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Case Report

MAGE Proteins and the Regulation of E2F Pathway

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Submitted: 04 April 2017

Accepted: 06 April 2017

Published: 08 April 2017

Keywords
- MAGE
- Transcription factors
- E2F1

Abstract

Melanoma Antigens Genes (MAGE) constitutes a mutagenic family divided in two subfamilies, MAGE-I and MAGE-II, according to its tissue pattern expression. While MAGE-I in adult humans are only expressed in testis and tumors tissues, those belonging to MAGE-II subfamily are ubiquitously expressed. During the last decade, functional characterization of MAGE proteins points to a role in transcription regulation.

E2F1 is a member of the E2F family and is among the transcription factors reported to be modulated by MAGE proteins. In this article we will focus on reported cases of E2F1 modulation by members of MAGE-I and MAGE-II subfamilies and the resulting biological consequences observed in normal and tumor cells.

ABBREVIATIONS

MAGE: Melanoma Antigens Genes; CDKs: Cyclin/Cyclin-Dependent Kinases; AR: Androgen Receptor; E1A: Human Adenoviral Early Region Protein E1A; HDM2: Human Double Minute 2; HDAC1: Histone Deacetylase 1; RPs: Ribosomal Proteins; p75NTR: p75 Neurotrophin Receptor; p75ICD: p75NTR Intracellular domain; PML: Promyelocytic Leukemia protein

INTRODUCTION

The first three members of the MAGE family were discovered in 1991, as a result of a screening performed to identify tumor specific antigens from melanoma cells [1]. Nowadays, MAGE family includes more than 50 genes containing a Mage Homology Domain (MHD), a highly conserved domain of approximately 200 amino acids.

MAGE family has been divided in two subfamilies, MAGE-I and MAGE-II, according to its tissue pattern expression. MAGE-I are tumor specific proteins and its expression in normal adult cells is restricted to testis and placenta. These genes are clustered in three different regions of the X chromosome, forming the MAGE-A, MAGE-B and MAGE-C groups [2]. Conversely, proteins belonging to MAGE-II subfamily are widely expressed in many embryonic and adult tissues, particularly in the nervous system [3], posses a less conserved MHD and their loci are not restricted to the X chromosome. MAGE-II proteins include Mage-D1/NRAGE/Dlxin-1, Mage-D2, MageD3/Trophinin/Magphinin, Mage-E1/MageD4, MageE2, Mage-F1, Mage-G1/Necdin-like 2, Mage-H1, MageL2 and Necdin.

Due to their highly conserved protein sequence, MAGE-I functions were *a priori* considered potentially redundant. These proteins were, at the beginning, mainly studied as possible antigens for cancer vaccines or as diagnostic and prognostic markers of cancer [4-6]. Molecular and cell biology studies aimed to shed light on their cellular functions arrived some years later. Fortunately, during the last decade, several functional characterizations of MAGE proteins were performed and their interaction and regulation with several transcription factors arise as the mechanism underlying their biological functions [7,8].

E2F1 is a member of the E2F family and is among the transcription factors reported to be modulated by MAGE proteins.

E2F1 specifically binds promoters and induces the expression of genes involved in G1/S-phase transition. This pathway is regulated by pocket proteins such as retinoblastoma protein (Rb), p107 and p130, which prevent E2F1 activation by binding to and directly blocking the E2F transactivation domain, or by recruiting chromatin-modifying proteins (i.e.; histone deacetylases, methyltransferases and Polycomb group proteins) to E2F-binding sites, thereby causing direct gene repression. When growth factor signaling activate cyclin/cyclin-dependent kinases (CDKs) complexes, pocket proteins became phosphorylated and their blocking action on E2F is released. As result, E2F1 is free to transactivate a number of target genes associated to cell-cycle progression through the S phase.

Besides, E2F1 is also able to trigger apoptosis under specific situations or processes, such as the normal brain development. During vertebrate development roughly half of all neurons generated die [9,10] and this process ultimately appears to play an important and beneficial role in the appropriate matching of

pre- and postsynaptic elements [11]. This neuron death requires the transcription-dependent induction of E2F1 pro-apoptotic pathway.

In this article we will focus on reported cases of E2F1 modulation by members of MAGE-I and MAGE-II subfamilies and the resulting biological consequences observed in normal and tumor cells.

MAGE-I proteins as positive regulators of E2F1 induced proliferation

MageA11 is a co-regulator of Androgen Receptor (AR), a ligand-dependent transcriptional factor which has a critical role in sexual development as well as tumor formation and progression in prostate cancer [12]. It has been recently reported that MAGEA11 is able to interact with endogenous hypophosphorylated form of E2F1 and cause an increased in E2F1 transcriptional activity [13]. Besides, MageA11 is also able to interact with members of the pocket protein family as p107 and, to a lesser extent, with pRB. Interaction of MAGEA11 with p107 results in p107 stabilization by inhibition of its ubiquitination. It has been suggested that MAGEA11-dependent increase in E2F1 transcriptional activity is due to MAGEA11/p107 interaction, which released transcriptional active E2Fs, similarly to that observed with the viral oncogene E1A (human adenoviral early region protein E1A) which is able to transform cells by activating E2 F1 through competitive interaction with proteins from the Rb family.

Authors also observed that E2F1 strongly associates with p107 in prostate cancer cell with endogenous expression of MAGEA11, and proposed MAGEA11 expression in prostate cancer cells could reverse p107 from a transcriptional repressor to a transcriptional activator of E2F1 [13].

More recently, it was reported that tumor suppressor $p14^{ARF}$ prevented MAGEA11 interaction with the E2F1, and inhibited the MAGEA11-induced increase in E2F1 transcriptional activity [14].

MAGEA11 is targeted for proteasomal degradation by human $p14^{ARF}$, independently of HDM2 E3 ubiquitin ligase or lysine ubiquitination. Degradation of MAGEA11 promoted by the human $p14^{ARF}$ tumor suppressor contributes to low levels of MAGEA11 in nontransformed cells, and higher levels of MAGEA11 associated with low levels of $p14^{ARF}$ increases AR and E2F1 transcriptional activity and promotes the development of castration-resistant prostate cancer [14].

MageB2 is a tumor-specific protein belonging to MAGE-I subfamily. This protein is expressed in tumors of diverse origin [15] and its expression is related with enhanced cell proliferation. Recently, we have reported that MageB2 enhances the activity of E2F transcription factors and promotes tumor cell proliferation [16].

No physical interaction between MageB2 and E2F1 was detected by performing *in vitro* and cellular binding assays. However, MageB2 strongly interacts with HDAC1, a transcriptional repressor able to complex with and inhibit E2F1 activity. Indeed, we observed MageB2 expression strongly reduced the amount of HDAC1 associated with the E2F1 complex, releasing E2F from such interaction and enhancing its free and transcriptionally active fraction. On the contrary to what we reported about MageA2-induced apoptosis resistance [17,18,7], MageB2-induced proliferation is independent of p53 [16].

When uncontrolled cellular proliferation occurs, cells triggers a ribotoxic stress response through regulation of master signaling pathways such as those involving E2F, p53, and c-myc proteins. Under this condition, specific ribosomal proteins (RPs) are released from the nucleoli to the nucleoplasm as a feedback loop to limit high translational demand and, consequently, cell proliferation. In the nucleus, ribosomal proteins have been found to inhibit E2F activity [19].We have reported that under ribotoxic stress, MageB2 expression could counteract the negative regulation of RPs on E2F, therefore avoiding part of their cell cycle-repressive function. As a consequence, MageB2 is capable of enhancing cell cycle progression even under stress conditions. This could explain, at least in part, the oncogenic potential of MageB2 [16].

MAGE-II proteins as negative regulators of E2F1 proapoptotic role in brain development and cell proliferation

As stated before, E2F1 is capable of inducing neuronal apoptosis, a physiological process which plays a critical role in the normal development of the mammalian brain [11]. E2F1 pro-apoptotic activity in brain is linked to p75 neurotrophin receptor (p75NTR). In response to ligand binding, p75NTR can release its intracellular domain (p75ICD) and induce E2F1 activity in differentiating retinal neurons [20].

Mage-II proteins are expressed in many embryonic and adult tissues, particularly in the nervous system [21,22]. Some members of MAGE-II subfamily were reported to be key elements in neurogenesis, being capable of modulating the pro-apoptotic signaling triggered by p75NTR and E2F1.

One of the better characterized MAGE-II proteins involved in regulation of brain development is Necdin, which was initially isolated from mouse embryonal carcinoma cells differentiated into neurons [23]. The mouse necdin gene is predominantly expressed in postmitotic neurons [24], and when expressed ectopically, it suppresses proliferation [25] and triggers neuronal differentiation [26] in different cell lines.

Another MAGE-II protein which plays a role in neurogenesis is the necdin homologue MageG1 [27]. Both human genes encoding necdin and MAGE-G1 are located in the proximal chromosome 15q, a region subject to genomic imprinting and implicated in various human neurological and mental disorders.

More recently, it was reported the existence of Mage protein in chicken, CMage, which is the only mage gene in this specie and shares close homology with the type II MAGE protein family [28].

For these three proteins, a common mechanism was proposed to explain how they regulate E2F1-dependent apoptosis in normal brain development.

Similarly to the E2F repressor Rb; Necdin, MAGE-G1 and CMage bind to the transactivation domain of the E2F1 transcription factor, repressed E2F1-dependent transcription

and antagonized E2F1-induced apoptosis in neuroblastoma cells. In addition, these proteins also interact with intracellular domain of p75 neurotrophin receptor (p75NTR). p75NTR can sequester Necdin and MageG1 to the cell membrane, preventing it interaction with E2F1 and consequently facilitating apoptosis in neuroblastoma cells [27-29].

Other MAGE-II proteins as MageD1 and MageH1 have been shown to interact with the intracellular domain of p75NTR, but their ability to interact and modulate E2F1 activity has not been reported [30,31].

Besides its role in apoptosis, ectopic expression of necdin strongly suppresses the proliferation of tumor-derived cell lines [25,28,32]. Necdin, also interacts with E2F1 on the Cdk1 promoter to repress the transcriptional activation of the Cdk1 gene [33]. Thus, necdin is likely to downregulate the expression of E2F dependent cell cycle-related genes in proliferative cells and exerts its anti-mitotic activity during neurogenesis [34].

DISCUSSION AND CONCLUSION

Functional characterization of MAGE proteins points to a role in the regulation of transcription through the regulation of specific transcription factors, as Skip by MageA1 [35], androgen receptor by MageA11 [36-39] and p53 and PML by MageA2 [18,19,40].

The regulation of E2F1 activity by MAGE proteins was first reported for different members of MAGE-II subfamily. First, Necdin was reported to regulate cell proliferation and apoptosis through direct binding and repression of E2F1 transcriptional activity [29]. Later, it was reported that MageG1 [27] and the only MAGE protein in chicken, CMage [28] also inhibited the E2F1 transcription factor through direct association, especially in post mitotic neurons, and resulting in E2F1 dependent apoptosis inhibition.

More recently, it was reported the regulation of E2F1 by specific members of MAGE-I subfamily. For their tissue pattern expression, MAGE-I regulation of E2F activity represents a highly relevant issue in cancer research. In this respect, it has been reported that MageA11 interacts with the pocket protein p107 and enhances E2F1 activity [13] as part of its pro oncogenic activity in prostate cancer. Besides, MageB2 interacts with transcriptional repressor HDAC1, weakening its recruitment to the E2F1 protein complex and, therefore, increasing the fraction of free and active E2F [16].

Concerning E2F1 activity, MAGE-I tumor specific proteins seem to play an opposite role of that reported for MAGE-II proteins. Meanwhile MAGE-I proteins enhance E2F1 pro-oncogenic activity, MAGE-II proteins act as E2F1 inhibitors, negatively regulating cell proliferation and contributing to normal brain development.

ACKNOWLEDGEMENTS

This work was supported by PICT2012 (2012-0866), PIP 2013 (112-201201-00411 CO) and UBACyT 2013 (20020120100013BA) Grants to MM.

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Cite this article

Fatima LM, Martin M (2017) MAGE Proteins and the Regulation of E2F Pathway. JSM Clin Oncol Res 5(1): 1052.