PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

World J Gastroenterol 2004;10(13):1849-1853
World Journal of Gastroenterology
 ISSN 1007-9327

• REVIEW •

Biological functions of melanoma-associated antigens

Jiang Xiao, Hong-Song Chen

Jiang Xiao, Hong-Song Chen, Hepatology Institute, People's Hospital, Peking University, Beijing 100044, China
Supported by National Natural Science Foundation, No. 30170047, the State "863" Program, No. 2001AA217151 and No. 2002AA217071 and "211" project of Peking University
Correspondence to: Dr. Hong-Song Chen, Hepatology Institute, People's Hospital, Peking University, Beijing 100044, China. chen2999@sohu.com
Telephone: +86-10-68314422 Ext. 5726 **Fax**: +86-10-68321900
Received: 2003-12-17 **Accepted:** 2004-01-08

Abstract

To date, dozens of melanoma-associated antigens (MAGEs) have been identified and classified into 2 subgroups, I and II. Subgroup I consists of antigens which expression is generally restricted to tumor or germ cells, also named as cancer/testis (CT) antigen. Proteins and peptides derived from some of these antigens have been utilized in promising clinical trials of immunotherapies for gastrointestinal carcinoma, esophageal carcinoma, pulmonary carcinoma and so on. Various MAGE family members play important physiological and pathological roles during embryogenesis, germ cell genesis, apoptosis, etc. However, little is known regarding the role of MAGE family members in cell activities. It is reasonable to speculate that the genes for subgroup I MAGEs, which play important roles during embryogenesis, could be later deactivated by a genetic mechanism such as methylation. In the case of tumor formation, these genes are reactivated and the resultant proteins may be recognized and attacked by the immune system. Thus, the subgroup I MAGEs may play important roles in the immune surveillance of certain tumor types. Here, we review the classifications of MAGE family genes and what is known of their biological functions.

Xiao J, Chen HS. Biological functions of melanoma-associated antigens. *World J Gastroenterol* 2004; 10(13): 1849-1853
http://www.wjgnet.com/1007-9327/10/1849.asp

INTRODUCTION

In 1991, researchers first isolated a melanoma-associated antigen (MAGE) gene, MAGE-A1[1]. This antigen, isolated from an MZ-2 human melanoma cell line, could be recognized by cytotoxic T lymphocytes (CTLs). In the following years, dozens of new MAGE gene were identified. MAGE proteins act as anti-tumoral immune targets, making these antigens a popular focus of immunotherapy researches on gastrointestinal carcinoma and other cancers[2]. Although much work has investigated the expression of MAGE genes and HLA-restricted epitopes, relatively little is known about the functions of the MAGE proteins. In this paper, we review the classifications of MAGE family genes and what is known of their biological functions.

CLASSIFICATIONS OF THE HUMAN MAGE PROTEIN FAMILY

In 1991, Terry Boon's laboratory established a method for identifying tumor antigens based on tumor-specific CTLs recognition[1]. Using this method, the group identified a new tumor antigen which they called MAGE-1. Subsequently, this and other screening methods were used to identify a large number of tumor-specific and tumor-related antigens, including dozens of MAGEs (Table 1)[3-7]. The MAGE family proteins share certain homologous regions, including the MAGE homology domain (MHD). Sequence comparison and analysis revealed 3 subgroups of acidic MAGEs, termed A, B, and C, and one basic subgroup, MAGE-D, which includes Necdin, Restin and others[8]. Based on expression patterns, the MAGEs were further classified as belonging to either subgroup I or II. Members of subgroup I, including MAGE-A, -B, and -C, are expressed in malignant tumors and testis, but not in other normal tissues. These members are also named as cancer/testis (CT) antigen and tumor-specific antigen. In contrast, subgroup II MAGEs are expressed in various normal adult human tissues[4,9]. Interestingly, testis germ cells do not express MHC I/II molecules and cannot present the MAGE proteins, so testis tissue is generally immune-exempt. Based on this, tumor-expressed MAGE-A, -B, and -C proteins have become important targets for cancer immunotherapy, and some clinical trials are ongoing for treating gastrointestinal carcinoma, esophageal carcinoma, pulmonary carcinoma and so on. Now researchers are continuing to identify additional MAGE genes in the hope of identifying better therapeutic targets.

CELLULAR FUNCTIONS OF MAGE PROTEINS

Regulation of subgroup I MAGE gene expression in normal somatic cells

In normal mature somatic cells, subgroup I MAGE genes are static. When cells become neoplastic, MAGE genes are activated and the corresponding proteins are expressed. Interestingly, some non-MAGE-A1-expressing tumor cells have been found to contain transcription factors capable of activating the MAGE-A1 promoter[10]. This suggests that the presence of the relevant transcription factor is not sufficient to trigger expression, indicating that alternative regulatory mechanisms may exist in terms of MAGE-1 gene activation.

Recent studies have shown that MAGE gene activation may be related to promoter demethylation[11]. The promoter of the MAGE-A1 gene contains several cis-regulatory sequences located from nt -792 to +47. Among them, the B and B' domains are critical for MAGE-1 gene expression. Both domains contain Ets transcription factor binding sites, including a critical CpG bi-nucleotide site. When this CpG is methylated, the Ets transcription factor cannot bind to the B and B' domains, and expression of MAGE-1 is inhibited. Therefore, promoter methylation inhibits the expression of MAGE-1[10]. Indeed, when non-MAGE-A1-expressing tumor cells were treated with the demethylating reagent, 5-aza-2-deoxycytidine (5DC), MAGE-A1 expression was induced[11]. When normally non-MAGE-expressing cells (i.e. fibroblasts) were treated with 5DC, some cells expressed MAGE genes[11], while others did not[12]. In explanation of this, the authors proposed that most normal cells possess strong methylating actions, and that demethylating reagents such as 5DC are insufficient to demethylate MAGE promoters to a degree that would allow for MAGE gene expression. Taken together, these studies suggest that both normal and tumor cells contain the transcription factors that activate subgroup I MAGEs, and that expression of these genes is regulated by promoter demethylation.

Table 1 The MAGE family genes

Subtype	Gene name	Access number	Expression status	Length of amino acids
MAGE-A	MAGE-A1	JC2358[27-29]	T	280
	MAGE-A2	AAH13098[30]	T	314
	MAGE-A3	AAH00340[30]	T	314
	MAGE-A4	BAA06841[31]	T	317
	MAGE-A5	NP_064402[32]	T	320
	MAGE-A6	AAA68875[5]	T	314
	MAGE-A7	NP_064677[33]	P	80
	MAGE-A8	AAH02455[30]	T	318
	MAGE-A9	P43362[5]	T	315
	MAGE-A10	AAA68869[5]	T	369
	MAGE-A11	P43364[5]	T	319
	MAGE-A12	AAF44789[34]	T	314
	MAGE-A13	U71148	P	–
	MAGE-A14	NT_011534.1	P	–
	MAGE-A15	NT_025309.1	P	–
MAGE-B	MAGE-B1	AAC23616[35]	T	347
	MAGE-B2	AAC23617[35]	T	319
	MAGE-B3	AAC23618[35]	T	346
	MAGE-B4	AAC23619[35]	T	346
	MAGE-B5	Q9BZ81[36]	T	111
	MAGE-B6	Q8N7X4[37]	T	407
	MAGE-B7	AC005297.1	P	–
	MAGE-B8	AC005297.1	P	–
	MAGE-B9	AC005297.1	P	–
	MAGE-B10	AC011693.5	T	348
	MAGE-B11	AC011693.5	P	–
	MAGE-B12	AC011693.5	P	–
	MAGE-B13	AC011693.5	P	–
	MAGE-B14	NT_025279.3	P	–
	MAGE-B15	NT_011752.1	P	–
	MAGE-B16	NT_025279.3	T	320
	MAGE-B17	NT_011766.3	T	203
MAGE-C	MAGE-C1	NP_005453[38-40]	T	1 142
	MAGE-C2	NP_057333[41-42]	T	373
	MAGE-C3	AAK00358[36]	T	346
	MAGE-C4	AL023279.1	T	115
	MAGE-C5	NT_025337.2	P	–
	MAGE-C6	NT_025337.2	P	–
	MAGE-C7	NT_025337.2	P	–
MAGE-D	MAGE-D1	AAG09704[25,43]	N	778
	MAGE-D2	CAB10841[44-46]	N	606
	MAGE-D3	Q12816[47,48]	N	1 387
	MAGE-D4	Q96JG8[49-52]	N	741
MAGE-E	MAGE-E1	Q96JG8[49-52]	N	741
	MAGE-E2	–	N	–
	MAGE-E3	NP_032879[53-55]	N	424
MAGE-F	MAGE-F1	NP_071432[56]	N	307
MAGE-G	MAGE-G1	AAG38607[57]	N	100
MAGE-H	MAGE-H1	AAG38608[58]	N	219
MAGE-I	MAGE-I1	NT_011638.3	P	–
	MAGE-I2	NT_011638.3	P	–
MAGE-J	MAGE-J1	NT_011577	P	–
MAGE-K	MAGE-K1	Z81311	P	–
MAGE-L2	MAGE-L2	NP_061939[59,60]	N	529
Necdin	Necdin	XM_007686[61-65]	N	321
Restin	Restin (Apr-1)	NP_054780[66-70]	N	219

T: Only expressed in tumor cells or germ cells. P: Pseudogene; N: Expressed in normal cells. -: Unknown.

The functions of MAGE genes in germ cells

Most MAGE genes are expressed in germ cells under physiological conditions, but their functions remain unclear. Early work has focused on the functions of the mouse MAGE-b4 gene during embryonic development[13]. The mouse MAGE-b4 gene is expressed in adult and fetal reproductive gland cells and shares high homology with members of the human MAGE-B subtype. The MAGE-b4 protein is located in the cytoplasm but not in the nucleus. In male testes, germ cells (gonocytes) continuously proliferate until they arrest at the G0/G1 stage, differentiate into foot cells and form the spermatic cords. After birth, the gonocytes differentiate into spermatogonia, which in turn undergo meiosis during adolescence and differentiate into sperm cells. The MAGE-b4 gene is highly expressed in germ cells suspended in G0/G1; in contrast, the gene is barely expressed in meiotic spermatogonia. Thus, MAGE-b4 likely plays an important role in male germ cells, perhaps by maintaining gonocytes in a non-proliferative state.

In female germ cells, MAGE-b4 is expressed prior to meiosis, and also during the pachytene and telophase portions of meiosis. Other cell cycle proteins have shown similar differences in expression in the two sexes. For instance, cyclin A1 deficiency in male germ cells inhibits meiosis but does not affect that in female germ cells[14]. Similarly, deficiencies of Hsp70-2 or A-myb suppress the development of male germ cells and male fertility but do not affect female development[15]. Therefore, we speculate that MAGE-b4 may similarly play an important role in the control of the cycle of male germ cells, while playing a less dramatic role in female germ cells.

Another member of the MAGE family Magphinin plays an important role in germ cell development. Mouse Trophinin is a membrane protein that plays an adhesive role in the process of zygotic implantation into the uterine endometrium; an alternative transcript of the Trophinin gene encodes Magphinin[16]. Although the Trophinin protein is not homologous with the MAGE family members, the mouse Magphinin protein shares high homology with Necdin, Dlxin and NRAGE. Of these, Necdin can bind transcription factor E_2F-1, which is responsible for inducing the expression of cyclin and promoting cell cycle progression from G1 to S. Thus, it is possible that the Necdin homolog, Magphinin, acts similarly to bind E_2F-1 and inhibit cell proliferation. Magphinin has three alternatively spliced forms: magphinin-a, β and γ. Northern blot analysis reveals that Magphinin protein is expressed in mouse brain, ovary, testis, and epididymis. Western blot analysis indicates that in mouse ovary and epididymis, Magphinin is derived from alternative translation of the Trophinin transcript beginning at the second start codon (AUG). The Magphinin protein sequence contains a nuclear localization signal that allows it to enter the nucleus and inhibit cell proliferation. Immunohistochemical studies suggest that the localization of Magphinin protein varies between male and female germ cells at various stages of the cell cycle. Before meiosis, Magphinin-β is mainly distributed in the cytoplasm of male germ cells. After the first meiosis, when primary spermatocytes differentiate into secondary spermatocytes, Magphinin is localized in both the cytoplasm and the nucleus, and after this nuclear translocation, cell division terminates. Based on this, we speculate that Magphinin regulates the cell cycle during the formation of male spermatocytes. In the female germ cell, the distribution of Magphinin is somewhat different. When the oocyte has only single- or double-layer vesicles, intracellular Magphinin (especially Magphinin-γ) is strictly cytoplasmic. When the oocyte has divided into multi-layer vesicles, the protein is only located in nucleus. In this case, meiosis terminates at stage G2, suggesting that Magphinin also controls the formation of the female ovum.

May these expression patterns and functional roles be generalized to other members of the MAGE family? This remains to be shown. Indeed, at present, little is known about the functions of MAGE family proteins inside germ cells. Further studies will show whether human MAGE proteins control the cell cycle in manners similar to the actions of MAGE-b4 and Magphinin.

Below, we will discuss several specific examples of MAGEs and what is known of their cellular functions.

MAGE-A4

In terms of cancer biology, yeast two-hybrid studies identified binding between the MAGE-A4 protein and a cancer protein: the gann ankyrin repeat protein (also called Gankyrin, PSMD10 or p28). This MAGE- A4-specific binding is mediated by its C-terminus[17]. Gankyrin, which consists of six gann ankyrin repeats and a 38 amino acid N-terminus, can bind the cancer-suppressing protein Rb, the S6 ATP enzyme subunit of the 26S protein, and cell cycle-dependent kinase 4 (Cdk4). Gankyrin expression is increased in the livers of hepatocellular carcinoma patients; this increase is seen at the earliest stages of tumor genesis[18,19]. Overexpression of Gankyrin can increase the phosphorylation and degradation of Rb, as well as immortalization of NIH/3T3 cells. In addition, the binding of Gankyrin to Cdk4 counteracts the cancer-inhibiting functions of $p16^{INK4A}$ and $p18^{INK4C}$[20]. Therefore, Gankyrin plays an important role in controlling cell cycle during liver cancer tumorigenesis. Studies have shown that exogenous MAGE-A4 can partly inhibit the adhesion-independent growth of Gankyrin-overexpressing cells *in vitro* and suppress the formation of migrated tumors from these cells in nude mice. This inhibition is dependent upon binding between MAGE-A4 and Gankyrin, suggesting that interactions between Gankyrin and MAGE-A4 inhibit Gankyrin-mediated carcinogenesis[17].

Necdin

Necdin, first identified in 1991, is the best-characterized member of the MAGE protein family. The gene was isolated from P19 neural cells; the encoded protein consists of 325 amino acids and shares about 30% homology with other MAGE proteins[21]. Studies have shown that overexpression of Necdin may induce cell cycle arrest in NIH3T3 and SAOS2 cell lines, suggesting that Necdin functions in cell cycle arrest and maintenance of cell stability. *In vivo*, Necdin can interact with cell cycle promoting proteins such as the SV40 big-T protein, adenovirus EIA and transcription factor E_2F-1, which acts as a cell cycle regulator by trans-activating the relevant genes in an Rb-regulated pathway. The latter binds to E_2F-1 protein during G1 stage and inhibits the binding ability of E_2F-1, leading to inhibition of gene activation. Similarly, Necdin can bind to E_2F-1 and initiate conformational changes to decrease E_2F-1 binding and inhibit cell growth[22]. In addition, Necdin can bind to and inhibit p53, which normally induces cell cycle arrest and cell death[23]. Thus, Necdin plays important roles in inhibiting cell growth and apoptosis through its interactions with E_2F-1 and p53.

The human Necdin gene is localized at $15q^{11-13}$, an area genetically associated with the neurological behavior disorder, Prader-willi Syndrome (PWS). Newborn PWS patients generally suffer respiratory failure and myasthenia, and adolescent PWS patients show psychonosema, sexual dysfunction and obesity. The PWS chromosomal region contains several genes, including Necdin and MAGE-L2. Indeed, knockouts of the mouse Necdin homolog show symptoms similar to human PWS[24], suggesting that Necdin plays a role in proper development, and that lack of Necdin may be involved in the pathogenesis of PWS.

MAGE-D1

The MAGE-D1, also named as NRAGE or Dlxin, plays important roles in mediating apoptosis and transcription. NRAGE interacts with $p75^{NTR}$, a TNF receptor responsible for binding the Trk receptor and forming a complex that facilitates binding of neurotrophin, which in turn activates the Trk receptor. $p75^{NTR}$ is also capable of mediating cell death. NRAGE- $p75^{NTR}$ binding, identified by yeast two-hybrid screening, occurs through an 80 amino acid intracellular segment of $p75^{NTR}$ located near the plasma membrane. Overexpression of NRAGE inhibited the interaction between $p75^{NTR}$ and the Trk receptor and induced cell apoptosis. In contrast, overexpression of the Trk receptor increased binding between Trk and $p75^{NTR}$, leading to inhibition of NRAGE-$p75^{NTR}$ complex-induced cell death. This indicates that NRAGE-$p75^{NTR}$ and Trk receptor-$p75^{NTR}$ binding are mutually exclusive.

Another NRAGE-binding protein, XIAP, can inhibit apoptosis by binding to activated Caspases. The NRAGE-XIAP complex accelerates the decomposition of XIAP, suggesting that NRAGE accelerates cell death by degrading XIAP and activating Caspases[4].

Indeed, members of the MAGE-D1 subgroup can bind to several proteins that control apoptosis and cell cycle[4]. The MAGE-D1 repeat regions can directly interact with Msx2, Dlx7 and Dlx5. Msx generally acts as a transcription inhibitor, whereas Dlx acts as a transcriptional activator. In addition, Msx1 and Msx2 play roles in the control of cell cycle, and Msx2 can promote cell death. The effect of MAGE-D1 on Msx remains unknown, but MAGE-D1 is necessary for Dlx5 to promote transcription.

CONCLUSION

Although subgroup I and II MAGE genes are expressed in different tissue-specific patterns, all family members contain the MAGE homology domain (MHD), suggesting some functional conservation. Biochemical analyses of these two subgroups have provided some insights into the physiological effects of the MAGE genes. Embryonic cells have much less CpG methylation at the MAGE genes than do somatic cells[25,26]. Similarly, MAGE genes in tumor cells are hypomethylated; indeed, the entire tumor genome is generally hypomethylated[10]. Thus, it is likely that MAGE gene expression in tumor tissues is the result of tumor genesis, not a cause. It is reasonable to speculate that this family of proteins functions during embryonic development, and that the genes are subsequently deactivated, perhaps by methylation. During neoplastic transformation, these genes are re-activated, expressed, and may become antigenic targets that are recognized and attacked by the immune system[8]. Therefore, MAGE genes take part in the immune process by targeting some early tumor cells for immune destruction. Consequently, these genes should be studied further in terms of their various functions as they relate to the pathogenic mechanism of tumors, immunotherapy, and other important fields.

REFERENCES

1 **van der Bruggen P**, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, Knuth A, Boon T. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science* 1991; **254**: 1643-1647

2 **Sadanaga N**, Nagashima H, Mashino K, Tahara K, Yamaguchi H, Ohta M, Fujie T, Tanaka F, Inoue H, Takesako K, Akiyoshi T, Mori M. Dendritic cell vaccination with MAGE peptide is a novel therapeutic approach for gastrointestinal carcinomas. *Clin Cancer Res* 2001; **7**: 2277-2284

3 **Chen YT**, Scanlan MJ, Sahin U, Tureci O, Gure AO, Tsang S, Williamson B, Stockert E, Pfreundschuh M, Old LJ. A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. *Proc Natl Acad Sci U S A* 1997; **94**: 1914-1918

4 **Barker PA**, Salehi A. The MAGE proteins: emerging roles in cell cycle progression, apoptosis, and neurogenetic disease. *J Neurosci Res* 2002; **67**: 705-712

5 **Chomez P**, De Backer O, Bertrand M, De Plaen E, Boon T, Lucas S. An overview of the MAGE gene family with the identification of all human members of the family. *Cancer Res* 2001; **61**: 5544-5551

6 **De Plaen E**, Arden K, Traversari C, Gaforio JJ, Szikora JP, De Smet C, Brasseur F, van der Bruggen P, Lethe B. Structure, chromosomal localization and expression of 12 genes of the MAGE family. *Immunogenetics* 1994; **40**: 360-369

7 http://www.ncbi.nlm.nih.gov/Genebank

8 **Zhao ZL**, Lu F, Zhu F, Yang H, Chai YB, Chen SM. Cloning and biological comparison of Restin, a novel member of Mage superfamily. *Sci China* 2002; **45**: 412-420

9 **Chiba T**, Yokosuka O, Kanda T, Fukai K, Imazeki F, Saisho H, Nishimura M, Saito Y. Hepatic graft-versus-host disease resembling acute hepatitis: additional treatment with ursodeoxycholic acid. *Liver* 2002; **22**: 514-527

10 **De Smet C**, Lurquin C, Lethe 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. *Mol Cell Biol* 1999; **19**: 7327-7335

11 **De Smet C**, De Backer O, Faraoni I, Lurquin C, Brasseur F, Boon T. The activation of human gene MAGE-1 in tumor cells is correlated with genome-wide demethylation. *Proc Natl Acad Sci U S A* 1996; **93**: 7149-7153

12 **Weber J**, Salgaller M, Samid D, Johnson B, Herlyn M, Lassam N, Treisman J, Rosenberg SA. Expression of the MAGE-1 tumor antigen is up-regulated by the demethylating agent 5-aza-2'-deoxycytidine. *Cancer Res* 1994; **54**: 1766-1771

13 **Osterlund C**, Tohonen V, Forslund KO, Nordqvist K. Mage-b4, a novel melanoma antigen (MAGE) gene specifically expressed during germ cell differentiation. *Cancer Res* 2000; **60**: 1054-1061

14 **Liu D**, Matzuk MM, Sung WK, Guo Q, Wang P, Wolgemuth DJ. Cyclin A1 is required for meiosis in the male mouse. *Nat Genet* 1998; **20**: 377-380

15 **Toscani A**, Mettus RV, Coupland R, Simpkins H, Litvin J, Orth J, Hatton KS, Reddy EP. Arrest of spermatogenesis and defective breast development in mice lacking A-myb. *Nature* 1997; **386**: 713-717

16 **Saburi S**, Nadano D, Akama TO, Hirama K, Yamanouchi K, Naito K, Tojo H, Tachi C, Fukuda MN. The trophinin gene encodes a novel group of MAGE proteins, magphinins, and regulates cell proliferation during gametogenesis in the mouse. *J Biol Chem* 2001; **276**: 49378-49389

17 **Meier S**, Haussinger D, Jensen P, Rogowski M, Grzesiek S. High-accuracy residual 1HN-13C and 1HN-1HN dipolar couplings in perdeuterated proteins. *J Am Chem Soc* 2003; **125**: 44-45

18 **Higashitsuji H**, Itoh K, Nagao T, Dawson S, Nonoguchi K, Kido T, Mayer RJ, Arii S, Fujita J. Reduced stability of retinoblastoma protein by gankyrin, an oncogenic ankyrin-repeat protein overexpressed in hepatomas. *Nat Med* 2000; **6**: 96-99

19 **Park TJ**, Kim HS, Byun KH, Jang JJ, Lee YS, Lim IK. Sequential changes in hepatocarcinogenesis induced by diethylnitrosamine plus thioacetamide in Fischer 344 rats: induction of gankyrin expression in liver fibrosis, pRB degradation in cirrhosis, and methylation of p16(INK4A) exon 1 in hepatocellular carcinoma. *Mol Carcinog* 2001; **30**: 138-150

20 **Li J**, Tsai MD. Novel insights into the INK4-CDK4/6-Rb pathway: counter action of gankyrin against INK4 proteins regulates the CDK4-mediated phosphorylation of Rb. *Biochemistry* 2002; **41**: 3977-8339

21 **Maruyama K**, Usami M, Aizawa T, Yoshikawa K. A novel brain-specific mRNA encoding nuclear protein (necdin) expressed in neurally differentiated embryonal carcinoma cells. *Biochem Biophys Res Commun* 1991; **178**: 291-296

22 **Taniura H**, Taniguchi N, Hara M, Yoshikawa K. Necdin, a postmitotic neuron-specific growth suppressor, interacts with viral transforming proteins and cellular transcription factor E2F1. *J Biol Chem* 1998; **273**: 720-728

23 **Taniura H**, Matsumoto K, Yoshikawa K. Physical and functional interactions of neuronal growth suppressor necdin with p53. *J Biol Chem* 1999; **274**: 16242-16248

24 **Nakada Y**, Taniura H, Uetsuki T, Inazawa J, Yoshikawa K. The human chromosomal gene for necdin, a neuronal growth suppressor, in the Prader-Willi syndrome deletion region. *Gene* 1998; **213**: 65-72

25 **Salehi AH**, Roux PP, Kubu CJ, Zeindler C, Bhakar A, Tannis LL, Verdi JM, Barker PA. NRAGE, a novel MAGE protein, interacts with the p75 neurotrophin receptor and facilitates nerve growth factor-dependent apoptosis. *Neuron* 2000; **27**: 279-288

26 **Reik W**, Dean W, Walter J. Epigenetic reprogramming in mammalian development. *Science* 2001; **293**: 1089-1093

27 **Ding M**, Beck RJ, Keller CJ, Fenton RG. Cloning and analysis of MAGE-1-related genes. *Biochem Biophys Res Commun* 1994; **202**: 549-555

28 **Suyama T**, Ohashi H, Nagai H, Hatano S, Asano H, Murate T, Saito H, Kinoshita T. The MAGE-A1 gene expression is not determined solely by methylation status of the promoter region in hematological malignancies. *Leuk Res* 2002; **26**: 1113-1118

29 **Chen YT**, Stockert E, Chen Y, Garin-Chesa P, Rettig WJ, van der Bruggen P, Boon T, Old LJ. Identification of the MAGE-1 gene product by monoclonal and polyclonal antibodies. *Proc Natl Acad Sci U S A* 1994; **91**: 1004-1008

30 **Strausberg RL**, Feingold EA, Grouse LH, Derge JG, Klausner RD, Collins FS, Wagner L, Shenmen CM, Schuler GD, Altschul SF, Zeeberg B, Buetow KH, Schaefer CF, Bhat NK, Hopkins RF, Jordan H, Moore T, Max SI, Wang J, Hsieh F, Diatchenko L, Marusina K, Farmer AA, Rubin GM, Hong L, Stapleton M, Soares MB, Bonaldo MF, Casavant TL, Scheetz TE, Brownstein MJ, Usdin TB, Toshiyuki S, Carninci P, Prange C, Raha SS, Loquellano NA, Peters GJ, Abramson RD, Mullahy SJ, Bosak SA, McEwan PJ, McKernan KJ, Malek JA, Gunaratne PH, Richards S, Worley KC, Hale S, Garcia AM, Gay LJ, Hulyk SW, Villalon DK, Muzny DM, Sodergren EJ, Lu X, Gibbs RA, Fahey J, Helton E, Ketteman M, Madan A, Rodrigues S, Sanchez A, Whiting M, Madan A, Young AC, Shevchenko Y, Bouffard GG, Blakesley RW, Touchman JW, Green ED, Dickson MC, Rodriguez AC, Grimwood J, Schmutz J, Myers RM, Butterfield YS, Krzywinski MI, Skalska U, Smailus DE, Schnerch A, Schein JE, Jones SJ, Marra MA. Mammalian Gene Collection Program Team. Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. *Proc Natl Acad Sci U S A* 2002; **99**: 16899-16903

31 **Imai Y**, Shichijo S, Yamada A, Katayama T, Yano H, Itoh K. Sequence analysis of the MAGE gene family encoding human tumor-rejection antigens. *Gene* 1995; **160**: 287-290

32 **Wang PJ**, McCarrey JR, Yang F, Page DC. An abundance of X-linked genes expressed in spermatogonia. *Nat Genet* 2001; **27**: 422-426

33 **De Plaen E**, De Backer O, Arnaud D, Bonjean B, Chomez P, Martelange V, Avner P, Baldacci P, Babinet C, Hwang SY, Knowles B, Boon T. A new family of mouse genes homologous to the human MAGE genes. *Genomics* 1999; **55**: 176-184

34 **Mallon AM**, Platzer M, Bate R, Gloeckner G, Botcherby MR, Nordsiek G, Strivens MA, Kioschis P, Dangel A, Cunningham D, Straw RN, Weston P, Gilbert M, Fernando S, Goodall K, Hunter G, Greystrong JS, Clarke D, Kimberley C, Goerdes M, Blechschmidt K, Rump A, Hinzmann B, Mundy CR, Miller W, Poustka A, Herman GE, Rhodes M, Denny P, Rosenthal A, Brown SD. Comparative genome sequence analysis of the Bpa/Str region in mouse and Man. *Genome Res* 2000; **10**: 758-775

35 **Lurquin C**, De Smet C, Brasseur F, Muscatelli F, Martelange V, De Plaen E, Brasseur R, Monaco AP, Boon T. Two members of the human MAGEB gene family located in Xp21.3 are expressed in tumors of various histological origins. *Genomics* 1997; **46**: 397-408

36 **Lucas S**, De Plaen E, Boon T. MAGE-B5, MAGE-B6, MAGE-C2, and MAGE-C3: four new members of the MAGE family with tumor-specific expression. *Int J Cancer* 2000; **87**: 55-60

37 **Ota T**, Suzuki Y, Nishikawa T, Otsuki T, Sugiyama T, Irie R, Wakamatsu A, Hayashi K, Sato H, Nagai K, Kimura K, Makita H, Sekine M, Obayashi M, Nishi T, Shibahara T, Tanaka T, Ishii S, Yamamoto J, Saito K, Kawai Y, Isono Y, Nakamura Y, Nagahari K, Murakami K, Yasuda T, Iwayanagi T, Wagatsuma M, Shiratori A, Sudo H, Hosoiri T, Kaku Y, Kodaira H, Kondo H, Sugawara M, Takahashi M, Kanda K, Yokoi T, Furuya T, Kikkawa E, Omura Y, Abe K, Kamihara K, Katsuta N, Sato K, Tanikawa M, Yamazaki M, Ninomiya K, Ishibashi T, Yamashita H, Murakawa K, Fujimori K, Tanai H, Kimata M, Watanabe M, Hiraoka S, Chiba Y, Ishida S, Ono Y, Takiguchi S, Watanabe S, Yosida M, Hotuta T, Kusano J, Kanehori K, Takahashi-Fujii A, Hara H, Tanase TO, Nomura Y, Togiya S, Komai F, Hara R, Takeuchi K, Arita M, Imose N, Musashino K, Yuuki H, Oshima A, Sasaki N, Aotsuka S, Yoshikawa Y, Matsunawa H, Ichihara T, Shiohata N, Sano S, Moriya S, Momiyama H, Satoh N, Takami S, Terashima Y, Suzuki O, Nakagawa S, Senoh A, Mizoguchi H, Goto Y, Shimizu F, Wakebe H, Hishigaki H, Watanabe T, Sugiyama A, Takemoto M, Kawakami B, Yamazaki M, Watanabe K, Kumagai A, Itakura S, Fukuzumi Y, Fujimori Y, Komiyama M, Tashiro H, Tanigami A, Fujiwara T, Ono T, Yamada K, Fujii Y, Ozaki K, Hirao M, Ohmori Y, Kawabata A, Hikiji T, Kobatake N, Inagaki H, Ikema Y, Okamoto S, Okitani R, Kawakami T, Noguchi S, Itoh T, Shigeta

K, Senba T, Matsumura K, Nakajima Y, Mizuno T, Morinaga M, Sasaki M, Togashi T, Oyama M, Hata H, Watanabe M, Komatsu T, Mizushima-Sugano J, Satoh T, Shirai Y, Takahashi Y, Nakagawa K, Okumura K, Nagase T, Nomura N, Kikuchi H, Masuho Y, Yamashita R, Nakai K, Yada T, Nakamura Y, Ohara O, Isogai T, Sugano S. Complete sequencing and characterization of 21,243 full-length human cDNAs. *Nat Genet* 2004; **36**: 40-45

38 **Jungbluth AA**, Chen YT, Busam KJ, Coplan K, Kolb D, Iversen K, Williamson B, Van Landeghem FK, Stockert E, Old LJ. CT7 (MAGE-C1) antigen expression in normal and neoplastic tissues. *Int J Cancer* 2002; **99**: 839-845

39 **Lim SH**, Bumm K, Chiriva-Intemati M, Xue Y, Wang Z. MAGE-C1 (CT7) gene expression in multiple myeloma: relationship to sperm protein 17. *Eur J Haematol* 2001; **67**: 332-334

40 **Chen YT**, Gure AO, Tsang S, Stockert E, Jager E, Knuth A, Old LJ. Identification of multiple cancer / testis antigens by allogeneic antibody screening of a melanoma cell line library. *Proc Natl Acad Sci U S A* 1998; **95**: 6919-6923

41 **Lucas S**, De Smet C, Arden KC, Viars CS, Lethe B, Lurquin C, Boon T. Identification of a new MAGE gene with tumor-specific expression by representational difference analysis. *Cancer Res* 1998; **58**: 743-752

42 **Gure AO**, Stockert E, Arden KC, Boyer AD, Viars CS, Scanlan MJ, Old LJ, Chen YT. CT10: a new cancer-testis (CT) antigen homologous to CT7 and the MAGE family, identified by representational-difference analysis. *Int J Cancer* 2000; **85**: 726-732

43 **Hennuy B**, Reiter E, Cornet A, Bruyninx M, Daukandt M, Houssa P, N'Guyen VH, Closset J, Hennen G. A novel messenger ribonucleic acid homologous to human MAGE-D is strongly expressed in rat Sertoli cells and weakly in Leydig cells and is regulated by follitropin, lutropin, and prolactin. *Endocrinology* 2000; **141**: 3821-3831

44 **Kurt RA**, Urba WJ, Schoof DD. Isolation of genes overexpressed in freshly isolated breast cancer specimens. *Breast Cancer Res Treat* 2000; **59**: 41-48

45 **Langnaese K**, Kloos DU, Wehnert M, Seidel B, Wieacker P. Expression pattern and further characterization of human MAGED2 and identification of rodent orthologues. *Cytogenet Cell Genet* 2001; **94**: 233-240

46 **Lucas S**, Brasseur F, Boon T. A new MAGE gene with ubiquitous expression does not code for known MAGE antigens recognized by T cells. *Cancer Res* 1999; **59**: 4100-4103

47 **Kikuno R**, Nagase T, Ishikawa K, Hirosawa M, Miyajima N, Tanaka A, Kotani H, Nomura N, Ohara O. Prediction of the coding sequences of unidentified human genes. XIV. The complete sequences of 100 new cDNA clones from brain which code for large proteins *in vitro*. *DNA Res* 1999; **6**: 197-205

48 **Fukuda MN**, Sato T, Nakayama J, Klier G, Mikami M, Aoki D, Nozawa S. Trophinin and tastin, a novel cell adhesion molecule complex with potential involvement in embryo implantation. *Genes Dev* 1995; **9**: 1199-1210

49 **Sasaki M**, Nakahira K, Kawano Y, Katakura H, Yoshimine T, Shimizu K, Kim SU, Ikenaka K. MAGE-E1, a new member of the melanoma-associated antigen gene family and its expression in human glioma. *Cancer Res* 2001; **61**: 4809-4814

50 **Kawano Y**, Sasaki M, Nakahira K, Yoshimine T, Shimizu K, Wada H, Ikenaka K. Structural characterization and chromosomal localization of the MAGE-E1 gene. *Gene* 2001; **277**: 129-137

51 **Wiemann S**, Weil B, Wellenreuther R, Gassenhuber J, Glassl S, Ansorge W, Bocher M, Blocker H, Bauersachs S, Blum H, Lauber J, Dusterhoft A, Beyer A, Kohrer K, Strack N, Mewes HW, Ottenwalder B, Obermaier B, Tampe J, Heubner D, Wambutt R, Korn B, Klein M, Poustka A. Toward a catalog of human genes and proteins: sequencing and analysis of 500 novel complete protein coding human cDNAs. *Genome Res* 2001; **11**: 422-435

52 **Nagase T**, Nakayama M, Nakajima D, Kikuno R, Ohara O. Prediction of the coding sequences of unidentified human genes. XX. The complete sequences of 100 new cDNA clones from brain which code for large proteins *in vitro*. *DNA Res* 2001; **8**: 85-95

53 **Sasaki A**, Masuda Y, Iwai K, Ikeda K, Watanabe K. A RING finger protein Praja1 regulates Dlx5-dependent transcription through its ubiquitin ligase activity for the Dlx / Msx-interacting MAGE / Necdin family protein, Dlxin-1. *J Biol Chem* 2002; **277**: 22541-22546

54 **Stork O**, Stork S, Pape HC, Obata K. Identification of genes expressed in the amygdala during the formation of fear memory. *Learn Mem* 2001; **8**: 209-219

55 **Mishra L**, Tully RE, Monga SP, Yu P, Cai T, Makalowski W, Mezey E, Pavan WJ, Mishra B. Praja1, a novel gene encoding a RING-H2 motif in mouse development. *Oncogene* 1997; **15**: 2361-2368

56 **Stone B**, Schummer M, Paley PJ, Crawford M, Ford M, Urban N, Nelson BH. MAGE-F1, a novel ubiquitously expressed member of the MAGE superfamily. *Gene* 2001; **267**: 173-182

57 **Kuwako K**, Taniura H, Yoshikawa K. Necdin-related MAGE proteins differentially interact with the E2F1 transcription factor and the p75 neurotrophin receptor. *J Biol Chem* 2004; **279**: 1703-1712

58 **Tcherpakov M**, Bronfman FC, Conticello SG, Vaskovsky A, Levy Z, Niinobe M, Yoshikawa K, Arenas E, Fainzilber M. The p75 neurotrophin receptor interacts with multiple MAGE proteins. *J Biol Chem* 2002; **277**: 49101-49104

59 **Lee S**, Kozlov S, Hernandez L, Chamberlain SJ, Brannan CI, Stewart CL, Wevrick R. Expression and imprinting of MAGEL2 suggest a role in Prader-willi syndrome and the homologous murine imprinting phenotype. *Hum Mol Genet* 2000; **9**: 1813-1819

60 **Boccaccio I**, Glatt-Deeley H, Watrin F, Roeckel N, Lalande M, Muscatelli F. The human MAGEL2 gene and its mouse homologue are paternally expressed and mapped to the Prader-Willi region. *Hum Mol Genet* 1999; **8**: 2497-2505

61 **Andrieu D**, Watrin F, Niinobe M, Yoshikawa K, Muscatelli F, Fernandez PA. Expression of the Prader-Willi gene Necdin during mouse nervous system development correlates with neuronal differentiation and p75NTR expression. *Gene Expr Patterns* 2003; **3**: 761-765

62 **Hu B**, Wang S, Zhang Y, Feghali CA, Dingman JR, Wright TM. A nuclear target for interleukin-1alpha: interaction with the growth suppressor necdin modulates proliferation and collagen expression. *Proc Natl Acad Sci U S A* 2003; **100**: 10008-10013

63 **Matsuda T**, Suzuki H, Oishi I, Kani S, Kuroda Y, Komori T, Sasaki A, Watanabe K, Minami Y. The receptor tyrosine kinase Ror2 associates with the melanoma-associated antigen (MAGE) family protein Dlxin-1 and regulates its intracellular distribution. *J Biol Chem* 2003; **278**: 29057-29064

64 **Ren J**, Lee S, Pagliardini S, Gerard M, Stewart CL, Greer JJ, Wevrick R. Absence of Ndn, encoding the Prader-Willi syndrome-deleted gene necdin, results in congenital deficiency of central respiratory drive in neonatal mice. *J Neurosci* 2003; **23**: 1569-1573

65 **Takazaki R**, Nishimura I, Yoshikawa K. Necdin is required for terminal differentiation and survival of primary dorsal root ganglion neurons. *Exp Cell Res* 2002; **277**: 220-232

66 **Zhu F**, Yan W, Zhao ZL, Chai YB, Lu F, Wang Q, Peng WD, Yang AG, Wang CJ. Improved PCR-based subtractive hybridization strategy for cloning differentially expressed genes. *Biotechniques* 2000; **29**: 310-313

67 **Sahin U**, Neumann F, Tureci O, Schmits R, Perez F, Pfreundschuh M. Hodgkin and Reed-Sternberg cell-associated autoantigen CLIP-170 / restin is a marker for dendritic cells and is involved in the trafficking of macropinosomes to the cytoskeleton, supporting a function-based concept of Hodgkin and Reed-Sternberg cells. *Blood* 2002; **100**: 4139-4145

68 **Ramchandran R**, Dhanabal M, Volk R, Waterman MJ, Segal M, Lu H, Knebelmann B, Sukhatme VP. Antiangiogenic activity of restin, NC10 domain of human collagen XV: comparison to endostatin. *Biochem Biophys Res Commun* 1999; **255**: 735-739

69 **Griparic L**, Keller TC. Identification and expression of two novel CLIP-170 / Restin isoforms expressed predominantly in muscle. *Biochim Biophys Acta* 1998; **1405**: 35-46

70 **Delabie J**, Bilbe G, Bruggen J, Van Leuven F, De Wolf-Peeters C. Restin in Hodgkin's disease and anaplastic large cell lymphoma. *Leuk Lymphoma* 1993; **12**: 21-26

Edited by Zhu LH **Proofread by** Xu FM