Transgelin interacts with PARP1 and affects Rho signaling pathway in human colon cancer cells

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Abstract

**Background:** Transgelin, an actin-binding protein, is associated with the cytoskeleton remodeling. Our previous studies found that transgelin was up-regulated in node-positive colorectal cancer versus in node-negative disease. Over-expression of *TAGLN* affected the expression of 256 downstream transcripts and increased the metastatic potential of colon cancer cells in vitro and in vivo. This study aims to explore the mechanisms that transgelin participates in the metastasis of colon cancer cells.

**Methods:** Immunofluorescence and immunoblotting analysis were used to determine the cellular localization of the endogenous and exogenous transgelin in colon cancer cells. Co-immunoprecipitation and subsequent high performance liquid chromatography/tandem mass spectrometry were performed to identify the proteins potentially interacting with transgelin. Bioinformatics methods were used to analyze the 256 downstream transcripts regulated by transgelin to discriminate the specific key genes and signaling pathways. By analyzing the promoter region of these key genes, GCBI tools were used to predict the potential transcription factor(s) for these genes. The predicted transcription factors were matching to the proteins that have been identified to potentially interact with transgelin. The interaction between transgelin and these transcription factors was verified by co-immunoprecipitation and immunoblotting.

**Results:** Transgelin was found to localize both in the cytoplasm and the nucleus of colon cancer cells. 297 proteins have been identified to interact with transgelin by co-immunoprecipitation and subsequent high performance liquid chromatography/mass spectrometry. Over-expression of *TAGLN* could lead to differential expression of 184 downstream genes. By constructing the network of gene-encoded proteins, 7 genes (*CALM1*, *MYO1F*, *NCKIPSD*, *PLK4*, *RAC1*, *WAS* and *WIPF1*) have been discriminated as key genes using network topology analysis. They are mostly involved in the Rho signaling pathway. Poly ADP-ribose polymerase-1 (PARP1) was predicted as the unique transcription factor for the key genes and concurrently matching to the DNA-binding proteins potentially interacting with transgelin. Immunoprecipitation validated that PARP1 interacted with transgelin in human RKO colon cancer cells.

**Conclusions:** The results of this study suggest that transgelin binds to PARP1 and regulates the
expression of the downstream key genes mainly involving Rho signaling pathway, thus participates in the metastasis of colon cancer.

**Background**

Colorectal cancer is a frequent malignant tumor in the gastrointestinal tract worldwide. Although the mechanisms of its tumorigenesis and metastasis have been extensively studied, it continues to have a high mortality rate, especially in patients with advanced disease[1]. Cytoskeleton plays a major role in cell migration. Once the expression of related genes or effector proteins is abnormal, it will lead to the activation of various signaling pathways, thus promoting the metastasis of tumors[2–5].

In our previous study, transgelin was found up-regulated in node-positive colorectal cancer versus in node-negative disease[6]. Transgelin (also known as 22 kDa actin-binding protein, protein WS3-10 or smooth muscle protein 22 alpha) has a molecular weight of 23 kDa and consists of 201 amino acids. It is encoded by TAGLN gene and is composed of an N-terminal calmodulin homologous (CH) domain and a C-terminal calmodulin like (CLIK) domain, which is closely related to actin binding activity[7]. It broadly expresses in vascular and visceral smooth muscle and is an early marker of smooth muscle differentiation[8]. Furthermore, it is associated with the remodeling of cytoskeleton in the cytoplasm. Therefore, we believe that it may serve as a biomarker for tumor metastasis.

In addition, we found that up-regulation of transgelin promoted the metastasis of colon cancer cells, while down-regulation substantially decreased the ability of cell invasion and metastasis[6, 9, 10].

Previously, gene expression profiling identified that over-expression of TAGLN affected the expression of 256 downstream transcripts, which were closely related to cell morphology, migration and invasion[9]. We also found that transgelin had nuclear localization in colon cancer cells[6]. Thus, we speculate that transgelin may play distinct roles in the nucleus and the cytoplasm, which collaboratively participate in the invasion and metastasis of colon cancer cells.

In this study, we confirmed the nuclear localization of transgelin in different colon cancer cell lines. Immunoprecipitation and high performance liquid chromatography/mass spectrometry found that 297 proteins could potentially interact with transgelin. Among these, 23 were DNA-binding proteins. We then analyzed the downstream target genes affected by over-expression of TAGLN and identified the
key genes using bioinformatics techniques. By analyzing the promoter regions of these key genes, a DNA-binding protein, poly ADP-ribose polymerase-1 (PARP1) has been predicted to be the transcription factor of the key genes. It also fell in the list of 23 DNA-binding proteins that have been detected to interact with transgelin. We then verified the interaction between transgelin and PARP1 by immunoprecipitation.

Materials And Methods

Cells

The human CRC cell lines RKO, SW480, HCT116 and LOVO were provided by Stem Cell Bank, Chinese Academy of Sciences (Shanghai, China). Cells were cultured in minimum Eagle’s medium (MEM, Gibco, USA), Mccoy’s 5A medium (Gibco, USA) and Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco, USA) with 10% fetal bovine serum (Gibco, USA). Cells were grown in an incubator at 37°C with 5% CO2.

Immunofluorescence

Localization of endogenous transgelin in RKO, SW480, HCT116 and LOVO cell lines was determined by immunofluorescence. The primary antibody (anti-transgelin, 1:500, Abcam, USA), secondary antibody (Alexa Flour 594 goat anti-rabbit IgG, 1:500, Invitrogen, USA), and the VECTASHIELD mounting medium (Vector Laboratories, USA) with 4′,6-diamidino-2-phenylindole (DAPI) were used. The immunofluorescence images were taken and preserved under the laser scanning confocal microscope (63× oil lens, Carl Zeiss, USA).

Transfection

SW480 and RKO cells were cultured in 12-well plates and transfected with pcDNA6/myc-His B-TAGLN-flag plasmid and pcDNA6/myc-His B-flag plasmid (Takara, Japan). In the validation experiment, we transfected the RKO cells with pENTER-TAGLN-Flag and pENTER-Flag control plasmid (Vigene Biosciences, USA). Transfection was conducted using Lipofectamine 2000/ Lipofectamine 3000 (Thermo Fisher Scientific, USA). The cells were harvested at 48 hours after transfection for further analysis.

Immunoblotting
Nuclear and plasma proteins from HCT116, SW480, LOVO and RKO cell lines were extracted using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific, USA). The cytoplasmic protein and nuclear protein extracted above were determined for protein concentration. Immunoblotting was carried out with the primary antibody anti-transgelin (1:500, Abcam, USA, or 1:500, R&D, USA), anti-GADPH (1:400, Abcam, USA or 1:500, Cell signaling technology, USA), anti-PARP1 (1:500, Cell signaling technology, USA), anti-Lamin B1(1:1000, Cell signaling technology, USA), anti-flag (1:500, Cell signaling technology, USA) and the secondary antibody (Horseradish Peroxidase (HRP)-conjugated goat anti-rabbit or anti-mouse IgG, 1:30000, Sigma-Aldrich, USA) or IgG Detector (IgG Detector Solution v2, HRP labeled, 1:1000, Takara, Japan). Antibody detection was performed using a chemiluminescence substrate and the protein bands were visualized with Syngene G:BOX Chemi XT4 fluorescence and chemiluminescence gel imaging system (Cambridge, UK).

**Immunoprecipitation**

RKO and SW480 cells were cultured conventionally and transfected with pcDNA6/ myc-His B-TAGLN-flag and pcDNA6/ myc-His B-flag plasmids. In the validation experiment, RKO cells were transfected with pENTER-TAGLN-Flag and pENTER-Flag control plasmids. After 48 hours, the culture medium was removed. According to the protocol of the Pierce Crosslink Immunoprecipitation Kit (Thermo Fisher Scientific, USA), antibody immobilization, cell lysis, pretreatment of cell lysate with control agarose resin, immunoprecipitation, immunoprecipitation elution, and immunoblotting analysis were performed in sequence. Anti-flag antibody (10ug, Sigma-Aldrich, USA for the subsequent mass spectrometry; 1:50, Cell signaling technology, USA for the validation experiment) and the control rabbit IgG (1:50, Cell signaling technology, USA) were used.

**Mass spectrometry**

A fraction of the protein samples after immunoprecipitation were handled by SDS-PAGE and silver staining. Another fraction of the samples was loaded for high performance liquid chromatography (EASY-nLC™, Thermo Fisher Scientific, USA) after filtered aided proteome preparation (FASP) and enzymatic hydrolysis. The samples were then analyzed by Q-Exactive Mass Spectrometer (Thermo Finnigan, USA). The mass charge/ratio of peptides and fragments of peptides were collected.
Maxquant 1.3.0.5 software was used to retrieve the Uniprot database by using the raw file as source. The search in the database was set up with specific parameters (Enzyme, trpsin; De-Isotopic, True; Max Missed Cleavages, 2; Fixed modifications, Carbamidomethyl (C); Variable modifications, Oxidation (M); First search ppm, 20ppm; Main search ppm, 6ppm; Decoy database pattern, reverse; Min. Reporter PIF, 0.75; Peptides false discovery rate (FDR) ≤0.01; Protein FDR≤0.01).

Bioinformatics

Identification of differential expression genes (DEGs), functional enrichment and signaling pathway enrichment analysis

According to our previous work[9], the relevant cDNA microarray data was obtained using Affymetrix microarray technique. Over-expression of TAGLN in RKO human colon cancer cells led to a total of 256 downstream transcripts that were differentially expressed with at least a 2-fold change (P<0.05). Among these, transcripts without gene symbols, gene database codes and duplicates were excluded. The remaining DEGs were screened for further bioinformatics analysis. Using the Metascape tool (www.metascape.org/), the screening parameters were set as the following: P < 0.01 or 0.001 (Biological Process), participating genes ≥3, and enrichment factor > 1.5. We conducted functional and signaling pathway enrichment analysis of the DEGs referring to the gene ontology (GO) database, Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway and Reactome Gene Sets databases.

Construction of the protein-protein interaction (PPI) network, topological analysis and key gene screening

The DEGs were simultaneously translated into proteins while STRING 10.0 (https://string-db.org/) [11] was used for PPI analysis. Subsequently, relevant data was imported into Cytoscape online software (www.cytoscape.org/) [12] and a PPI network was constructed. In this study, CytoHubba plug-ins were used to calculate the degree centrality and intermediate centrality of the DEGs. Those with values that are 2-fold higher than the overall average value were selected as the core genes in the network. In addition, we obtained the core modules by using an MCODE plug-in (k-core=2), and defined the core genes and the genes included in the core modules as key genes. Key genes were further
analyzed with Metascape for signaling pathway enrichment in KEGG Pathways and Reactome Gene Sets database using the same parameters mentioned above.

**Prediction of the transcription factors for the key genes**
The transcription factor (TF) evaluation model within the GCBI tools (https://www.gcbi.com.cn/) was used to predict the TFs for the key genes. Those with medium or high recommendation were selected, and the potential TFs were selected for further analysis. We then compared these potential TFs to the DNA-binding proteins identified in the mass spectrometry analysis.

**Nuclear localization signal analysis**
The sequences of selected potential TF(s) were obtained from Uniprot database (https://www.uniprot.org/)[13]. The cNLS Mapper (www.nls-mapper.iab.keio.ac.jp/) [14] was used to detect the nuclear localization signal of the potential TF(s).

**Statistics**
The statistical analysis was carried out by SPSS 20.0 software. The relevant values were expressed as mean ± standard deviation, and the significance of the differences between two groups was determined by Student’s t test. P<0.05 (bilateral) was considered to be statistically significant.

**Results**

**Localization of transgelin in human colon cancer cell lines**
The expression of transgelin in colon cancer cell lines (HCT116, SW480, RKO and LOVO) was detected by immunofluorescence and immunoblotting analysis. Both cytoplasmic and nuclear localization of endogenous transgelin were observed (Figure 1A and 1B). Further, pcDNA6/myc-His B-TAGLN-flag plasmid and pcDNA6/myc-His B-flag control plasmid were transiently transfected into RKO and SW480 cells. Immunoblotting analysis showed that exogenous transgelin-flag protein could be detected both in the cytoplasm and the nucleus of the associated RKO and SW480 cells, although mainly in the cytoplasm (Figure 1C). In RKO-TAGLN-FLAG cells, the expression of transgelin-flag protein (1.00±0.05) was significantly increased compared with the control group (0.13±0.03, P<0.0001, Figure 2A) and the wild type (WT) RKO cells (0.08±0.02, P<0.0001).

**Identification of proteins potentially interacted with transgelin in RKO cells**
To explore the proteins that were potentially interacting with transgelin, we performed immunoprecipitation in RKO-TAGLN-FLAG and control cells using anti-flag monoclonal antibody. As shown in Figure 2B, we observed a clear band in RKO-TAGLN-FLAG group, ranging from 20.1 to 31kb in the silver staining gel. To further identify the proteins in the samples, we performed the high performance liquid chromatography coupled with tandem mass spectrometry. Results showed that 725 proteins were identified in the RKO-CTRL-FLAG group, while 717 were in the RKO-TAGLN-FLAG group (Additional file 1, Additional file 2). We further analyzed the data in the two groups and found that 297 proteins were uniquely present in the RKO-TAGLN-FLAG group (Figure 2C, Supplementary Table 1 in Additional file 3). As shown in Figure 2D, gene ontology (GO) functional enrichment analysis suggested these 297 proteins in the RKO-TAGLN-FLAG group were mainly involved in translation, RNA processing, enzyme activity and cell junction adhering. Among these, 23 proteins were DNA-binding proteins (Table 1).

Table 1. DNA-binding proteins that were potentially interacted with Transgelin [FDR≤0.01]
| gi number | Name of the protein                                      | Molecular weight (Dalton) |
|-----------|---------------------------------------------------------|---------------------------|
| gi|124494254 | Proliferation-associated protein2G4                  | 43786                     |
| gi|114205460 | HIST1H2BC protein                                    | 13833                     |
| gi|21361745 | Spermatid perinuclear RNA-binding protein             | 73651                     |
| gi|4827071  | Cellular nucleic acid-binding protein                 | 19462                     |
| gi|156523968| poly (ADP-ribose) polymerase family, member 1 (PARP1)| 113084                    |
| gi|29612542 | Histone H2A                                           | 13162                     |
| gi|6912616  | Histone H2A                                           | 13508                     |
| gi|323650782| HMGA2 fusion protein                                  | 13811                     |
| gi|297262894| High mobility group protein                           | 12714                     |
| gi|4506491  | Replication factor C subunit 4                       | 36877                     |
| gi|4502747  | Cyclin-dependent kinase 9                            | 42777                     |
| gi|345783096| Barrier-to-autointegration factor                     | 10058                     |
| gi|7661672  | Polymerase delta-interacting                          | 42032                     |
| gi|98986457 | Host cell factor 1                                    | 208730                    |
| gi|32129199 | SAP domain-containing Ribonucleo protein              | 23670                     |
| gi|57530065 | CCR4-NOT transcription complex subunit 7             | 32744                     |
| gi|302699237| Eukaryotic translation initiation factor 4 gamma 1    | 158643                    |
| gi|5730027  | KH domain-containing, RNA-binding, signal transduction-associated protein 1 | 48226                    |
| gi|238066755| Disrupted in schizophrenia 1isoform 49                | 21427                     |
| gi|351694577| Activated RNA polymerase II transcriptional coactivator p15 | 13993                    |
| gi|119607091| DNA replication licensing factor MCM4                 | 11656                     |
| gi|7673373  | SCAN-related protein RAZ1                             | 23430                     |
| gi|4758356  | Flap endonuclease 1                                   | 42592                     |

**Effects of TAGLN over-expression on downstream genes and signaling pathways**

In our previous study, over-expression of **TAGLN** in RKO cells led to the differential expression of 256 transcripts in the Affymetrix cDNA microarray [9]. Among them, 68 with undefined gene symbols and gene database codes, and 4 with duplicated identification were eliminated. A total of 184 DEGs were obtained (92 DEGs were up-regulated and 92 DEGs were down-regulated) for further analysis.
Functional enrichment and signaling pathway enrichment analysis were performed using the Metascape tool (Fig. 3A). Data indicated that the 184 DEGs were mainly involved in the cytoskeleton, protein kinase binding, regulation of cytoskeleton remodeling and Rho GTPase activation.

A protein-protein interaction (PPI) network was composed with the proteins encoded by the 184 DEGs using the STRING tool. The topological properties of the network were analyzed, which were composed of 167 nodes and 70 edges. The data of the PPI network was introduced into Cytoscape (Figure 3B). CytoHubba plug-ins were used to calculate the degree centrality and intermediate centrality of the DEGs. The mean value of global centrality was 4.375, and that of intermediate centrality was 153.375. Four genes were selected as the core genes in this network, including CALM1, RAC1, PLK4 and MYO1F (Fig. 3B in red circles). An MCODE plug-in was utilized to analyze the interaction within the network. By k kernel analysis (k=2), a core module with 4.5 points was selected (Fig. 3B in blue circle), consisting of 5 nodes (RAC1, WAS, WIPF1, NCKIPSD, MYO1F) and 9 edges.

STRING tool also proposed that there have been complex interactions between the gene-coding proteins.

The core genes and the genes included in the core module were combined and 7 discrete genes were selected as key genes, namely CALM1, PLK4, RAC1, WAS, WIPF1, NCKIPSD and MYO1F. Signaling pathway enrichment analysis of the key genes was performed. Three entries with significant differences were obtained, mainly involving the Rho GTPase signaling pathway (Fig. 3C).

**Prediction of the transcription factor for the key genes and validating its interaction with transgelin**

We then analyzed the promoter regions of the 7 key genes (CALM1, PLK4, RAC1, WAS, WIPF1, NCKIPSD, MYO1) to explore if they share the same transcription factor(s) using GCBI tools through Ensembl, Transfac, COSMIC, and dbSNP database. As the description from the GCBI website (http://college.gcbi.com.cn/archives/2437), a computational model was utilized (Fig. 4A). PARP1 was eventually predicted as the transcription factor for the 7 key genes (Fig. 4B and 4C) and it was also included in the 23 DNA-binding proteins potentially interacting with transgelin-flag fusion protein (Table. 1). CNLS Mapper was used to predict the possible existence of nuclear localization signal in
PARP1 protein. The results showed that it does have NLS (Figure 4D).

To validate the interaction between PARP1 and transgelin, we transiently transfected pENTER-TAGLN-FLAG plasmid and the control plasmid into RKO cells. Immunoblotting analysis indicated that over-expression of transgelin could be observed in RKO-TAGLN-FLAG cells (Fig. 4E) as compared to the control groups. Its expression level (0.89±0.02) was higher than the RKO-CRTL group (0.48±0.02) and the wild type RKO group (0.49±0.02) (P<0.0001, Fig. 4E). The immunoprecipitation followed by immunoblotting showed that PARP1 could be specifically immunoprecipitated by anti-flag antibody in the RKO-TAGLN-FLAG cells which confirmed its binding to transgelin-flag fusion protein (Fig. 4F).

Discussion

**Transgelin in colon cancer metastasis**

Tumor metastasis, in which cancer cells move from one site to another, is a complex process associated with remodeling of the cytoskeleton. The intracellular cytoskeleton requires a high degree of functional integration and coordination of actin (microfilament), microtubules and intermediate filaments. However, cancer cells can metastasize once the proteins related to cytoskeleton are abnormal [3, 15].

Transgelin is an actin-binding protein presumably existed in the cytoplasm of smooth muscle cells. In this study, we found that both endogenous and exogenous transgelin were expressed in the cytoplasm and the nucleus of colon cancer cells (Fig. 1). These indicate that transgelin is likely to have nuclear-cytoplasmic shuttling and performs its biological functions in different cellular compartments. Recent studies have shown that actin-binding proteins not only regulate actin nucleation, cellulose capping, fragmentation, monomer and other functions in the cytoplasm, but is also involved in the formation of transcription complexes[16]. Our previous study found that transgelin was able to increase the metastatic potential of colon cancer cells by remodeling the cytoskeleton in the cytoplasm[10], it also altered the expression of metastasis-related genes[9], thereby promoting the formation of metastatic phenotypes in tumor cells. Since many actin binding proteins have been proven to have different biological functions in the cytoplasm and nucleus[17-20], we hypothesized that transgelin may play an important role in invasion and metastasis of colon
cancer cells through specific mechanisms in different cellular localization. In addition, the immunoprecipitation and protein mass spectrometry analysis suggested that transgelin interacted with a variety of metabolic-related enzymes, transport proteins, transcription factors, and cytoskeletal proteins (Supplementary Table 1, Additional file 3).

**Transgelin and Rho signaling pathway in colon cancer cells**

At present, studies on actin and its interacting molecules mainly focus on specific signaling pathways, such as Rho GTPases and its downstream effector proteins, which mediates tumor cell migration, invasion and metastasis through cytoskeleton (reviewed in [15]). Based on the expression profiling data from our previous study[9], we obtained 184 DEGs and selected 7 key genes, including *CALM1*, *MYO1F*, *NCKIPSD*, *PLK4*, *RAC1*, *WAS* and *WIPF1* by bioinformatics. These key genes are associated with signaling pathways related to tumor formation and metastasis[21-29]. The Rho GTPases activation pathway was identified as the key signaling pathway. Therefore, it may be an important pathway for transgelin to participate in colon cancer metastasis. Although some of the DEGs identified from the same cDNA microarray were validated by qRT-PCR in another cell line (DLD-1) [9], the effects of transgelin on Rho signaling pathway warrant further experiments to fully uncover the underlying mechanism.

Rho GTPase has been found to play an important role in controlling cytoskeletal dynamics, directional sensing, cell-cell assembly (disassembly), and integrin matrix adhesion in a variety of potential migration pathways. Rho GTPase, a family of 20 small G proteins, interacts with downstream proteins to influence cell cycle, polarity, and migration by regulating cytoskeleton[30]. In addition, various studies have suggested that increased expression of Rho GTPase gene is associated with increased cell invasiveness and metastatic phenotype (reviewed in [15]). Rho GTPase interacts with Rho, Rac, and Cdc42 in eukaryotic cells to regulate the assembly and remodeling of actin cytoskeleton. Rho can recruit Rho kinase (ROCK) and phosphorylate various cytoskeletal proteins, thus promoting the formation of actin fiber stress and generating contractile force. Rho kinase, a major downstream effector of Rho GTPase family proteins, is a small GTPase effector protein that can participate in the regulation of actin remodeling by phosphorylation of cofolin and myosin light chain (MLC) [31, 32].
**Transgelin interacts with PARP1 in colon cancer cells**

As we found that transgelin had nuclear localization, manipulation of its expression resulted in the differential expression of a variety of genes, and affected the biological behaviors of the colon cancer cells in vitro and in vivo[9]. It can potentially bind to 297 proteins, but it did not directly bind to RNA polymerase II (Supplementary Table 1, Additional file 3). Neither does it have a nuclear localization signal. All of these lead us to the hypothesis that transgelin may interact with other partner(s) to regulate the downstream target genes thereby affecting colon cancer metastasis. By analyzing the promoter region of the key genes downstream of transgelin to predict their potential transcription factor(s) (Fig. 4) and comparing the 23 DNA-binding proteins identified by mass spectrometry that were potentially binding to transgelin (Table 1), PARP1 was the only one mapping to both. Poly ADP-ribose polymerase-1 is encoded by PARP1 and the molecular weight is 113kDa. It is involved in DNA repair, cell cycle, cell death, tumorigenesis and other cellular processes[33-36]. Its N-terminal has the DNA binding domain consisting of two zinc finger motifs and a nuclear localization sequence[37]. Most importantly, PARP1 can regulate the transcription of genes by directly binding to promoters[38-40].

PARP1 has been found over-expressed in some malignant tumors and promoting tumor metastasis in soft tissue sarcoma[41] and non-small cell lung cancer[42]. Moreover, the nuclear localization of PARP1 can affect the chemo-sensitivity of hepatocellular carcinoma to oxaliplatin [43]. PARP1 has also been reported to play an important role in the early development of colorectal cancer[44]. Another study has shown that PARP1 expression was associated with colorectal cancer progression [45]. However, Dorsam et al. [46] found that PARP1 can reduce the nitroso compounds (NOC)-induced tumorigenesis, while it also drives intestinal inflammation through innate immune response and promotes colorectal tumor growth. All of these studies suggest that PARP1 could be a promising target for malignant tumor intervention, including colon cancer. In the current study, we validated the interaction between transgelin and PARP1 by immunoprecipitation (Fig. 4F). Although, our prospects are to fully delineate the mechanisms of how transgelin interacting with PARP1 influences the Rho signaling pathway and participates in colon cancer metastasis, there are still works to be finished in a
while. We believe that transgelin participates through two different mechanisms: the cytoplasmic transgelin takes part in the cytoskeletal remodeling when cancer cells received the micro-environmental signals; Meanwhile, cytoplasmic transgelin binds to PARP1 and translocates into the nucleus where the complex regulates the expression of the key genes, and subsequently affects the Rho GTPase activation pathway in the cytoplasm and initiates cytoskeletal remodeling (Fig. 5). These two mechanisms may simultaneously promote colon cancer metastasis.

Conclusions

The results support a hypothesis that transgelin interacts with PARP1 and regulates the expression of the downstream key genes (CALM1, MYO1F, NCKIPSD, PLK4, RAC1, WAS and WIPF1) mainly involving Rho signaling pathway in human RKO colon cancer cells.

List Of Abbreviations

PARP1, Poly ADP-ribose polymerase-1; CH, calmodulin homologous; CLIK, C-terminal calmodulin like; MEM, minimum Eagle’s medium; RPMI, Roswell Park Memorial Institute; HRP, Horseradish Peroxidase; FASP, filtered aided proteome preparation; FDR, false discovery rate; DEGs, differential expression genes; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, protein-protein interaction; TF, transcription factor; NLS, nuclear localization signal; ROCK, Rho kinase; MLC, myosin light chain; NOC, nitroso compound.

Declarations

Ethics approval and consent to participate

All procedures performed in studies were in accordance with the ethical standards of Sun Yat-sen Memorial Hospital. Written informed consents were obtained from all individual participants included in the study.

Consent for publication

Not applicable.

Availability of data and material

The microarray datasets analyzed in this manuscript have been deposited in NCBI's Gene Expression Omnibus (Zhou et al., 2013) and are accessible through GEO Series accession number GSE48998 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE48998). Other datasets generated and/or
analyzed during the current study are included within the article and its additional files.

**Competing interests**

The authors declare that there are no competing interests associated with the manuscript.

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**Author’s contribution:**

Substantial contribution to the conception and design of the work: Ying Lin, Hui-min Zhou; Analysis and interpretation of the data: Zhen-xian Lew, Yuan-yuan Fang, Zhen Ye; Drafting the manuscript: Zhen-xian Lew, Wa Zhong, and Zhong Yu; Revising the work critically for important intellectual content: Xin-yi Yang, Dan-yu Chen, Si-min Luo, and Li-fei Chen; Collecting of grants: Ying Lin and Hui-min Zhou. All authors read and approval the final manuscript.

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Figures
The localization of transgelin in different human colon cancer cell lines. A. transgelin was observed by immunofluorescence in colon cancer cell lines RKO, SW480, HCT116 and LOVO. Panels show transgelin immunostaining, 4',6-diamidino-2-phenylindole DNA staining (DAPI), and a merged image as indicated. B. The distribution of transgelin in the cells of SW480, LOVO, HCT116 and RKO was identified by immunoblotting. C. The distribution of transgelin-flag fusion protein in the RKO and SW480 cells that were transiently transfected with pcDNA6/myc-His B-TAGLN-flag plasmid and control plasmid were detected by immunoblotting. N is the fraction of nuclear protein, and C is the fraction of cytoplasmic protein. Lamin B1 is a marker of nuclear protein, while GAPDH is a marker of cytoplasmic protein.
Identification of proteins potentially interacted with transgelin in RKO cells. A. Transgelin-flag protein was expressed in RKO cells transiently transfected with plasmids. Expression of transgelin in RKO wild-type (WT), RKO-CTRL-FLAG and RKO-TAGLN-FLAG cells was detected by immunoblotting. **** P<0.0001. B. Proteins extracted from RKO-CTRL-FLAG and RKO-
TAGLN-FLAG cells were immunoprecipitated by anti-flag antibody, respectively, and visualized by silver staining. C. Relationship between proteins that were immunoprecipitated by anti-flag antibody in RKO-CTRL-FLAG and RKO-TAGLN-FLAG cells. D. Functional enrichment analysis of the 297 proteins potentially interacted with transgelin-flag fusion protein.
Figure 3

Effects of TAGLN overexpression on other genes and signaling pathways in RKO cells. A. Functional enrichment (including cellular components, molecular functions, biological processes) and signaling pathway analysis were performed for the DEGs. B. The topology analysis of constructing the network illustrating the relationship of the proteins encoded by the DEGs. Genes in the red circle were core genes. The blue circle was the core module. The combination of the genes in the red circle and in the blue circle are the key genes. C. Signaling pathway enrichment analysis of the key genes identified the Rho signaling pathway.
Prediction of the transcription factor(s) for the key genes and validation of transgelin-PARP1 interaction. A. Illustration of the computational model for predicting the transcription factors for the key genes (http://college.gcbi.com.cn/archives/2437). B. Prediction of the transcription factors for the key genes (partially illustrated). PARP1 protein was circled by a blue frame. C. The recommendation degree of PARP1 as the transcription factor for the 7 key genes downstream of transgelin. D. The protein sequence of PARP1. The red fonts represented the corresponding sequences of possible nuclear localization signal in the PARP1 protein. E. Immunoblotting analysis of transgelin and flag protein expressed in RKO-TAGLN-FLAG, RKO-CTRL and wild type RKO cells, ****P<0.0001. F. The interaction between transgelin-flag fusion protein and PARP1. RKO-CTRL was the control group. Normal rabbit
IgG was used as the control antibody.

Figure 5

Model proposed for mechanisms of transgelin in promoting colon cancer metastasis.

Cytoplasmic transgelin participates in the cytoskeletal remodeling when cancer cells received the micro-environmental signals. It also binds to PARP1 protein and the complex translocates into the nucleus and regulates the expression of the key genes. Subsequently, the Rho signaling pathway is aroused and initiating the cytoskeletal remodeling which results in promoting colon cancer metastasis.

Supplementary Files
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