ubiquitination, a post-translational modification process, involves attaching a small protein, ubiquitin, to target proteins to prepare them for degradation or regulate their activity (16). The ubiquitination process occurs in three consecutive steps. First, ubiquitin (Ub) is activated by the E1 enzyme, which consumes ATP. In this step, the C-terminus domain of Ub binds to the active site of the E1 enzyme. The next step involves the E2 attachment of Ub through formation of the thioester bond, which requires the mediating action of the E3 ligase. During the ubiquitination process, a thioester bond forms between ubiquitin and E2. E3 Ub-Ligase is the only factor that can accurately identify and provide a surface for the transfer of Ub to the substrate. E3 uses two different paths, namely direct and indirect. The HECT3 ligase, homologous to E6-AP carboxy terminus, acts indirectly by first transferring Ub to the cysteine of its active site before finally delivering it to the substrate. In contrast, the E3 rings act differently by directly attaching Ub to the substrate. It is noteworthy that MAGE proteins enhance the ligase activity of ring E3s (16, 17). MAGE proteins can attach to E3 hrough their MAGE homology domains (MHDs),

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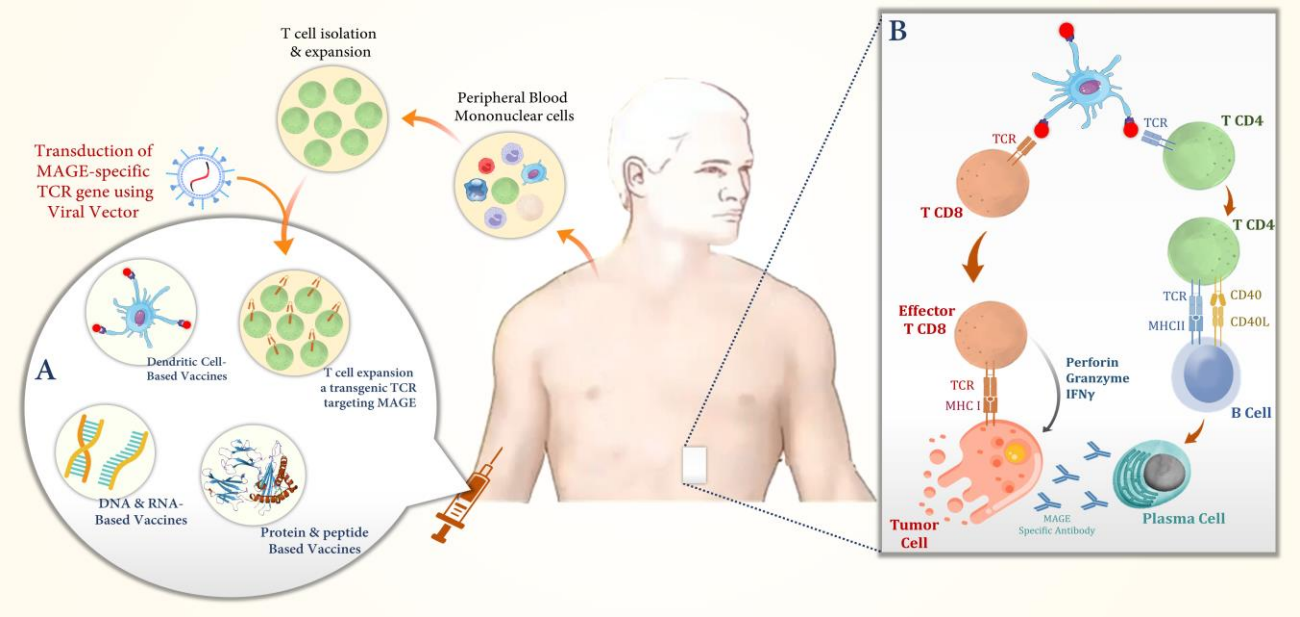

Figure 1. Schematic depiction of MAGE-directed cancer immunotherapy. **(A)** Many types of MAGE-directed cancer immunotherapy have been developed, including protein or peptide vaccines, DNA or RNA vaccines, dendritic-based vaccines, and adoptive T cell therapy. **(B)** The immune response to a cancer vaccine consists of several steps: Antigen-presenting cells (APC) capture injected MAGE (whether DNA, RNA, or peptides) and present them to stimulate CD4 and CD8 T cells. CD4 T cells coordinate immune responses by communicating with other cells and inducing B cells to differentiate into plasma cells. Finally, effector T cells, B cells, antibodies, and some cytokines have either a direct or indirect antitumor effect on cancer cells.

which are present in all MAGE proteins of type I and II and consist of approximately 170 amino acids. The substrate's fate is determined based on the number and manner of ubiquitin attachment, such as mono/multi-mono or poly-Ub. Mono-ubiquitination is involved in processes such as DNA damage repair through RING-type proteins of E3 ligase, transcription, and protein transportation. Multi-mono-ubiquitination is responsible for inducing substrate degradation in proteasomes (18). The performance and function of the substrate may vary depending on the connection through each specific domain of Ub. For instance, Ubs connected linearly through the K63 domain cause signal transductions, while connections through other domains can lead to DNA damage repair or proteasome degradation (17). Ubiquitination is essential in several cellular processes, including apoptosis, cell cycle, and proliferation (see **Figure 2**).

MAGE proteins, particularly those of type I, are expressed in the testis and placenta. Initially, MAGE-A, -B, and -C were thought to be predominantly expressed in the testis, earning them the name cancer-testis antigens (CTAs). They were believed to play roles in gametogenesis, the development of premeiotic germ cells, embryonic (8), and spermatogonial stem cells (19). Previous studies have suggested that MAGE I type proteins (such as MAGE-B4 and -B16) are mainly found in males. However, all MAGE II proteins are expressed in the ovary. During meiosis and primary stages of oocytogenesis, oocytes express MAGE-B4. MAGEs A-10, -B3, and -B7 have roles in later stages of follicle maturation (8). Moreover, MAGEs are involved in gene expression regulation and nervous system development. **Table 1** provides a summary of MAGE protein functions in normal tissues and cells.

2.3. Transcriptional and epigenetic regulation of MAGE can cause changes of its expression in different cancers

MAGE genes were first described in melanoma and have since been found to be expressed in numerous tumors of various stages of progression and histological types (11, 20). Due to their widespread expression in various cancers, many studies seek to understand and identify the mechanisms leading to the abnormal expression of MAGEs in cancer. As a CTA subfamily, MAGE gene expression is specific to the male germline and certain tumor types, suggesting that a combination of epigenetic modifications is required for sustained transcriptional activation of MAGE genes. However, the exact regulatory

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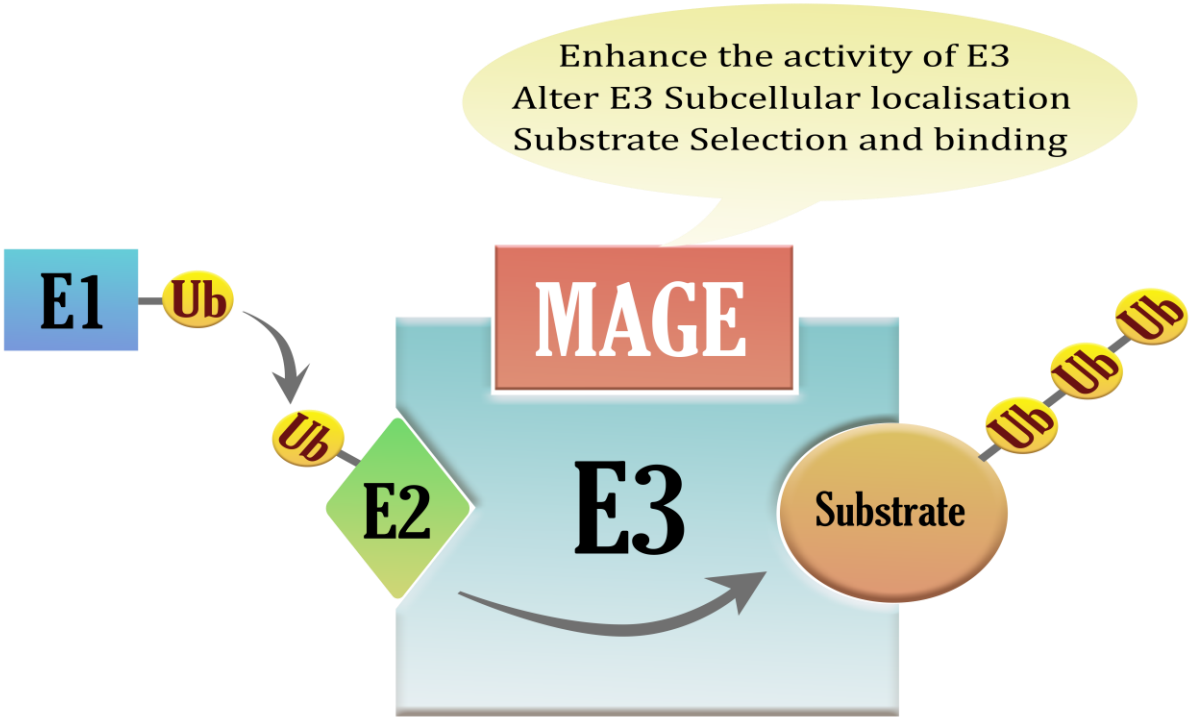

Figure 2. By regulating the activity of E3 ubiquitin ligases, MAGE proteins participate in a range of cellular processes, including protein degradation, DNA repair, and cell cycle regulation. They act as substrate adaptors that guide the ubiquitination of specific target proteins. E3 ubiquitin ligases are responsible for recognizing particular target proteins and catalyzing the transfer of ubiquitin molecules onto them. They play a critical role in determining the specificity and efficiency of the ubiquitination process. MAGE proteins and E3 ubiquitin ligases are involved in regulating protein abundance, protein degradation, and cellular processes such as autophagy.

mechanisms that are not yet fully understood (16, 21, 22). Herein, we have delineated certain transcriptional and epigenetic mechanisms that regulate MAGE, potentially resulting in alterations to its expression across various types of cancer.

2.3.1. DNA methylation

MAGE genes display a high level of sequence similarity, but their promoter regions show lower similarity. These promoter regions contain binding sites for transcription factors, and excessive methylation of these sites may repress MAGE gene expression (25, 26). There are two families of DNA methyltransferases (DNMTs) in mammals that regulate DNA methylation: the DNMT3 family, responsible for de novo methylation, and the DNMT1 family, which maintains the methylation pattern (27). MAGE-I expression is often repressed by DNA methylation, which can prevent the binding of transcription factors and other proteins involved in gene activation (28). Methyl-CpG-binding domain (MBD) proteins, which can bind to methylated DNA, act as regulators of MAGE-A gene silencing (24). Most hypermethylated promoters are bound by MBD proteins, while unmethylated promoters usually lack MBDs. The use of demethylating agents results in the hypomethylation of CpG islands, the release of MBDs, and the reactivation of genes, further supporting the idea that MBDs bind to methylated promoters based on the presence of methylation (29). Among all MBD-containing proteins, MBD1 is unique due to its distinct structure and specialized role in gene regulation. In addition to the conserved MBD domain located at its N-terminal, MBD1 also has a transcriptional repression domain (TRD) at its C-terminal (30). MBD1 uses its MBD domain to interact with other proteins, which facilitates its binding to methylated DNA. Depending on the isoform of MBD1, it has two or three CXXC domains, with the first two enabling binding to methylated DNA and the third allowing binding to DNA regardless of its methylation status (31). MBD1 can bind to both methylated and unmethylated MAGE-A gene promoters, leading to their

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repression. Repression of methylated genes requires the MBD domain, while repression of unmethylated genes depends on the third CXXC domain. MBD1mut lacks the MBD domain and has a non-functional TRD, so it does not impact MAGE-A gene expression (24). In contrast, MBD1v1 contains an additional third CXXC domain and can repress MAGE-A gene promoters regardless of their methylation status. MBD1v3, while lacking the third CXXC domain, still weakly represses unmethylated MAGE-A gene promoters, indicating that the other two CXXC domains may also contribute to the repression of unmethylated MAGE-A promoters, albeit with less effectiveness. The ability of MBD1 to bind to both methylated and unmethylated DNA allows it to play a role in different epigenetic regulations for MAGE-A genes. (32).
The recent discovery that the BORIS protein, which normally removes DNA methylation at specific locations during germ cell development, can also lead to the abnormal activation of MAGE genes in human cancers supports the idea that the regulatory mechanisms that activate MAGE genes in normal male germ cells and cancer cells are connected with respect to CpG methylation. BORIS, also known as 'Brother of the Regulator of Imprinted Sites', plays a crucial role in controlling the timing and positioning of epigenetic changes in germ cells. However, it was found to drive abnormal activation of MAGE genes in human tumors (33, 34). As a cancer-testis gene, BORIS highlights the potential link between the regulatory mechanisms that activate MAGE genes in normal male germ cells and cancer cells. The role of BORIS suggests that inappropriate activation of MAGEs may not be a result of widespread loss of DNA methylation in cancer, as previously thought. Thus, the presence of the BORIS protein in male germ cells coincides with the expression of multiple MAGE genes during the development of spermatogonia to spermatocytes, and its expression is also associated with the removal of methylation patterns across the entire genome (34).The inappropriate activation of BORIS protein is linked to increased expression of multiple MAGE genes in cancer cells , suggesting that aberrant MAGE activation may involve focused epigenetic changes. However, in melanoma, the expression of CTA genes like MAGE-A1 can occur even in the absence of activated BORIS protein, indicating that the regulation of these genes is more complex and involves other factors as well (35).
Methylation of the CpG-binding domain, a binding site for the ETS and SP1 transcription factors, in the MAGE-A1 gene promoter may lead to inhibition of promoter activity and regulation of transcriptional activation (24, 32). MAGE-A1 gene's promoter region is significantly methylated in somatic tissues, whereas the promoter is mostly unmethylated in male germ cells and tumor cells expressing this gene (36). Furthermore, studies have shown that the use of demethylating agents can induce the expression of MAGE-A1 in cells that do not normally express this gene, indicating that DNA methylation plays a crucial role in suppressing MAGE-A1 expression in somatic cells. In colon cancer cells, the removal of DNMT1 resulted in a moderate increase in the expression of X-linked cancer/germline (CG-X) genes, including MAGE-A1, NY-ESO-1, and XAGE-1, whereas the removal of DNMT3b had little effect. However, the simultaneous removal of DNMT1 and DNMT3b led to a significant decrease in promoter methylation and a strong induction of these CG-X genes (37). Similarly, in MZ2-MEL cells, the suppression of DNMT1 activated the MAGE-A1 gene, while the down-regulation of DNMT3A and DNMT3B had little effect. These findings suggest that DNMT1 plays a more significant role than DNMT3A/B in maintaining the methylation pattern of the MAGE-A1 gene (38). The discovery that 5'-aza-2'- deoxycytidine (DAC), a methyltransferase inhibitor, is capable of activating the MAGE-A1 family suggests that DNA methylation plays a significant role in the abnormal expression of MAGE genes in cancer and the silencing of these genes in normal tissues (39). The degree of hypermethylation of promoter sites of various MAGE genes correlates with their silencing, and DNMT1 is the primary methyltransferase involved in effective CpG island hypermethylation (38, 40). Although the potential role of DNA methylation in controlling MAGE expression during spermatogenesis is largely unexplored, the observed methylation reprogramming during gametogenesis suggests that it may be involved in the cell type-specific regulation of MAGEs (41).
In addition, the expression of MAGE-A11 is increased during the progression of prostate cancer and in the regrowth of cancer after castration. This increase in expression is associated with a decrease in methylation of CpG sites in proximity to the transcription start site of the MAGE-A11 gene. The expression of MAGE-A11 has also been linked to a reduction in DNA methylation at its TSS in epithelial ovarian cancer, which is consistent with the overall reduction in DNA methylation levels throughout the genome in this type of cancer (40). The methyl-CpG binding domain protein 2 (MeCP2), a protein that binds to methylated DNA, has been discovered to regulate the expression of MAGE-A11 during the progression of esophageal squamous cell carcinoma (42).

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Table 1. Functions of MAGEs in normal tissues.

Family	Sub-family	Tissue	Main role	Normal functions
MAGE-A	MAGE-A1	Testis, stomach, fat, spleen, esophagus	Potent transcriptional repressor via interactions with Ski interacting protein and the deacetylase HDAC1	Enables histone deacetylase and protein binding, negative regulation of Notch signaling pathway, and transcription by RNA polymerase II
	MAGE-A2	Testis, placenta, fat, brain, stomach	Inhibits p53 ubiquitination and induces cell death	Enables DNA-binding transcription factor, histone deacetylase, protein, and ubiquitin protein ligase binding, involved in cellular senescence. negative regulation of protein acetylation, protein sumoylation, transcription by RNA polymerase II, positive regulation of ubiquitin-protein transferase activity, involved in protein catabolic process, and signal transduction by p53 class mediator
	MAGE-A3	Testis, fat, placenta, brain, stomach	Unknown	Enables caspase, histone deacetylase, and protein binding, negative regulation of autophagy, cysteine-type endopeptidase activity, endoplasmic reticulum stress-induced intrinsic apoptotic signaling pathway, protein processing, transcription by RNA polymerase II involved in apoptotic process
	MAGE-A4	Testis, placenta, lymph node	promoting cell growth and inhibiting growth arrest and apoptosis in normal cells, but further investigations is still in need	Enables histone deacetylase and protein binding, and also enables molecular function negative regulation of apoptotic process, and transcription by RNA polymerase II, positive regulation of cell cycle
	MAGE-A5	Placenta, testis, skin, gall bladder, endometrium, duodenum, fat, Colon	Enhances the resistance to genotoxic stress during spermatogenesis and promotes cell survival	Enables molecular function, and regulation of chromosome segregation
	MAGE-A6	Testis, fat, brain, spleen, placenta	promotes anchorage-independent growth of normal diploid colonic epithelial cells	Enables histone deacetylase binding, molecular function, and protein binding, negative regulation of autophagy, and transcription by RNA polymerase II
	MAGE-A7		Unknown	- (pseudogene)
	MAGE-A8	Placenta, testis, Skin	Unknown	Enables histone deacetylase and protein binding, and molecular function. negative regulation of transcription by RNA polymerase II. involved in biological process,
	MAGE-A9	Testes, placenta, fat, lymph node, appendix	Embryonic development	Enables histone deacetylase and protein binding, and molecular function. negative regulation of transcription by RNA polymerase II. involved in biological process.
	MAGE-A10	Placenta, testis, fat	Unknown	Enables histone deacetylase binding, negative regulation of transcription by RNA polymerase II
	MAGE-A11	Testis, placenta adrenal, spleen	Unknown	Enables histone deacetylase binding and protein binding, negative regulation of transcription by RNA polymerase II
	MAGE-A12	Testis, brain, fat, Stomach, spleen, `endometrium, small intestine	Unknown	Enables histone deacetylase binding, enables molecular function, enables protein binding, involved in biological process, negative regulation of transcription by RNA polymerase II

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MAGE-B	MAGE-B1	Testis, lung	Unknown	Negative regulation of transcription by RNA polymerase II
	MAGE-B2	Testis, placenta, Lung	Normal spermatogenesis	Enables protein binding Negative regulation of transcription by RNA polymerase II
	MAGE-B3	Testis	Unknown	Enables protein binding Negative regulation of transcription by RNA polymerase II
	MAGE-B4		Spermatogenesis and embryonic development	Enables protein binding Negative regulation of transcription by RNA polymerase II
	MAGE-B5	Testis	Germ cell development and stress response pathways	Enables molecular function Negative regulation of transcription by RNA polymerase II Involves in biological process
	MAGE-B6	Testis	Germ cell development and stress response pathways	Enables molecular function and protein binding Negative regulation of transcription by RNA polymerase II Involves in biological process
	MAGE-B7	(Rat)	Germ cell development and stress response pathways	-
	MAGE-B8, B9	(House mouse)	Germ cell development and stress response pathways	-
	MAGE-B10	Testis	Germ cell development and cell-cell interaction	Enables protein binding Negative regulation of transcription by RNA polymerase II
	MAGE-B11	(House mouse and rat)	Germ cell development and stress response pathways	-
	MAGE-B16	Testis	Germ cell development and pluripotent stem cell differentiation	Negative regulation of transcription by RNA polymerase II
	MAGE-B17	Almost all tissues (Testis, appendix, prostate)	Germ cell development	Negative regulation of transcription by RNA polymerase II
	MAGE-B18	Testis	Germ cell development	Enables protein binding Negative regulation of transcription by RNA polymerase II
MAGE-C	MAGE-C1	Testis	Germ cell development and Aberrant expression in cancers is positively correlated with tumorigenesis progress and p53 ubiquitination	Enables protein binding Negative regulation of transcription by RNA polymerase II
	MAGE-C2	Testis	Germ cell development and regulation of cell cycle	Enables protein, and ubiquitin protein ligase binding Negative regulation of transcription by RNA polymerase II Positive regulation of ubiquitin-protein transferase activity Involved in protein catabolic process
	MAGE-C3	Almost all tissues (Testis, brain, spleen)	Germ cell development	Negative regulation of transcription by RNA polymerase II
	MAGE-C4-7	Not characterized	Germ cell development	Not characterized
MAGE-D	MAGE-D1	Almost all tissues (Brain, placenta, adrenal)	Upregulates p53 and inhibits the proliferation and migration of breast cancer cells, Neuronal functions and CNS development, embryonic development	Enables identical protein binding and transcription co-activator activity, circadian regulation of gene expression,

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				Negative regulation of epithelial cell proliferation, protein localization to nucleus, transcription by RNA polymerase II, Positive regulation of DNA-templated transcription, MAP kinase activity, apoptotic signaling pathway, and branching involved in ureteric bud morphogenesis, regulation of apoptotic process and circadian rhythm
	MAGE-D2	Almost all tissues (Ovary, prostate, placenta)	Cell cycle regulation and signaling pathway, protection against apoptosis	Enables protein binding Negative regulation of transcription by RNA polymerase II, and renal sodium ion absorption involved in female pregnancy
	MAGE-D3	Not characterized	Unknown	Not characterized
	MAGE-D4, D4B	Almost all tissues (Brain, endometrium, ovary)	Unknown	Enables protein binding, Negative regulation of transcription by RNA polymerase II Involved in biological process
MAGE-E	MAGE-E1		Unknown	Enables protein binding, Negative regulation of transcription by RNA polymerase II Involved in biological process
	MAGE-E2		Unknown	Negative regulation of transcription by RNA polymerase II
	MAGE-E3	Not characterized	Unknown	Not characterized
MAGE-F	MAGE-F1		Unknown	Enables protein binding, Negative regulation of double-strand break repair via homologous recombination, transcription by RNA polymerase II. Positive regulation of ubiquitin-dependent protein catabolic process, involved in protein maturation by iron-sulfur cluster transfer, and in protein ubiquitination
MAGE-H	MAGE-H1		Germ cell development, stress response pathway	Enables protein binding, Negative regulation of transcription by RNA polymerase II Involved in biological process
MAGE-L	MAGE-L2		Unknown	Enables protein binding, and ubiquitin-protein transferase activity, Negative regulation of transcription by RNA polymerase II, and DNA-templated transcription Positive regulation of actin nucleation, and regulation of circadian rhythm, involved in protein K63-linked ubiquitination, retrograde transport (endosome to Golgi) involved in Arp2/3 complex-mediated actin nucleation.

In summary, the significant roles of DNMTs and BORIS in spermatogenesis, their expression at specific stages of male germ cell development, and their involvement in cancer suggest that DNA methylation plays a critical role in the expression of MAGE genes in both germ cells and cancer cells. Additionally, the different patterns of methylation marks in male and female gametes may contribute to the differences in MAGE gene expression between male and female gonads (41). The activity of MAGE-A11 can be promoted by processes such as DNA demethylation, histone acetylation, and histone methylation (32). These findings help explain why the activation of MAGE-A genes is observed following promoter demethylation. DNA methylation is typically the primary epigenetic mechanism responsible for the repression of CTA genes, including MAGE genes (43). Nevertheless, there is a need for further research to provide more data about methylation in MAGE-B, C and other members of MAGE family and also further solid

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experimental evidence for clarifying the molecular mechanisms that underlie this regulation.

2.3.2. Histone modifications

The methylation of DNA in MAGE gene promoters is closely associated with the post-translational modification of histones, and both processes work together to increase the expression of MAGE genes in cancer cells (42, 44). Cancer cells with high levels of MAGE-A1 and -A3 genes exhibit more activation marks and fewer repressive marks (45). G9A, also known as Euchromatic Histone Lysine N-methyltransferase 2 (EHMT2), attaches methyl groups to a specific amino acid (Lysine-9) on histone H3 in certain regions of the MAGE-A2, A6, and A8 genes. This modification leads to the formation of a silent and tightly packed chromatin structure around these genes, causing their repression (46). In pituitary tumors and thyroid cancer, fibroblast growth factor receptor 2-IIIb (FGFR2-IIIb) can reactivate and suppress MAGE-A3/A6 gene expression by increasing histone methylation and histone deacetylation (47, 48). Conversely, in pituitary tumors of female patients, the hormone estradiol promotes the acetylation of histone H3 and the expression of MAGE-A3 genes (25, 48). The inhibition of DNA methyltransferases and histone deacetylases (HDACs) leads to the activation of MAGE-A11 gene expression, indicating that DNA methylation and histone modifications jointly regulate the expression of MAGE genes (49). However, more research is needed to establish concrete experimental evidence and elucidate the molecular mechanisms behind this regulation. Research has revealed that the suppression of MAGE-A1, A2, A3, and A12 gene expression is caused by the removal of acetyl groups from histones. Histone lysine methylation has also been shown to impact the expression of MAGE genes in cancer cells (45). Several studies propose that diverse epigenetic mechanisms are responsible for regulating the activation and deactivation of MAGE genes in cancer cells. However, the extent to which these epigenetic processes regulate MAGE gene expression in the germline is mostly unknown. Further research is necessary to explore how epigenetic modifications regulate MAGE gene expression, particularly since epigenetic drugs are being combined with immunotherapy to improve the response of cancer patients (45, 50).

2.3.3. Transcription factors and signal transduction pathways

MAGE genes exhibit cell-specific expression during sperm development and are also expressed in various cancers, indicating that their regulation involves specific transcription factors. Although epigenetic regulation of MAGE genes has been explored, the transcription factors and upstream signaling pathways involved remain largely unknown. Analysis of the promoter regions of some MAGE genes has revealed binding sites for ETS and SP1 transcription factors. Deletion studies and searches for transcription factor binding sites demonstrated that ETS transcription factors can bind to these sites and activate the expression of MAGE-A1 (51). Further research revealed that methylation of the ETS and SP1 binding elements in multiple MAGE-A promoters inhibits MAGE-A expression. Methylation hinders the binding of ETS and SP1 transcription factors and instead attracts methyl-CpG binding domain proteins (52). The DNA sequences of MAGE-A gene promoters contain ETS motifs, and previous research has shown that the ETS transcription factor is responsible for the significant activation of MAGE-A1 gene expression (53). Increased levels of ETS-1 can activate MAGE-A gene promoters. Nevertheless, MBD-1's inhibitory effect on MAGE-A gene expression cannot be reversed by the transcriptional activator ETS-1. This suggests that the binding of MBD1 to the unmethylated MAGE-A gene promoter leads to the repression of gene expression, which cannot be counteracted by ETS-1 (24).

In certain cancers, the expression of MAGE-A and MAGE-C genes is stimulated by the overactive KIT tyrosine kinase, a cancer-causing enzyme, along with demethylation of their promoter regions (16). Treatment of mast cells, which rely on the KIT protein tyrosine kinase, with the tyrosine kinase inhibitor imatinib results in a reduction in the expression of various genes from the MAGE-A and MAGE-C families (54). Interestingly, Mage-A gene expression reaches its highest level in the seminiferous cycle following the rise in retinoic acid that switches on KIT signaling and activates the differentiation of male germ cells. Consistent with this expression pattern, MAGE-A protein levels are the highest in STRA8/KIT-positive spermatogonia and increase in cultured primary spermatogonia after a spike in retinoic acid, suggesting that KIT may also help control the expression of MAGE-A genes during sperm cell development (21).

MAGE expression may also be influenced by other signaling mechanisms. For instance, fibronectin and FGFR2 have been shown to increase MAGE-A3 expression

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in certain types of cancers. (55). MAGE-A3 expression can also be augmented by carcinogens such as helicobacter pylori or cigarette smoking and is affected by miRNAs and lncRNAs (56-59). In summary, the available evidence suggests that the MAGE family of genes is suppressed in normal somatic cells by multiple epigenetic modifications and the actions of transcription factors and signaling pathways that become simultaneously disrupted in cancer.

3. MAGE IN MM

3.1. Abnormal cellular functions of MAGE proteins in MM

The aberrant expression of MAGEs in cancers can contribute to tumorigenesis by inducing ubiquitination and other modifications, resulting in alterations in cellular processes and signaling pathways (60). As mentioned earlier, one of the functions of MAGEs is to activate ubiquitin ligases. For instance, a ubiquitin ligase such as TRIM-28 can cause spontaneous ubiquitination, leading to apoptosis by MAGE-A. Mei et al. demonstrated that by knocking down MAGE A3 in the MM cell line (HMCL), they observed an increase in the expression of pro-apoptotic and cyclin-dependent kinase (CDK) inhibitors. They also found that the sensitivity of the cells to alkylating agents increased with this approach. Therefore, they concluded that MAGE-A3 plays a role in inhibiting apoptosis and promoting cell proliferation in MM (61). Additionally, other MAGEs of type I, such as A1 and A2, have been shown to be involved in the survival of myeloma cells and their drug resistance (10). MAGE-B has also been found to have oncogenic functions and is associated with certain tumors. MAGE-B2 has been identified as one of the most common CTAs in both newly diagnosed and relapsed MM patients. The expression of MAGE-C1/CT7 is believed to be associated with higher disease stages, cell-cycle progression, and is crucial for MM cell survival (62). The presence of MAGE genes, such as MAGE-A3 and MAGE-C1, in MM suggests their involvement in therapy resistance and disease progression.

MAGE-A3, -A6, and -C2 are among the most common CTAs in human cancer. They are expressed in healthy tissue only in immune-privileged sites but are also expressed in cancers (63). The expression of MAGEs, especially MAGE-A3/6, has been associated with more aggressive disease progression, poorer patient prognosis, metastasis, and reduced overall survival. Cancer cells that inappropriately activate MAGE-A3/6 become reliant on them, as the depletion of MAGE-A3/6 leads to reduced cell viability and clonogenicity (8). In MM, MAGE-A3 acts as an apoptosis suppressor by inhibiting the P53-dependent mechanism. Generally, the p53-dependent apoptosis pathway is a tightly regulated process that eliminates damaged or potentially cancerous cells, thereby preserving genome integrity and preventing the onset of cancer. The p53 protein is a crucial factor that plays a critical role in controlling the cell cycle and preventing the onset of cancer. Upon detection of DNA damage, p53 is activated and initiates a range of cellular responses, such as apoptosis, to eliminate any damaged or cancerous cells from the body (64). The P53-dependent apoptosis involves several steps, resulting in mitochondrial cytochrome C release into the cytoplasm and proteasome formation through APAF-1. The apoptosome activates caspase-9 and -3, resulting in biochemical and morphological changes in apoptosis (65). In MM, MAGEs (-A3, -C1, and C2) activate various mechanisms to inhibit apoptosis. For example, MAGE-A3 can prevent the interaction of p53 with TRIM28 (also known as KAP1), a RING E3 ubiquitin ligase that catalyzes the ubiquitination and inactivation of p53, thus inhibiting its function. Based on this information, Nardiello et al. tried silencing MAGE-A3, and they observed the induction of apoptosis through caspase-dependent and intrinsic pathways. Additionally, they have reported the activation of proapoptotic proteins, namely Bax, and a reduction in the protein levels of antiapoptotic elements, such as Survivin. These alterations ultimately result in mitochondrial depolarization and a rise in the number of apoptotic cells through both p53-dependent and independent mechanisms. The study concluded that MAGE-A3 plays a crucial role in promoting the survival of proliferating myeloma cells by inhibiting apoptosis through multiple mechanisms (66). This general apoptosis mechanism is mediated by MAGE-A3 and MAGE-C3, both of which are present in MM. Mei et al. demonstrated that the silencing of MAGE-As induces apoptosis through the intrinsic pathway (67). Therefore, when they are silenced by RNAi transfection, it results in pro-apoptotic effects on the proliferation of myeloma cells (68). Despite the homology of MAGE-A1 and MAGE-A3, inhibition assays through shRNA transfection have shown that MAGE-A3 plays a crucial role in this process (69). A study analyzing the protein-protein interactions of CT-7 with TRIM28, STAT1, and PIASy revealed that CT-7 is involved in the regulation of STAT-1 activity. MAGE-C1 negatively regulates phosphorylated-STAT1 activity by trapping the transcription factor out of the nucleus through small Ubi-like modification (SUMO). The study suggests that some MAGE-I proteins may possess SUMO

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ligase activity and identifies STAT1 as a novel biochemical pathway regulated by these genes independent of p53 (66).

3.2. Prognostic values of MAGE genes in MM

Researchers have assessed the efficacy of various cancer/testis antigens (CTAs) in both MM samples and cell lines, as reported in reference (70). Longitudinal studies have uncovered a robust correlation between the expression of CTAs and the clinical progression of MM. Specifically, the research indicates that only a small fraction of patients who achieve complete remission exhibit any indication of CTA expression, while, on the other hand, approximately half of the patients who are in partial remission have been observed to express CTAs (71). Previously, Andrade et al. conducted a study which demonstrated that three CTAs located on the X chromosome, namely MAGEC1/CT7, MAGE-A3/6, and LAGE-1, are frequently expressed in MM, indicating their potential as targets for immunotherapy. The research also revealed that the CTA gene MAGE-C1/CT7 was the most commonly expressed CTA in MM and is believed to have a role in predicting overall survival (72).

According to research, the expression of MAGE-A1 and MAGE-A2 occurs at similar rates in both newly diagnosed and relapsed cases of MM (70). Studies have suggested that the MAGE-A3 gene plays a vital role in the survival of myeloma cells by reducing drug-induced apoptosis (68). The knockdown of MAGE-A3 leads to a delay in the growth of plasma cell precursors, which is considered a poor prognostic factor in MM (73). The expression level of MAGE-A3 was found to be high in relapsed MM patients compared to newly diagnosed patients (66). Moreover, research indicates that more than half of MM patients with over 10% plasma cell infiltration in the bone marrow express the MAGE-A3 gene (71). In a study by Amberly et al., which analyzed 565 primary MM patient tissue samples at the time of diagnosis, MAGE-A3 expression was detected in 25% of MM patients, but the rate was much higher, 50%, in relapsed patients and even higher, 80%, in those with highly proliferative disease (74). Recent studies have found that newly diagnosed MM patients who express MAGE-A6 and MAGE-A9 have a shorter progression-free survival and overall survival, respectively. Furthermore, MAGE-A9 expression has also been observed in relapse patients (70). MAGE-B2, a testis-specific antigen, is frequently found in both newly diagnosed and relapsed MM patients, with a higher incidence in newly diagnosed cases. However, it has also been detected in a subset of normal plasma cells. Likewise, MAGE-B1 and MAGE-B4 have been observed to be present in both groups of MM patients (70). The MAGE-C1 gene is located in the Xq26-27 region and its protein has been detected in both the cytoplasm and nucleus of the cell through staining techniques. The research also revealed that the CTA gene MAGE-C1/CT7 was the most commonly expressed CTA in MM and is believed to have a role in predicting overall survival (72).Furthermore, MAGE-C1/CT7 is known to physically interact with the NY-ESO-1 protein, which results in their coordinated expression in various tumor types, including MM (75). MAGE-C1 is among the most prevalent CTA found exclusively in the testis, and it has been detected in both newly diagnosed and relapsed MM patients, with expression in a small proportion of normal plasma cells (76, 77). Notably, the MAGE-C1 protein has been identified in the majority of MM, medullary plasmacytoma, and extramedullary plasmacytoma samples (76). The expression of MAGE-C1/CT7 appears to occur in the initial stages of MM progression and may play a role in the early stages of the disease, as well as in the proliferation of plasma cells (72). Curioni-Fontecedro et al. conducted an examination to determine the ability of the MAGE-C1/CT7 antigen to stimulate an immune response in vivo. Their research revealed that this CTA elicited high levels of specific IgG antibodies in MM patients, and they also observed a specific immune response against MAGE-C1/CT7, indicating that an antimyeloma immune response can be induced in individuals with this disease (78). The involvement of MAGE-C1/CT7 in the survival of malignant MM cells has been demonstrated in two separate studies aimed at silencing MAGE-C1/CT7. Both studies have indicated that the expression of MAGE-C1/CT7 in MM reduces drug-induced apoptosis (79, 80). Atanackovic et al. proposed that both MAGE-C1/CT7 and MAGE-A3 cancer-testis antigens are involved in the survival of myeloma cells, reducing the apoptosis induced by chemotherapy, through temporary silencing of these genes. In addition to their previous findings, Atanackovic et al. also demonstrated that transient silencing of MAGE-C1/CT7 in MM cell lines affected the expression of MAGE-C2/CT10, suggesting a potential interaction between these two genes (79). Furthermore, de Carvalho et al. silenced MAGE-C1/CT7 through short hairpin RNA in MM cell lines and found that this cancer-testis antigen is involved in promoting the survival of malignant plasma cells. MAGE-C1/CT7 protects myeloma cells from spontaneous and drug-induced apoptosis, with the drug used in the study being bortezomib, an inhibitor of the 26S proteasome. The authors suggest that this CTA may have a role in the cell cycle and speculate that silencing MAGE-C1/CT7 could be a useful therapeutic approach for MM,

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especially in combination with proteasome inhibitors. However, the precise function of the MAGE-C1/CT7 protein in the pathophysiology of MM remains undefined (80).

The presence of MAGE-C1/CT7 and MAGE-C2/CT10 expression has been commonly observed in osteolytic lesions of patients with MM. A correlation between MAGE-C1/CT7 expression and 17p13 deletion was suggested, which results in the decrease and loss of the tumor suppressor gene TP53 and an increase in CTA expression particularly in those with advanced disease stages. Additionally, there is a correlation between the proportion of myeloma cells expressing MAGE-C1/CT7 and a higher rate of cell proliferation (77, 81). Furthermore, the expression of MAGE-C1/CT7 has been shown to increase after treatment and in instances of recurrence (70). It has been demonstrated that if a patient shows MAGE-C1/CT7 expression at any point, the probability of its expression during relapse is nearly 100%. This finding is significant in selecting MAGE-C1/CT7 as a target for immunotherapy. Since MAGE-C1/CT7 has been confirmed to be present even during the remission phase, it is a suitable target for immunotherapy in minimal residual disease (71). Several studies have investigated the relationship between MAGE-C1/CT7 expression and disease stage, patient prognosis, and survival. Studies have demonstrated that the expression of MAGE-C1/CT7 is associated with the progression of MM, as MM stage III patients exhibit higher levels of MAGE-C1/CT7 expression compared to individuals with MGUS or lower stages (72, 77). Recent research has also indicated that MAGE-C1/CT7 expression is elevated in MM patients compared to those with MGUS, and this increased expression is linked to reduced survival rates (82). Moreover, the subcellular distribution of MAGE-C1/CT7 has been found to be associated with prognosis, where its presence solely in the cytoplasm is linked to a more favorable prognosis compared to cases where it is found in both the nucleus and cytoplasm, or solely in the nucleus (83). Furthermore, the presence of MAGE-C1/CT7 in malignant plasma cells in the bone marrow is indicative of a higher likelihood of early relapse and poorer overall survival following an allogeneic hematopoietic stem cell transplant (alloSCT). This expression also correlates with the extent of disease after treatment (71). In a separate study, the presence of MAGE-C1/CT7 was found to be the only prognostic factor in MM patients who had not undergone a transplant (72). Additionally, the frequency of MAGE-C1/CT7 expression was found to be higher in newly diagnosed MM cases compared to relapsed cases (70). Furthermore, specific T lymphocytes that target MAGE-C1/CT7 have been identified in patients with MM, indicating that this antigen could be a viable option for immunotherapy strategies. Anderson et al. identified immunogenic CD8+ T cell epitopes of MAGE-C1/CT7 and demonstrated that these epitopes are naturally processed and presented by tumor cells (84-86). The MAGE-C1 gene also serves as a regulator of the expression of other cancer-testis antigens (71).

MAGE-C2 is an antigen that is primarily expressed in the testis and brain and is more frequently observed in newly diagnosed MM patients than those in relapse (70). Moreover, the expression of MAGE-C2 has been commonly identified in bone-lytic lesions of individuals with MM (81), with approximately two-thirds of MM patients with bone marrow plasma cell infiltration of 10% or higher expressing this antigen (71). The high frequency of MAGE-C2 expression in MM suggests that it could be a potential target for cancer vaccines, especially considering previous research demonstrating its ability to induce both humoral and CD8+ T cell responses in patients with solid tumors expressing MAGE-C2 (87). Although MAGE expression is considered a prognostic factor in MM cases, it is only one of the many factors that should be considered. Other factors that could influence prognosis include the patient's age, disease stage, minimal residual disease (MRD), health status, and response to treatment. Therefore, it is crucial to evaluate all these prognostic factors in MM patients when developing a personalized treatment plan for them (88).

3.3. Application of MAGE proteins in staging of MM

MM is subjected to staging in order to ascertain the extent of the disease and to formulate an appropriate treatment plan. There are two staging systems that are commonly utilized by doctors, namely the Durie-Salmon staging system (DSS) and the international staging system (ISS) (89). However, the revised international staging system (R-ISS) is now more frequently employed to classify MM. The R-ISS is based on data collected from individuals with MM from various regions of the world. The system comprises three stages, which are determined by measuring serum albumin, lactase dehydrogenase (LDH), and serum beta-2 microglobulin (β2-M), as well as by identifying high-risk chromosomes using the fluorescence in situ hybridization (FISH) test (90). On the other hand, the ISS stages MM by analyzing the results of two blood tests, namely the albumin level and the beta-2-microglobulin level. The DSS, on the other hand, calculates the myeloma stage by measuring hemoglobin concentration, level of blood calcium, and the

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presence of bone lesions on imaging studies, in order to determine the extent of the myeloma, amount of the M protein in the blood and urine, and level of kidney function. It introduces three stages of myeloma: Stage I, Stage II, and Stage III. The stage depends on factors including The higher the stage number, the larger the amount of the myeloma in the body (91).

The expression of various types of MAGEs can differ not only based on the type of tumor but also on different stages of MM. Previous studies have demonstrated that MAGE expression can vary depending on the stage of MM. For instance, one study found that MAGE-A1 and MAGE-A3 were expressed in only 5% of bone marrow (BM) samples from patients with monoclonal gammopathy of undetermined significance (MGUS). However, 50% of patients with early-stage MM (IA and smoldering) and 40% of patients with advanced-stage MM expressed MAGE-A1 or MAGE-A3. Thus, it was confirmed that there is a correlation between MAGE expression and the early or late stage of MM. As MAGE-A1 and MAGE-A3 expression increases from the early stage to the advanced stage, they can be utilized to predict prognosis and monitor the progression of MM (92). Also, according to microarray analysis, MAGE-A2, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A8, and MAGE-12 are detected in MM patient samples and cell lines but not in MGUS (82). Additionally, the expression level of MAGE-A3 was found to be high in relapsed MM patients compared to newly diagnosed patients. While MAGE-C1 and C2 were typically detected in relapsed patients, their expression levels did not increase in these patients. However, MAGE-C1 expression increased during the progression phase of MM. In contrast to MAGE-C1/CT7 and MAGE-C2/CT10, other CTAs such as MAGE-A1, MAGE-A4, and NY-ESO-1 were detected in less than 20% of patients in either group (66, 77). A study analyzing specimens from patients with stage-III MM found that approximately 80% and 70% of samples expressed MAGE-C1/CT7 and MAGE-A3/6, respectively. Additionally, MAGE-C1 and MAGE-A mRNAs were detected in 87% and 100% of stage-III MM samples, respectively. The study concluded that a higher level of MAGE-C1 protein is associated with poor prognosis in MM. Moreover, these results suggest that MAGE-A3/6 and C1 may be potential therapeutic targets for developing cancer vaccines (93).

3.4. Therapeutic potentials of MAGE vaccines for treatment of MM

3.4.1. A brief overview of cancer vaccines

Cancer vaccines have begun to exhibit indications of effectiveness and the potential to assist patients who are unresponsive to other conventional immunotherapies, after a prolonged period of time (94). They are a type of immunotherapy that work to boost the body's immune system against cancer. There are two main types of cancer vaccines: preventive cancer vaccines and therapeutic cancer vaccines (95). The first group is utilized in case the patients are infected with viruses and in need of prevention from getting certain cancers caused by viruses. This type of vaccine will only work if a person gets the vaccine before they are infected with the virus. However, the therapeutic ones, are used as a treatment in patients who already have cancer. They may prevent a cancer or tumor from relapsing, growing and/or spreading. Therapeutic cancer vaccines expose the immune system to antigens, that are associated with a specific type of cancer. These vaccines enable the immune system to recognize and attack cancer cells. The vaccine can also be made by removing the patient's own immune cells and exposed to antigens in the laboratory and finally reinjecting the cells to create the vaccine. More data about types of antigens utilized in cancer vaccine development is provided in **Figure 3.**

Scientists are studying many different types of cancer vaccines and how they work in different ways. More research is needed before they have a full picture of how well this type of treatment works and which cancers it could treat. Researchers around the world are looking at the following types of cancer vaccines: protein or peptide vaccines, DNA and RNA vaccines, whole cell vaccines, dendritic cell vaccines, and virus vaccines (96, 97). The advantages and disadvantages of different types of cancer vaccines are summarized in **Table 2**.

3.4.2. MAGE as a promising antigen in cancer vaccination of MM

Antigen selection is a critical process in the design of cancer vaccines. It is essential to ensure that the chosen antigen is immunogenic enough to elicit a robust immune response and ultimately lead to clinical efficacy. The three features of high immunogenicity, specific expression in cancer cells, and necessity for cancer cell survival are important to ensure that the cancer cannot evade the new robust immune system after cancer vaccine therapy (26, 99). For MM, different targets are under evaluation for cancer vaccines. Survivin, a cell death preventer, telomerase, an enzyme for sustaining the integrity of DNA, WT1, an abnormal mutated protein in cancers, and MAGEs are some of them. MAGE-targeted immunotherapy has shown promises in early clinical trials

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for the treatment of MM as long as MAGEs are mostly expressed in myeloma cells rather than normal ones and have the previous mentioned appropriate targets in MM vaccine therapies. There are several clinical trials that emphasize on the usage of MAGE-based cancer vaccines in MM. An interventional phase II clinical trial (NCT01245673) reported that by injecting Poly-ICLC/GM-CSF-primed MAGE-A3 vaccine the cellular immune response of the post autologous stem cell transplanted MM patients were increased and MAGE-A3-specific CD8 T cells were observed in 88% of patients. Also, it was reported to be well-tolerated and safe with a high frequency of T-cell responses (102). Another phase I clinical trial (NCT01380145) investigated the safety, clinical and immunologic outcomes of autologous lymphocyte infusion combined with recombinant MAGE-A3. Thirteen MM patients undergone autoSCT and then were injected with autologous lymphocyte infusion and MAGE vaccination. Combination immunotherapy resulted in appropriate humoral and cellular immunity, antigen-specific CD4+ T-cell responses in all subjects. In 23% of subjects, CD8+ T-cell responses were also observed. These results demonstrated that MAGE-A3 vaccination has the potential to be a therapeutic option in MM (103). Due to the diverse expression of MAGE-A3 and the limited presence of anti-MAGE-A3 precursor CTLs, some other therapies are based on using inhibitors of epigenetic changes. These include epigenetic modification of MAGE-A3 antigen expression in MM using the demethylation agent 5-azacitidine and the histone deacetylase inhibitor MGCDO103, and enhancing MAGE-A3 antigen delivery and uptake by DCs through antibody-opsonized MAGE-A3 protein or bortezomib-induced immunogenic apoptosis (74, 104).

Other methods of targeting MAGE in MM are through the use of genetically engineered MAGE-specific T cells that can recognize and eliminate cancer cells expressing MAGE antigens. Nonetheless, the process of engineering T cells can cause side effects such as fever, chills, and hypotension (105, 106). Generally, for MM treatments, the ongoing studies lie on MAGE-A3 and CT7 more than other MAGEs. The safety and efficacy of the MAGE vaccines are confirmed by mentioned trials however the possible side-effects and challenges make the MAGE vaccines to progress slower in further clinical trial phases.

3.4.3. Safety and challenges of MAGE vaccines

MAGE-targeted immunotherapy holds promise for treating MM, but it can also give rise to adverse effects. The infusion of T cells, for instance, may trigger an immune response that can damage healthy tissues, leading to side effects like rash, diarrhea, and liver damage. vaccines may cause local reactions at the injection site, such as swelling and pain, as well as systemic reactions like fever, fatigue, and muscle pain. Clinical trials have investigated the safety and efficacy of cancer vaccines, and on the whole, most vaccines are well-tolerated and have limited toxicity (107). However, the safety of these vaccines depends on several factors, such as the type of vaccine (DNA, RNA, protein, and peptide), the adjuvants used, and whether they are autologous or heterologous. Although cancer vaccines may cause flu-like symptoms such as fever, chills, weakness, dizziness, nausea or vomiting, muscle or joint pain, and headache (108), their primary objective is to bolster the body's natural defenses against cancer. These vaccines are specifically designed to prompt the immune system to recognize and react to tumor-associated antigens (26). In a non-randomized clinical trial, Nooka et al. assessed the safety and immunogenicity of the PVX-410 multipeptide vaccine in patients with smoldering multiple myeloma (SMM). The vaccine was found to be well-tolerated, with mild-to-moderate injection site reactions and constitutional symptoms being the most common adverse events. (109). Despite significant progress in the development of cancer vaccines, most of them are still in the preclinical and clinical research phase, and the development of more specific antigens and vaccine platforms is necessary (107). Although cancer vaccines have demonstrated safety and tolerability, they may cause flu-like symptoms.

Although these side effects may be concerning, they can generally be managed with proper supportive care. Before deciding on the most appropriate cancer treatment, it is critical to carefully weigh the potential benefits and drawbacks of MAGE-targeted immunotherapy for each patient. Close monitoring of patients is essential, and treatment plans should be adjusted as necessary to minimize the risk of complications (110). Researchers are exploring various strategies, such as antibody-based and tumor antigens-peptide-based approaches, as well as genetically engineered and CARs-transfected T cell therapy, in clinical trials for MM patients. The transfer of autologous T cells that have been engineered to express the MAGE-A3 TCR is a matter of concern due to its potential to cause severe cardiovascular toxicity. This is because the transferred T cells may recognize an epitope from an unrelated protein that is present in normal cardiac tissue, resulting in adverse effects (111). Despite, the trials that have shown promising results, indicating that

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Table 2. Advantages and disadvantages of different types of cancer vaccines

Type of vaccine	Advantage	Disadvantage	References
Protein or peptide	• Easy to produce and store • Well-tolerated and have suitable responses	• Largely ineffective when it is used as a standalone intervention • Not successful in the trials for various reasons	(98)
DNA and RNA	Simple and inexpensive production Not HLA-specific	Tripping in immune tolerance mechanisms	(99, 100)
Whole cell	Stimulates both T cell and antibody responses	Challenging to standardize and replicate them consistently	(98)
Dendritic cell	• Stimulates both T cell and antibody responses • Can be personalized to each patient's tumor cells	Challenging to standardize and replicate them consistently	(98)
Virus	• Stimulates both T cell and antibody responses • Can be effective against virus-associated cancer	Limited to virus-associated cancers	(101)

immunotherapy can trigger both immune and clinical responses with a favorable safety profile, adverse effects were also reported. For instance, after injecting a peptide vaccine of MAGE-A3 or NY-ESO-1 peptide and GM-CSF adjuvant in combo with chemotherapy in high-risk MM patients, van Rhee et al. observed in a phase II and III clinical trial (NCT00090493) that 75% (n=4) of their cases showed deep vein thrombosis (DVT) and infection with staphylococcus (112). However, while the data are encouraging, the findings from these trials are still limited, and more extensive and long-term studies are necessary to determine the full clinical effectiveness of immunotherapy in MM. It is also important to note that, despite the progress made in this area, some MM patients may still experience persistent or recurrent disease after undergoing immune therapies (113).

Therapies aimed at targeting the immune system have been developed to reduce the side effects associated with traditional cancer treatments. However, these immunotherapies have also been found to have harmful effects. Most of these patients' responses have been linked to on-target and off-target toxicity, and a few patients have experienced fatal problems (114). The nature and severity of the effects may vary depending on the specific treatment, the patient's health status, and other factors. It's worth noting that certain cancer-testis antigens, including NY-ESO-1, are expressed in normal stem cells, which may contribute to some of the potential side effects (10). In addition, substances utilized in the preparation of T cells expanded outside the body, such as intricate media, serum, and cytokines, as well as genetic modifications, can increase the risk of infusion-related reactions. Dendritic cell-based immunization has been shown to have low side effects (110) . Administering custom-made antibodies can result in immune reactions such as overactive responses, serum sickness, and the emergence of antibodies that are associated with various adverse effects related to their specific targets (115).

Immunotherapies like all other treatments may not always have desired results. The failure of immunotherapy as a challenge, can be traced back to several underlying factors. Firstly, the patient's condition may be the primary cause. Secondly, inadequate recognition of tumor antigens (TAs) and changes in tumor cells due to immune pressure, coupled with qualitative and/or quantitative issues in antigen presenting cells (APCs) and T cells, can also influence patient outcomes. Moreover, the tumor microenvironment plays a critical role in determining the adverse events and not desired outcomes associated with immunotherapy in clinical settings. Numerous endeavors have been undertaken to surmount hurdles and achieve a potent and significant immune response against MM. These endeavors encompass refining the vaccine system, inhibiting factors that impede the immune system, augmenting immunity through engineering, and combining therapies such as adoptive cellular therapy and/or immunomodulatory molecules (116).

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4. CONCLUSION

This comprehensive review delves into the crucial role played by MAGE family proteins in cancer, with a specialized focus on their involvement in MM. The findings of this study explore the intricate expression patterns of MAGE proteins in different tissues and their intimate association with several critical cellular processes, such as cell cycle progression, apoptosis, and gene expression regulation. The data presented in this review provide a profound understanding of the structure and function of MAGE proteins and their potential utility in cancer immunotherapy. Here we underlined the potential of MAGEs in facilitating the prognosis of MM cases. Furthermore, despite, we emphasized the promising potential of MAGE-based cancer vaccines in the prevention and treatment of MM. Due to the small number of conducted trials, the MAGE-based cancer vaccines are in an area of limited attention, while the evidences showed that MAGE-A3 and C1/CT7 can be suitable and potentiated antigens to get in use. However, it is evident that further comprehensive research is required in this area, and more extensive clinical trials must be conducted to evaluate the effectiveness and potency of MAGE antigens

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Conflict of interest

The authors declare that they have no conflict of interests.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: a cancer journal for clinicians. 2021;71(3):209-49.

2. Monteith BE, Sandhu I, Lee AS. Management of Multiple Myeloma: A Review for General Practitioners in Oncology. Current oncology (Toronto, Ont). 2023;30(5):4382-401.

3. Dobosz P, Dzieciątkowski T. The intriguing history of cancer immunotherapy. Frontiers in immunology. 2019:2965.

4. Saxena M, van der Burg SH, Melief CJM, Bhardwaj N. Therapeutic cancer vaccines. Nature reviews Cancer. 2021;21(6):360-78.

5. Cancer Vaccines: The Types, How They Work, and Which Cancers They Treat [Available from: https://www.mskcc.org/cancer-care/diagnosis-treatment/cancer-treatments/immunotherapy/cancer-vaccines.

6. What are Cancer Vaccines? 2020 [Available from: https://www.cancer.net/navigating-cancer-care/how-cancer-treated/immunotherapy-and-vaccines/what-are-cancer-vaccines.

7. Li S, Shi X, Li J, Zhou X. Pathogenicity of the MAGE family. Oncology letters. 2021;22(6):844.

8. Florke Gee RR, Chen H, Lee AK, Daly CA, Wilander BA, Fon Tacer K, et al. Emerging roles of the MAGE protein family in stress response pathways. The Journal of biological chemistry. 2020;295(47):16121-55.

9. Schooten E, Di Maggio A, van Bergen En Henegouwen PMP, Kijanka MM. MAGE-A antigens as targets for cancer immunotherapy. Cancer Treat Rev. 2018;67:54-62.

10. Ghafouri-Fard S. Expression of cancer-testis antigens in stem cells: is it a potential drawback or an advantage in cancer immunotherapy. Asian Pacific journal of cancer prevention : APJCP. 2015;16(7):3079-81.

11. van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, et al. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science (New York, NY). 1991;254(5038):1643-7.

12. Sang M, Wang L, Ding C, Zhou X, Wang B, Wang L, et al. Melanoma-associated antigen genes - an update. Cancer letters. 2011;302(2):85-90.

13. Weon JL, Potts PR. The MAGE protein family and cancer. Current opinion in cell biology. 2015;37:1-8.

14. Wei X, Chen F, Xin K, Wang Q, Yu L, Liu B, et al. Cancer-Testis Antigen Peptide Vaccine for Cancer Immunotherapy: Progress and Prospects. Translational Oncology. 2019;12(5):733-8.

15. Yang SW, Huang X, Lin W, Min J, Miller DJ, Mayasundari A, et al. Structural basis for substrate recognition and chemical inhibition of oncogenic MAGE ubiquitin ligases. Nature Communications. 2020;11(1):4931.

16. Lee AK, Potts PR. A Comprehensive Guide to the MAGE Family of Ubiquitin Ligases. Journal of molecular biology. 2017;429(8):1114-42.

17. Metzger MB, Pruneda JN, Klevit RE, Weissman AM. RING-type E3 ligases: master manipulators of E2 ubiquitin-conjugating enzymes and ubiquitination. Biochimica et biophysica acta. 2014;1843(1):47-60.

18. Fleming MC, Chiou LF, Tumbale PP, Droby GN, Lim J, Norris-Drouin JL, et al. Discovery and structural basis of the selectivity of potent cyclic peptide inhibitors of MAGE-A4. Journal of medicinal chemistry. 2022;65(10):7231-45.

19. Wang Z, Xu X, Li J-L, Palmer C, Maric D, Dean J. Sertoli cell-only phenotype and scRNA-seq define PRAMEF12 as a factor essential for spermatogenesis in mice. Nature Communications. 2019;10(1):5196.

20. De Plaen E, Traversari C, Gaforio JJ, Szikora J-P, De Smet C, Brasseur F, et al. Structure, chromosomal localization, and expression of 12 genes of the MAGE family. Immunogenetics. 1994;40(5):360-9.

[DOI: 10.61186/ijbc.16.1.1]

21. Fon Tacer K, Montoya MC, Oatley MJ, Lord T, Oatley JM, Klein J, et al. MAGE cancer-testis antigens protect the mammalian germline under environmental stress. Science advances. 2019;5(5):eaav4832.

22. Weon JL, Potts PR. The MAGE protein family and cancer. Current Opinion in Cell Biology. 2015;37:1-8.

23. Pitcovski J, Shahar E, Aizenshtein E, Gorodetsky R. Melanoma antigens and related immunological markers. Critical reviews in oncology/hematology. 2017;115:36-49.

24. Wischnewski F, Friese O, Pantel K, Schwarzenbach H. Methyl-CpG binding domain proteins and their involvement in the regulation of the MAGE-A1, MAGE-A2, MAGE-A3, and MAGE-A12 gene promoters. Molecular cancer research. 2007;5(7):749-59.

25. Wischnewski F, Pantel K, Schwarzenbach H. Promoter demethylation and histone acetylation mediate gene expression of MAGE-A1, -A2, -A3, and -A12 in human cancer cells. Molecular cancer research : MCR. 2006;4(5):339-49.

26. Liu J, Fu M, Wang M, Wan D, Wei Y, Wei X. Cancer vaccines as promising immuno-therapeutics: platforms and current progress. Journal of Hematology & Oncology. 2022;15(1):28.

27. Elliott EN, Sheaffer KL, Kaestner KH. The 'de novo' DNA methyltransferase Dnmt3b compensates the Dnmt1-deficient intestinal epithelium. Elife. 2016;5.

28. Almutairi MH, Alotaibi MM, Alonaizan R, Almutairi BO. Expression Profile of MAGE-B1 Gene and Its Hypomethylation Activation in Colon Cancer. BioMed Research International. 2022;2022.

29. Lopez-Serra L, Ballestar E, Fraga MF, Alaminos M, Setien F, Esteller M. A profile of methyl-CpG binding domain protein occupancy of hypermethylated promoter CpG islands of tumor suppressor genes in human cancer. Cancer research. 2006;66(17):8342-6.

30. Ng HH, Jeppesen P, Bird A. Active repression of methylated genes by the chromosomal protein MBD1. Molecular and cellular biology. 2000;20(4):1394-406.

31. Jørgensen HF, Ben-Porath I, Bird AP. Mbd1 is recruited to both methylated and nonmethylated CpGs via distinct DNA binding domains. Molecular and cellular biology. 2004;24(8):3387-95.

32. Liu S, Liu F, Huang W, Gu L, Meng L, Ju Y, et al. MAGE-A11 is activated through TFCP2/ZEB1 binding sites de-methylation as well as histone modification and facilitates ESCC tumor growth. Oncotarget. 2018;9(3):3365.

33. Vatolin S, Abdullaev Z, Pack SD, Flanagan PT, Custer M, Loukinov DI, et al. Conditional expression of the CTCF-paralogous transcriptional factor BORIS in normal cells results in demethylation and derepression of MAGE-A1 and reactivation of other cancer-testis genes. Cancer research. 2005;65(17):7751-62.

34. Schwarzenbach H, Eichelser C, Steinbach B, Tadewaldt J, Pantel K, Lobanenkov V, et al. Differential regulation of MAGE-A1 promoter activity by BORIS and Sp1, both interacting with the TATA binding protein. BMC Cancer. 2014;14(1):796.

35. Martin-Kleiner I. BORIS in human cancers–a review. European journal of cancer. 2012;48(6):929-35.

36. De Smet C, Lurquin C, Lethé B, Martelange V, Boon T. DNA methylation is the primary silencing mechanism for a set of germ line-and tumor-specific genes with a CpG-rich promoter. Molecular and cellular biology. 1999;19(11):7327-35.

37. James SR, Link PA, Karpf AR. Epigenetic regulation of X-linked cancer/germline antigen genes by DNMT1 and DNMT3b. Oncogene. 2006;25(52):6975-85.

38. Loriot A, De Plaen E, Boon T, De Smet C. Transient down-regulation of DNMT1 methyltransferase leads to activation and stable hypomethylation of MAGE-A1 in melanoma cells. The Journal of biological chemistry. 2006;281(15):10118-26.

39. Weber J, Salgaller M, Samid D, Johnson B, Herlyn M, Lassam N, et al. Expression of the MAGE-1 tumor antigen is up-regulated by the demethylating agent 5-aza-2′-deoxycytidine. Cancer research. 1994;54(7):1766-71.

40. Karpf AR, Bai S, James SR, Mohler JL, Wilson EM. Increased expression of androgen receptor coregulator MAGE-11 in prostate cancer by DNA hypomethylation and cyclic AMP. Molecular cancer research : MCR. 2009;7(4):523-35.

41. Trasler JM. Epigenetics in spermatogenesis. Molecular and cellular endocrinology. 2009;306(1-2):33-6.

42. Lian Y, Meng L, Ding P, Sang M. Epigenetic regulation of MAGE family in human cancer progression-DNA methylation, histone modification, and non-coding RNAs. Clinical epigenetics. 2018;10:1-11.

43. Akers SN, Odunsi K, Karpf AR. Regulation of cancer germline antigen gene expression: implications for cancer immunotherapy. Future Oncol. 2010;6(5):717-32.

44. Timp W, Feinberg AP. Cancer as a dysregulated epigenome allowing cellular growth advantage at the expense of the host. Nature Reviews Cancer. 2013;13(7):497-510.

45. Rao M, Chinnasamy N, Hong JA, Zhang Y, Zhang M, Xi S, et al. Inhibition of Histone Lysine Methylation Enhances Cancer–Testis Antigen Expression in Lung Cancer Cells: Implications for Adoptive Immunotherapy of Cancer. Cancer Research. 2011;71(12):4192-204.

46. Tachibana M, Sugimoto K, Nozaki M, Ueda J, Ohta T, Ohki M, et al. G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis. Genes & development. 2002;16(14):1779-91.

47. Kondo T, Zhu X, Asa SL, Ezzat S. The Cancer/Testis Antigen Melanoma-Associated Antigen-A3/A6 Is a Novel Target of Fibroblast Growth Factor Receptor 2-IIIb through Histone H3 Modifications in Thyroid Cancer. Clinical Cancer Research. 2007;13(16):4713-20.

48. Zhu X, Asa SL, Ezzat S. Fibroblast Growth Factor 2 and Estrogen Control the Balance of Histone 3 Modifications Targeting MAGE-A3 in Pituitary Neoplasia. Clinical Cancer Research. 2008;14(7):1984-96.

49. James SR, Cedeno CD, Sharma A, Zhang W, Mohler JL, Odunsi K, et al. DNA methylation and nucleosome occupancy regulate the cancer germline antigen gene MAGEA11. Epigenetics. 2013;8(8):849-63.

[DOI: 10.61186/ijbc.16.1.1]

50. Chiappinelli KB, Zahnow CA, Ahuja N, Baylin SB. Combining Epigenetic and Immunotherapy to Combat Cancer. Cancer Research. 2016;76(7):1683-9.

51. De Smet C, Courtois SJ, Faraoni I, Lurquin C, Szikora JP, De Backer O, et al. Involvement of two Ets binding sites in the transcriptional activation of the MAGE1 gene. Immunogenetics. 1995;42(4):282-90.

52. Serrano A, García A, Abril E, Garrido F, Ruiz-Cabello F. Methylated CpG points identified within MAGE-1 promoter are involved in gene repression. Int J Cancer. 1996;68(4):464-70.

53. De Smet C, Courtois SJ, Faraoni I, Lurquin C, Szikora J-P, De Backer O, et al. Involvement of two Ets binding sites in the transcriptional activation of the MAGE1 gene. Immunogenetics. 1995;42:282-90.

54. Yang B, Wu J, Maddodi N, Ma Y, Setaluri V, Longley BJ. Epigenetic control of MAGE gene expression by the KIT tyrosine kinase. The Journal of investigative dermatology. 2007;127(9):2123-8.

55. Liu W, Cheng S, Asa SL, Ezzat S. The melanoma-associated antigen A3 mediates fibronectin-controlled cancer progression and metastasis. Cancer Res. 2008;68(19):8104-12.

56. Fukuyama T, Yamazaki T, Fujita T, Uematsu T, Ichiki Y, Kaneko H, et al. Helicobacter pylori, a carcinogen, induces the expression of melanoma antigen-encoding gene (Mage)-A3, a cancer/testis antigen. Tumour Biol. 2012;33(6):1881-7.

57. Chen X, Wang L, Liu J, Huang L, Yang L, Gao Q, et al. Expression and prognostic relevance of MAGE-A3 and MAGE-C2 in non-small cell lung cancer. Oncol Lett. 2017;13(3):1609-18.

58. Wu F, Liu F, Dong L, Yang H, He X, Li L, et al. miR-1273g silences MAGEA3/6 to inhibit human colorectal cancer cell growth via activation of AMPK signaling. Cancer Lett. 2018;435:1-9.

59. Pan SJ, Ren J, Jiang H, Liu W, Hu LY, Pan YX, et al. MAGEA6 promotes human glioma cell survival via targeting AMPKα1. Cancer Lett. 2018;412:21-9.

60. Li S, Shi X, Li J, Zhou X. Pathogenicity of the MAGE family. Oncology letters. 2021;22(6):1-7.

61. Mei AH, Tung K, Han J, Perumal D, Laganà A, Keats J, et al. MAGE-A inhibit apoptosis and promote proliferation in multiple myeloma through regulation of BIM and p21(Cip1). Oncotarget. 2020;11(7):727-39.

62. Wienand K, Shires K. The use of MAGE C1 and flow cytometry to determine the malignant cell type in multiple myeloma. PloS one. 2015;10(3):e0120734.

63. Gordeeva O, editor Cancer-testis antigens: Unique cancer stem cell biomarkers and targets for cancer therapy. Seminars in cancer biology; 2018: Elsevier.

64. Murray-Zmijewski F, Slee EA, Lu X. A complex barcode underlies the heterogeneous response of p53 to stress. Nature reviews Molecular cell biology. 2008;9(9):702-12.

65. Nardiello T, Jungbluth AA, Mei A, DiLiberto M, Huang X, Ely SA, et al. MAGE-A Inhibits Apoptosis In Proliferating Multiple Myeloma Cells. Blood. 2010;116(21):785.

66. Nardiello T, Jungbluth AA, Mei A, Diliberto M, Huang X, Dabrowski A, et al. MAGE-A inhibits apoptosis in proliferating myeloma cells through repression of Bax and maintenance of survivin. Clinical cancer research : an official journal of the American Association for Cancer Research. 2011;17(13):4309-19.

67. Mei AH-C, Tung K, Han J, Perumal D, Laganà A, Keats J, et al. MAGE-A inhibit apoptosis and promote proliferation in multiple myeloma through regulation of BIM and p21Cip1. Oncotarget. 2020;11(7):727.

68. Atanackovic D, Hildebrandt Y, Jadczak A, Cao Y, Luetkens T, Meyer S, et al. Cancer-testis antigens MAGE-C1/CT7 and MAGE-A3 promote the survival of multiple myeloma cells. Haematologica. 2010;95(5):785.

69. Doyle JM, Gao J, Wang J, Yang M, Potts PR. MAGE-RING protein complexes comprise a family of E3 ubiquitin ligases. Molecular cell. 2010;39(6):963-74.

70. van Duin M, Broyl A, de Knegt Y, Goldschmidt H, Richardson PG, Hop WC, et al. Cancer testis antigens in newly diagnosed and relapse multiple myeloma: prognostic markers and potential targets for immunotherapy. Haematologica. 2011;96(11):1662-9.

71. Atanackovic D, Luetkens T, Hildebrandt Y, Arfsten J, Bartels K, Horn C, et al. Longitudinal analysis and prognostic effect of cancer-testis antigen expression in multiple myeloma. Clinical cancer research : an official journal of the American Association for Cancer Research. 2009;15(4):1343-52.

72. Andrade VC, Vettore AL, Felix RS, Almeida MS, Carvalho F, Oliveira JS, et al. Prognostic impact of cancer/testis antigen expression in advanced stage multiple myeloma patients. Cancer immunity. 2008;8:2.

73. Takahashi T, Lim B, Jamal N, Tritchler D, Lockwood G, McKinney S, et al. Colony growth and self renewal of plasma cell precursors in multiple myeloma. Journal of Clinical Oncology. 1985;3(12):1613-23.

74. Moreno-Bost A, Szmania S, Stone K, Garg T, Hoerring A, Szymonifka J, et al. Epigenetic modulation of MAGE-A3 antigen expression in multiple myeloma following treatment with the demethylation agent 5-azacitidine and the histone deacetlyase inhibitor MGCD0103. Cytotherapy. 2011;13(5):618-28.

75. Cho HJ, Caballero OL, Gnjatic S, Andrade VC, Colleoni GW, Vettore AL, et al. Physical interaction of two cancer-testis antigens, MAGE-C1 (CT7) and NY-ESO-1 (CT6). Cancer immunity. 2006;6:12.

76. Dhodapkar MV, Osman K, Teruya-Feldstein J, Filippa D, Hedvat CV, Iversen K, et al. Expression of cancer/testis (CT) antigens MAGE-A1, MAGE-A3, MAGE-A4, CT-7, and NY-ESO-1 in malignant gammopathies is heterogeneous and correlates with site, stage and risk status of disease. Cancer immunity. 2003;3:9.

77. Jungbluth AA, Ely S, DiLiberto M, Niesvizky R, Williamson B, Frosina D, et al. The cancer-testis antigens CT7 (MAGE-C1) and MAGE-A3/6 are commonly expressed in multiple myeloma and correlate with plasma-cell proliferation. Blood. 2005;106(1):167-74.

78. Curioni-Fontecedro A, Knights AJ, Tinguely M, Nuber N, Schneider C, Thomson CW, et al. MAGE-C1/CT7 is the dominant

[DOI: 10.61186/ijbc.16.1.1]

cancer-testis antigen targeted by humoral immune responses in patients with multiple myeloma. Leukemia. 2008;22(8):1646-8.

79. Atanackovic D, Hildebrandt Y, Jadczak A, Cao Y, Luetkens T, Meyer S, et al. Cancer-testis antigens MAGE-C1/CT7 and MAGE-A3 promote the survival of multiple myeloma cells. Haematologica. 2010;95(5):785-93.

80. de Carvalho F, Costa ET, Camargo AA, Gregorio JC, Masotti C, Andrade VC, et al. Targeting MAGE-C1/CT7 expression increases cell sensitivity to the proteasome inhibitor bortezomib in multiple myeloma cell lines. PloS one. 2011;6(11):e27707.

81. Pabst C, Zustin J, Jacobsen F, Luetkens T, Kröger N, Schilling G, et al. Expression and prognostic relevance of MAGE-C1/CT7 and MAGE-C2/CT10 in osteolytic lesions of patients with multiple myeloma. Experimental and molecular pathology. 2010;89(2):175-81.

82. Condomines M, Hose D, Raynaud P, Hundemer M, De Vos J, Baudard M, et al. Cancer/testis genes in multiple myeloma: expression patterns and prognosis value determined by microarray analysis. Journal of immunology (Baltimore, Md : 1950). 2007;178(5):3307-15.

83. Tinguely M, Jenni B, Knights A, Lopes B, Korol D, Rousson V, et al. MAGE-C1/CT-7 expression in plasma cell myeloma: sub-cellular localization impacts on clinical outcome. Cancer science. 2008;99(4):720-5.

84. Nuber N, Curioni-Fontecedro A, Matter C, Soldini D, Tiercy JM, von Boehmer L, et al. Fine analysis of spontaneous MAGE-C1/CT7-specific immunity in melanoma patients. Proceedings of the National Academy of Sciences of the United States of America. 2010;107(34):15187-92.

85. Lendvai N, Gnjatic S, Ritter E, Mangone M, Austin W, Reyner K, et al. Cellular immune responses against CT7 (MAGE-C1) and humoral responses against other cancer-testis antigens in multiple myeloma patients. Cancer immunity. 2010;10:4.

86. Anderson LD, Jr., Cook DR, Yamamoto TN, Berger C, Maloney DG, Riddell SR. Identification of MAGE-C1 (CT-7) epitopes for T-cell therapy of multiple myeloma. Cancer immunology, immunotherapy : CII. 2011;60(7):985-97.

87. Atanackovic D, Arfsten J, Cao Y, Gnjatic S, Schnieders F, Bartels K, et al. Cancer-testis antigens are commonly expressed in multiple myeloma and induce systemic immunity following allogeneic stem cell transplantation. Blood. 2007;109(3):1103-12.

88. Hanbali A, Hassanein M, Rasheed W, Aljurf M, Alsharif F. The Evolution of Prognostic Factors in Multiple Myeloma. Advances in hematology. 2017;2017:4812637.

89. Rajkumar SV. Updated diagnostic criteria and staging system for multiple myeloma. American Society of Clinical Oncology Educational Book. 2016;36:e418-e23.

90. D'agostino M, Cairns DA, Lahuerta JJ, Wester R, Bertsch U, Waage A, et al. Second revision of the International Staging System (R2-ISS) for overall survival in multiple myeloma: a European Myeloma Network (EMN) report within the HARMONY project. Journal of clinical oncology. 2022;40(29):3406-18.

91. Encinas C, Hernandez-Rivas J-Á, Oriol A, Rosiñol L, Blanchard M-J, Bellón J-M, et al. A simple score to predict early severe infections in patients with newly diagnosed multiple myeloma. Blood cancer journal. 2022;12(4):68.

92. Necasova J, Kadlecova J, Spesna R, Penka M, Hajek R. Expression of MAGE-A1 and MAGE-A3 in bone marrow from monoclonal gammapathy to myeloma patients. American Society of Hematology; 2007.

93. Jungbluth AA, Ely S, DiLiberto M, Niesvizky R, Williamson B, Frosina D, et al. The cancer-testis antigens CT7 (MAGE-C1) and MAGE-A3/6 are commonly expressed in multiple myeloma and correlate with plasma-cell proliferation. Blood. 2005;106(1):167-74.

94. Lin MJ, Svensson-Arvelund J, Lubitz GS, Marabelle A, Melero I, Brown BD, et al. Cancer vaccines: the next immunotherapy frontier. Nature cancer. 2022;3(8):911-26.

95. Aikins ME, Xu C, Moon JJ. Engineered nanoparticles for cancer vaccination and immunotherapy. Accounts of chemical research. 2020;53(10):2094-105.

96. Lollini P-L, Cavallo F, Nanni P, Quaglino E. The promise of preventive cancer vaccines. Vaccines. 2015;3(2):467-89.

97. Gilboa E, Nair SK, Lyerly HK. Immunotherapy of cancer with dendritic-cell-based vaccines. Cancer Immunology, Immunotherapy. 1998;46:82-7.

98. Donninger H, Li C, Eaton JW, Yaddanapudi K. Cancer Vaccines: Promising Therapeutics or an Unattainable Dream. Vaccines (Basel). 2021;9(6).

99. Hollingsworth RE, Jansen K. Turning the corner on therapeutic cancer vaccines. npj Vaccines. 2019;4(1):7.

100. Stephens AJ, Burgess-Brown NA, Jiang S. Beyond just peptide antigens: the complex world of peptide-based cancer vaccines. Frontiers in Immunology. 2021;12:696791.

101. Zhao Y, Baldin AV, Isayev O, Werner J, Zamyatnin AA, Bazhin AV. Cancer Vaccines: Antigen Selection Strategy. Vaccines. 2021;9(2):85.

102. Rapoport AP, Aqui NA, Stadtmauer EA, Vogl DT, Xu YY, Kalos M, et al. Combination immunotherapy after ASCT for multiple myeloma using MAGE-A3/Poly-ICLC immunizations followed by adoptive transfer of vaccine-primed and costimulated autologous T cells. Clinical cancer research : an official journal of the American Association for Cancer Research. 2014;20(5):1355-65.

103. Cohen AD, Lendvai N, Gnjatic S, Jungbluth AA, Bertolini S, Pan L, et al. Recombinant (rec) MAGE-A3 protein immunotherapy and peripheral blood lymphocyte (PBL) reconstitution induce strong antigen-specific humoral and cellular immune responses in patients undergoing autologous stem cell transplantation (ASCT) for consolidation of multiple myeloma (MM). Blood. 2014;124(21):1184.

104. Moeller I, Spagnoli GC, Finke J, Veelken H, Houet L. Uptake routes of tumor-antigen MAGE-A3 by dendritic cells determine priming of naïve T-cell subtypes. Cancer immunology, immunotherapy : CII. 2012;61(11):2079-90.

105. Badieyan ZS, Hoseini SS. Adverse Effects Associated with Clinical Applications of CAR Engineered T Cells. Archivum Immunologiae et Therapiae Experimentalis. 2018;66(4):283-8.

[DOI: 10.61186/ijbc.16.1.1]

106. CAR T Cells: Engineering Patients' Immune Cells to Treat Their Cancers 2022 [updated 2022. Available from: https://www.cancer.gov/about-cancer/treatment/research/car-t-cells.

107. Zhao J, Chen Y, Ding ZY, Liu JY. Safety and Efficacy of Therapeutic Cancer Vaccines Alone or in Combination With Immune Checkpoint Inhibitors in Cancer Treatment. Frontiers in pharmacology. 2019;10:1184.

108. institute Nc. Cancer Treatment Vaccines. 2019.

109. Nooka AK, Wang ML, Yee AJ, Kaufman JL, Bae J, Peterkin D, et al. Assessment of safety and immunogenicity of PVX-410 vaccine with or without lenalidomide in patients with smoldering multiple myeloma: a nonrandomized clinical trial. JAMA oncology. 2018;4(12):e183267-e.

110. Cruz CR, Hanley PJ, Liu H, Torrano V, Lin YF, Arce JA, et al. Adverse events following infusion of T cells for adoptive immunotherapy: a 10-year experience. Cytotherapy. 2010;12(6):743-9.

111. Linette GP, Stadtmauer EA, Maus MV, Rapoport AP, Levine BL, Emery L, et al. Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. Blood. 2013;122(6):863-71.

112. Study of MAGE-A3 and NY-ESO-1 Immunotherapy in Combo With DTPACE Chemo and Auto Transplantation in Multiple Myeloma, NCT00090493. 2013.

113. Klippel ZK, Chou J, Towlerton AM, Voong LN, Robbins P, Bensinger WI, et al. Immune escape from NY-ESO-1-specific T-cell therapy via loss of heterozygosity in the MHC. Gene therapy. 2014;21(3):337-42.

114. Stauss HJ, Morris EC. Immunotherapy with gene-modified T cells: limiting side effects provides new challenges. Gene therapy. 2013;20(11):1029-32.

115. Hansel TT, Kropshofer H, Singer T, Mitchell JA, George AJ. The safety and side effects of monoclonal antibodies. Nature reviews Drug discovery. 2010;9(4):325-38.

116. Wang L, Jin N, Schmitt A, Greiner J, Malcherek G, Hundemer M, et al. T cell-based targeted immunotherapies for patients with multiple myeloma. International journal of cancer. 2015;136(8):1751-68.

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