MAGI1 inhibits cancer cell migration and invasion of hepatocellular carcinoma via regulating PTEN

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Abstract: **Objective** To explore the biological function and molecular mechanism of membrane associated guanylate kinase, WW and PDZ domain containing 1 (MAGI1) in hepatocellular carcinoma. **Methods** HepG2^{MAGI1} stable cell line was constructed by transfecting HepG2 cells with pcDNA3.1-MAGI1 plasmid. Wound healing and invasion assay were performed to compare the migration and invasion ability of HepG2^{MAGI1} and HepG2 cells. Furthermore, the expression of MAGI1 and phosphatase and tensin homolog deleted on chromosome ten (PTEN) was also examined by Western blot and the relationship was analyzed. **Results** The wound healing assay showed that the closure of HepG2^{MAGI1} cells was significantly slower than that of HepG2 cells [(90 ± 10)% vs. (50 ± 15)%, $P < 0.05$], and the invasion assay showed that the number of HepG2^{MAGI1} cells that passed through the matrigel was fewer than HepG2 cells (68 ± 18 vs. 150 ± 30, $P < 0.05$). The protein expression level of PTEN was significantly elevated in HepG2^{MAGI1} cells compared with HepG2 cells (1.40 ± 0.32 vs. 0.28 ± 0.15, $P < 0.05$). MAGI1 and PTEN protein expression levels were positively correlated ($r = 0.913$, $P < 0.01$). **Conclusion** MAGI1 may inhibit the cancer cell migration and invasion of hepatocellular carcinoma via regulating PTEN.

Key words: hepatocellular carcinoma; MAGI1; PTEN; invasion

MAGI1 通过调节 PETN 抑制肝癌细胞的侵袭运动

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[**摘要**] **目的**:探讨 *MAGI1* 基因在肝癌侵袭转移中的作用及分子机制。**方法**:将 MAGI1 过表达载体,稳定转染 HepG2 细胞(HepG2^{MAGI1}),应用划痕愈合实验和基质胶侵袭实验检测 HepG2^{MAGI1} 和对照组细胞(HepG2)在细胞运动侵袭能力上的差别。采用 Western 印迹方法检测 MAGI1 和 PTEN 的表达,并分析其相关性。**结果**:HepG2^{MAGI1} 细胞的划痕愈合能力明显弱于 HepG2 细胞[(90 ± 10)% vs. (50 ± 15)%, $P < 0.05$], HepG2^{MAGI1} 细胞穿过基质胶的细胞数亦明显少于 HepG2 细胞(68 ± 18 vs. 150 ± 30, $P < 0.05$)。HepG2^{MAGI1} 细胞中 PTEN 的蛋白表达水平明显高于 HepG2 细胞(1.40 ± 0.32 vs. 0.28 ± 0.15, $P < 0.05$)。相关分析显示 MAGI1 和 PTEN 存在高度正相关($r = 0.913$, $P < 0.01$)。**结论**:MAGI1 可能通过调节 PTEN 蛋白表达抑制肝癌的侵袭运动能力。

[**关键词**] 肝癌; MAGI1; PTEN; 侵袭

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Hepatocellular carcinoma (HCC) is one of the most common malignant cancers threatening the health of Chinese people. In statistics, people died of HCC in China takes up about 55% of those in the world, and the mortality rate of male was 37.9/100 000, female 14.2/100 000, which was the top of all countries[1]. Surgery is the first choice for treatment of HCC, but the rate of recurrence and metastasis after 5 years is as high as 80% – 100%[2]. Recurrence and metastasis have been a major factor of restraining general HCC treatment effect.

In our previous study, we found that membrane associated guanylate kinase, WW and PDZ domain containing 1 (MAGI1) significantly decreased in HCC and was closely related to the disease-free survival after surgery, indicating that MAGI1 might play an important role in recurrence and metastasis of HCC (based on unpublished data). Recent researches have proved that MAGI1 can combine with the tumor suppressor gene phosphatase and tensin homolog deleted on chromosome ten (*PTEN*), block cell detachment, and inhibit the progression and metastasis of cancer cells[3]. So far, there is no report on the effect of MAGI1 in recurrence and metastasis of HCC or whether it can regulate the expression of *PTEN* gene. This research aimed to study the effect in HCC metastasis, and analyze the relationship between MAGI1 and PTEN by using gene cloning, transfection, wound healing assay, matrigel invasion assay, and so on.

1 MATERIALS AND METHODS

1.1 Materials

Cells: HepG2 cells were bought from Cell Institute of Central South University, which were cultured in high glucose Dulbecco's modified Eagle media (DMEM) supplemented with 10% fetal bovine serum and incubated in 5% CO_2 at 37 ℃.

Main reagents: Rat anti-human MAGI1 and Rabbit anti-human PTEN polyclonal antibodies, and goat anti-rat and anti-rabbit secondary antibodies were bought from Santa Cruz Biotechnology (Santa Cruz, CA, USA); fetal bovine serum and DMEM high-glucose medium, lipofectamine 2000, G418, RIPA lysis buffer and matrigel were purchased from Invitrogen Corporation (Carlsbad, CA, USA); pcDNA3.1-MAGI1 carrier was from Shanghai Jikai Biotechnology Company.

1.2 Methods

1.2.1 Cell transfection

pcDNA3.1-MAGI1 vectors or empty vectors were transferred into HepG2 cells in log phase (the cell density was 70%) using lipofectamine 2000. Later, the medium was changed after 12 h and the cells were cultured for another 24 h. Then, the cells were digested with 0.25% trypsin, and transferred into a new culture dish (the cell density was 20%). G418 (final concentration, 500 μg/mL) was added into the medium for screening until all the untransfected HepG2 cells died. The survival cells were cultured and amplified. The HepG2 cells stably overexpressing MAGI1 were named $HepG2^{MAGI1}$, and those transfected with empty vector were named HepG2 cell.

1.2.2 Western blot

The total protein was extracted using RIPA lysis buffer. A total of 80 μg protein was loaded into 8% denaturing polyacrylamide gel well, and underwent electrophoresis at 100 V for 90 min. The protein were transferred onto the nitrocellulose membrane and blocked in 5% (*w/v*) defatted milk for 1 h. Incubated with the primary antibody (1∶1 000) at 4 ℃ over night, and rinsed with PBST for 3 times, 5 min each, then with secondary antibody (1∶3 000) at the room temperature for 1 h. After rinsed with PBST for 3 times, 15 min each, it was developed and photographed with the SuperSignal fluorescent reagent. Quantity one software (Bio-rad) was utilized to conduct the quantitative analysis of the bands. The protein expression level was presented as the product of absorbance and area of protein band.

1.2.3 Wound healing assay

$HepG2^{MAGI1}$ and HepG2 cells in log phase were cultured until the cell density reached 100%. A line was scratched straightly in the middle of the cells with a 15 μL tips. Then the cells were rinsed 3 times to eliminate the scratched cells, and cultured with serum-free DMEM high-glucose medium in a incubator of 37℃ and 5% CO_2. They were observed and photographed every 12 hours.

1.2.4 Matrigel invasion assay

A thin cover of matrigel was applied in Transwell chambers with 8 μm pore. $HepG2^{MAGI1}$ and HepG2 cells in log phase were incubated and diluted with serum-free DMEM high-glucose medium to the density of 1×10^6/mL. The 200 μL of the cell suspension was added into each chamber, and the

chambers were placed into a 24-well plate and cultured in an incubator of 37 ℃ and 5% CO_2. The chambers were taken out 48 h after of the incubation, and removed the upper-layer cells with cotton swab. Then the cells were stained and photographed, and the number of cells past through the matrigel and transwell pores were counted.

1.3 Statistical analysis

The data were analyzed with the SPSS 11.0 software. The quantitative data were presented as mean ± standard deviation ($\bar{x} \pm s$), and analyzed by t test (unpaired). The correlation of MAGI1 and PTEN was tested by Pearson's correlation. $P < 0.05$ was referred to as statistical significance.

2 RESULTS

2.1 Construction of MAGI1 stably over-expressed HepG2 cell line

The Western blot results showed that the expression of MAGI1 was significantly higher in $HepG2^{MAGI1}$ cells than in HepG2 cells (1.20 ± 0.25 vs. 0.20 ± 0.10, $P < 0.05$; Fig. 1), suggesting the stable cell line was constructed successfully.

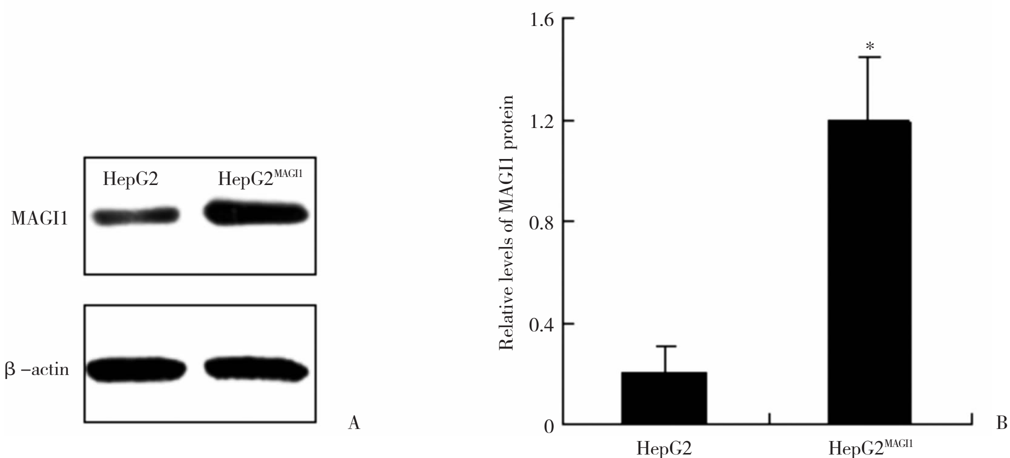

Fig. 1 Expression of MAGI1 protein in $HepG2^{MAGI1}$ and HepG2 cells. A: Level of MAGI1 protein was detected by Western blot. β-actin was involved as control; B: Histogram of the levels of MAGI1 protein. Compared with the HepG2 cells, $*P < 0.05$.

2.2 Role of MAGI1 in HCC metastasis

The wound healing assay indicated that the wound closure of $HepG2^{MAGI1}$ cells was significantly slower than that of HepG2 cells [$(90 \pm 10)\%$ vs. $(50 \pm 15)\%$, $P < 0.05$; Fig. 2]. Besides, the matrigel invasion assay proved that the number of $HepG2^{MAGI1}$ cells past through the matrigel and trans well pore was obviously fewer than that of HepG2 cells (68 ± 18 vs. 150 ± 30, $P < 0.05$, Fig. 3).

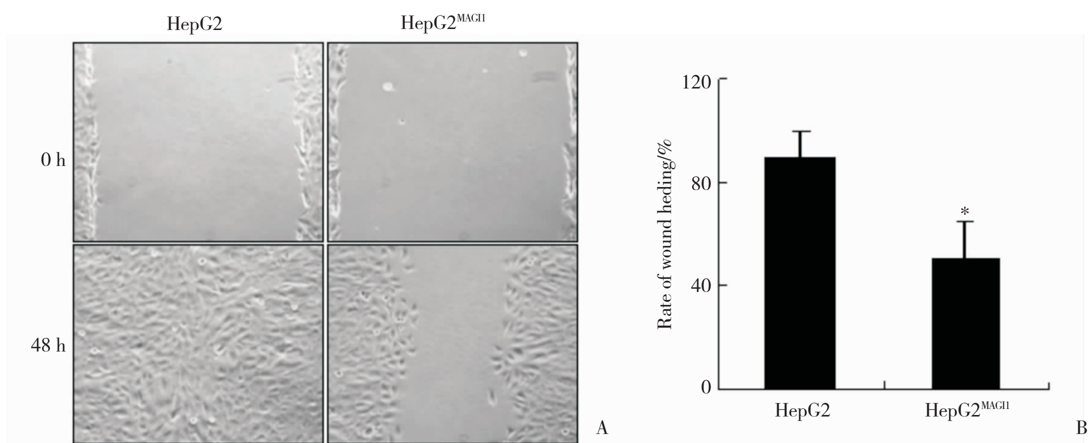

Fig. 2 Wound healing assay. A: Wound healing photos of $HepG2^{MAGI1}$ and HepG2 cells; B: Histogram of the closure percentage of $HepG2^{MAGI1}$ and HepG2 cells after 48 h. Compared with the HepG2 cells, $*P < 0.05$.

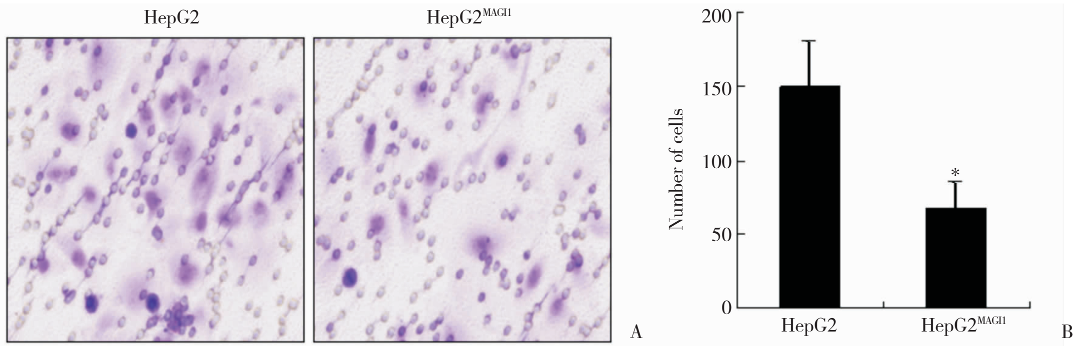

Fig. 3 Invasion assay. A: Cells staining photos shows cells invaded through the matrigel and transwell pores at the 48th hour (×400); B: Histogram of the number of the cells. Compared with the HepG2 cells, $*P<0.05$.

2.3 Correlation of MAGI1 and PTEN

The protein expression level of PTEN was significantly higher in HepG2^{MAGI1} cells than that in HepG2 cells (1.40 ± 0.32 vs. 0.28 ± 0.15, $P<0.05$; Fig. 4), indicating over-expression of MAGI1 could up-regulate the expression of PTEN protein. Furthermore, the expression level of PTEN and MAGI1 had positive correlation ($r=0.913$, $P<0.01$).

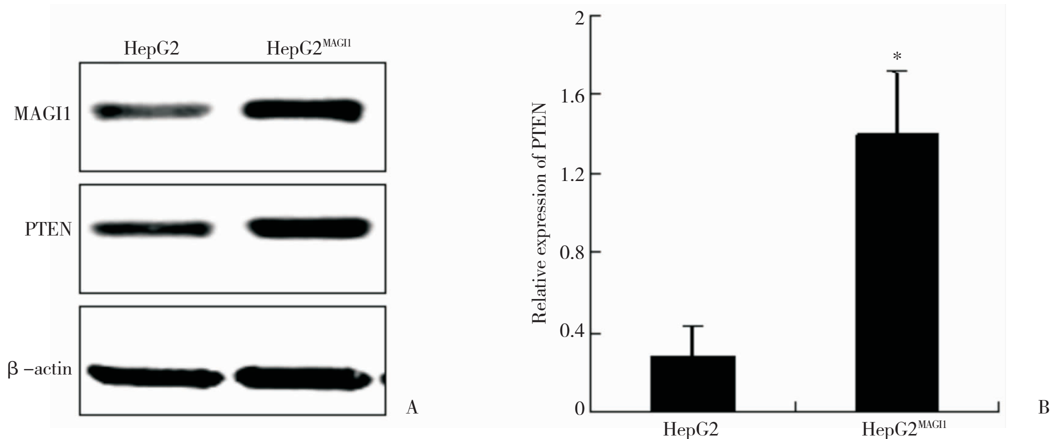

Fig. 4 Expression of MAGI1 and PTEN protein. A: Levels of MAGI1 and PTEN protein detected by Western blot. β-actin was involved as control; B: Histogram of the levels of PTEN protein. Compared with the HepG2 cells, $*P<0.05$.

3 DISCUSSION

The invasion and metastasis of HCC is a complicated process involving multiple factors and procedures, in which the cancer cell detachment from primary sites is the first step[4]. It was found that the intercellular adherence was regulated by a variety of adhesion molecules[5]. *MAGI1* is a newly found gene that regulates intercellular adherence, locates at 3p14.1, and encodes a scaffolding protein which belongs to the membrane-associated guanylate kinase homologue (MAGUK) family[6]. *MAGI1* contains several protein structural domains like SH3, WW and PDZ domain[7]. It has been proven that MAGI1 can recruit PTEN to the junctional complex, stabilize cell-to-cell conjunction and prevent cell dissociation[3,8]. Previously, we have found that MAGI1 expression was significantly decreased in HCC, and its expression level was closely correlated with HCC vascular invasion. Furthermore, the post-operative overall survival and disease-free survival of HCC patients with high MAGI1 expression were significantly shorter than those with low MAGI1 expression. The multivariable Cox regression analysis indicated that MAGI1 was an independent prognostic factor for HCC. These results suggested that MAGI1 might play a critical role in recurrence and metastasis of HCC.

PTEN, which locates at 10q23.3, containing 9 exons and 8 introns, encoded a protein containing 403 amino acids, and is a dual-specificity phosphatase[9]. Li, et al.[10] proved that PTEN can inhibit cell adherence, migration and infiltration by regulating substrates like focal adhesion kinase (FAK)

and Scr homology collagen protein through phosphorylation. Recent studies found that PTEN participated in the recurrence and metastasis regulation of several cancers. For example, colorectal cancer was closely related to PTEN[11]; low expression of PTEN was correlated with metastasis of gallbladder carcinoma and served as an prognostic fator[12]; the inactivation of *PTEN* gene was frequently observed in breast cancer and associated with its invasion and metastasis, but not angiogenesis[13]. In study conducted by Cheng, et al.[14], they detected the expression of PTEN protein in 62 paraffin-embedded HCC tissues using immunohistochemical staining, and found that the expression of PTEN was significantly decreased in HCC specimens, and was much higher in enveloped and non-invasive cancers than in non-enveloped and invasive ones, indicating that PTEN might play an important role in carcinogenesis and development of HCC.

However, the effect of MAGI1 and its mechanism in invasion and metastasis of HCC was still unknown. In our study, we proved that MAGI1 can inhibit the migration and invasion of HepG2 cells in vitro. Considering MAGI1 can combine PTEN, we investigated the relationship MAGI1 and PTEN. Our results showed that MAGI1 up-regulated PTEN expression, and there was postive correlation between MAGI1 and PTEN. The research from Kotelevets, et al.[3] showed that MAGI1 can recruit PTEN to the cell conjunction by combining with its PDZ domain, which locates in the C terminal of PTEN protein and play important rloe in regulating the degradation of PTEN protein[15]. So, MAGI1 probably up-regulates the expression of PTEN by combining with PTEN protein and inhibits its degradation. However, the detailed mechanism by which MAGI1 regulated PTEN expression in HCC need further investigation.

In a word, MAGI1 may inhibit invasion of HCC by regulating PETN, and serve as a new target for treating recurrence and metastasis of HCC.

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