Original Article

Effect of RAB25 gene on proliferation of human breast cancer cell line MCF-7 in vivo and in vitro

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Abstract: Rab25 is a newly discovered member of the Rab11 subfamily of the Rab protein family. The purpose of this study was to investigate the proliferation of derivatives of the human breast cancer cell line MCF-7 with different levels of expression of the Rab25 gene and their tumorigenicity in nude mice. The effect of different expression levels of the Rab25 gene on various biological activities of the human breast cancer cells was also investigated. Cells in the logarithmic phase of growth were harvested and subcutaneously implanted into nude mice to evaluate tumor induction. The mRNA expression of Rab25 in tumors in nude mice was evaluated using quantitative RT-PCR. The results showed that stable expression of the Rab25 gene after transfection significantly increased the proliferation activity of MCF-7 breast cancer cells. The tumorigenicity in nude mice was also increased. In addition, interference with the expression of the Rab25 gene significantly inhibited the proliferation of MCF-7 breast cancer cells and attenuated their tumorigenicity in nude mice. Finally, Rab25 was discovered to be an oncogene. A high level of expression could directly increase the proliferation and invasion abilities of breast cancer cells and their tumorigenicity in nude mice. The RNA interference technology is expected to provide new approaches for tumor targeted gene therapy of breast cancer.

Keywords: Breast cancer, Rab25 gene, human breast cancer cell line MCF-7, biological activity, nude mice

Introduction

The Rab25 protein is a newly discovered member of the Rab11 subfamily of the Rab protein family. The Rab25 gene is located at 1q21.2 and encodes a low molecular weight protein consisting of 213 amino acid residues [1]. Many domestic and international studies have shown that Rab25 expression is upregulated in a variety of epithelial malignant tumors. In recent years, expression of Rab25 has been extensively reported in breast cancer, gastric cancer, colon cancer, esophageal cancer, and ovarian cancer [2]. However, there are different opinions about the biological functions of Rab25 in breast cancer. Current studies are mostly at the cellular level; there are few studies of the effect of Rab25 on the occurrence and development of solid tumors. By upregulating and silencing Rab25 gene expression in MCF-7 breast cancer cells, we established a series of MCF-7 derivatives with different Rab25 expression levels to observe the effect of Rab25 expression on breast cancer xenografts in nude mice and to investigate the effect of the Rab25 gene on the proliferation of the cells in vivo and in vitro.

Materials and methods

Materials

E. coli DH-5α competent cells and the In-Fusion PCR Cloning Kit (which provided linearized pDNR-Dual plasmid and Sall and HindIII restriction endonucleases) were purchased from Clontech. The human MCF-7 breast cancer cell line was purchased from the Cell Bank of the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. The MCF-7 cells transfected with the recombinant plasmid pDNR-Dual-Rab25, the MCF-7 cells transfected with Rab25 siRNA, and the MCF-7 cells transfected with a pDNR-Dual empty vector were cryopreserved in the Laboratory for Gastrointestinal Gland Surgery, First Affiliated Hospital of Guangxi Medical University [3]. SPF grade BALB/c nude mice at four to six weeks of age with a body weight of 18-22 g were purchased.
from the Shanghai Experimental Animal Center, Chinese Academy of Sciences. The PCR reagent kit was purchased from Promega. The Trizol RNA extraction kit, the Lipofectamine™ 2000 transfection reagent kit, and the primers for Rab25 mRNA and β-actin were purchased from Invitrogen. The primers for Rab25 DNA were synthesized by Shanghai Sangon Biotech.

Cell culture technique and methods

A series of cell lines previously constructed by our group, including normal parental MCF-7 cells, Rab25 transfected cells, cells transfected with Rab25 siRNA, and cells transfected with the empty vector were recovered from storage [3]. The cells were cultured and passaged, and some cells were prepared as cell suspensions to detect cell proliferation and adjust cell concentrations. Cell suspensions were centrifuged at a low speed, mixed thoroughly with DMEM, and transferred to a 100 ml culture flask. Cells were cultured in a 5% CO₂ incubator at 37°C. The culture medium was replaced the next day, and the incubation was continued. After the cell confluence reached 90%, the cells were subcultured. After several passages, some cells were cultured for amplification and some cells were cryopreserved to ensure a source of cells to facilitate subsequent studies.

Detection of Rab25 in the cell lines studied

The Trizol method was used for the extraction of total RNA from MCF-7 and the three derivatives. The target fragment (152 bp) of the Rab25 gene was amplified using the RT-PCR reagent kit as shown below. Primers were designed according to the Rab25 coding sequences (GM ID57111) in GenBank: upstream, 5′-CCATCACCTCGGCGTACTATC-3′ and downstream, 5′-TTTGTTACCCACGAGCATGAC-3′. RT-PCR products from the four cell lines were detected using 1% agarose gels.

Amplification of the full length Rab25 cDNA. Rab25 total RNA from the parental MCF-7 cells was used as a template to amplify the full-length Rab25 cDNA (649 bp). PCR primers were designed according to the Rab25 mRNA sequence (NCBI Accession No. NM_020387) provided by GenBank. The primers were as follows: upstream, 5′-AAGATGGGGAATGGAACTGA-3′; downstream, 5′-AAGGCTAGGCGCTGATGACA-3′. RT-PCR was performed, and the products were detected using 1% agarose gels.

Detection of cell proliferation activity using the MTT assay. The cells were divided into four groups: parental cells, cells transfected with the recombinant Rab25 plasmid, cells transfected with Rab25 siRNA, and cells transfected with the empty vector. Cells in the logarithmic phase of growth were prepared as single-cell suspensions using DMEM containing 10% fetal bovine serum (FBS) and inoculated onto 96-well culture plates. The blank control group was in serum-free L-15 culture medium. Each well contained 0.2 ml, and the culture time was one to six days, after which 20 µl 0.5% MTT was added. After 4 h, the supernatants in the wells were aspirated, and 150 µl DMSO was added to each well. The plates were vortexed for 10 min, and the absorbance value (A value) of each well was determined at 490 nm using a plate reader. Cell growth curves were plotted using time as the horizontal axis and A490 as the vertical axis. Experiments were repeated three times, and three replicate wells were used. The mean value was used as the final result.

Inoculation

A total of 32 four- to six-week-old female BALB/c nude mice with a body weight of 18-22 g were randomly divided into four groups with eight animals in each group. Each group was inoculated with parental MCF-7 cells, Rab25 transfected cells, Rab25 siRNA transfected cells, or empty vector transfected cells. The skin of the axilla of each mouse was disinfected using povidone-iodine complex. Tumor cell suspensions were aspirated using a syringe with a no. 6 needle and injected subcutaneously into the axilla of each nude mouse. Each inoculation site was injected with 0.2 ml containing approximately 10⁷ live cells. After the needle was withdrawn, the site was clipped using tweezers to prevent leakage of tumor cells.

Observation and measurement

The behavior, feeding, and activity of the mice were regularly observed every day. The length and the maximum width of each tumor nodule were measured using a Swiss WHB vernier caliper. After six weeks of observation, the mice were sacrificed, and the tumors were collected, weighed, and recorded. The maximum diameter, a, and the minimum diameter, b, were measured and the tumor volume was calculated based on the formula: V (mm³) = a*b²/2. The
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tumor volume was calculated, and the growth curves of the xenograft tumors were plotted. The results were statistically analyzed.

Calculation and fluorescence analysis of quantitative RT-PCR standard curves of Rab25 mRNA in xenograft tumors in a series of nude mice.

The cDNA from MCF-7 cells that showed Rab25 gene expression was removed from a -80°C ultra-low freezer and used as a standard. β-Actin was used as the quantitative template for the internal control. The following primers were used:

Rab25: upstream, 5'-GGGTTGAGGTCATTGAGC-3', downstream, 5'-GAGGTATTTGTGATAGGGCA-3', probe, 5'-AGATTGGTCTTCCCCACACTGATT-3'; β-actin: upstream, 5'-GTCACTACCATGCGCAATGAG-3', downstream, 5'-CGTCACACTTCTAGATGGAGTT-3', probe, 5'-TCCTGGCCATGGAGTGATCC-TG-3'.

The amplification length was 881 bp. The 5' and 3' ends of the probes were labeled with fluorescein FAM and TAMRA, respectively. The standard cDNA and the internal control template (β-actin) were diluted at the ratios of 1:10-10^9. The reactions contained 25 μl, including 10 μl buffer, 1 μl of primers, fluorescence probe, and dNTPs, 2.5 U Taq polymerase, and 2 μl cDNA from the reverse transcribed products (equivalent to 100 ng). Real-time quantitative RT-PCR was performed. The reaction conditions were 94°C denaturation for 4 min followed by 40 cycles of 94°C for 30 s, 55°C for 30 s, and 70°C for 30 s. With the temperature controlled at 79°C, the A_{260}/A_{280} absorbance values of RNA were measured. The amplification curves were plotted using the number of cycles as the horizontal axis and the values of the fluorescence signals as the vertical axis. The threshold values (CT, the minimum number of cycles required for the fluorescence signal in the reaction tube to reach the set threshold value) were confirmed according to the amplification curve. Two standard curves were plotted using logarithmic values of the gradient concentrations of Rab25 mRNA and β-actin as the horizontal axis and CT values as the vertical axis. The levels of Rab25 and β-actin in the samples were calculated from the standard curves according to the CT values. Three duplicate tubes were used for each sample for detection, and the mean value was calculated.

The double standard curve method was performed for relative quantitation. The levels of the Rab25 gene and the internal quantitative template β-actin were measured to estimate the level of Rab25 gene relative to β-actin. Finally, comparisons of the relative levels of the samples in all the experimental groups were performed, and the Rab25 mRNA expression in the tumors was determined. In addition, the CT values for the samples and the β-actin internal control were compared to ascertain whether the linear amplification efficiency (E) of the two genes was basically consistent (E = 10^{-1/K-1}), where K is the slope of the standard curve, and to control for possible experimental error.

Statistical methods
The data are presented as the mean ± the standard deviation (X ± s). The SPSS13.0 software was used for the analysis of variance (ANOVA) and for the t test. Values of P < 0.05 indicated statistical significance.

Results
Observation under a microscope of the four groups of cells
The MCF-7 cell line and three derivatives prepared in previous studies were recovered from storage and cultured. Cells from the parental MCF-7 cell line, cells transfected with Rab25, and cells transfected with the empty vector were viewed with an inverted microscope and did not show any significant differences. These cells all showed monolayer growth, regular morphology, and polygonal or long spindle shapes. The cell nuclei were round or oval, the cytoplasm was transparent and bright, and the dendrites were long. However, cells transfected with Rab25 siRNA showed irregular shapes, increased cell body size, wider intercellular spaces, decreased brightness of the cytoplasm, increased number of dendrites (which were short and thick), and decreased cell attachment, indicating a decrease in cell growth (Figure 1).

Analysis of Rab25 expression in MCF-7 and its derivatives
After RT-PCR, the target fragment (152 bp) of the Rab25 gene was not amplified in the MCF-7 cells transfected with Rab25 siRNA, whereas it was amplified in the MCF-7 cells transfected
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Figure 1. A: Cells from the parental MCF-7 cell line, cells transfected with Rab25, and cells transfected with the empty vector were viewed with an inverted microscope and did not show any significant differences. These cells all showed monolayer growth, regular morphology, and polygonal or long spindle shapes. The cell nuclei were round or oval, the cytoplasm was transparent and bright, and the dendrites were long. B: Cells transfected with Rab25 siRNA showed irregular shapes, increased cell body size, wider intercellular spaces, decreased brightness of the cytoplasm, increased number of dendrites (which were short and thick), and decreased cell attachment, indicating a decrease in cell growth.

Amplification of full-length of Rab25 cDNA

The total RNA of MCF-7 cells was extracted and used as the template. As shown in (Figure 3A), the brighter band was the 28S RNA, and the relatively weaker band below it was the 18S RNA. This result indicated that the extracted RNA was intact and without DNA contamination. After RT-PCR amplification, a single specific band (649 bp) was observed (Figure 3B), which was consistent with the length of the Rab25 gene sequence provided by GenBank.

Results of cell proliferation assays

Absorbance values (A values) of the cultured cells were measured. The A values for MCF-7 cells with different levels of Rab25 are shown in (Table 1). The growth curve of each cell line was plotted based on the A values (Figure 4). Figure 4 shows that the proliferation of cells transfected with Rab25 increased rapidly starting from day 3 (P < 0.05) and that the proliferation of cells transfected with Rab25 siRNA was significantly slower (P < 0.05). The proliferation of cells transfected with the empty vector was not significantly different from that of the parental cells or cells transfected with Rab25 siRNA (P > 0.05).

Growth of the different cell lines in nude mice

The tumor incidence in mice for all the cell lines after inoculation was 100%. The incubation
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Figure 3. A: Electrophoresis results of total RNA extracted from MCF-7 cells. B: Electrophoresis results of the PCR products of the Rab25 cDNA which show that the extracted total RNA was intact and without DNA contamination. B indicates that the length of the detected gene was consistent with the Rab25 gene sequence provided by GenBank.

Table 1. Detection by absorbance of the effect of Rab25 on MCF-7 cells (X ± s)

<table>
<thead>
<tr>
<th>Groups</th>
<th>1 d</th>
<th>2 d</th>
<th>3 d</th>
<th>4 d</th>
<th>5 d</th>
<th>6 d</th>
<th>7 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal parental MCF-7 cells</td>
<td>0.15 ± 0.02</td>
<td>0.18 ± 0.03</td>
<td>0.22 ± 0.02</td>
<td>0.26 ± 0.01</td>
<td>0.32 ± 0.01</td>
<td>0.28 ± 0.02</td>
<td>0.26 ± 0.03</td>
</tr>
<tr>
<td>Rab25 transfected cells</td>
<td>0.15 ± 0.02</td>
<td>0.18 ± 0.03</td>
<td>0.34 ± 0.01</td>
<td>0.46 ± 0.02</td>
<td>0.54 ± 0.03</td>
<td>0.62 ± 0.02</td>
<td>0.58 ± 0.01</td>
</tr>
<tr>
<td>Cells transfected with Rab25 siRNA</td>
<td>0.16 ± 0.02</td>
<td>0.17 ± 0.02</td>
<td>0.19 ± 0.04</td>
<td>0.23 ± 0.03</td>
<td>0.24 ± 0.02</td>
<td>0.20 ± 0.01</td>
<td>0.21 ± 0.03</td>
</tr>
<tr>
<td>Cells transfected with the empty vector</td>
<td>0.16 ± 0.03</td>
<td>0.18 ± 0.02</td>
<td>0.22 ± 0.03</td>
<td>0.26 ± 0.02</td>
<td>0.33 ± 0.02</td>
<td>0.29 ± 0.01</td>
<td>0.31 ± 0.01</td>
</tr>
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</table>

Figure 4. Growth curves of all the cell lines based on A values. The proliferation of cells transfected with Rab25 was significantly more rapid than that of cells transfected with the empty vector, the parental cell line group, or the interference group.

The incubation period for tumor growth in mice of cells transfected with Rab25 was approximately 14-15 days. The xenograft tumors in the nude mice were observed every day, and tumor diameters were measured once every week. The results showed that the tumors in the mice inoculated with the parental cell line, with cells transfected with Rab25, or with cells transfected with the empty vector gradually increased with time and that there were local ulcers. In contrast, the mice inoculated with cells transfected with Rab25 siRNA only developed small, palpable subcutaneous tumors,
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and the tumor growth was slow (Figure 5). The animals were sacrificed after six weeks. The mean tumor volumes of mice inoculated with the parental cell line, cells transfected with Rab25, or cells transfected with the empty vector were all larger than 100 mm$^3$, whereas the mean tumor volume of mice inoculated with cells transfected with the Rab25 siRNA was <250 mm$^3$. The records of the tumor weights and volumes of the xenograft tumors from the nude mice in each group are shown in (Table 2). Growth curves of the xenograft tumors in nude mice with different Rab25 expression levels are plotted according to data from the weekly measurements (Figure 6).

Figure 5. Gross morphology of xenograft tumors in nude mice induced by a series of breast cancer cells at week 6. A: Parental cell line group; B: Transfection group; C: Interference group; D: Empty vector group. It was visible to the unaided eye that the transfection group had the largest tumors.

Table 2. Weight and volume of xenograft tumors in nude mice in all groups ($\bar{x} \pm s, n = 8$)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Volume (mm$^3$)</th>
<th>Tumor weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal parental MCF-7 cells</td>
<td>1073.12 ± 419.59</td>
<td>1.196 ± 0.307</td>
</tr>
<tr>
<td>Rab25 transfected cells</td>
<td>2232.25 ± 621.56</td>
<td>1.855 ± 0.346</td>
</tr>
<tr>
<td>Cells transfected with Rab25 siRNA</td>
<td>246.50 ± 73.83</td>
<td>0.656 ± 0.053</td>
</tr>
<tr>
<td>Cells transfected with the empty vector</td>
<td>1052.60 ± 336.75</td>
<td>1.146 ± 0.185</td>
</tr>
</tbody>
</table>

Note: Results were analyzed using analysis of variance (ANOVA) for completely randomized design. Pairwise comparison of volume and weight of xenograft tumors was performed using the LSD method. N: number of samples. Significance level: $\alpha = 0.05$. F = 31.362 and 30.827. Comparison between the transfection group and the parental cell line group, $P < 0.01$; comparison between the interference group and the parental cell line group, $P < 0.01$; comparison between the empty vector transfection group and the parental cell line group, $P > 0.05$.

Quantitative RT-PCR measurements of Rab25 expression levels in xenograft tumors induced by derivatives of MCF-7

Reactions with four groups of samples and the standard were performed at the same time. Each sample was measured three times. The mean CT values obtained for Rab25 mRNA and
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β-actin were separately introduced into the regression equation of the standard curve to calculate the individual amount of the initial template. β-actin was used as the control reference gene to normalize the results from all the samples (RNA calibration). The quantitative result for Rab25 mRNA divided by the quantitative result for β-actin was the calibrated value. To facilitate the comparison of expression levels of samples between groups, the Rab25 mRNA expression level in tumors from mice inoculated with the parental cell line was set to “1” to calculate the relative levels in other groups. Comparisons of Rab25 mRNA expression levels in samples from the different groups were then performed (Table 3). Finally, to present the differences in relative expression levels of samples in all the groups more intuitively, the calculated relative levels were plotted in histograms (Figure 7). As shown in Figure 7, the Rab25 mRNA expression level in the tumors from mice inoculated with cells transfected with Rab25 was 4.986 times that in tumors from mice inoculated with the parental cell line. The Rab25 mRNA expression level in tumors from mice inoculated with cells transfected with Rab25 siRNA was only 0.082 times that in tumors from mice inoculated with the parental cell line. The expression levels in tumors from the empty vector group and the parental cell line group were similar (0.993).

### Discussion

The occurrence and development of breast cancer is a process involving multiple genes and multiple steps. Currently, studies of gene mutations in p53, BRCA1, and BRCA2 only explain some of the etiology of breast cancer; there may be more unknown genes participating in the development of breast cancer. The study of Calero et al. showed that the Rab25 gene might be associated with the development and invasion of breast cancer [4]; however, the biological function of Rab25 in the occurrence and development of breast cancer is still unknown. The groups of Cheng KW and Cheng JM published different results on the effect of Rab25 on the development of breast cancer. Cheng et

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**Table 3.** Data for the real-time quantitative PCR standard curve of Rab25 mRNA and β-actin in xenograft tumors in nude mice

<table>
<thead>
<tr>
<th></th>
<th>MCF-7/Rab25 mRNA</th>
<th>MCF-7/β-actin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CT</td>
<td>K</td>
</tr>
<tr>
<td>Normal parental MCF-7 cells</td>
<td>24.66</td>
<td>-3.49</td>
</tr>
<tr>
<td>Rab25 transfected cells</td>
<td>14.92</td>
<td>-3.48</td>
</tr>
<tr>
<td></td>
<td>14.55</td>
<td>-3.42</td>
</tr>
<tr>
<td></td>
<td>15.12</td>
<td>-3.49</td>
</tr>
<tr>
<td>Cells transfected with Rab25 siRNA</td>
<td>36.49</td>
<td>-3.41</td>
</tr>
<tr>
<td></td>
<td>36.15</td>
<td>-3.40</td>
</tr>
<tr>
<td></td>
<td>36.24</td>
<td>-3.47</td>
</tr>
<tr>
<td>Cells transfected with the empty vector</td>
<td>24.52</td>
<td>-3.50</td>
</tr>
<tr>
<td></td>
<td>24.76</td>
<td>-3.43</td>
</tr>
<tr>
<td></td>
<td>24.32</td>
<td>-3.46</td>
</tr>
</tbody>
</table>

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**Figure 6.** Growth curves of xenograft tumors in nude mice with different Rab25 expression levels. The figure shows that the xenograft tumor volume in nude mice induced by cells from the transfection group was significantly higher than in the empty vector group, the parental cell line group, or the interference group.
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al. [5] considered that increased DNA copy numbers and RNA levels of the Rab25 gene decreased the disease-free survival and the overall survival of breast cancer patients. In cell experiments, overexpression of Rab25 increased the proliferation activity and invasiveness of breast cancer cells. However, the study of Cheng et al. [6] showed that overexpression of Rab25 in breast cancer cell lines inhibited cell growth. In addition, detection of expression in mammary tissues using RT-PCR showed that Rab25 was extensively expressed in normal mammary tissues and that approximately 33% of breast cancer tissues lost expression. These results indicated that loss of Rab25 might play an important role in the pathogenic process of breast cancer. Since then, studies of the biological behavior of the Rab25 gene in breast cancer have been gradually implemented.

Although studies of the function of Rab25 protein were begun 30 years ago, its function is currently not completely clear. Rab25 is expressed in a variety of tumor cells as well as some tissues, including gastrointestinal mucosa, lung, kidney, and mammary gland. Culiné et al. [7] showed that Rab25 was upregulated in a variety of epithelial malignant tumors. Cao et al. [8] detected Rab25 protein expression in gastric cancer tissues using immunohistochemistry and showed that Rab25 protein expression in gastric cancer tissues was higher than in adjacent tissues, the expression in stage III-IV patients was higher than in stage I-II patients, and the expression in patients with lymph node and distant metastasis was higher than in patients without metastasis; the differences were all statistically significant. Interference with Rab25 expression in MGC80-3 cells using siRNA significantly decreased the invasion ability of the cells. The study indicated that high levels of Rab25 expression could promote the occurrence and development of gastric cancer. The study of Li [9] confirmed that high levels of Rab25 expression were closely associated with the classification of renal cell carcinoma invasion, lymph node metastasis, and pathological stage. Interference with Rab25 protein expression could inhibit renal cell carcinoma proliferation, migration, and invasion. However, the study of Tong et al. showed the opposite results. In esophageal squamous cell carcinoma, Tong et al. [10] showed that Rab25 was a tumor suppressor. Real-time quantitative PCR and immunohistochemistry results showed that Rab25 expression in esophageal squamous cell carcinoma was significantly downregulated compared with that in non-tumor tissues. Hypermethylation of the promoter region of the Rab25 gene in esophageal cancer caused the decrease or loss of the expression of this gene in tumor tissues. In addition, the loss of Rab25 expression in nude mice inhibited tumor development and angiogenesis. The Rab25 gene mainly exerts its biological function through the MAPK/ERK signal transduction pathway. The study of Nam [11] in nude mice showed that loss of Rab25 expression in nude mice could promote the occurrence and development of intestinal cancer, which resembles colorectal cancer in humans.

For mammary glands, Gonzalez-Angulo [12] used reverse phase protein array (RPPA) and showed that the combination of three factors, CHK1pS345, Caveolin1, and Rab25, in patients with primary breast cancer recurrence could be used as an independent prognosis model. In

Figure 7. To present the differences of relative expression levels in xenograft tumor samples from nude mice more intuitively, the calculated relative levels are presented as histograms.
addition, different levels of Rab25 expression had significantly different 3-year recurrence-free survival rates, and the Rab25 overexpression group had the worst prognosis. However, the subsequent study of Cheng et al. [13] showed that Rab25 overexpression had significant inhibitory effects on the development of triple negative breast cancer. Chen et al. [14] also showed that Rab25 gene could reduce the invasiveness of breast cancer and that Rab25 was a tumor suppressor gene. Currently, there is no consistent conclusion about changes in the proliferation activity and invasiveness of tumor cell lines caused by low or high levels of Rab25 expression. In this study, a series of experiments was performed to understand the changes in proliferation activity and invasiveness of a breast cancer cell line by silencing or expressing high expression levels of Rab25. In addition, the influence of Rab25 on solid tumor formation and whether Rab25 could be an independent prognostic factor and a new therapeutic target for breast cancer were studied. A series of MCF-7 breast cancer cell lines with different Rab25 expression levels were constructed. Rab25 gene expression in the parental MCF-7 cells, cells transfected with Rab25, and cells transfected with the empty vector was confirmed by many repetitions of RT-PCR. Cells transfected with Rab25 siRNA showed little or no Rab25 expression. Breast cancer cell line proliferation was detected using the MTT assay. Statistical analysis showed that the proliferation activity of MCF-7 breast cancer cells significantly increased after the transfected Rab25 gene was stably expressed, suggesting that upregulation of Rab25 gene expression increased the proliferation of breast cancer cells. In addition, after interference with Rab25 gene expression, the proliferation activity was significantly inhibited, which allowed us to confirm from another angle that downregulation of the Rab25 gene attenuates the proliferation ability of breast cancer cells.

Rab25 participates not only in tumor cell proliferation but also in solid tumor formation. In this study, after breast cancer cells with different Rab25 expression levels were transplanted into 32 nude mice, all the mice developed tumors. After six weeks of observation, the mean tumor volumes in the mice in the parental cell line group, the transfection group, and the empty vector group were all larger than 1000 mm$^3$. With the progression of time, the tumors gradually became larger, and the tumors in some nude mice showed local ulcers and necrosis; however, after the mice in the interference group were observed for six weeks, only palpable subcutaneous tumors with smaller volumes developed. The mean tumor volume was < 250 mm$^3$, and the tumors no longer grew with the progression of time but showed signs of decreasing in size. In addition, Rab25 mRNA expression in the tumors was detected quantitatively using real-time quantitative PCR. The results showed that the Rab25 mRNA expression level in the tumors in the transfection group was significantly higher than that in the parental cell line group, whereas the Rab25 mRNA expression level in the tumors in the interference group was significantly lower than that in the parental cell line group. These results suggested that high levels of Rab25 expression promoted the formation and progression of xenograft tumors in nude mice, while inhibition of Rab25 expression could effectively inhibit the growth and formation of tumors in nude mice.

In summary, changes in a variety of biological characteristics in MCF-7 cells and xenograft tumors in nude mice suggested that high levels of Rab25 expression played a role in the promotion of growth and the increase in the malignancy of breast cancer cells as well as in the promotion of the development and progression of solid tumors. The inhibition of Rab25 expression significantly decreased the proliferation activity of breast cancer cells and effectively inhibited the growth and formation of xenograft tumors. These results suggested that the Rab25 gene is an oncogene for breast cancer. High Rab25 gene expression levels resulted in disorders of the biological behavior of breast cancer cells, i.e., transport disorders of cytoplasmic vesicles; therefore, relevant functional proteins could not accurately reach the target location, and disorders of normal proliferation, differentiation, and metabolism of the cells occurred, which finally caused malignant changes of the cells and typical tumor cell features of malignant proliferation and invasion. These results were consistent with the studies of Gonzalez-Angulo et al. and Cheng et al. Therefore, although it has been confirmed that Rab25 participates in the process of transport and endocytosis in cells, more studies are needed.
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required to elucidate and confirm how Rab25 and the related biological processes exert their roles in tumor formation, ascertain whether Rab25 is an oncogene or a tumor suppressor gene, and determine the intermediate, complex mechanisms involved. These goals can be used as the direction of future studies, which are expected to provide new approaches to determine the role of Rab25 gene in the signaling transduction pathways in breast cancer and cancer therapy.

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Disclosure of conflict of interest

None.

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