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The MAGE protein family and cancer

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The Melanoma Antigen Gene (MAGE) protein family is a large, highly conserved group of proteins that share a common MAGE homology domain. Intriguingly, many MAGE proteins are restricted in expression to reproductive tissues, but are aberrantly expressed in a wide variety of cancer types. Originally discovered as antigens on tumor cells and developed as cancer immunotherapy targets, recent literature suggests a more prominent role for MAGEs in driving tumorigenesis. This review will highlight recent developments into the function of MAGEs as oncogenes, their mechanisms of action in regulation of ubiquitin ligases, and outstanding questions in the field.

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Introduction

The Melanoma Antigen Gene (MAGE) family has garnered growing interest as biomarkers in cancer and targets of immunotherapies because a subset of these >40 human proteins are classified as cancer-testis antigens (CTAs), which have restricted expression to the testis (and occasionally ovary and placenta) and are aberrantly re-expressed in cancer where they can be immunogenic (reviewed in [1]). Collectively, MAGEs have been found to be broadly expressed in many tumor types, including colon [2], melanoma [3,4], brain [5], lung [6–8], prostate [9], and breast [10,11], among others. For many years, the focus on MAGE CTAs was centered on their potential for cancer immunotherapy. However, this approach has had little success and met recent challenges. Detailed functional studies of these proteins have started to emerge and suggest that their expression in cancer is not simply due to non-specific, progressive promoter demethylation due to global genomic instability in cancer. MAGE genes are associated with hallmarks of aggressive cancers, including worse clinical prognosis, increased tumor growth, metastases, and enrichment in stem cell-like populations. Importantly, functional studies have shown that some MAGE CTAs can have non-overlapping oncogenic driver activity. Thus, MAGE CTAs may provide a novel means to develop cancer-specific therapeutics to treat a broad range of cancers.

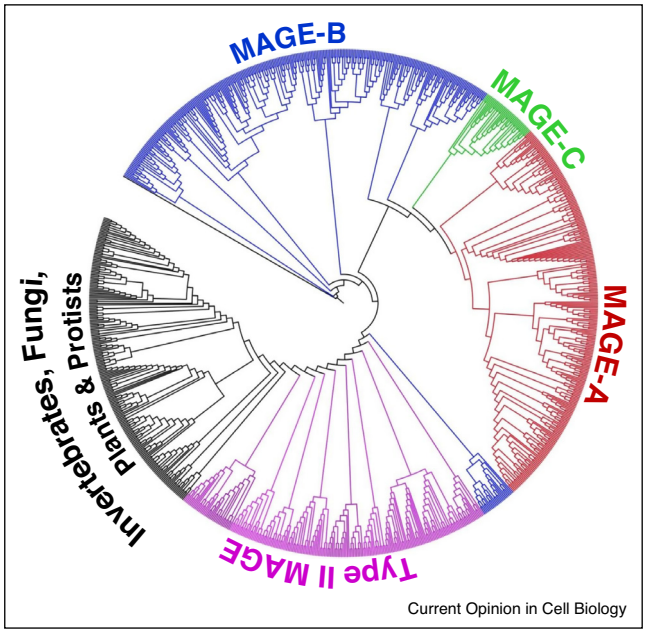
MAGE family of proteins

MAGE genes are conserved in all eukaryotes and have rapidly expanded in gene number in mammals (Figure 1). Members of the human MAGE family can be divided into two categories based on tissue expression pattern: Type I MAGEs are considered CTAs and in humans include the MAGE-A, -B, and -C subfamily members which are clustered on the X-chromosome (reviewed in [1,12]). Type II MAGEs (MAGE-D, -E, -F, -G, -H, -L subfamilies and Necdin) are expressed throughout many tissues in the body and are not restricted to the X chromosome (reviewed in [12]). Both type I and type II MAGEs contain a MAGE homology domain (MHD) that is approximately 170 amino acids (Figure 2A), which on average is 46% conserved amongst all human MAGEs [13]. Structural studies have revealed that the MHD consists of tandem winged helix motifs (Figure 2B-D) [13]. Our lab has shown that a defining biochemical function of MAGEs is their ability to bind to specific E3 RING ubiquitin ligases through their MHDs (Table 1), which may alter the relative orientation of the two winged helix motifs (compare Figure 2B-C with D) [13]. Importantly, we and others have determined that MAGEs can regulate the ubiquitination of proteins through modulating the activity of their cognate E3 ligase. This includes enhancing general ligase activity, binding to and specifying novel substrates for ubiquitination by the E3 ligase complex, and altering the subcellular localization of E3 ligases to dictate substrates (Figure 2E) [13,14•,15••]. Thus, aberrant expression of MAGEs in tumor cells can lead to alterations in cellular processes and signaling pathways through ubiquitination and potentially other activities to contribute to tumorigenesis.

MAGEs are associated with poor clinical prognosis

Extensive studies of MAGE expression in various cancers (Table 2) have shown their predictive association with poor clinical outcomes. For example, MAGE-A3 and -A9 expression is significantly correlated with decreased survival in non-small cell lung cancers [7,16] and MAGE-A3, -A6, and -C2 expression in breast cancers is significantly associated with estrogen receptor-negative or progesterone receptor-negative status, higher grade tumors, and

Figure 1



The MAGE protein family. Phylogenetic tree of eukaryotic MAGEs. Note that all non-mammalian MAGEs cluster together (black). Mammalian MAGEs have dramatically expanded in number and cluster into type II (pink) MAGEs that are anatomically broadly expressed and type I (MAGE-A, MAGE-B, and MAGE-C subfamilies) that are primarily restricted in expression to testis, ovary, and placenta.

correlated with worse outcome [11,17]. In ovarian cancers, MAGE-A1, -A9, and -A10 expression are associated with worse prognosis [18•,19•]. MAGEs are also associated with increased rates of recurrence after therapy. In gastric carcinoma, MAGE-A1-6 expression in peritoneal washes after cancer resection correlated with a significant decreased disease-free survival rate [20], and in hepatocellular carcinoma MAGE-A9 expression was significantly correlated with decreased disease-free survival, advanced tumor grade, metastasis, portal vein invasion, and overall survival [21].

MAGE CTAs have oncogenic activity

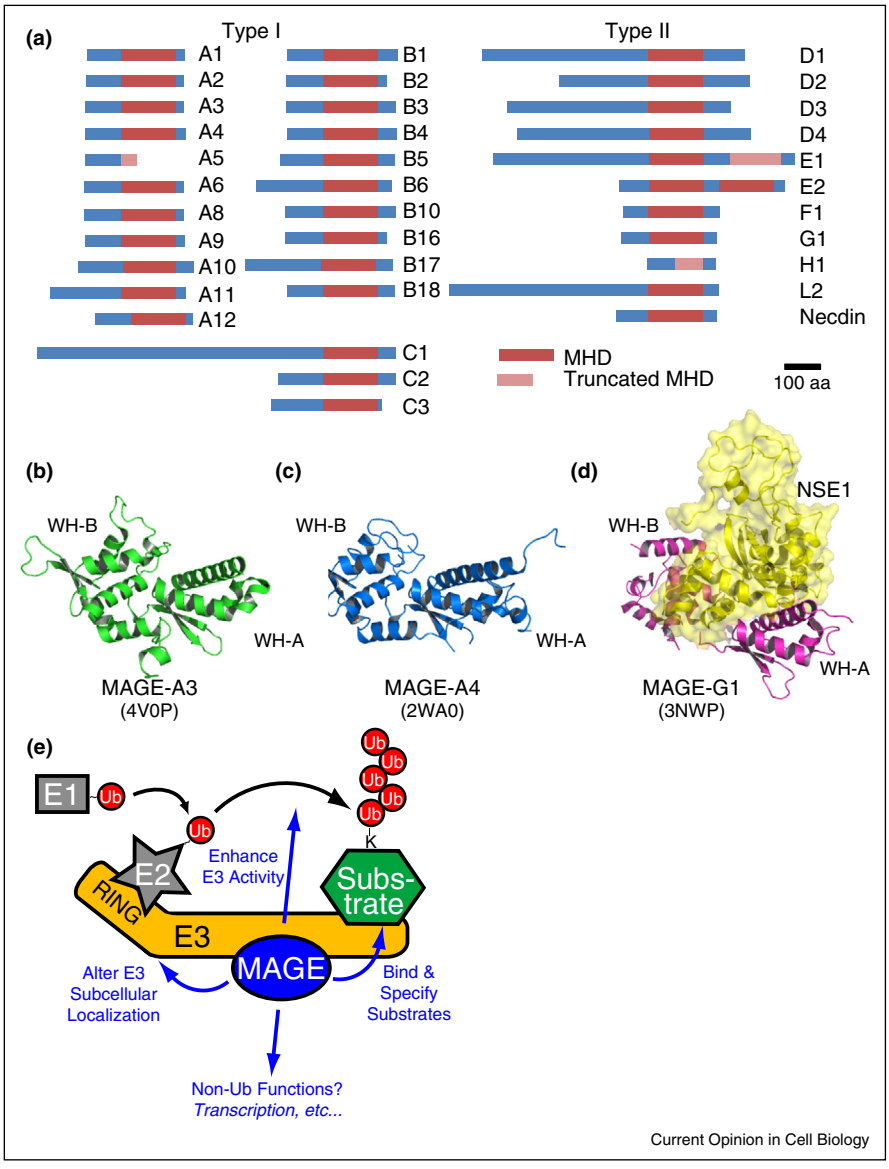
MAGEs are not only associated with poor clinical prognosis, but recent reports suggest they function as drivers of tumorigenesis. Upon expression, multiple cancer types become addicted to MAGEs for viability, such as MAGE-As or MAGE-Cs in breast [15••], lung [15••], colon [15••], mast cells [22], multiple myeloma [23], and melanoma [24]. MAGE-A3 and -C2 expression in cancer lines has been shown to increase invasive potential *in vitro* [17,25]. Furthermore, MAGE-A3 and -A6 promote the transformation of fibroblasts and increase soft agar growth of cancer cells [15••]. More impressively, MAGE-A6 promotes anchorage-independent growth of normal diploid colonic epithelial cells [15••]. In addition, MAGEs enhance tumor formation *in vivo*. Orthotopic xenografts of MAGE-A3 overexpressing human thyroid carcinoma cells exhibited increased tumor growth and metastases to the lung [25], and MAGE-C knockdown delayed tumor formation of metastatic melanoma *in vivo* [26•]. Furthermore, MAGE-B knockdown suppressed the growth of melanoma cells in a syngeneic mouse tumor model [24]. These studies suggest that MAGE CTAs may have oncogenic activity and additional rigorous studies in genetically engineered models of cancer in mice are warranted. Additionally, the contribution and activities of specific MAGEs (including many MAGE-B genes) needs further investigation.

Mechanism in cancer development and progression

Although mechanistic studies of MAGEs are limited, there is a growing body of evidence for their interactions with other proteins, especially E3 ubiquitin ligases. The MHDs of MAGE-A2, -A3, -A6, and -C2 can bind to the coiled-coil domain of the TRIM28/KAP1 ubiquitin ligase [13,24]. MAGE-C2 increases phosphorylation of TRIM28/KAP1 and improves DNA repair after double-stranded breaks, possibly by enhancing complex formation between TRIM28/KAP1 and ATM [26•]. Our lab and others have shown that MAGE binding can enhance TRIM28/KAP1's ubiquitin ligase activity against p53, resulting in its degradation in a proteasome-dependent manner [13,27]. In the presence of wild-type p53, knockdown of MAGE-A genes appears to increase p53 recruitment to target promoters [28] and increase mRNA levels of p53 transcriptional targets [27,28]. Others have also suggested that MAGE-A binds to p53's DNA-binding domain directly which may prevent its transcriptional activity [28,29]. Additionally, MAGE-As and -C2 may downregulate p53 activity through preventing its acetylation [24] at promyelocytic leukemia (PML) nuclear bodies by recruiting HDAC3 [29] and blocking p300-mediated PML acetylation [30].

However, the relevance of MAGE-As in cancer is not limited in scope to modulating p53 function. Expression of MAGE-A3 or -A6 does not correlate with p53 mutation status in multiple tumor types [15••]. Most recently, our lab has determined that MAGE-A3-TRIM28 and MAGE-A6-TRIM28 ligase complexes can ubiquitinate the alpha catalytic subunit (PRKAA1) of the tumor suppressor AMPK that functions as the master cellular energy sensor and regulator [15••,31,32]. This event leads to AMPK degradation and reduction of overall AMPK protein levels in tumors [15••]. Furthermore, downregulation of AMPK by MAGE-A3 and -A6 led to significantly decreased autophagy levels and upregulation of mTOR signaling [15••], which may provide the optimal environment for early tumor formation and growth [33–35]. Importantly, use of AMPK agonists significantly decreased MAGE-A6-mediated anchorage-independent growth *in vitro* [15••].

Figure 2



MAGE proteins structure and function. (A) List of human MAGE proteins and their known common domain, the MAGE homology domain (MHD). Note that a few MAGEs have truncated MHDs. MAGEs that are likely pseudogenes are not listed. Crystal structures of MAGE-A3 **(B)**, MAGE-A4 **(C)**, and MAGE-G1 in complex with the NSE1 E3 ubiquitin ligase **(D)** are shown. The two winged-helix motifs (WH-A and WH-B) that form the MHD are noted. Note the change in the relative orientation of WH-A and WH-B motifs in MAGE-A3/-A4 compared to MAGE-G1 bound to NSE1. **(E)** Summary of known biochemical and cellular functions of MAGEs.

Because AMPK agonists (*e.g.* metformin) and mTOR inhibitors (*e.g.* everolimus) are already in use in the clinic [36,37], an immediate applicable point of these results may be to utilize MAGE-A3 and -A6 as a biomarkers for effective use of these drugs [15••].

MAGE-A11 is unique among the type I MAGEs in that it is known to be involved in the regulation of hormonal signals in prostate cancer [9,38]. Binding of MAGE-A11 to the N-terminal FXXLF motif of the androgen receptor (AR) facilitates SRC/p160 co-activator binding [38]. Transcriptional activity of AR was also enhanced by epidermal growth factor (EGF)-mediated phosphorylation and ubiquitination of MAGE-A11 [39]. In addition to modulating hormone signaling, MAGE-A11 may play a role in mediating survival of tumors in stressful conditions (such as when tumors outgrow their blood supply) by stabilizing HIF-1alpha levels, possibly by binding to and inhibiting PHD2, a prolyl 4-hydroxylase which modulates HIF-1alpha stability [40].

Table 1

Summary of high confidence interactions between MAGEs and E3 ubiquitin ligases.

MAGE	E3 ubiquitin ligase	Target
MAGE-A1	TRIM31 [64]	Unknown
MAGE-A2	MDM2 [65]	Inhibit MDM2-mediated ubiquitination of MDM4 [65]
MAGE-A2, -A3, -A6, -C2	TRIM28/KAP1 [13,24]	Ubiquitination and degradation of p53 [13]
MAGE-A3	TRIM28/KAP1 [13,24]	Ubiquitination and degradation of KZNFs containing A + B box KRAB domains [66]
MAGE-A3, -A6	TRIM28/KAP1 [13,24]	Recruit AMPKα1 subunit for ubiquitination and degradation [15••]
MAGE-A4	TRIM69 [67]	Unknown
MAGE-B18	LNX1 [13]	Unknown
MAGE-D1	Praja1 [68]	MAGE-D1 is ubiquitinated [68,69] and modulates Dlx5 transcription factor activity [68]
MAGE-D1	Unknown	Ubiquitination of the serotonin transporter SERT [72]
MAGE-D1	XIAP [70,71]	Unknown
MAGE-G1	NSE1 [13,73]	Unknown
MAGE-L2	TRIM27 [14•]	K63-ubiquitination of WASH for proper actin assembly and endosomal protein recycling [14•]
Necdin	Unknown	Ubiquitination and degradation of PIAS1 [74]

MAGEs and cancer stem cells

In addition to their ability to function as oncogenes, MAGEs are enriched in the stem cell population of certain cancers. MAGE-A3 has much higher expression in a cancer stem cell-like side population in bladder cancer, which compared to the main population, exhibited more robust tumor growth *in vivo* [41]. Additionally, MAGE-A2, -A3, -A4, -A6, -A12, and -B2 are highly enriched in the stem cell-like side population of multiple cancer cell lines [42]. Furthermore, analysis across the maturation stages of B-cells demonstrated that MAGE-C1 is expressed with high frequency in CD34+ stem cells and early to immature B-cells (CD10+ or CD19+) [43•], suggesting that MAGE-C1 may be intimately related to the initiating cell population in this disease. Consistent with this data, MAGE-C1 correlates with decreased time to relapse after allogeneic stem cell transplant [44] and decreased overall survival [44,45]. Whether MAGEs contribute to maintenance of cancer stem cells within tumors will need to be examined.

Transcriptional regulation of MAGEs in cancer

Determining the regulatory processes controlling the aberrant re-expression of MAGEs in cancer may provide insight into potential drug targets for MAGE-expressing tumors. The use of demethylating agents such as 5-aza-2-deoxycytidine (5DC) can induce expression of MAGE-A1 in cell lines derived from malignant tumors [46,47], and this effect can be augmented through the use of trichostatin A, an HDAC inhibitor [48,49]. This suggests that type I MAGEs are not normally expressed in somatic cells due to methylation of CpG islands in their promoter regions. Mechanisms proposed for the demethylation of type I MAGE promoters include the deregulation of KIT tyrosine kinase activity [50] and the FGFR2-IIIb which was found to be a putative upstream regulator of MAGE-A3/6 expression [51]. Fibronectin knockdown also led to increased MAGE-A3 expression [25]. Fibronectin signaling through integrin receptors, FGFR2 signaling, and the c-KIT pathway all involve the PI3K/Akt [52–54] and Ras pathways [54–56], suggesting that these pathways may be the key to understanding how type I MAGEs are turned on in cancer cells.

In addition to CpG promoter demethylation, several studies have implicated additional transcriptional regulation of MAGEs in cancer. In one study, 5DC was not able to induce MAGE-A1 in several normal diploid cell lines [46], indicating that there may be more involved in the regulation of these CTAs than simply CpG demethylation. However, 5DC was able to induce MAGE-A1, -A2, -B1, and -B4 expression in normal XY karyotype human dermal fibroblasts [57]. Brother of the Regulator of Imprinted Sites (BORIS) is a CTA and transcription factor that was found to induce the expression of MAGE CTAs in normal human dermal fibroblasts and cancer lines [48,57]. In addition, the Ets-1 and Sp1 transcription factors may potentially play a role in promoting expression of MAGE CTAs, but MAGE promoter demethylation is a prerequisite [58,59]. The relevance of these factors in the regulation of MAGE expression is intriguing and should be further defined in the context of cancer progression.

MAGE-based therapy: from immunotherapies to direct targeting

The relatively restricted expression of MAGEs and their antigenicity has spurred research into utilizing them as targets for immunotherapies. In the largest therapeutic trial in lung cancer, MAGRIT (MAGE-A3 as Adjuvant Non-Small Cell Lung Cancer Immunotherapy), recombinant MAGE-A3 protein was injected in approximately 2300 patients after lung cancer tumor resection [60]. Although the MAGE-A3 vaccine was well-tolerated by

Table 2

Summary of select cancer subtypes with percent MAGE-positive patient tumors.

Cancer type	MAGE gene	Percent	References
Lung, non-small cell	MAGE-A1	27-46%	[6–8]
	MAGE-A3	38-55%	[6–8]
	MAGE-A4	19-35%	[6–8]
	MAGE-A6	26%	[6]
	MAGE-A10	14-27%	[6–8]
	MAGE-C1	19%-27%	[7,75]
Melanoma	MAGE-A1, primary tumor	16-20%	[3,4]
	MAGE-A1, metastases	48-51%	[3,4]
	MAGE-C1, primary tumor	24%	[76]
	MAGE-C1, metastases	40%	[76]
	MAGE-C2, primary tumor	33%	[76]
	MAGE-C2, metastases	40%	[76]
Breast	MAGE-A1	6%	[10]
	MAGE-A2	19%	[10]
	MAGE-A3/6	10-15%	[10,11]
	MAGE-A4	13%	[10]
	MAGE-A9	45%	[77]
	MAGE-A11	67%	[77]
	MAGE-A12	9%	[10]
	MAGE-C1	14%	[78]
	MAGE-C2	8%	[78]
Ovarian	MAGE-A1	15%-54%	[18•,79]
	MAGE-A3	36%-37%	[18•,79]
	MAGE-A4	47%	[18•]
	MAGE-A9	37%	[19•]
	MAGE-A10	52%	[18•]
	MAGE-C1	16%	[18•]
Colon	MAGE-A1	12%-30%	[2,80]
	MAGE-A2	28%	[2]
	MAGE-A3	20-27%	[2,80]
	MAGE-A4	22%	[80]
Multiple myeloma	MAGE-A1	<10-26%	[27,45]
	MAGE-A2	36%	[45]
	MAGE-A3/6	37-41%	[27,45]
	MAGE-A3/6, after relapse	77%	[27]
	MAGE-A12	21%	[45]
	MAGE-C1	77%	[27,45]
	MAGE-C2	50%	[27]

patients, the phase III clinical trial failed to demonstrate an increase in disease-free survival *versus* placebo [61••]. Additionally, there have been unexpected deaths in anti-MAGE T-cell-based therapies due to cross-reactivity to unrelated proteins and to certain MAGEs found at low levels in normal brain [62,63•]. These examples demonstrate the critical need to further pursue rigorous, detailed studies of MAGE expression across normal tissues and suggest that mechanistic studies of the MAGE proteins may offer valuable, alternative approaches to targeting a wide spectrum of cancers. For example, targeting MAGE-E3 ligase interactions, inhibiting MAGE-A11 binding to the androgen receptor in prostate cancers, and disrupting MAGE-A3/6 interaction with AMPKα1 or using AMPK agonists or mTOR inhibitors in MAGE-A positive cancers are all potential new routes to target the activity of MAGEs.

Conclusions

MAGEs are expressed in a wide-variety of cancers, but the transcriptional programs controlling their aberrant expression are unclear. MAGEs can drive tumor progression through various mechanisms, which ultimately result in more aggressive, metastatic tumors that have greater chance of recurrence. They are attractive targets of cancer therapy and more mechanistic studies of MAGE function in cancer will facilitate the development of targeted therapy across multiple types of cancers. Further study of MAGEs may facilitate determining their physiological function in the testis and may elucidate the role of a conserved gametogenic program in the context of cancer.

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