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Research article

Decorin impeded the advancement of thyroid papillary carcinoma by thwarting the EGFR/ FER/ SHP2 signaling-induced sustenance of early endosomes

Yaping Liu [a], Yunchao Xin [b], Tianyun Lv [c], Zhicheng Chang [c], Gang Xue [b], Xiaoling Shang [b,*]

[a] *Otorhinolaryngology, Head and Neck Surgery, Hebei North University.Medical Insurance Office, The Fourth Hospital of Hebei Medical University, Zhangjiakou, Hebei, China*
[b] *Otorhinolaryngology, Head and Neck Surgery, The First Affiliated Hospital of Hebei North University, Zhangjiakou, Hebei, China*
[c] *Otorhinolaryngology, Head and Neck Surgery, Hebei North University, Zhangjiakou, Hebei, China*

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ABSTRACT

Objective: This study explores the inhibition of papillary thyroid carcinoma proliferation by Decorin via the EGFR/SHP2/FER pathway.
Method: ology: Thirty-two pairs of papillary thyroid carcinoma tissues and adjacent normal tissues were collected for immunohistochemical analysis. Thyroid cancer cell lines with overexpressed or silenced Decorin were employed in subcutaneous tumor formation experiments in nude mice. Cell membrane proteins were extracted for Western blot and immunofluorescence analyses.
Results: Reduced Decorin expression in human papillary thyroid carcinoma was associated with inhibited formation of the EGFR/SHP2/FER complex. Immunohistochemical analysis revealed lower Decorin levels in carcinoma tissues compared to adjacent normal tissues, corroborated by decreased Decorin and PTEN levels in carcinoma as shown by Western Blot. Overexpression of Decorin in mouse models diminished tumor growth, an effect reversed by Decorin silencing and mitigated by FER inhibition. Decorin modulated Rab5-GTP and Rab7-GTP levels, impacting endosome transition and subsequent signaling pathways.
Conclusion: Decorin inhibits papillary thyroid carcinoma proliferation by disrupting the EGFR/SHP2/FER pathway and modulating endosomal transport.

1. Introduction

Papillary thyroid carcinoma is a relatively common type of thyroid cancer, classified among thyroid malignancies. This carcinoma generally progresses slowly and is associated with a relatively favorable prognosis [1]. Common symptoms include a neck lump, hoarseness, difficulty swallowing, and abnormal thyroid hormone levels. Treatment options typically include surgical removal, radiation therapy, and/or radioactive iodine treatment [2].

Decorin (DCN) is the most extensively studied member of the small leucine-rich proteoglycan (SLRP) family and functions as an extracellular matrix (ECM) protein. Named for its role in “decorating” collagen fibers within the ECM, decorin plays a crucial role in

* Corresponding author.
E-mail address: liuyaping6298@163.com (X. Shang).

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maintaining tissue structural stability and regulating cellular behavior [3]. In mammals, decorin comprises a 42 kDa monomeric protein core and a single chondroitin/dermatan sulfate glycosaminoglycan (GAG) chain. In physiological solutions, DCN exists as a dimer and is among the most thoroughly understood members of the expanding SLRP family. Structurally, it features a domain with tandem leucine-rich repeats (LRRs, totaling 12 LRRs) flanked by cysteine-rich regions on either side. The N-terminus of the decorin dimer structure is located at the "center" of the "anti-parallel homodimer" [4].

Research into decorin's role in cancer genesis and progression is increasing, revealing its potential therapeutic value. Decorin can impact cancer in the following ways:

1. Inhibition of tumor growth: Decorin can inhibit tumor cell proliferation and promote apoptosis by directly interacting with specific receptors on tumor cell surfaces. For instance, it can inhibit the growth of breast and liver cancer cells [5–8].
2. Anti-angiogenesis: Decorin can impede tumor angiogenesis by inhibiting the expression of key angiogenic factors such as VEGF (vascular endothelial growth factor), thus suppressing tumor growth and metastasis [9–12].
3. Modulation of the tumor microenvironment: Decorin can alter the interactions between tumor cells and their surrounding microenvironment, including the ECM composition and cellular communication within the tumor microenvironment, significantly affecting tumor growth and metastasis [13–15].
4. Inhibition of tumor growth through signaling pathways: Decorin has been found to interfere with several tumor-related signaling pathways, including TGF-β (transforming growth factor-β) and EGFR (epidermal growth factor receptor), inhibiting tumor proliferation and metastasis [16–18].

Endosomes are essential membrane-bound structures within cells, playing a critical role in material transport and signal transduction. Formed via endocytosis, endosomes primarily function in sorting, transporting, and processing ingested substances. They are present in eukaryotic cells, including both animal and plant cells.

Early Endosomes: These are the initial sorting stations for substances internalized through endocytosis. Here, ingested materials are preliminarily sorted. Some substances are returned to the cell membrane, while others are transported to subsequent endosomes. The pH of early endosomes is slightly acidic compared to the extracellular environment, facilitating the release of certain ligands from receptors. Rab5 is a marker of early endosomes and regulates their transport.

Late Endosomes: Materials transported from early endosomes move to late endosomes. The increased acidity of late endosomes aids in material degradation. Fusion with lysosomes typically occurs at this stage, allowing lysosomal enzymes to degrade the contents of the late endosomes. Rab7 is a marker of late endosomes and plays a critical role in their transport regulation.

Recycling Endosomes: Some substances from endocytosis are not destined for degradation but need to be returned to the cell membrane or other cellular locations for reuse. Recycling endosomes facilitate this process. Rab11 is a marker of recycling endosomes and is involved in their transport, closely associated with various developmental processes [19,20].

EGF can bind to EGFR and thereby activate downstream signaling pathways. Following EGF activation, EGFR binds to FER and activates it. FER overexpression increases EGFR phosphorylation, activates ERK, and activates NF-κB. EGF rapidly activates EGFR and FER, and FER's phosphorylation may occur through its SH2 domain binding to phosphorylated tyrosine residues on EGFR. Overexpression of FER enhances its binding to EGFR, thereby activating EGFR, ERK, and NF-κB. Simultaneously, FER phosphorylation strengthens GAB1 phosphorylation and indirectly promotes SHP2 phosphorylation. After internalization, EGFR co-localizes with RAB5 and then progresses to late endosomes (RAB7 positive) and lysosomes [21–25].

The protein complex of EGFR/FER/SHP2 in early endosomes can enhance the phosphorylation state of PKCδ. Elevated levels of pY374-PKCδ are linked to the activation of receptor tyrosine kinases such as EGFR and IGF1R. Additionally, high levels of pY374-PKCδ are associated with an increase in RAB5-RAB7 positive transitional endosomes and may correlate with defects in endosomal transport.

Phosphorylation of PKCδ can facilitate the downstream conversion of Rab5 and Rab11 from their GDP-bound forms to GTP-bound forms, thereby inhibiting the maturation of early endosomes into late endosomes. Concurrently, it promotes the recycling and reutilization of EGFR at the cell membrane and inhibits EGFR degradation [26]. Furthermore, the protein complex of EGFR/FER/SHP2 can stimulate the phosphorylation of PI3K and NOX1-mediated oxidative stress during early endosome formation. Excessive ROS production can inhibit the phosphorylation state of PTEN [27,28]. Through the phosphorylation and activation of PIKfyve, AKT promotes the production of PI(3,5)P2, which subsequently facilitates the transport of EGFR from early endosomes to multivesicular bodies and late endosomes, thereby promoting EGFR degradation. Inhibition of AKT reduces EGFR recycling, leading to its accumulation in early endosomes, prolonging the EGFR signal, and enhancing the activation of ERK and RSK. AKT inhibition also diminishes EGFR transport to lysosomes, resulting in decreased EGFR degradation [29]. PTEN, via its protein phosphatase activity, dephosphorylates RAB7, promoting its transport to the late endosome membrane, thus facilitating the maturation of late endosomes and the transport of EGFR from early to late endosomes, thereby promoting EGFR degradation [30].

Decorin can competitively inhibit the receptor-ligand binding of EGF and EGFR, and based on the mechanisms described, Decorin can block the phosphorylation of FER and SHP2, thus inhibiting the formation of early endosomes, their transition to late endosomes, and the recycling function of recycling endosomes. The aim of this discourse, augmented with the provided illustration, is to elucidate the inhibitory role of Decorin in the receptor-ligand binding interface of EGF and EGFR. By delving into the molecular intricacies, it underscores how Decorin impedes the phosphorylation of FER and SHP2, thereby obstructing the formation and transition of early endosomes to late endosomes and their subsequent recycling functions. Notwithstanding this, the nuanced mechanism through which Decorin hinders the progression of thyroid papillary carcinoma via the FER/SHP2/PTEN pathway, by disrupting early-to-late endosomal transition, remains an enigma yet to be unraveled.

However, the specific mechanism by which Decorin impedes the progression of thyroid papillary carcinoma through the FER/

SHP2/PTEN pathway by inhibiting the conversion of early endosomes to late endosomes remains unclear.

2. Materials and methods

2.1. Cell cultivation

The human papillary thyroid carcinoma cellular lineage, IHH4, was acquired from Procell Life Science&Technology Co.,Ltd., and nurtured in 1640 medium complemented with 10 % exquisite fetal bovine serum and 1 % antibiotics, maintaining a steady temperature of 37 °C in an environment composed of 95 % oxygen and 5 % carbon dioxide. The FER inhibitor DS21360717 and the SHP2 inhibitor PHPS1 were obtained from MCE.

2.2. Immunohistochemistry

Thirty-two pairs of clinical papillary thyroid carcinoma samples and adjacent normal tissues were collected. The samples were first treated with xylene I for 15 min, followed by xylene II for another 15 min. This was succeeded by a series of ethanol dehydration steps (100 % ethanol I and II for 5 min each, then 90 %, 80 %, and 70 % ethanol for 5 min each), and PBS for 5 min, repeated three times. Subsequently, permeabilization was performed using 0.5 % TritonX-100 in ultrapure water at room temperature for 15 min, followed by three PBS washes for 5 min each. The slides were then subjected to antigen retrieval in a microwave within a beaker until boiling, accompanied by a water bath preheated to 99 °C for 8–12 min, and then allowed to cool naturally, followed by PBS washes. Alternatively, high-pressure antigen retrieval was executed by placing the slides and antigen retrieval solution in an autoclave at 125 °C for 5 min, followed by cooling down to 90 °C and then to room temperature naturally, followed by PBS washes. Samples were incubated with 3 % H2O2 for 10 min or 0.3 % H2O2 for 30 min to inhibit endogenous peroxidase activity, followed by washing and blocking with serum at room temperature for 2 h without rinsing off the blocking solution. The sections were incubated with primary antibodies diluted in the blocking solution at 4 °C overnight. The decorin primary antibody was used at a dilution of 1:50. After incubation, the sections were washed and incubated at room temperature for 1 h, followed by development with DAB and counterstaining with hematoxylin. Dehydration was then conducted through graded alcohols, clearing in xylene, and mounting with neutral resin. Finally, photographs were taken.

2.3. Stable cell line generation

I generated lentiviruses, specifically LV-OE-NC, LV-OE-Decorin, LV-KD-NC, and LV-KD-Decorin, in our laboratory and concentrated them. The Decorin CDS region was sourced from NCBI, and shRNA sequences targeting the Decorin gene were meticulously designed via the GPP Web Portal. The synthesized full-length fragments and shRNA were cloned into the pLKO_005 vector using direct chemical synthesis and annealing extension methods. Subsequently, these constructs were assembled into the pLKO-U6-shRNA-EGFP-puro vector through PCR and T4 ligase connection. The assembled plasmids, along with psPAX2 and pMD2.G, were co-transfected into 293T cells using Lipo8000. The medium was replaced with DMEM containing 10 % FBS the following day. After 72 h, the viral supernatant was collected, centrifuged at 3000 rpm for 15 min at 4 °C, and stored at −80 °C. This procedure yielded four stable cell lines: IHH4-OE-NC, IHH4-OE-Decorin, IHH4-KD-NC, and IHH4-KD-Decorin.

2.4. Subcutaneous tumor formation in nude mice

Thirty-two six-week-old BALB/c-nu mice, weighing between 18 and 20 g, were obtained from Henan Scebeis Biotechnology Co., Ltd., and maintained under standard laboratory conditions (25 ± 1 °C, 50%–60 % humidity, and a 12-h light/dark cycle) with adequate food and water. For constructing the tumor model, 36 BALB/c-nu nude mice were randomly assigned into six groups, with six mice per group. IHH4 cells in the logarithmic phase were collected, adjusted to a cell density of 6 × 10^7 cells/ml in 0.5 ml of matrix gel, and injected subcutaneously into the right axillary region of the mice. Tumor size was measured daily using calipers, and after four weeks, the mice were anesthetized with isoflurane. The tumors were excised and photographed after 28 days.

2.5. Western Blot analysis

Cell membrane proteins were isolated by adding 2 μl of protease inhibitor cocktail to each 500 μl of cold Protein Extraction Solution A, mixing thoroughly, and keeping on ice. A similar procedure was followed for Membrane Protein Solubilization Solution C. Between 5–10 × 10^6 cells were collected by centrifugation at 500×*g* for 5 min at 4 °C, and the supernatant was removed. The cells were washed twice with cold PBS. Cell lysates were prepared by adding 200 μl–400μl of cold Solution A and mixing vigorously. The lysates were then oscillated at 2–8 °C for 20–30 min until fully lysed, followed by centrifugation at 12,000×*g* for 5 min at 4 °C. The supernatant was transferred to a clean centrifuge tube and incubated in a 37 °C water bath for 5–10 min. After centrifugation at 37 °C and 1000×*g* for 5 min, the solution was separated into two layers, with the lower layer containing the membrane proteins (approximately 30–50 μl). The upper layer was carefully removed, and the lower layer was dissolved in 150–200 μl of cold Solution B, mixed thoroughly, and incubated on ice for 2 min, followed by a 37 °C water bath for 5–10 min. After centrifugation at 37 °C and 1000×*g* for 5 min, the lower layer containing membrane proteins was collected and dissolved in 50–200 μl of cold Solution C. The proteins were subjected to SDS-PAGE, transferred to a PVDF membrane, and incubated with primary antibodies at 4 °C overnight. The Western blotting was performed

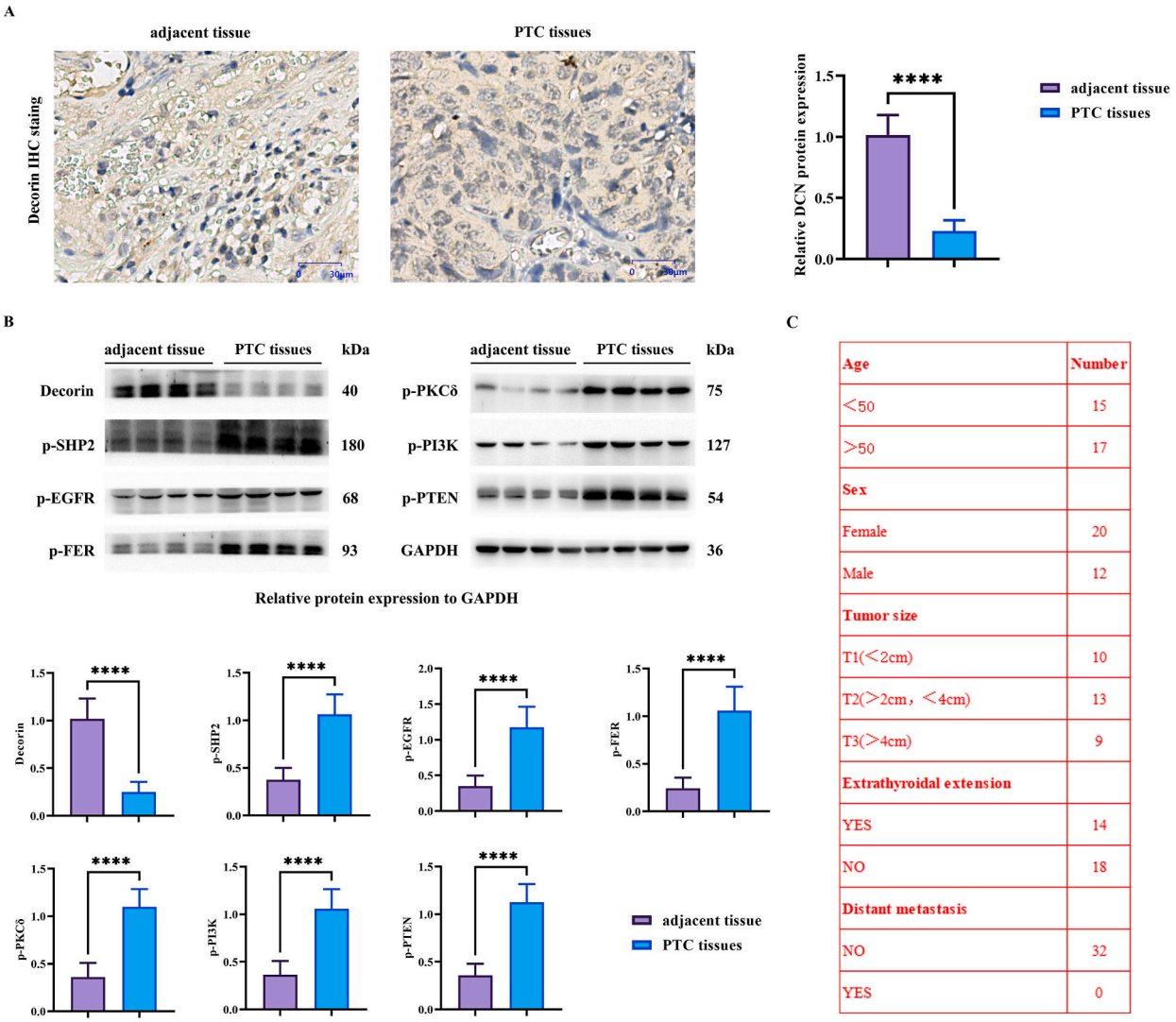
using primary antibodies for Decorin, PTEN, p-EGFR, p-SHP2, p-FER, p-PKCδ, p-PI3K, EGFR, Rab5-GTP, Rab11-GTP, Rab7-GTP, all sourced from Abcam. Visualization was done using an EL5000 exposure unit.

2.6. Immunofluorescence

For immunofluorescence staining, the sections were permeabilized with Triton-X and blocked with goat serum for 30 min. They were then incubated overnight at 4 °C with primary antibodies against p-FER and RAB7. After washing with PBST, the sections were treated with Alexa Fluor-tagged secondary antibodies (1:400) for 3 h at room temperature. DAPI staining was performed, followed by sealing the sections with glycerol. Imaging was conducted using confocal microscopy.

2.7. Flow cytometry apoptosis assay

IHH4 adherent cells were gently collected into a centrifuge tube. The cells were digested with trypsin without EDTA until they could be gently dislodged with a pipette, then the culture medium was added to dislodge all adherent cells, and the cells were



Age	Number
<50	15
>50	17
Sex	
Female	20
Male	12
Tumor size	
T1(<2cm)	10
T2(>2cm，<4cm)	13
T3(>4cm)	9
Extrathyroidal extension	
YES	14
NO	18
Distant metastasis	
NO	32
YES	0

Fig. 1. The differential expression of Decorin and associated proteins influences EGFR/SHP2/FER complex formation in human papillary thyroid carcinoma. (A) Immunohistochemical staining reveals a diminished expression of Decorin on carcinoma cell membranes compared to adjacent normal thyroid tissue, with reduced spotted distribution and lower photodensity values in carcinoma samples; N = 32, P < 0.0001. (B) Western Blot analysis indicates downregulation of Decorin and PTEN in carcinoma tissues, while the phosphorylated states of EGFR, SHP2, FER, PKCδ, and PI3K are upregulated compared to adjacent normal tissues; N = 32, ****P < 0.0001. (C)Statistical analysis was performed on the age, gender, tumor size, extrathyroidal extension, and distant metastasis of patients clinically diagnosed with papillary thyroid carcinoma; N = 32.

resuspended. The cells were collected by centrifugation at around 1000 rpm for 5 min, and the supernatant was aspirated, leaving approximately 50 μl of medium. About 1 ml of precooled 4 °C PBS was added to resuspend the cells, followed by another centrifugation. The supernatant was carefully aspirated, and cells were resuspended in 1x Binding Buffer (2 ml of 10x Binding Buffer diluted with 18 ml of deionized water), adjusting the concentration to 1–5 × 10^6/ml. A 100 μl aliquot of cell suspension was taken in a 5 ml flow cytometry tube, to which 5 μl of Annexin V/FITC was added, mixed, and incubated in the dark at room temperature for 5 min.

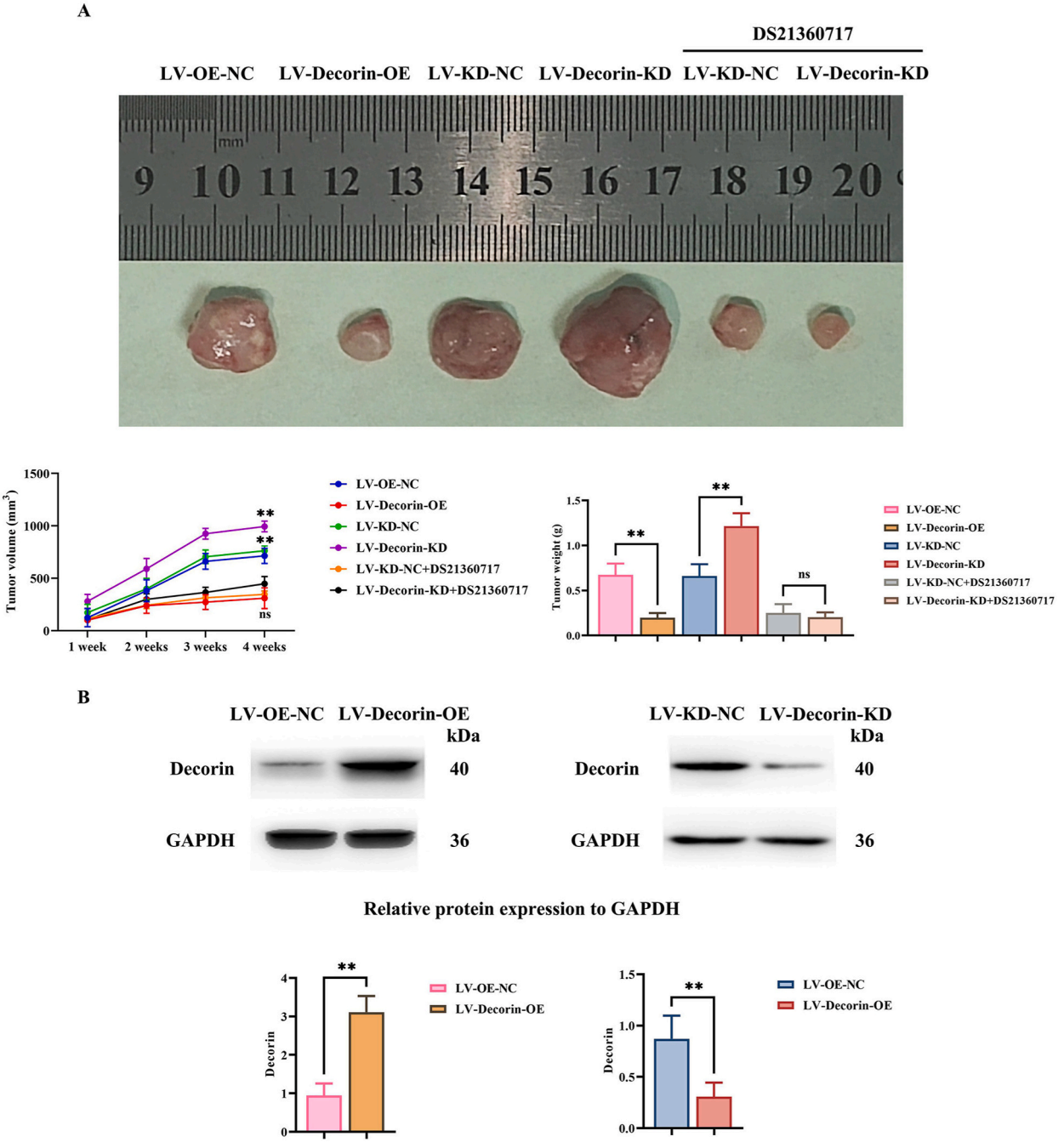


Fig. 2. Modulation of tumor growth in athymic nude mice through lentiviral-mediated overexpression and silencing of Decorin and the impact of FER kinase inhibition. (A) Tumor weight analysis shows a significant decrease in the LV-Decorin-OE group versus control and an increase in the LV-Decorin-KD group, which is abrogated following DS21360717 treatment, with no discernible difference between treated LV-KD-NC and LV-Decorin-KD groups; N = 8, ****P < 0.0001; **P < 0.01; ^{ns}P > 0.05. (B) Western Blot demonstrates changes in Decorin protein levels, with an increase in the overexpression group, a decrease in the knockdown group, and subsequent reduction upon FER inhibitor treatment in the LV-Decorin-KD + FER inhibitor group; N = 8, ****P < 0.0001; ***P < 0.001.

Then, 5 μl of Propidium Iodide (PI) solution was added, followed by 400 μl of PBS, and the samples were immediately analyzed using a CytoFLEX S Flow Cytometer.

2.8. Statistical methods

Statistical analysis was conducted using GraphPad 8.0. Data were presented as mean ± standard deviation (S.D.). Pairwise comparisons between groups are pragmatically conducted using the *t*-test. Statistical methods for more than four groups involve employing one-way analysis. A p-value of less than 0.05 was considered statistically significant.

3. Results

1. The diminished expression of Decorin in human papillary thyroid carcinoma inhibits the formation of the EGFR/SHP2/FER complex.

To investigate the expression patterns of Decorin in papillary thyroid carcinoma and adjacent normal thyroid tissue using immunohistochemical methods, we collected a total of 32 matched pathological specimens. Immunohistochemical analysis revealed that Decorin accumulated on the cell membranes of adjacent tissues, displaying a spotted distribution and strong positivity. In contrast, carcinoma tissue samples exhibited subdued cellular membrane expression, less distinct spotted distribution, and weak positivity. The photodensity values of adjacent tissue were significantly higher than those of the carcinoma (Fig. 1A).

To explore the expression levels of p-EGFR, p-SHP2, p-FER, p-PKCδ, and p-PI3K in cell membrane proteins, we extracted these proteins, excluding the cytoplasm and nucleus, and conducted protein detection operations. Cell membrane proteins were extracted from human papillary thyroid carcinoma and adjacent normal thyroid tissue for Western Blot analysis. Compared to adjacent tissue, expression levels of Decorin and PTEN in papillary thyroid carcinoma were notably reduced, whereas p-EGFR, p-SHP2, p-FER, p-PKCδ, and p-PI3K were conspicuously elevated (Fig. 1B).

Later, we conducted statistical analysis on the age, gender, tumor size, extrathyroidal extension, and distant metastasis of patients with papillary thyroid carcinoma clinically (Fig. 1C).

2. Lentiviral-mediated overexpression and silencing of Decorin suppress tumor growth in athymic nude mouse models of papillary thyroid carcinoma through FER-mediated pathways.

To investigate the impact of combined administration of DS21360717 (FER inhibitor) on tumor volume and tumor weight in nude mice with Decorin overexpressing and IHH4-knockdown cell lines, a lentiviral system was established for the augmentation and suppression of Decorin expression. This lentiviral system subsequently infected IHH4 cells with lentivirus LV-Decorin-OE and LV-Decorin-KD, along with their respective controls, LV-OE-NC and LV-KD-NC. The infection of IHH4 cells led to the establishment of four stably transfected cell lines: Decorin-NC-IHH4, Decorin-OE-IHH4, Decorin-NC-IHH4, and Decorin-KD-IHH4. These stable transfectants were cultured to maintain an equal cell number and logarithmic phase, then implanted subcutaneously into athymic nude mice at a quantity of 10^6 cells per mouse. Five days post-termination of the Decorin-NC-IHH4 and Decorin-KD-IHH4 groups, the mice were orally administered the FER tyrosine kinase inhibitor DS21360717 once daily for seven days. Tumor weight analysis revealed a significant reduction in the LV-Decorin-OE group compared to the LV-OE-NC group, while the LV-Decorin-KD group showed a significant increase in tumor weight compared to the LV-KD-NC group. Following the administration of DS21360717, both the LV-KD-NC and LV-Decorin-KD groups exhibited a notable decrease in tumor weight with no significant difference between the two groups (Fig. 2A).

To authenticate the normal function of stable cell lines and the expression level of Decorin protein, cell membrane protein processing methods akin to those employed in human studies were employed for animal experiments. After tumor induction, cell membrane proteins were harvested for Western Blot analysis. Results indicated that compared to the LV-OE-NC group, the LV-Decorin-OE group showed a significant increase in Decorin protein expression; conversely, the LV-Decorin-KD group showed a marked reduction when compared to the LV-KD-NC group. Post-administration of DS21360717, the LV-Decorin-KD + FER inhibitor group displayed a significant decrease in Decorin protein levels (Fig. 2B).

3. Decorin inhibits EGFR degradation by restraining Rab5-GTP and favoring Rab7-GTP.

Following tumor formation, cell membrane proteins were extracted for Western blot analysis to assess vesicle transport under varying levels of Decorin protein and in combination with FER inhibitors. The findings indicated that, relative to the LV-OE-NC group, the LV-Decorin-OE group had noticeably reduced levels of EGFR, Rab5-GTP, and Rab11-GTP proteins, yet an increase in Rab7-GTP protein levels. Conversely, the LV-Decorin-KD group, compared to the LV-KD-NC group, displayed elevated levels of EGFR, Rab5-GTP, and Rab11-GTP proteins, but a reduction in Rab7 protein levels. Following the administration of DS21360717, the LV-KD-NC and LV-Decorin-KD groups showed concomitant reductions in EGFR, Rab5-GTP, and Rab11-GTP protein levels, while displaying an increase in Rab7 protein levels, with no notable difference between the groups (Fig. 3A&B).

4. Decorin modulates the cell membrane protein signaling pathway FER/SHP2, impacting endosome transition.

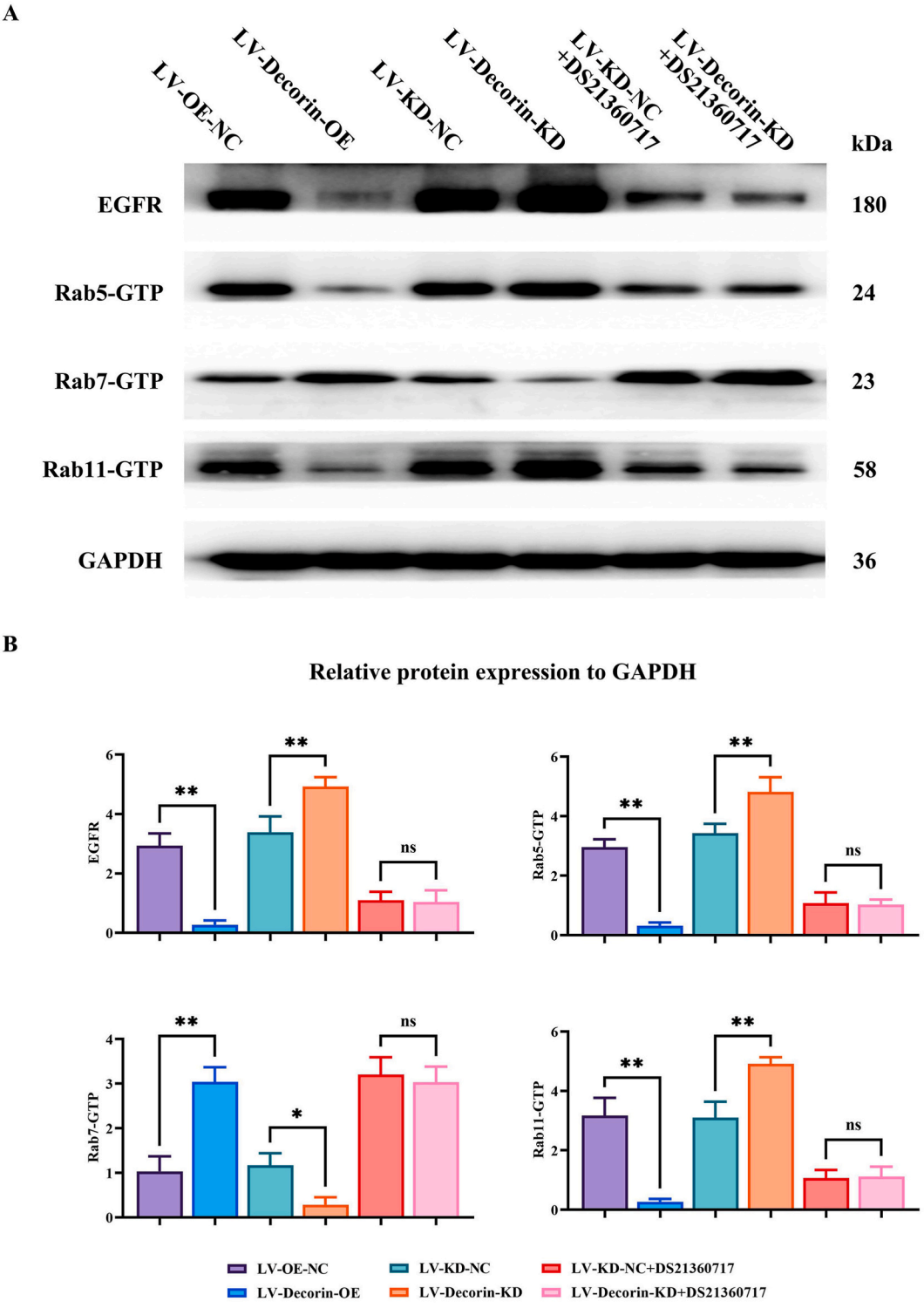


Fig. 3. Decorin's role in EGFR trafficking and degradation by modulating Rab5-GTP and Rab7-GTP levels. (A) Western Blot analysis post-tumor formation reveals a decrease in EGFR, Rab5-GTP, and Rab11-GTP, associated with an increase in Rab7-GTP in the LV-Decorin-OE group as opposed to the control. The LV-Decorin-KD group exhibits the inverse pattern. Both LV-KD-NC and LV-Decorin-KD groups post-DS21360717 treatment display reductions in EGFR, Rab5-GTP, and Rab11-GTP levels alongside an increase in Rab7-GTP, with no significant differences noted between treated groups; N = 3, ****P < 0.0001; ***P < 0.001; *P < 0.05; ^{ns}P > 0.05.

To scrutinize the vesicular trafficking of RAB7 and FER downstream, stably transfected IHH4 cells underwent immunofluorescence staining, with p-FER highlighted by green fluorescence and Rab7-GTP by red fluorescence. Results revealed that compared to the LV-OE-NC group, the LV-Decorin-OE group manifested reduced green fluorescence intensity for p-FER, while the intensity for Rab7-GTP's red fluorescence increased. In the LV-Decorin-KD group, relative to the LV-KD-NC group, the intensity for p-FER's green fluorescence strengthened, whereas the red fluorescence intensity for Rab7 diminished (Fig. 4A).

For a more thorough exploration, let's delve into how downstream genes are modulated by FER and SHP2. Furthermore, cell membrane proteins were extracted from the stably transfected IHH4 cells for Western Blot analysis. Outcomes showed that compared

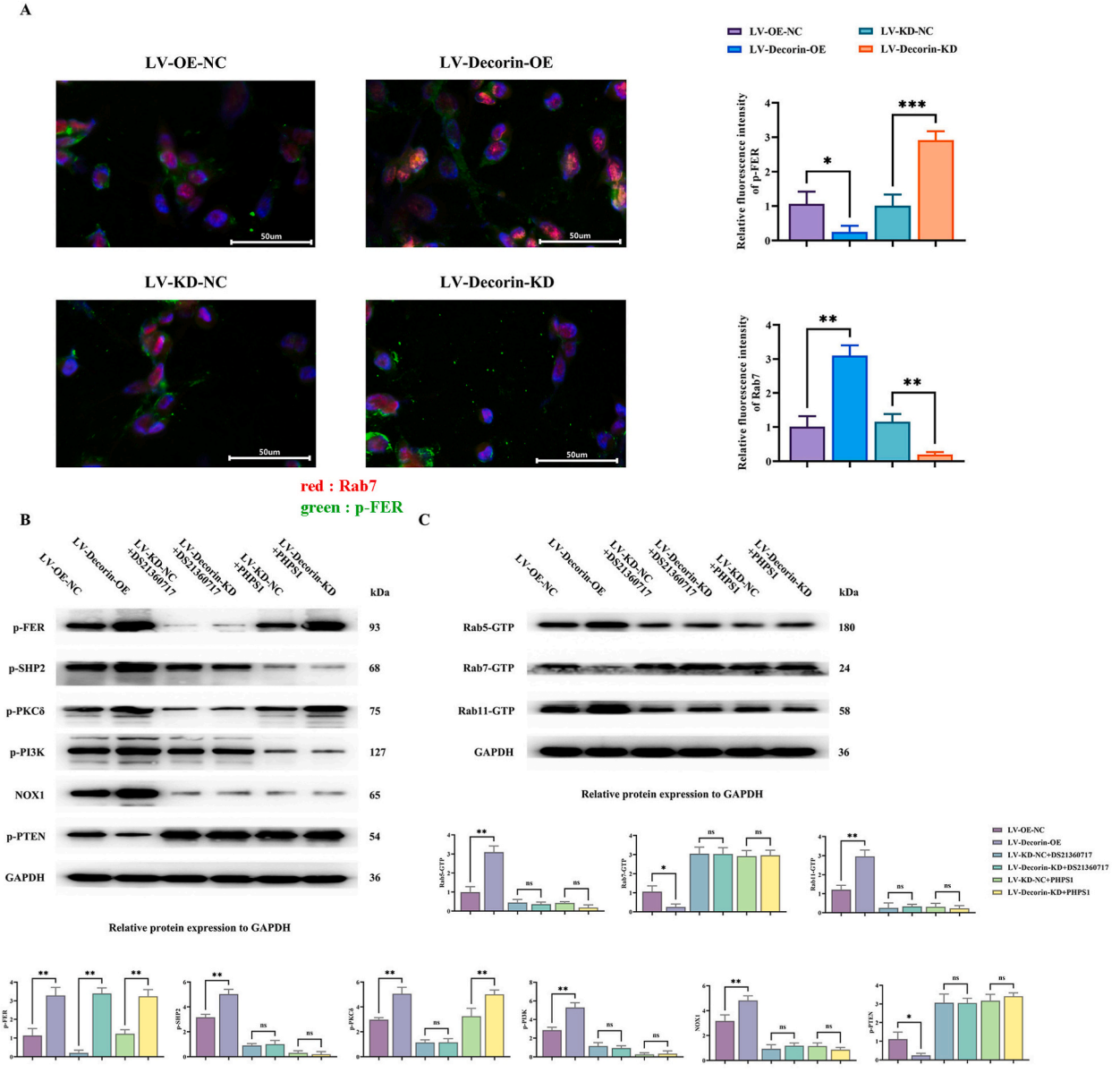


Fig. 4. Impact of Decorin on the cell membrane protein signaling pathway FER/SHP2 and endosome transition. (A) Immunofluorescence staining demonstrates a decrease in p-FER (green fluorescence, $P < 0.05$) and an increase in Rab7-GTP (red fluorescence, $P < 0.01$) in the LV-Decorin-OE group relative to the control. The LV-Decorin-KD group shows increased p-FER and decreased Rab7-GTP fluorescence compared to the control group; $N = 3$. (B) Western Blot analysis following treatment with the FER inhibitor (and SHP2 inhibitor PHPS1) shows a significant reduction in p-FER, p-SHP2, p-PKCδ, p-PI3K, and NOX1 levels while increasing PTEN levels in both treated LV-KD-NC and LV-Decorin-KD groups irrespective of the inhibitor used, highlighting the impact on protein signaling pathways; $N = 3$, ****$P < 0.0001$; ***$P < 0.001$; *$P < 0.05$; $^{ns}P > 0.05$. (C) Western Blot analysis following treatment with the FER inhibitor (and SHP2 inhibitor PHPS1) shows a significant reduction in Rab5-GTP and Rab11-GTP levels while increasing Rab7-GTP levels in both treated LV-KD-NC and LV-Decorin-KD groups irrespective of the inhibitor used, highlighting the impact on protein signaling pathways; $N = 3$, ****$P < 0.0001$; *$P < 0.05$; $^{ns}P > 0.05$.

to the LV-OE-NC group, the LV-Decorin-OE group saw a significant elevation in the expression levels of p-FER, p-SHP2, p-PKCδ, p-PI3K, NOX1, p-PTEN, Rab5-GTP, Rab7-GTP and Rab11-GTP proteins, while p-PTEN and Rab7-GTP protein levels notably decreased. Upon addition of the FER inhibitor to both groups, the LV-KD-NC and LV-Decorin-KD groups manifested exceedingly faint p-FER protein expressions, with concurrent reductions in p-SHP2, p-PKCδ, p-PI3K, NOX1, Rab5-GTP and Rab11-GTP protein levels, yet an increase in p-PTEN and Rab7-GTP protein levels, exhibiting no discernible differences between the two groups. Additionally, when the SHP2 inhibitor PHPS1 was introduced, both the LV-KD-NC and LV-Decorin-KD groups saw a slight elevation in the latter's p-FER and p-PKCδ

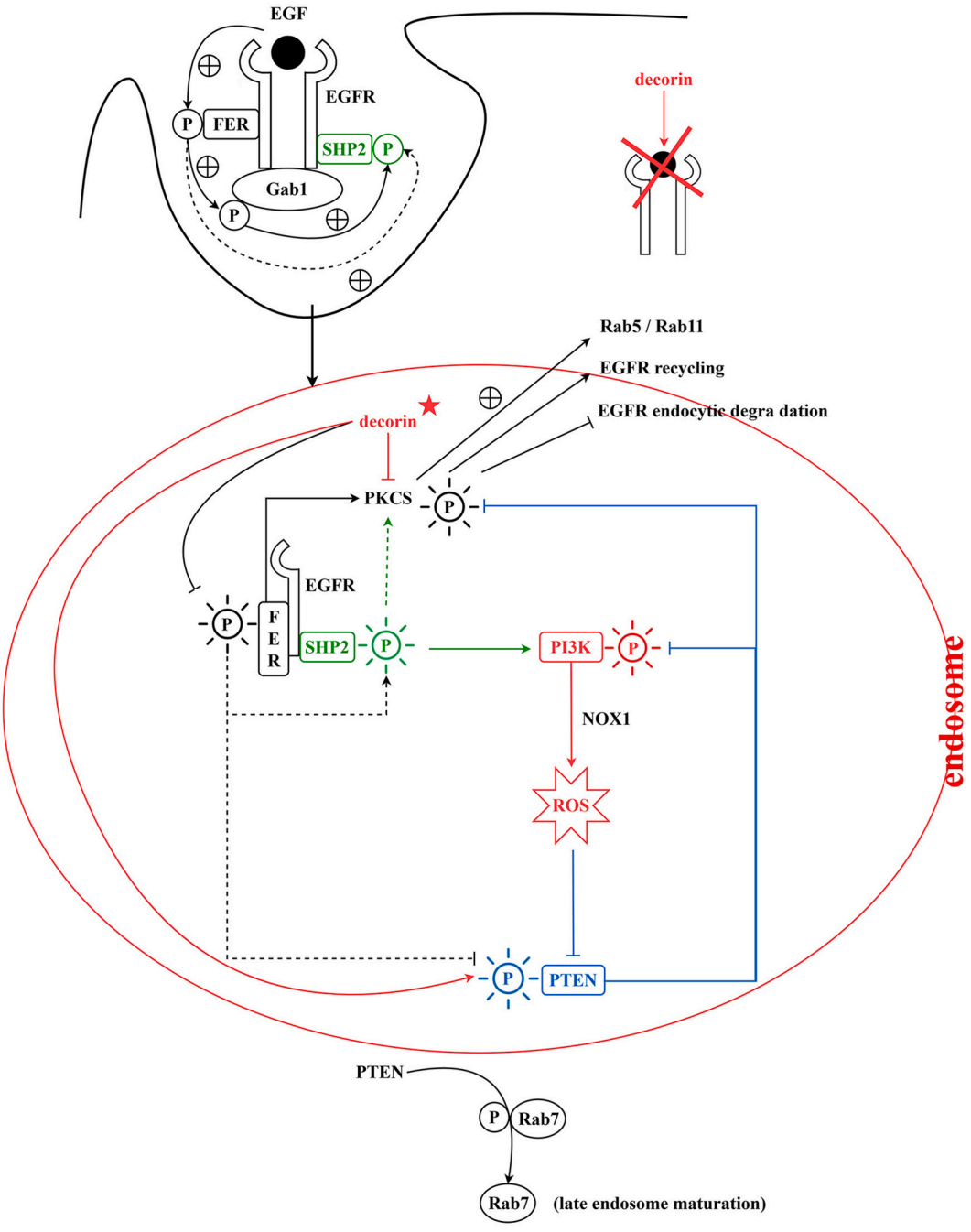


Fig. 5. Illustration of Decorin's modulation of vesicle transport via the EGF/EGFR pathway. Upon EGF binding to EGFR, a cascade activates FER, resulting in enhanced EGFR phosphorylation which leads to ERK and NF-κB activation. Decorin inhibits this sequence by obstructing EGF's binding to EGFR, thereby impeding the phosphorylation events of FER and SHP2. This interference disrupts early endosome formation, transition to late endosomes, and hampers the recycling of EGFR, delineating a pivotal mechanism in regulating EGFR-mediated signal transduction and vesicle trafficking.

protein levels, with both manifesting significant reductions in p-PI3K and p-SHP2 protein levels appearing faint, but NOX1, Rab5-GTP and Rab11-GTP protein levels markedly decreased, whereas p-PTEN and Rab7-GTP protein levels noticeably increased, with no notable differences between the groups (Fig. 4B and C).

5. The function of Decorin within the schematic representation of vesicle transportation.

Epidermal Growth Factor (EGF) binding to the Epidermal Growth Factor Receptor (EGFR) triggers downstream signaling cascades, wherein EGF stimulation induces EGFR engagement and subsequent activation of FER. Amplification of FER results in heightened EGFR phosphorylation, instigating the activation of ERK and NF-κB. FER phosphorylation likely facilitated through the interaction between its Src homology 2 (SH2) domain and EGFR's phosphotyrosine residues, facilitates the recruitment of Growth Factor Receptor-Bound Protein 2 (GAB1) to EGFR's intracellular domain on the plasma membrane. This, in turn, reinforces GAB1 phosphorylation, indirectly escalating the phosphorylation of Src homology region 2 domain-containing phosphatase 2 (SHP2). Following EGFR internalization, it colocalizes with RAB5 before translocating to late endosomes and lysosomes. The EGFR/FER/SHP2 complex within early endosomes augments the phosphorylation status of Protein Kinase C delta (PKCδ). Elevated levels of phosphorylated PKCδ, correlated with the activation of receptor tyrosine kinases and increased transition from RAB5 to RAB7-endosomes, could suggest defects in endosomal transportation. PKCδ phosphorylation converts Rab5 and Rab11 from GDP to GTP forms, arresting the transition from early to late endosomes and facilitating the recycling of EGFR to the plasma membrane, thus averting its degradation. Additionally, the EGFR/FER/SHP2 complex promotes the phosphorylation of Phosphoinositide 3-kinase (PI3K) induced by early endosome formation and NOX1-mediated oxidative stress. Excessive Reactive Oxygen Species (ROS) may impede the phosphorylation of Phosphatase and Tensin Homolog (PTEN). The activity of AKT, which generates Phosphatidylinositol 3,5-bisphosphate (PI(3,5)P2) via PIKfyve, facilitates the transportation of EGFR from early endosomes to later stages, thus facilitating its degradation. Inhibition of AKT diminishes EGFR recycling, leading to its accumulation in early endosomes, prolonging EGFR signaling, and enhancing ERK and Ribosomal S6 Kinase (RSK) activation. It also attenuates EGFR's transport to lysosomes, reducing its degradation. Dephosphorylation of RAB7 by PTEN enhances the transport and maturation of late endosomal membranes, facilitating EGFR's endosomal transport and degradation. Decorin competitively inhibits the binding of EGF to EGFR, thereby impeding the phosphorylation of FER and SHP2, consequently hindering early endosome formation, late endosome transition, and the function of recycling endosomes (Fig. 5).

4. Discussion

Decorin exerts inhibitory effects on the proliferation of thyroid papillary carcinoma cells by suppressing the EGFR/SHP2/FER pathway and influencing intracellular trafficking processes. Specifically, the downregulation of Decorin expression in human papillary thyroid carcinoma cells is associated with inhibition of EGFR/SHP2/FER complex formation. Immunohistochemical analysis reveals a lower level of Decorin presence in cancerous tissue compared to adjacent normal tissue, further supported by Western Blot analysis, indicating decreased levels of Decorin and PTEN in cancer cells. Overexpression of Decorin reduces tumor growth in nude mouse models, an effect reversed by Decorin silencing and attenuated by FER inhibitors. Additionally, Decorin modulates the levels of Rab5-GTP and Rab7-GTP, affecting endosomal trafficking and downstream signaling. Thus, Decorin inhibits the proliferation of thyroid papillary carcinoma cells through suppression of the EGFR/SHP2/FER pathway and modulation of intracellular trafficking.

Endocytosis, alternatively termed cellular engulfment, epitomizes the mechanism through which cellular entities internalize extracellular substances via their plasma membrane. This intricate process, replete with dynamism and energy requisites, facilitates the cellular assimilation of vital nutrients, fluids, proteins, and assorted macromolecules. Additionally, it assumes a pivotal role in the removal of cell surface receptors and modulation of cellular interactions with the external milieu. Receptor-mediated endocytosis, marked by its high degree of specificity, hinges upon cell surface receptors discerning and binding with cognate ligands (e.g., proteins, lipoproteins, iron transport proteins, etc.). After this binding event, the cell aggregates these receptor-ligand complexes locally, culminating in the formation of vesicular structures termed clathrin-coated pits, which subsequently bud off to form internalized vesicles recognized as endosomes. These endosomes embark upon a journey to diverse subcellular destinations, wherein ligands may be utilized, degraded, or, in certain scenarios, recycled back to the extracellular milieu.

The efficient sorting of substances internalized via endocytosis represents a fundamental aspect of key cellular processes and constitutes a primary, if not exclusive, transit pathway within mammalian cells. The trafficked entities—solutes, receptors and their cargos, lipids, and even pathogens—are directed to their respective destinations at two principal sorting stations within mammalian cells: early and late endosomes. Early endosomes serve as an initial sorting nexus, receiving a plethora of materials from the plasma membrane and the Golgi apparatus. This pivotal role involves routing molecules either back to the cell surface via recycling endosomes, retrogradely to the *trans*-Golgi network, or onwards to the late endosomes/lysosomes. Additionally, early endosomes regulate cellular signaling by downregulating internalized receptors, which become encapsulated within intraluminal vesicles formed via invagination of the limiting membrane. These multivesicular regions either segregate from the early endosomes or evolve into free endocytic transport vesicles/multivesicular bodies, facilitating cargo conveyance to late endosomes. Late endosomes serve as a central nexus for inbound traffic from endosomal, biosynthetic, and autophagic pathways, while also facilitating outbound traffic to lysosomes, the Golgi complex, or the plasma membrane. Moreover, they operate as a critical sensing/signaling platform, providing the cell with pertinent information regarding its nutritional status.

Decorin, a diminutive leucine-rich proteoglycan abundantly distributed within the extracellular matrix, comprises a core protein adorned with one or multiple glycosaminoglycan (GAG) chains, typically featuring dermatan sulfate. This multifaceted entity assumes a pivotal role in an array of physiological and pathological processes, including tissue regeneration, cellular proliferation, apoptosis,

and tumorigenesis. Furthermore, Decorin modulates the activity of various growth factors and receptors, including the epidermal growth factor receptor (EGFR) and transforming growth factor-β (TGF-β), thereby exerting influence upon cell signaling cascades [31–33].

The interplay between Decorin and endosomal pathways is principally evident in the pathways of cellular endocytosis and signaling. For example, Decorin can bind to tyrosine kinase receptors (TKRs) such as the EGF receptor (EGFR), inducing their aggregation and internalization, followed by degradation via the endosome-lysosome pathway. After material uptake, substances initially enter early endosomes, then maybe transported to late endosomes, and ultimately reach lysosomes for degradation, or be recycled back to the cell surface [34]. Through endocytosis of its bound receptors, Decorin indirectly influences the intracellular distribution and degradation of these proteins, thereby affecting the dynamics of cell signaling. Sometimes considered to have anti-tumor effects, Decorin is believed to modulate receptor internalization and downstream signaling by interacting with cell surface receptors, such as through inhibiting growth-factor-activated signaling and limiting tumor cell proliferation and dissemination [35–37]. This study found that overexpression of Decorin can inhibit the phosphorylation of proteins such as EGFR, FER, and SHP2, while Decorin knockdown promotes their phosphorylation, suggesting that Decorin may inhibit the transition from early to late endosomes, thus impeding EGFR degradation. Overexpression of Decorin suppresses the expression of Rab5-GTP and Rab11-GTP while promoting Rab7-GTP expression, indicating Decorin might inhibit early to late endosomal transition.

Numerous documents have chronicled the role of Decorin within the tumor microenvironment. Decorin, a diminutive polysaccharide-rich in leucine, pervades the extracellular matrix, exerting pivotal roles in various physiological and pathological processes, notably in tumorigenesis, cellular proliferation, migration, invasion, and extracellular matrix modulation. Within diverse cancer types, Decorin typically manifests as a tumor suppressor, regulating an array of receptors associated with cellular growth and survival. Diminishing Decorin expression may attenuate the extracellular matrix, enhancing receptor efficacy, thereby fostering tumor dissemination [38–40]. Decorin is believed to influence the development and metastasis of tumors by modulating the interaction between tumor stroma and immune cells. For instance, Decorin can recruit mesenchymal stem cells to damaged tissue sites through its N-terminal fragment, which generates a novel chemotactic factor called MayDay, potentially impacting the tumor immune microenvironment. In a prostate cancer bone metastasis model, Decorin, along with immune cells, hematopoietic progenitor cells, and bone-resident cells in the tumor microenvironment, collectively shapes the tumor microenvironment, affecting bone structure and function [41,42]. Decorin possesses the capability to selectively bind with various types of collagen, notably type I collagen, thereby modulating the formation and organization of collagen fibers. This interaction is pivotal in maintaining the mechanical strength and integrity of tissues. Moreover, Decorin exhibits an affinity for multiple growth factors, such as Transforming Growth Factor-β (TGF-β). Through this binding, Decorin orchestrates the activity of these growth factors, thereby influencing processes such as cell proliferation, differentiation, and tissue repair. Additionally, Decorin interacts with other extracellular matrix proteins such as fibronectin, thus impacting cellular adhesion and migration [43–46].

EGFR, a cell surface receptor tyrosine kinase, governs cell growth, differentiation, and more. Upon binding to its ligands like the epidermal growth factor (EGF), EGFR is activated and phosphorylated, thereby triggering downstream signaling pathways like RAS/RAF/MEK/ERK and PI3K/AKT, promoting cell proliferation. After activation, EGFR is internalized into the cell, passing through early to late endosomes, hence facilitating downstream signaling. Within the late endosomes, EGFR may be ubiquitinated and targeted for lysosomal degradation, or recycled back to the cell surface [47,48]. Decorin competes with EGF for binding to EGFR, thus inhibiting the activation of EGFR and subsequent downstream signaling pathways.

FER is a cytoplasmic tyrosine kinase involved in regulating cell motility, polarity, and cancer metastasis. p-FER refers to the phosphorylated, active form of FER [49]. SHP2, a protein tyrosine phosphatase with SH2 domains, partakes in various signaling pathways such as the RAS/MAPK pathway, regulating cellular differentiation, proliferation, and migration [50,51]. PKCδ, a member of the protein kinase C (PKC) family, is involved in a host of biological processes, including cell survival, differentiation, and apoptosis. p-PKCδ indicates the phosphorylated, active state of PKCδ [52–54]. PI3K, an enzyme activated to phosphorylate phosphatidylinositol and generate the signaling molecule PIP3, activates AKT and is integral to cell proliferation and survival pathways [55–58].

This report reveals a significant underexpression of Decorin in papillary thyroid carcinoma tissues and elevated expression in adjacent normal tissues, suggesting Decorin's involvement in the development of this cancer. The xenograft model demonstrated that overexpression of Decorin hinders the proliferation of papillary thyroid carcinoma cells, while its knockdown enhances proliferation. Western Blot analysis revealed that overexpression of Decorin inhibits the phosphorylation of proteins such as EGFR, FER, and SHP2 while silencing Decorin boosts their phosphorylation. The findings imply that Decorin could suppress the EGFR signaling pathway and regulate endosomal transport to inhibit the growth of papillary thyroid carcinoma. This provides theoretical justification for Decorin as a potential target in the treatment of this type of cancer. Understanding Decorin's regulatory mechanisms could aid in the development of novel therapeutic strategies.

The research is constrained by limited samples, potentially affecting the generalizability and statistical significance of the findings. Specific experimental designs, such as in vitro experiments or animal models, may not entirely mimic human disease conditions. The outcomes are contingent upon particular data analysis methods, restricting the breadth of interpretive possibilities. The long-term inhibitory effects of Decorin on tumor progression remain unexplored and necessitate further investigation. Validation of these findings in clinical applications requires additional clinical trials to ascertain their safety and efficacy. There may be other unexplored signaling pathways or molecular mechanisms involved in the action of Decorin on tumor cells.

Ethics approval

This study has been reviewed and approved by the Laboratory Animal Ethics Committee of Hebei North University. Ethics Number:

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Yaping Liu: Writing – review & editing, Writing – original draft, Data curation. **Yunchao Xin:** Writing – review & editing, Data curation. **Tianyun Lv:** Writing – review & editing, Data curation. **Zhicheng Chang:** Funding acquisition, Data curation. **Gang Xue:** Writing – review & editing, Data curation. **Xiaoling Shang:** Writing – review & editing, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33358.

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