Original Article
Characterization of MAGED1 in ten human solid tumors using the cancer genome atlas

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Abstract: MAGED1, an unorthodox member of MAGE family, is a very complicated protein with ubiquitous expression in a numerous of normal tissues and contrarily regulates cell growth in different tumor cells. Although MAGED1 has been discovered for more than ten years, to our knowledge, there are few studies on the risk of the copy number variations (CNVs) and the single nucleotide polymorphisms (SNPs) of MAGED1 in tumorigenesis. As a consequence, it is quite necessary to comprehensively analyze the bioinformatics of MAGED1 in a series of tumors, providing new directions for the future studies of MAGED1 in tumorigenesis. In the present study, using data from The Cancer Genome Atlas (TCGA), we systematically analyze the mRNA expression, the CNVs, the SNPs and the networks of MAGED1 in ten popular solid tumors, including breast invasive carcinoma (BRCA), esophageal carcinoma (ESCA), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), pancreatic adenocarcinoma (PAAD), prostate adenocarcinoma (PRAD), skin cutaneous melanoma (SKCM), stomach adenocarcinoma (STAD) and uterine carcinosarcoma (UCS). Above all, to some extent, our results will shed light on the discovery of novel targets of MAGED1 in specific tumor diagnosis, prognosis and therapy in the future.

Keywords: MAGED1, mRNA, CNV, SNP, TCGA

Introduction

MAGED1, also known as NRAGE and DIxin-1, is firstly identified as an unorthodox member of MAGE gene family with ubiquitous expression, the same as another MAGE member, Necdin, in a large number of normal tissues [1], which is quite different from the canonical MAGE members, for instance, MAGE-A, MAGE-B, MAGE-C, which are tumor specific antigens [2]. MAGED1 protein contains three functional domains, including the DNA polymerase subunit domain (DNAPIII, 50aa-290aa), the interspersed hexapeptide repeat domain (IRD, 291aa-425aa) and the MAGE homology domain (MHD, 475aa-674aa) [3, 4]. Our previous studies have shown that MAGED1 binds with PCNA via its DNAPIII domain to positively regulate cell proliferation in esophageal tumorigenesis [4]. The MHD domain of MAGED1 has been reported to interact with a lot of proteins, such as Praja1 (PJA1) [3], UNC5H1 [5], Necdin [6], to positively participate in cell apoptosis regulation. As for the IRD of MAGED1, Msx1 and Msx2 have been

confirmed to specifically and directly bind with it to negatively regulate myogenic differentiation of C2C12 cells [6]. It seems that the three domains of MAGED1 function quite differently from each other in biological processes, suggesting that MAGED1 is more complicated than we have expected. As a result, a systematic analysis of MAGED1 in a wide range of tumors is quite necessary for further exploration of its specific roles in tumorigenesis and cancer gene individual therapy.

TCGA database makes the CNVs, the gene mRNA expression, the microRNA expression, the DNA methylation, the SNPs, the networks and the signaling pathways available for a numerous of genes in a series of tumors and adjacent normal tissues [7, 8]. Data from TCGA database have become an extraordinary resource for basic, translational, and clinical researches and have the potential to shape cancer diagnostic and treatment strategies [9]. Rachel Wevrick and his team have evaluated the MAGE gene expression in human cancers

using TCGA data and demonstrated that the overexpression of MAGE genes in a subset of cancers is greatly associated with reduced survival, thus they have drawn a conclusion that the expression of MAGE genes could inform individualized treatment in cancers [7].

Overexpression of specific genes at both mRNA and protein levels in specific tumor tissues are quite valuable for identifying the potential biomarkers for diagnosis, therapy, prognosis and clinical decision making in individualized cancer treatments. For example, RAD51, which has been observed to be strongly upregulated and closely related with poor survival in patients with pancreatic cancer, serves as an effective and sensitive biomarker for early detection of pancreatic cancer [10]. CNVs refer to a form of genomic structural variations, including gene amplification, gain, loss and deletion. DNA CNV is an important influential factor for the expression of both protein-coding and non-coding genes, affecting the activity of various signaling pathways [11]. Therefore, the CNVs of key regulators in tumorigenesis carry specific emphasis for the diagnosis, prognosis and therapy of specific tumors. In addition, the SNP rs1465618 in thyroid adenoma associated (THADA) has been identified as being associated with prostate cancer (PCa) risk in European and Chinese populations, suggesting that this SNP of THADA impacts the susceptibility and progression of PCa [12]. Above all, we think that identification of SNPs and CNVs of tumor-related genes is critical for their translational applications in cancer gene therapy in the future.

As a consequence, in the study, using the data from TCGA, we systematically analyzed the mRNA expression, the CNVs, the SNPs and the networks of MAGED1 in ten solid tumors, including BRCA, ESCA, LIHC, LUAD, LUSC, PAAD, PRAD, SKCM, STAD and UCS. Our analytical results not only provide consistent results with previous researches, but also shed light on the specific applications of MAGED1 in cancer gene diagnosis and therapy in different tumors, which could guide the following studies of MAGED1 in these tumors.

Materials and methods

Cell culture

Cells of HEK-293, C2C12, HSF, MSF, Hela, HepG2, MDM-MB-231 and MCF-7 were purchased from Shanghai Cell Bank of the Chinese

Academy of Sciences and the oesophageal cancer cell lines including EC9706, EC109 and TE1 were obtained from American Type Culture Collection (ATCC). All the cells were maintained in Dulbecco's modified Eagle's medium with 12% fetal bovine serum, 100 U/ml penicillin and 100 U/ml streptomycin at 37°C in a humidified incubator, containing 5% CO_2.

Data collection

The mRNA expression data, CNVs, somatic mutation data and SNPs array data of MAGED1 were primarily downloaded from TCGA database (http://cancergenome.nih.gov/) and then subjected to the following analysis in the study.

mRNA expression analysis

Two different ways were employed to analyze the mRNA expression of MAGED1 in ten human solid tumors. First, we manually excluded the data with less than three samples in either the normal or the tumor tissues. Afterwards, the DEseq, which is an R package to analyze count data from high-throughput sequencing assays, were used to analyze the differential mRNA expression of MAGED1 in the cancer tissues with statistical significance. The mRNA expression change criteria were defined as follows: Fold change ≤0.5 or ≥1.5 and p-value <0.05. On the other hand, the visual tool in TCGA, termed the cBio Cancer Genomics Portal (cBio-Portal) (http://www.cbioportal.org/public-portal/index.do) [13], was used to analyze the mRNA expression of MAGED1 in cancer tissues and the significant expression was determined as the Fold change ≥2 (up) or ≤-2 (down).

Somatic mutation analysis

The somatic mutation data from TCGA database was manually analyzed to identify the SNPs. Afterwards, the cBioPortal tool was used to visually analyze the somatic mutations in MAGED1 gene, especially in the functional domains, including the DNAPIII, the IRD and the MHD [3, 4].

Copy number variant analysis

Data from TCGA database were analyzed for the CNVs using the visual tool cBioPortal. And the correlation between mRNA expression of MAGED1 and its CNVs was depicted using the box plot in ten cancers, in which the Y-axis represented the LOG_2 value of mRNA expression and the X-axis was the CNVs.

Network and GO analysis

The cBioPortal tool integrating the data from Human Reference Protein Database (HPRD), Reactome, National Cancer Institute (NCI)-Nature and the Memorial Sloan-Kettering Cancer Center (MSKCC) Cancer Cell Map was used for the network analysis. In the network, the circle represents a specific gene; the color of the circle represents the change (such as mutation, amplification and deletion) of the gene (the redder the circle is the more frequent change of the gene). The green lines between two genes mean that A gene affects the change of B gene, the brown line represents that A and B genes compose a complex, whereas the purple line represents other types of the interactions among these genes, for instance, the protein-protein interaction. For the GO analysis, the genes in the network were analyzed using the Panther database (http://go.pantherdb.org/).

Immunofluorescence

Cells on the slides were firstly fixed with 4% paraformaldehyde for 15 min at room temperature, followed by the incubation with MAGED1 antibody (Santa Cruz) at 4°C overnight. Then the slides were washed and incubated with the corresponding secondary antibody at 37°C for 20 min, followed by the incubation with DAPI (Thermo Scientific) for the nucleus staining. Finally, the slides were sealed and pictured under the confocal fluoresence microscopy (Carl Zeiss). The intensity of MAGED1 staining across the red line was automatically evaluated and showed by the software of the microscopy.

Statistical analysis

The DEUs of MAGED1 in the specific organs from the GeneHub-Gepis database was statistically using the Z-test. Moreover, the fold change of MAGED1 in the ten solid tumors was analyzed using the hypergeometric distribution statistical analysis. The statistical significance was indicated as *P<0.05 and **P<0.01.

Results

mRNA expression of MAGED1 in ten solid tumors

It has been reported that MAGED1 has a very contradictive role in tumorigenesis, inducing cell apoptosis [14] as well as inhibiting cell proliferation [4]. Using the immunofluorescence

(IF) assays, we found that MAGED1 differently localized in normal and tumor cell lines. In normal cell lines, such as HEK-293, C2C12, HSF, MSC, MAGED1 mainly localized in the cytoplasm (Figure S1A, 1B); whereas in tumor cell lines, for instance, Hela, HepG2, MDM-MB-231, MCF-7, MAGED1 predominantly localized in the nucleus (Figure S1C, 1D), suggesting that MAGED1 played different roles due to its subcellular localization. Moreover, we examined the cellular localization of MAGED1 in three esophageal carcinoma (EC) cell lines, namely EC9706, EC109 and TE1, results also showed that MAGED1 mainly localized in the nucleus (Figure S1E). We have previously demonstrated that MAGED1 significantly promotes cell growth of EC cells [4], consequently, we propose that the nuclear localized MAGED1 is of great possibility to positively regulate tumor cell proliferation.

It is widely recognized that the biological functions of proteins are closely related with their expressions in tumor tissues. Therefore, to primarily predict the role of MAGED1 in tumorigenesis, we evaluated the mRNA expression of human MAGED1 using the EST-based digital expression levels (DEU), correlating with mRNA transcript levels, in the GeneHub-GEPIS database (http://research-public.gene.com/cgi-bin/genentech/genehub-gepis). Interestingly, we found that MAGED1 were greatly overexpressed in 72% (13/18) tumor tissues (Figure S1F and Table S1). Consistently, MAGED1 has been reported to be significantly overexpressed in esophageal carcinoma [4], colon cancer, melanoma, prostate cancer, breast cancer and lung cancer [15].

Subsequently, we analyzed the mRNA expression of MAGED1 in ten solid tumors using data from TCGA database by means of two different analytical skills. The ten selected tumors are BRCA, ESCA, LIHC, LUAD, LUSC, PAAD, PRAD, SKCM, STAD and UCS, respectively. As shown in **Table 1**, MAGED1 was strongly upregulated in eight tumors, except that no usable data about ESCA and UCS were available. The detailed fold change and case ID of upregulated and downregulated MAGED1 in specific tumors in cBioPortal were shown in Table S2.

Functional SNPs of MAGED1 in the ten solid tumors

An increasing number of reports declaim that the frequencies of SNPs are greatly correlated

Table 1. The mRNA expression of MAGED1 in the ten solid tumors evaluated by two different ways

Tumor type	Fold change	*P*-value	Upregulated ratio in cBioPortal	Downregulated ratio in cBioPortal	Expression
BRCA	2.177962255	1.29E-20	25/988	0/988	up
ESCA	—	—	—	—	—
LIHC	—	—	7/134	0/134	up
LUAD	1.935889	1.13E-06	11/230	1/230	up
LUSC	1.566501	0.01056	—	—	up
PAAD	—	—	1/40	0/40	up
PRAD	1.503609	3.12E-07	6/195	0/195	up
SKCM	—	—	12/332	0/332	up
STAD	—	—	3/35	0/35	up
UCS	—	—	—	—	—

with gene expression and clinical factors [16]. Wu et al. has reported that the SNP loci rs5970360 and rs5925210 of MAGE-A3 gene could be an predictive marker for epidermal growth factor receptor (EGFR) mRNA expression levels and be helpful for the selection of patients for EGFR targeted immuotherapy [17]. Moreover, the rs1465618 in THADA has been reported to impact Pca susceptibility and progression [12]. To our present knowledge, there has been no report on the study of MAGED1 polymorphisms in tumorigenesis. As a result, using the TCGA database, we downloaded and analyzed the SNPs of MAGED1 in the ten solid tumor tissues. Interestingly, except that no data were available for PAAD and UCS, we found that the number of SNPs in other eight tumors were quite different. The types of SNP and their corresponding frequency in the ten solid tumors were summarized in **Figure 1A**. The first three polymorphisms with high frequencies were G>A (15 cases), C>T (14 cases) and G>T (8 cases), respectively, implicating that these SNPs were of greater possibility in regulating the biological functions of MAGED1 in tumorigenesis.

We have previously reported that there are three functional domains of human MAGED1 protein, the DNAPIII, the IRD and the MHD [4, 5]. It is well-known that the SNPs in the domains of proteins are quite important to affect their biological functions. Therefore, we analyzed the SNPs of MAGED1 leading to missense mutations in its three domains. As shown in **Figure 1B**, there were five tumors having functional SNPs in the domains of MAGED1 and these SNPs were quite different from each other. The

BRCA had two SNPs in the DNAPIII domain (S68L, D171V), the LUAD had three SNPs (Q321K, A337T in the IRD and Y509N in MHD, respectively), the LUSC had two SNPs (N105D, V508I), the SKCM had three SNPs (P119Q in DNAPIII, P401S in IRD and L607F in MHD, respectively), the STAD had all the three SNPs (P177S, D215N, R245W) in the DNAPIII domain. The detailed information about SNPs in the above five tumors in the TCGA database were shown in Table S3.

CNVs analysis of MAGED1 in solid tumors

The DNA CNV is a kind of fragment of DNA ranging from 1 Kb to 3 Mb that is present in a variable number of cancer related genes, leading to activation of oncogenes or inactivation of tumor suppressors in tumorigenesis by changing gene mRNA expression, activities in signaling pathways and epigenetic phenotypes [20]. The CNV is classified into four subgroups, including amplification, gain, homozygous deletion and homozygous loss. The detection of CNVs in chromosomal or mitochondrial DNA from tissue or blood samples may assist the diagnosis, prognosis and targeted therapy of cancer [11]. However, the CNVs of MAGED1 in carcinogenesis have never been reported by now. Taking advantage of the TCGA database, we predicted the CNVs in MAGED1 gene in the ten selected tumor tissues using the cBioPortal visual tool. As shown in **Table 2**, except that there were no data available for ESCA, LUSC, PRAD and USC, the CNVs of MAGED1 occurred quite more frequently in BRCA, LIHC, LUAD, SKCM and STAD, in comparison with PAAD. Additionally, compared with the homozygous loss [Homdel(-2)] and the amplification [Amp(2)], the incidence rate of the homozygous deletion [Het loss(-1)] and the Gain [Gain (1)] were apparently higher.

Afterwards, we further analyzed the correlation of MAGED1 mRNA expression with the CNVs in the specific tumors, using the Log_2 value of the MAGED1 RNAseq expression data as Y-axis,
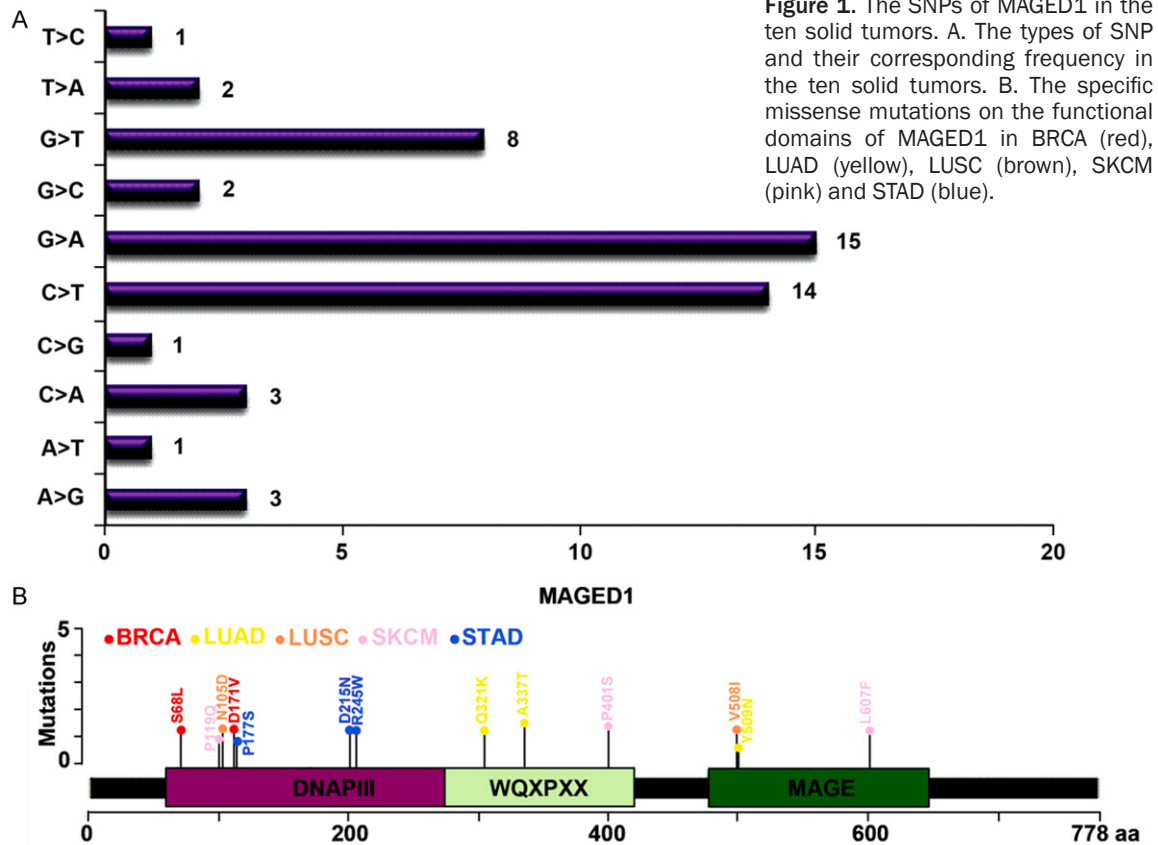
Figure 1. The SNPs of MAGED1 in the ten solid tumors. A. The types of SNP and their corresponding frequency in the ten solid tumors. B. The specific missense mutations on the functional domains of MAGED1 in BRCA (red), LUAD (yellow), LUSC (brown), SKCM (pink) and STAD (blue).

Table 2. The number of MAGED1 CNVs in the tumor samples available from TCGA

Cancer Type	Homozygous Deletion	Homozygous Deletion	Gain	Amplifi-cation
BRCA	4/988	174/988	171/988	6/988
ESCA	—	—	—	—
LIHC	2/139	31/139	17/139	0/139
LUAD	2/229	22/229	12/229	1/229
LUSC	—	—	—	—
PAAD	1/50	7/50	0/50	0/50
PRAD	—	—	—	—
SKCM	1/336	84/336	51/336	1/336
STAD	3/293	37/293	42/293	0/293
UCS	—	—	—	—

the putative CNVs from GISTIC in the tumors as X-axis, as depicted in **Figure 2**. In the LUAD, the mRNA expression of MAGED1 greatly increased with the amplification of its CNVs. Unfortunately, the expression of MAGED1 did not show significant correlation with its CNVs in BRCA, LIHC, PAAD, PRAD, SKCM and STAD.

Network analysis of MAGED1 in the ten solid tumors

Using the cBioPortal visual tool integrated data from HPRD, NCI-Nature and MSKCC, we carried out the network analysis of MAGED1 in the nine tumors, except for the UCS. As shown in **Figure 3**, the expression of a lot of genes, directly or indirectly interacting with MAGED1, were affected by MAGED1 in BRCA, indicated by the color of the circle (the redder the circle is, the more frequent change of the gene). For instance, MAGED1 interacted with and significantly affected the expressions of TRAF4, CYCS, AATF, SIRT7, SH2B1, XIAP, DLX4, PLK1, TUBA4A, ROR2, PJA2, MPC1, MAPK8. In ESCA, according to the network result, we found that MAGED1 bound with a lot of proteins, analogous to those in BRCA, but without apparently affecting their expression. While in LIHC, MAGED1 interacted with SIRT7 and obviously regulated its expression. Moreover, it also bound with RFX1 and slightly influenced its
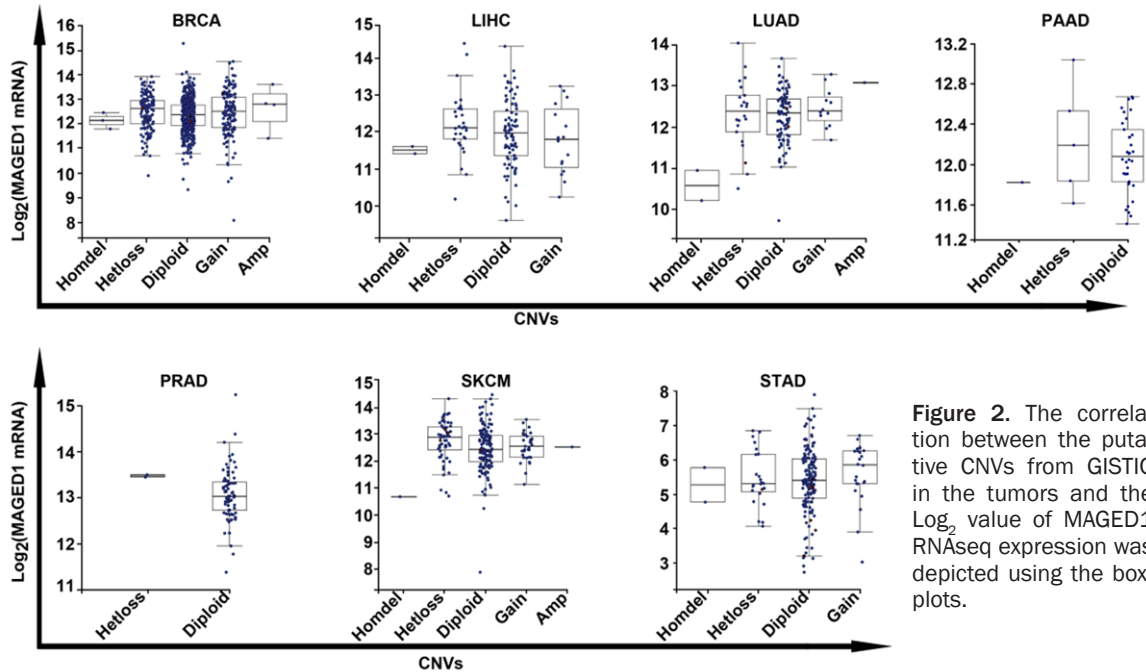
Figure 2. The correlation between the putative CNVs from GISTIC in the tumors and the Log_2 value of MAGED1 RNAseq expression was depicted using the boxplots.

expression. In LUAD, MAGED1 independently bound with SIRT7 and CYCS, the expression of which were significantly regulated. In addition, MAGED1 also bound with and slightly affected the expression of the following proteins, including NGF, NGFR, DLX4, DLX5, UNC5A and MPC1. In LUSC and PRAD, MAGED1 could only interact with the proteins similar to those in ESCA, with no interruption on their expressions. In case of PAAD, MAGED1 interacted with and affected the expressions of MSX2, UNC5A, CYCS, NGFR, DLX5, PJA1 and MPC1. In SKCM, MAGED1 interacted with and significantly regulated the expression of SIRT7, accompanied with the slight regulation on the expression of DLX4, MPC1, UNC5A, CYCS, EIF3J and MPC1. As for STAD, MAGED1 apparently regulated the expressions of DLX5, AATF, CYCS and MPC1.

Furthermore, we analyzed the biological process, the molecular function, cellular component, protein class and pathway of these MAGED1 interacted with proteins using the PANTHER database (http://go.pantherdb.org). As shown in **Figure 4**, the interacted proteins with MAGED1 in the center were mostly involved in cellular process (21.7%), metabolic process (18.8%) and developmental process (14.5%) in the biological process category (**Figure 4A**); and functioned mainly in binding (35.7%), catalytic activity (21.4%) and the nucleic acid bind-

ing transcription factor activity (17.9%) in the molecular function category (**Figure 4B**); and formed cell part (50%), organelle (30%) and macromolecular complex (20%) in the cellular component category (**Figure 4C**); the classification of these proteins were transcription factor (20.8%), nucleic acid binding (20.8%) and receptor (16.7%) in the protein class category (**Figure 4D**); these proteins dominantly participated in the apoptosis signaling pathway (12.5%) (**Figure 4E**), combined with other pathways, such as metabotropic glutamate receptor pathway, the oxidative stress response, the FAS signaling pathway.

Discussion

In the study, we primarily characterized the properties of MAGED1 in ten selected solid tumors (including BRCA, ESCA, LIHC, LUAD, LUSC, PAAD, PRAD, SKCM, STAD, UCS) using the TCGA database, and interestingly found that it is highly overexpressed in eight of the tumors, the SNPs mostly occured in its functional domains are the G>A, C>T and G>T, its CNVs occurred more frequently in BRCA, LIHC, LUAD, SKCM and STAD, compared with PAAD, and the genes and their coding proteins strikingly regulated by it in different tumors were quite different. Taken all these bioinformatics into consideration, we think that MAGED1 plays
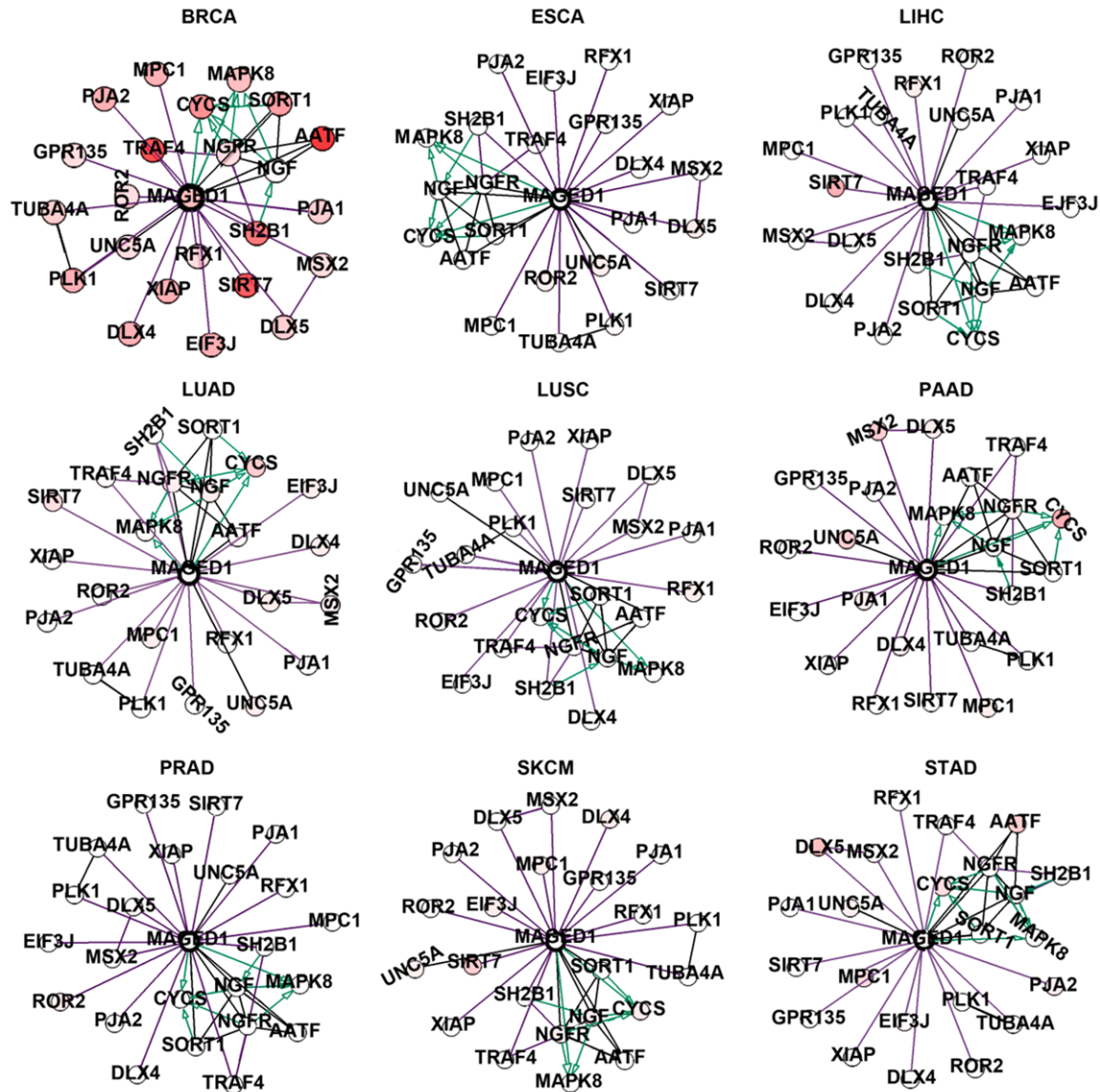
Figure 3. Network analysis of MAGED1 in the tumors.

quite different roles in tumorigenesis depending on the phenotype of the cancers. Therefore, it is urgent to elucidate the actual roles of MAGED1 in different tumors in order to properly put it into practice in tumor diagnosis and targeted-therapy.

In the process of exploring the expression of MAGED1 in the above ten tumors, we discovered that it was dramatically overexpressed in eight tumors, except that no usable data about ESCA and UCS were available. According to previous studies, there is a strong correlation between the biological roles in tumorigenesis

and the differential expressed genes [23]. Moreover, the upregulated genes in tumor tissues could mostly be an effective predictor for the development of the tumors in the diagnosis, and also be a potential targeted molecular in tumor genetic therapy [10]. As a result, we consider that the widely upregulated gene MAGED1 could be potentially applied into the field of tumor diagnosis and targeted-therapy in the future.

More and more studies show that SNPs of some key regulators are greatly correlated with gene expression and clinical factors (16). In the

A **Biological process**

- □ apoptotic process (GO:0006915)
- ■ biological regulation (GO:00065007)
- □ cellular component (GO:0071840)
- ■ cellular process (GO:00099987)
- ■ developmental process (GO:0032502)
- ■ immune system process (GO:0002376)
- ■ localization (GO:0051179)
- ■ metabolic process (GO:0008152)
- □ organismal process (GO:0032501)
- □ response to stimulus(GO:0050896)

B **Molecular function**

- ■ binding (GO:0005488)
- □ catalytic activity (GO:0003824)
- □ enzyme regulator activity (GO:0030234)
- ■ nucleic acid binding transcription factor activity (GO:0001071)
- □ receptor activity (GO:0004872)
- ■ structural molecule activity (GO:0006198)
- □ transporter activity (GO:0005215)

C **Cellular component**

- ■ cell part (GO:0044464)
- ■ macromolecular complex (GO:0032991)
- □ organelle (GO:0043226)

D **Protein class**

- □ cytoskeletal protein (PC00085)
- ■ enzyme modulator (PC00095)
- □ hydrolase (PC00121)
- □ kinase (PC00137)
- □ ligase (PC00142)
- ■ nucleic acid binding (PC00171)
- □ receptor (PC00197)
- ■ signaling molecule (PC00207)
- ■ transcription factor (PC00218)
- □ transferase (PC00220)
- □ transporter (PC00227)

E **Pathway**

- □ ATP synthesis (P02721)
- ■ alzheimer disease-amyloid secretase pathway (P00003)
- ■ angiogenesis (P00005)
- ■ apoptosis signaling pathway (P00006)
- ■ axon quidance mediated by netrin (P00009)
- ■ B cell activation (P000010)
- ■ CCKR signaling map (P06959)
- □ FAS signaling pathway (P00020)
- □ gonadotropin releasing hormone receptor pathway (P06664)
- □ huntington disease (P00029)
- □ integrin signaling pathway (P00034)
- □ interferon-gamma signaling pathway (P00035)
- □ ionotropic glutamate receptor pathway (P00037)
- ■ metabotropic glutamate receptor group I pathway (P00041)
- ■ metabotropic glutamate receptor group III pathway (P00039)
- □ oxidative stress response (P00046)
- ■ PDGF signaling pathway (P00047)
- □ parkinson disease (P00049)
- ■ Ras pathway (P04393)
- □ T cell activation (P00053)
- ■ TGF-beta signaling pathway (P00052)
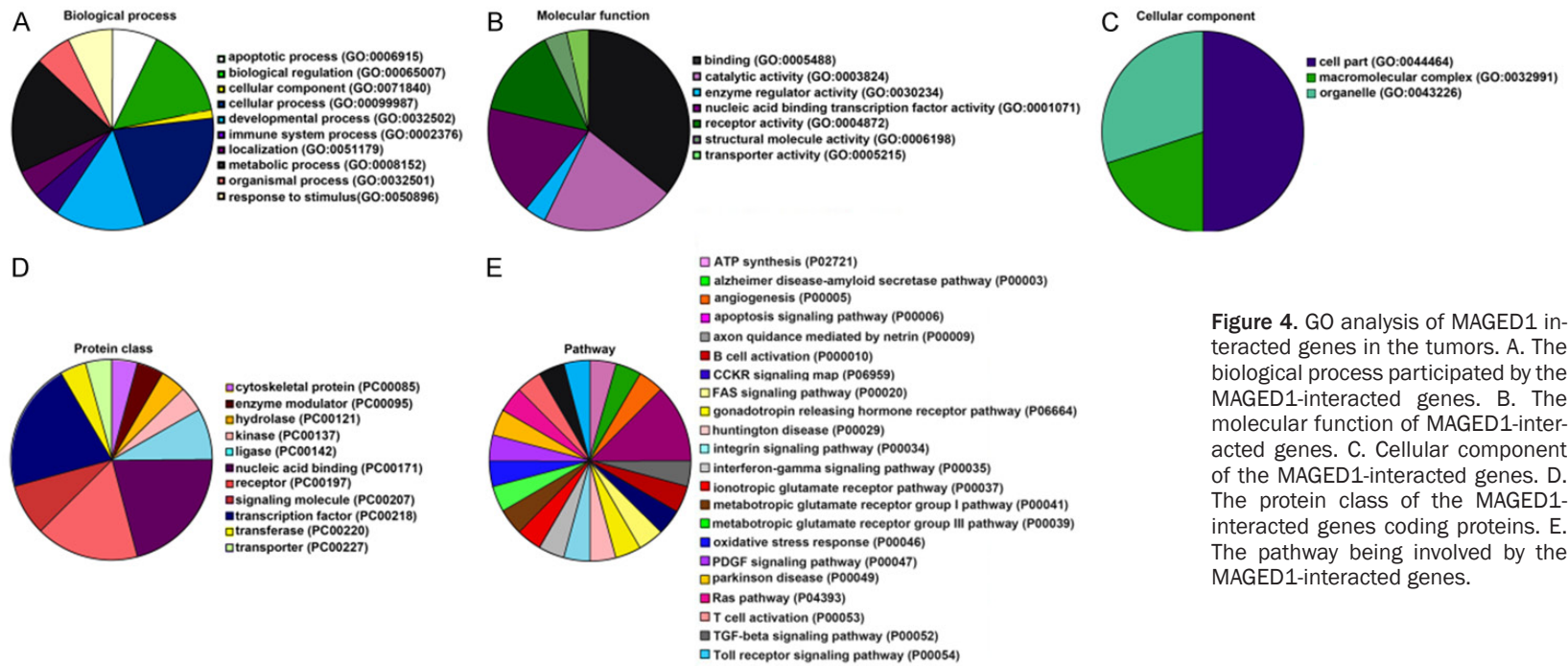- □ Toll receptor signaling pathway (P00054)

Figure 4. GO analysis of MAGED1 interacted genes in the tumors. A. The biological process participated by the MAGED1-interacted genes. B. The molecular function of MAGED1-interacted genes. C. Cellular component of the MAGED1-interacted genes. D. The protein class of the MAGED1-interacted genes coding proteins. E. The pathway being involved by the MAGED1-interacted genes.

study, using the TCGA database, we found that the first three polymorphisms with high occurrence were G>A, C>T and G>T. However, there were only five tumors having functional SNPs in the domains of MAGED1 and these SNPs were quite different from each other. We have previously reported that MAGED1 interacts with PCNA via its DNAPIII domains to promote esophageal tumorigenesis [4]. Other groups have reported that MAGED1 binds with apoptosis related proteins via its MHD domain to mediate the cell apoptosis in tumorigenesis and simultaneously regulates cell differentiation by interacting with Msx1/2 via its IRD [6]. It seems that the three domains of MAGED1 act quite differently in the process of tumor development. Consequently, we speculated that the SNPs in different domains of MAGED1 were likely to lead to different consequences. However, the biological functions of the specific SNPs in the five tumor tissues are required to be further studied in a wide range of clinical tumor samples by genotyping [18], which could possibly provide clues for predicting cancer risks and making therapeutic decisions for the clinicians [19].

In the process of analyzing the CNVs of MAGED1 in the above ten solid tumors, we found that they are more frequently occurred in BRCA, LIHC, LUAD, SKCM and STAD than PAAD. Moreover, we analyzed the relationship between CNVs and mRNA expression of MAGED1 in the specific tumors, and discovered that its mRNA expression greatly increased with the amplification of its CNVs in LUAD. Whereas it did not show significant correlation with its CNVs in BRCA, LIHC, PAAD, PRAD, SKCM and STAD. As a result, we considered that the CNVs of MAGED1 played a more important role in LUAD than those in other tumors. Whatever, further detailed studies on the biological functions of MAGED1 CNVs in LUAD are required to confirm our speculation.

Subsequently, using the cBioPortal visual tool integrated data from HPRD, NCI-Nature and MSKCC, we carried out the network analysis of MAGED1 in the nine tumors, except for the UCS. Intriguingly, we found that the genes and their coding proteins strikingly regulated by MAGED1 in different tumors were quite different, suggesting that the role of MAGED1 in specific tumors was mediated through different partners and the corresponding biological func-

tions were required to further specifically explored. Additionally, we found that MAGED1 bound with most of the same proteins in these nine tumors, but regulated the expression of different proteins and comprised different complexes. It has been previously reported that MAGED1 interacts with p75NTR [21], MSX2 [6], DLX5 [3], XIAP [22], PJA1 [3] to regulate cell apoptosis. However, the interaction of MAGED1 with CYCS and SIRT7 in most of these tumors has never been reported and needs to be further consolidated by immunoprecipitation or the yeast two-hybrid screen.

Taken together, we thought that MAGED1 played very complicated or contrary roles in tumorigenesis by binding with different proteins in these signaling pathways to differently regulate the biological processes in specific tumors. However, all these bioinformatics about MAGED1 in different tumors have to be further consolidated in a wide range of clinical samples both in vivo and in vitro, which will specifically promote the translational application of MAGED1 as an efficient cancer therapeutic target in the long run.

Disclosure of conflict of interest

None.

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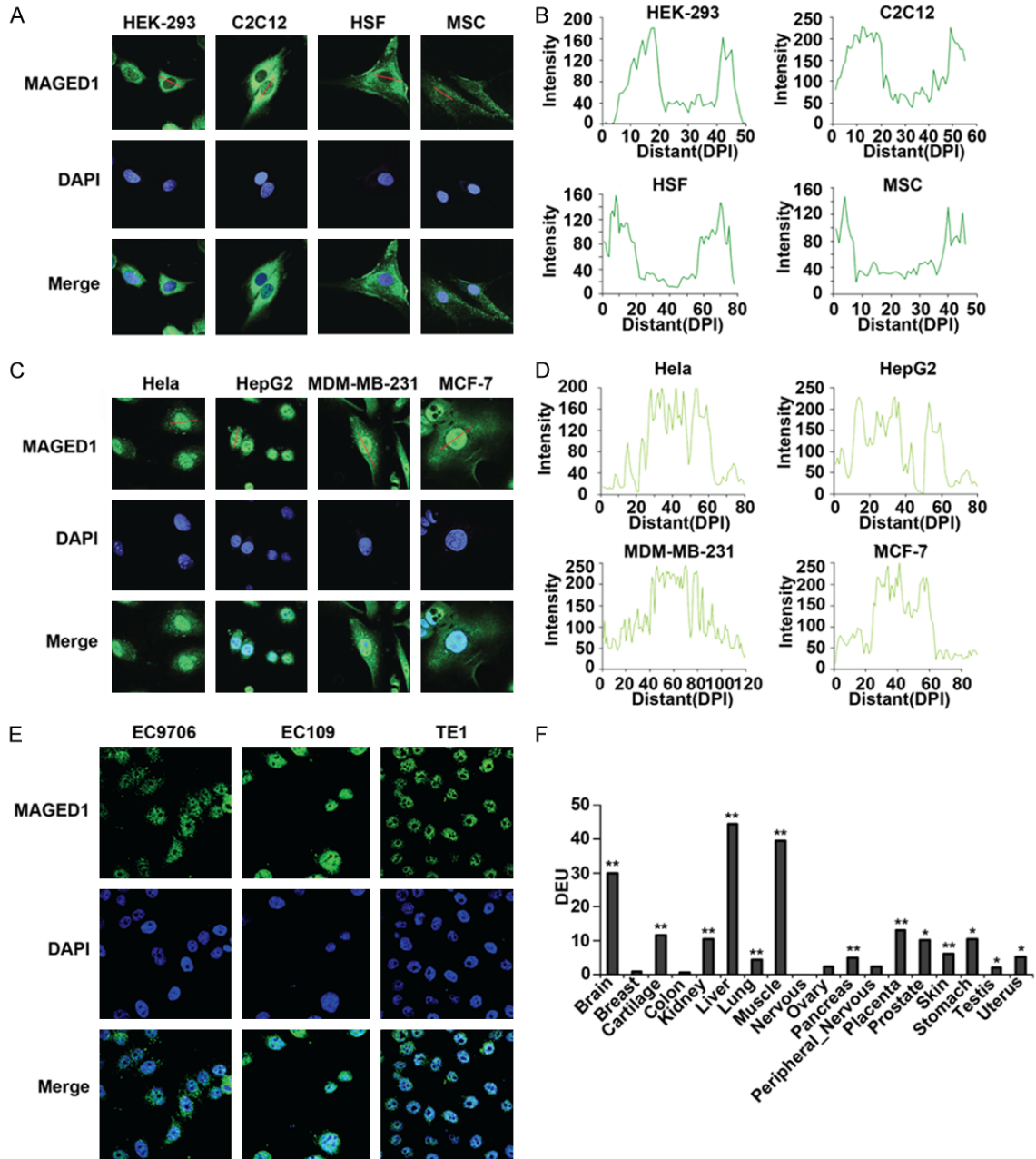
Figure S1. A. The subcellular localizations of MAGED1 (Green) in the normal cell lines were detected using the immunofluorescence (IF) assays. The nucleus was stained with DAPI (Blue). B. The MAGED1 staining intensity across the red line in the normal cells was automatically evaluated and depicted by the microscopy. C. The subcellular localizations of MAGED1 (Green) in a series of tumor cell lines were detected using the IF. And the nucleus was indicated with DAPI staining (Blue). D. The MAGED1 staining intensity across the red line in the tumor cells was automatically evaluated and depicted by the microscopy. E. The subcellular localization of MAGED1 (Green) in the esophageal cancer cells (EC9706, EC109, TE1) were examined by the IF. F. The relative expression of MAGED1, evaluated by the DEU parameter, in a number of organs was significantly upregulated.

Table S1. The detailed DEUs of MAGED1 in specific organs, which were statistically analyzed using the *Z*-test

Organ	DEU (T)	DEU (N)	DEU (T)/DEU (N)	T/N Z-test	Significance
Brain	527.35	17.44	30.23	5.38E-53	**
Brest	95.24	67.75	1.41	2.75E-01	
Cartilage	348.45	0	12.1	8.73E-03	**
Colon	98.46	78.53	1.25	3.58E-01	
Kidney	241.32	21.84	11.05	1.59E-03	**
Liver	410.79	9.2	44.66	5.80E-11	**
Lung	471.26	93.49	5.04	2.88E-06	**
Muscle	567.7	0	39.93	5.22E-06	**
Nervous	0	433.5	0	1.00E+00	
Ovary	315.66	112.33	2.81	1.44E-01	
Pancreas	411.57	73.94	5.57	8.58E-07	**
Peripheral nerve	834.72	298.63	2.8	1.60E-01	
Placenta	621.99	45.36	13.71	7.35E-18	**
Prostate	82.77	0	10.67	1.28E-02	*
Skin	753.07	113.44	6.64	8.70E-07	**
Stomach	159.8	0	10.88	1.11E-02	*
Testis	135.59	52.4	2.59	2.55E-02	*
Uterus	280.4	0	5.75	4.58E-02	*

Table S2. The detailed information of MAGED1 mRNA expression change in the indicated tumors

Tumor Type	Case ID	Fold change	Expression
BRCA	TCGA-A8-A06Q	2.0034	up
	TCGA-D8-A1JK	2.0158	up
	TCGA-A8-A07R	2.0351	up
	TCGA-D8-A146	2.0354	up
	TCGA-D8-A1XR	2.0601	up
	TCGA-A2-A0D2	2.0769	up
	TCGA-BH-A0BV	2.0817	up
	TCGA-A7-A13F	2.302	up
	TCGA-BH-A0DG	2.3242	up
	TCGA-A8-A09G	2.3502	up
	TCGA-A2-A04X	2.4329	up
	TCGA-GM-A2DD	2.446	up
	TCGA-EW-A3U0	2.4467	up
	TCGA-A7-A0CE	2.4469	up
	TCGA-A7-A4SD	2.7615	up
	TCGA-A7-A26I	2.7716	up
	TCGA-A2-A04W	2.9133	up
	TCGA-AC-A2BK	2.9267	up
	TCGA-A7-A13D	2.9477	up
	TCGA-C8-A26V	3.0547	up
	TCGA-BH-A1EV	3.1697	up
	TCGA-AC-A3YJ	5.9454	up
	TCGA-A2-A0YE	7.5951	up
	TCGA-OL-A5S0	9.8617	up
	TCGA-D8-A1JN	10.6729	up

LIHC	TCGA-EP-A3RK	2.0032	up
	TCGA-CC-5258	2.1432	up
	TCGA-FV-A3I0	2.1493	up
	TCGA-DD-A118	2.4844	up
	TCGA-BC-A5W4	3.9196	up
	TCGA-DD-A39W	4.8095	up
	TCGA-ED-A459	5.1653	up
PRAD	TCGA-HC-7230	2.1802	up
	TCGA-G9-6332	2.5587	up
	TCGA-HC-A48F	2.6549	up
	TCGA-CH-5737	3.2089	up
	TCGA-HC-7210	3.4254	up
	TCGA-CH-5741	7.7293	up
SKCM	TCGA-GF-A2C7	2.0664	up
	TCGA-FW-A5DX	2.2361	up
	TCGA-EB-A5KH	2.3149	up
	TCGA-EE-A2MQ	2.5233	up
	TCGA-DA-A1IC	2.6064	up
	TCGA-FR-A3YO	2.8735	up
	TCGA-HR-A5NC	2.9049	up
	TCGA-EE-A2MH	3.1896	up
	TCGA-DA-A1I7	3.5891	up
	TCGA-FS-A1ZQ	3.619	up
	TCGA-BF-A3DL	4.0663	up
	TCGA-DA-A1IA	4.2114	up
STAD	TCGA-CG-4460	2.1519	up
	TCGA-CG-4449	2.4565	up
	TCGA-CG-4443	3.0162	up
PAAD	TCGA-HZ-8001	3.5729	up
LUAD	TCGA-49-4506	-2.0304	down
	TCGA-69-7980	2.0444	up
	TCGA-78-7536	2.1206	up
	TCGA-91-6840	2.1863	up
	TCGA-69-7760	2.3192	up
	TCGA-05-4424	2.4388	up
	TCGA-05-5715	2.4394	up
	TCGA-80-5608	3.1051	up
	TCGA-75-6212	3.1666	up
	TCGA-78-7163	3.9328	up
	TCGA-55-6980	4.5686	up
	TCGA-05-4396	4.8141	up

Table S3. The detailed information of MAGED1 SNPs in the indicated tumors

Tumor Type	Variant Classification	Reference Allele	Tumor Seq Allele1	Tumor Seq Allele2	AA change	Sample Barcode
BRCA	Missense_Mutation	C	C	T	S68L	TCGA-A8-A0A2
	Missense_Mutation	A	A	T	D171V	TCGA-D8-A1JI
LUAD	Missense_Mutation	C	A	A	Q321K	TCGA-50-6594
	Missense_Mutation	T	A	A	Y509N	TCGA-49-6767
LUSC	Missense_Mutation	C	C	A	P119Q	TCGA-46-3768
	Missense_Mutation	G	G	A	V508I	TCGA-39-5030
SKCM	Missense_Mutation	A	G	G	N105D	TCGA-D3-A2JL
	Missense_Mutation	C	T	T	L607F	TCGA-EE-A3AC
STAD	Missense_Mutation	C	T	T	P177S	TCGA-BR-8591
	Missense_Mutation	G	A	A	D215N	TCGA-HU-A4H3
	Missense_Mutation	C	T	T	R245W	TCGA-BR-8680