The expression and the tumor suppressor role of CLDN6 in colon cancer

Huinan Qu1 · Min Wang1 · Miaomiao Wang1 · Yuanyuan Liu1 · Chengshi Quan1

Received: 17 November 2021 / Accepted: 24 April 2022 / Published online: 14 June 2022
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2022

Abstract
As a member of the tight junction family, CLDN6 is a tumor suppressor in breast cancer, but its role in colon cancer is unknown. In this research, we aimed at revealing the function of CLDN6 in colon cancer. We found that colon cancer tissues lowly expressed CLDN6, and the expression of CLDN6 was negatively correlated with lymph node metastasis. Similarly, CLDN6 was lowly expressed in the colon cancer cell line SW1116, and overexpression of CLDN6 inhibited cell proliferation in vitro and in vivo. Consistently, the migration and invasion abilities of cells were significantly inhibited after CLDN6 overexpression. In addition, we demonstrated that CLDN6 may inhibit the migration and invasion abilities by activating the TYK2/STAT3 pathway. Therefore, our data indicated that CLDN6 acted as a tumor suppressor and had the potential to be regarded as a biomarker for the progression of colon cancer.

Keywords CLDN6 · Proliferation · Migration · Invasion · Colon cancer · TYK2/STAT3

Introduction
Colon cancer is the fourth most deadly cancer with about 900,000 deaths every year in the world [1]. Approximately 25% of colon cancer patients have hepatic metastasis on initial diagnosis, and about 50% would develop hepatic metastasis within 3 years of the primary surgery [2]. Therefore, searching for new biomarkers related to tumor development and clarifying underlying molecular mechanisms could be critical for screening patients at high risk of recurrence and identifying novel therapeutic targets.

Claudins (CLDNs) are major tight junction proteins and the alteration of CLDNs is one of the mechanisms for the loss of cell adhesion, which is an important step in tumor metastasis [3]. In addition, CLDNs regulate cancer cells proliferation [4], apoptosis [5, 6], autophagy [7–9], drug resistance [10], and so on. Several CLDNs are associated with the malignant phenotypes of colon cancer. Studies have shown that CLDN1 increased the metastatic behavior of colon cancer by the promotion of EMT[11, 12]. On the contrary, Resnick et al. [13] found that lower expression of CLDN1 was associated with higher tumor grade, lymphovascular invasion and poor survival in stage II colon cancer. Loss of CLDN3 increased the abilities of migration and invasion of colon cancer cells [14], and CLDN7 correlated with venous invasion and liver metastasis of colon cancer [15]. However, the role and mechanism of CLDN6 in colon cancer progression were unclear.

The protein tyrosine kinase 2 (TYK2) is the first identified member of the Janus kinase (JAK) family and is aberrantly activated in various types of cancer [16]. TYK2 is required to transduce the signaling from growth factors, cytokines, and oncogenes to the signal transducers and activators of transcription (STAT) family, including STAT3. STAT3 is an important transcription factor that plays a crucial role in the development of cancer by regulating the expression of genes related to the cell cycle, motility, apoptosis, and metastasis [17]. For that, it is regarded as an oncogene. However, studies have found that STAT3 could be either an oncogene or tumor suppressor under different conditions [18].

Signaling transduction involving CLDN6 has received much attention in recent years. There is a PDZ binding motif at the carboxy terminus of CLDN6 that can bind to proteins that contain PDZ domains [19]. In this way, CLDN6 is involved in cell signaling transduction that regulates
multiple biological behaviors of tumors. The signaling pathways involved in tumors that CLDN6 participates in include but are not limited to the SFK/PI3K/AKT pathway [20], the AF6/ERK pathway [21], and the ASK1/p38/JNK pathway [22] in breast cancer, the YAP/Snail pathway in gastric cancer [23], and the PI3K/AKT/mTOR pathway in endometrial carcinoma [24]. We previously performed RNA sequencing of MCF-7/CLDN6 cells and found that the differentially expressed genes were enriched in the TYK2/STAT3 pathway. Therefore, we hypothesized that CLDN6 may regulate the TYK2/STAT3 pathway in colon cancer.

In this research, we examined the expression of CLDN6 in colon cancer tissues and analyzed the relationship between CLDN6 expression and clinicopathological characteristics of colon cancer patients. Also, we investigated the effect of CLDN6 on the biological characteristics of colon cancer cells. Moreover, we explored the potential mechanism by which CLDN6 regulated the migration and invasion abilities of colon cancer cells. Our study suggested that CLDN6 might be a useful marker to identify the progression of colon cancer.

Materials and methods
Clinical samples and data collection
Paraffin sections were collected from 107 patients pathologically with colon cancer who were treated with surgery at the Eastern Division of the First Hospital of Jilin University from January 2012 to June 2013. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Jilin University and informed consent was obtained from all patients. There were 66 males and 41 females with an average age of 58.3 years old. Among them, 11 tumors were well-differentiated, 65 tumors were moderately-differentiated and the other 31 tumors were poorly-differentiated. There were 49 cases with lymph node metastasis and 58 cases without metastasis. 107 cases of normal colonic tissues adjacent to the cancer were taken as control specimens.

Immunohistochemistry (IHC)
IHC was performed as described [25]. Tissue sections were immunostained with CLDN6 antibody (Santa Cruz, CA, USA). Diaminobenzidine (DAB) was used for color development. CLDN6 expression was indicated in brown and was expressed in the cell membrane. Scoring was performed as follows: negative, < 10% positive tumor cells; positive, ≥ 10% positive tumor cells. Positive or negative reactions were determined in five random fields of each sample.

Cell culture and reagents
Human colon cancer cell line SW1116 and immortalized colon epithelial cell line NCM460 were cultured in H-DMEM medium (Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS; HyClone Laboratories, Logan, UT, USA) and 100 units/mL penicillin and 100 μg/mL streptomycin (Gibco, Carlsbad, CA, USA) in a 5% CO₂ humidified incubator at 37 °C. AG490 (Sigma-Aldrich Crop., St. Louis, MO, USA) was dissolved in DMSO at a store concentration of 1 mM.

Plasmid and transfection
A CLDN6 overexpression plasmid was established as previously described [26]. Cells were transfected with plasmid by using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), following the procedure recommended by the manufacturer. The single clone was screened out by G418 and then cultured for amplification.

RNA extraction and RT-PCR analysis
Total RNA was extracted using TRizol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) and reverse-transcribed with M-MLV reverse transcriptase (Takara Bio Inc, Shiga, Japan). RT-PCR was performed to analyze CLDN6 mRNA expression (Applied Biosystems, Carlsbad, CA, USA). The primer sequences of CLDN6:

F-(5′-TTC ATC GGC AAC AGC ATC GT-3′)
R-(5′-GGT TAT AGA AGT CCC GGA TGA-3′)

Western blot (WB) analysis
RIPA lysis buffer (Beyotime, Shanghai, China) was used to extract protein from cells. The protein concentration was determined by a BCA protein assay kit (Beyotime). 40 μg of each sample were applied to 12% SDS–polyacrylamide gel, then proteins were transferred onto a nitrocellulose membrane (Millipore, California, USA) and incubated with each primary antibody overnight at 4 °C, followed by incubation for 1 h at room temperature with HRP-conjugated secondary antibody.

Colony formation assay
Single-cell suspensions were prepared and plated into six-well plates. The visible colonies appeared after 3 weeks and were fixed with methanol for 20 min, and then stained with Giemsa solution for 5 min. Cell colonies with a diameter > 0.5 mm were counted and the colony formation
efficiency was calculated with the following formula: % of colony formation = (number of colonies formed/number of cells inoculated) × 100%.

**Cell viability assay**

The viability of cells was monitored using the Cell Counting Kit-8 (CCK-8) assay (Dojindo, Kumamoto, Japan) according to the manufacturer’s instructions. The optical density (OD) at 450 nm of each group was measured at 12, 24, 48, 72, and 96 h on a Microplate Reader (Thermo, Schwerte, Germany).

**Subcutaneous xenograft models**

A total number of twelve 6-week-old BALB/c nude mice (body weight: 16–20 g) were randomly divided into two groups and subcutaneously injected with SW1116/vec or SW1116/CLDN6 cells (5 × 10⁶ cells per mouse) into the hind flank. Calipers were used to measure the length (L) and width (W) of the tumors every 4 days. The tumor volume was estimated by the formula \( V = 0.52 \times L \times W^2 \). All animal experiments and their care were approved by the Experimental Animal Ethical Committee of Jilin University and were carried out following relevant institution guidelines.

**Wound-healing assay**

When the cell grew to about 80% confluence, a single scratch was produced by dragging a 10 μL pipette tip. Then these cells were washed with PBS three times to remove cell debris. The width of the wounded areas was measured by microscopy (OLYMPUS, Tokyo, Japan) at 0 h (W₀) and 24 h (W₂₄) after injury. The migration rate was calculated as \((W₀ - W₂₄)/W₀\).

**Transwell chamber assay**

Matrigel invasion assay was performed using Transwells containing 8.0 μm pore membranes (Corning, Lowell, MA, USA). Cells were placed in the upper chamber of the Transwell for 48 h. Then the chambers were washed twice with PBS. The filter side of the upper chamber was cleaned with a cotton swab. Next, the remained cells in the upper chamber of the membrane were cut out of the insert. Cells were fixed in methanol and stained with 5% Giemsa for 30 min at room temperature. The invaded cells were photographed and counted under a light microscope.

**Statistical analysis**

Statistical analysis was performed using GraphPad Prism 8.0 (GraphPad, San Diego, CA, USA). All data were presented as mean ± SD. Statistical significance was determined by the Student’s t-test or one-way analysis of variance (ANOVA). The protein expression levels and clinicopathologic features were compared by the chi-square test. Differences between groups were considered statistically significant if \( P < 0.05 \).

**Results**

**CLDN6 is lowly expressed in colon cancer**

To detect the expression of CLDN6 in colon cancer, we performed an IHC assay using colon cancer tissues. As shown in Fig. 1a, positive expression of CLDN6 in adjacent normal tissues was found mainly in the plasma membrane. The positive expression ratio of CLDN6 in adjacent tissues was 75.70% (81/107) and that in colon cancer tissues was only 26.16% (28/107) (Table 1). Besides, the expression of CLDN6 in cancer tissues was negatively associated
with lymph node metastasis (Table 2). We also detected the expression of CLDN6 in the colon cancer cell line SW1116. Both mRNA (Fig. 1b, c) and protein levels (Fig. 1d, e) of CLDN6 were significantly lower in SW1116 cells than that in NCM460 cells. Collectively, these results suggested that CLDN6 was lowly expressed in colon cancer and it may have an important role in the progression of colon cancer.

CLDN6 inhibits proliferation, migration and invasion of SW1116 cells

To better understand the function of CLDN6, we stably overexpressed CLDN6 in SW1116 cells. RT-PCR and WB were performed to measure the mRNA and protein levels of CLDN6 (Fig. 2a–c). As shown in Fig. 2d, e, the colony formation ability of SW1116/CLDN6 cells was significantly lower than that of SW1116/vec cells. Using CCK8 assay, we found that SW1116/CLDN6 cells showed lower cell viability compared with SW1116/vec cells (Fig. 2f). To evaluate whether CLDN6 inhibited cell proliferation in vivo, we injected subcutaneously tumor cell suspensions in nude mice and observed the situation of tumor growth. All six mice injected with SW1116/vec cells formed a transplanted tumor, as in the SW1116/CLDN6 group only five mice formed a transplanted tumor (Fig. 2g). Transplanted tumors formed by SW1116/CLDN6 cells had a lighter weight (Fig. 2h) and slower growth rate (Fig. 2i) than that generated by SW1116/vec cells. IHC assay also verified the higher expression of CLDN6 in the SW1116/CLDN6 xenografts (Fig. 2j). These results suggested that CLDN6 inhibited the proliferation of SW1116 cells in vitro and in vivo.

We also found that the cell morphology changed from fusiform to polygon after CLDN6 overexpression (Fig. 3a). Next, we performed a wound-healing assay to evaluate the effect of CLDN6 on cell migration. The results showed that the SW1116/CLDN6 cells had a significantly decreased wound-healing ability compared with SW1116/vec cells (Fig. 3b, c). SW1116/CLDN6 cells elicited a significant reduction of invading cells compared with SW1116/vec cells detected by transwell chamber assay (Fig. 3d, e). Taken together, these results indicated that CLDN6 suppressed the migration and invasion abilities of SW1116 cells.

CLDN6 suppresses the migration and invasion abilities of SW1116 cells via activating the TYK2/STAT3 pathway

Our previous studies have shown that MCF-7/CLDN6 cells have significantly changed genes enriched in the TYK2/STAT3 pathway. We next accessed the effect of CLDN6 on the TYK2/STAT3 pathway in SW1116 cells. As shown in Fig. 4a and b, p-TYK2 and p-STAT3 expressed significantly higher in SW1116/CLDN6 cells, indicating that CLDN6 activated the TYK2/STAT3 pathway.

To demonstrate whether CLDN6 exerted its function on cell migration and invasion abilities via the TYK2/STAT3 pathway, SW1116/CLDN6 cells were treated with AG490, an inhibitor of the TYK2/STAT3 pathway. After treating SW1116/CLDN6 cells with different concentrations of AG490, the optimal concentration was found to be 50 μM (Fig. 4c, d). Besides, SW1116/CLDN6 cells treated with AG490 for 48 h showed a significantly lower p-STAT3 expression (Fig. 4e, f). Thus, we used the most appropriate concentration and time of AG490 to process the following research. AG490 treatment returned the SW1116/CLDN6 cells to the original fusiform (Fig. 4g). The wound-healing assay showed that cells treated with AG490 had a significantly increased wound healing ability compared with the
Figure 2 Effects of CLDN6 on the proliferation of SW1116 cells in vitro and in vivo. a–c Transfection efficacy was confirmed by RT-PCR and WB. d, e Detection of cell proliferation in both SW1116/vec and SW1116/CLDN6 cells by colony formation assay. f Detection of cell viability in both SW1116/vec and SW1116/CLDN6 cells by CCK8. g Xenograft tumors in nude mice formed by SW1116/vec and SW1116/CLDN6 cells. h Tumor tissues of SW1116/vec and SW1116/CLDN6 groups were weighed. i Volumes of xenograft tumors measurements in each group every 4 days. j Representative images of negative staining of CLDN6 in SW1116/vec group and positive staining of CLDN6 in SW1116/CLDN6 group. *P < 0.05; Scale bars, 50 µm

Discussion

Dysregulated CLDN6 plays an oncogenic or tumor-suppressive role depending on target tissues or cell types. Studies have shown that CLDN6 was upregulated and promoted tumor progression in gastric cancer [27], non-small-cell lung cancer [28], atypical teratoid/rhabdoid tumors [29], control (Fig. 4h, i), indicating AG490 reversed the inhibition of migration by CLDN6. The invasion assay conducted using matrigel-coated transwell chambers obtained similar results (Fig. 4j, k). Collectively, these data indicated that CLDN6 might suppress the migration and invasion abilities of SW1116 cells via activating the TYK2/STAT3 pathway.
and hepatocellular carcinoma [30], which made CLDN6 a possible therapeutic target for these tumors. In the present study, we found that CLDN6 was lowly expressed in colon cancer, and this was consistent with our previous study of low expression of CLDN6 in breast cancer [26]. In breast cancer, the reduction of CLDN6 mRNA is associated with the methylation of CLDN6’s promoter [31, 32]. The underlying mechanism of CLDN6 expression in colon cancer should be clarified in the future.

To evaluate the role of CLDN6, we overexpressed CLDN6 in SW1116 cells and examined the effect of CLDN6 on the proliferation of SW1116 cells by in vitro and in vivo experiments. The in vitro experiments showed that CLDN6 inhibited the colony formation ability and cell viability of SW1116 cells, suggesting that CLDN6 inhibited colon cancer cell proliferation. The in vivo experiments showed that the volume and weight of transplanted tumors in the SW1116/CLDN6 group were significantly lower than those in the SW1116/vec group, suggesting that CLDN6 inhibited the growth of transplanted tumors in nude mice. We noted that all six mice in the SW1116/vec group had tumors, while only five mice in the SW1116/CLDN6 group had tumors, which we speculate may be related to individual differences in nude mice or the manipulation of the cell suspension when it was inoculated.

The JAK/STAT pathway is considered to be the core pathway of tumors and directly contributes to all hallmarks of tumors [33, 34]. Previous studies have mostly focused on the relationship between JAK1-3 and tumors, and as research has progressed, the impact of TYK2 on tumors has received increasing attention, especially its relationship with tumor metastasis [35, 36]. Abundant evidence indicates that TYK2/STAT3 pathway is persistently activated in several cancers, with a crucial position in tumor onset and progression [37, 38]. Notably, recent studies have shown that a high level of CLDN9 or CLDN17 enhanced the metastatic potential of

![Fig. 3 Effects of CLDN6 on the migration and invasion abilities of SW1116 cells. a] Comparison of cell morphology before and after CLDN6 overexpression. b, c] The migration ability of SW1116/vec and SW1116/CLDN6 cells was assessed by wound-healing assay. d, e] The invasion ability of SW1116/vec and SW1116/CLDN6 cells was assessed by transwell assay. *P < 0.05.](image-url)
hepatocytes via activating the TYK2/STAT3 pathway [39, 40]. Consistent with this, in colon cancer cells, we found that the TYK2/STAT3 pathway was activated following CLDN6 overexpression. However, the activated TYK2/STAT3 pathway played an important role in CLDN6-mediated inhibition of proliferation, migration, and invasion, contrary to its role in CLDN9 or 17-mediated promoting the metastatic potential of hepatocellular carcinoma. Similar to our results, several studies have demonstrated the anticancer effect of STAT3 or p-STAT3. For example, Bekki et al. [41] found that in undifferentiated pleomorphic sarcoma, p-STAT3 expression was lower in tumors than in normal tissues, and positivity for p-STAT3 was significantly correlated with a better prognosis. Wu et al. [42] reported that high STAT3 expression may predict a better overall outcome for breast cancer patients. It’s speculated that different cells or tissues, and different cellular states or microenvironments may affect the role of the TYK2/STAT3 pathway.

There are still certain limitations in this study. First, although we have observed that the expression of CLDN6 in colon cancer tissue was negatively related to colon cancer lymph node metastasis, we have only verified it in vitro, and in vivo experiments are needed to verify our results. Second, we found that CLDN6 overexpression activated the TYK2/STAT3 pathway, and we used AG490 to inhibit the activation of the signaling and detect cell migration, and invasion. However, although AG490 was considered as a pan-JAK family inhibitor in some studies [43–45], it has also been reported to be a specific inhibitor of JAK2 [46–48]. So we can’t exclude its inhibitory effect on the JAK2/STAT3 pathway. Finally, the mechanism by which CLDN6 increased the expression level of p-TYK2 was not clear. CLDNs play a decisive role in maintaining the integrity of the barrier function of epithelial cells. After the redistribution or expression of CLDNs change, the tight junctions between cells are destroyed and the barrier function of epithelial cells is impaired, which in turn changes the permeability between epithelial cells and mediates cellular inflammation. The JAK/STAT pathway is regulated by a variety of cytokines including distinct interleukin (ILs) and interferons (IFNs), which mediate the pathway activation by binding to corresponding receptors. It is reported that the loss of CLDN3 promotes the IL-6/STAT3 signaling [14]. Besides, IL-12 and IL-23 specifically activate the TYK2/STAT3 pathway [49]. Whether CLDN6 overexpression regulates the TYK2/STAT3 pathway by affecting cytokines including IL-12 or IL-23 deserves further exploration. Besides, the PDZ binding motif of CLDN6 may play an important role in activating the TYK2/STAT3 pathway. Importantly, a recent study showed that CLDN6 directly activated the SFK/PI3K/AKT signaling depending on ECL2 and Y196/200 to transmit cell adhesion signals to the nucleus and regulate gene expression [20]. To expand upon the results of the present study, our future investigations will attempt to elucidate the mechanism by which CLDN6 activates the TYK2/STAT3 pathway.

Conclusion

CLDN6 expression was low in colon cancer and negatively correlated with lymph node metastasis in patients. CLDN6 inhibited the proliferation of SW1116 cells in vitro and in vivo. CLDN6 may suppress the migration and invasion abilities of SW1116 cells by activating the TYK2/STAT3 pathway. Our finding suggested that CLDN6 acted as a tumor suppressor and could be a potential biomarker for colon cancer progression.
**Fig. 4** CLDN6 suppresses the migration and invasion abilities of SW1116 cells by activating the TYK2/STAT3 pathway. a, b WB analysis of proteins in the TYK2/STAT3 pathway of SW1116/vec and SW1116/CLDN6 cells. c, d SW1116/CLDN6 cells were treated with AG490 at indicated concentrations for 24 h to find the optimum treatment dose. e, f SW1116/CLDN6 cells were treated with 50 μM AG490 for indicated hours to find the optimum treatment time. g The morphology of SW1116/CLDN6 cells before and after AG490 treatment. h, i The effect of AG490 on the migration ability of SW1116/CLDN6 cells by wound-healing assay. j, k The effect of AG490 on the invasion ability of SW1116/CLDN6 cells by transwell assay. *P < 0.05

**Author contributions** The first draft of the manuscript was written by HQ. Cell and animal experiments, data analysis were performed by MW. Molecular biology experiments were performed by YL. Immunochemistry experiments were performed by YL. Study design, funding acquisition, and supervision were completed by CQ.

**Funding** This work was supported by the National Natural Science Foundation of China [Grant Numbers 81772816] and the Natural Science Foundation of Jilin Province [Grant Numbers 20210101329JC].

**Data availability** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Declarations**

**Conflict of interest** The authors have no relevant financial or non-financial interests to disclose.

**Ethical approval** Tumor tissues were obtained from the Eastern Division of the First Hospital of Jilin University. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Jilin University and informed consent was obtained from all patients. All animal experiments and their care were approved by the Experimental Animal Ethical Committee of Jilin University and were carried out following relevant institution guidelines.

**References**

1. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB (2019) Colorectal cancer. Lancet 394:1467–1480. https://doi.org/10.1016/S0140-6736(19)32319-0
2. DeSantis CE, Lin CC, Mariotto AB, Siegel RL, Stein KD, Kramer JL, Alteri R, Robbins AS, Jemal A (2014) Cancer treatment and survivalship statistics, 2014. CA Cancer J Clin 64:252–271. https://doi.org/10.3322/caac.21235
3. Tabarriés S, Siegel PM (2017) The role of claudins in cancer metastasis. Oncogene 36:1176–1190. https://doi.org/10.1038/onc.2016.289
4. Shen Z, Song W, Qian L, Zhu J, Li Y, Li M, Zhang T, Zhao W, Zhou Y, Yang X (2021) Effect of claudin 1 on cell proliferation, migration and apoptosis in human cervical squamous cell carcinoma. Oncol Rep 45:606–618. https://doi.org/10.3892/or.2020.7889
5. Zhang X, Ruan Y, Li Y, Lin D, Quan C (2015) Tight junction protein claudin-6 inhibits growth and induces the apoptosis of cervical carcinoma cells in vitro and in vivo. Med Oncol 32:148. https://doi.org/10.1007/s12032-015-0600-4
6. Lu YZ, Li Y, Zhang T, Han ST (2020) Claudin-6 is down-regulated in gastric cancer and its potential pathway. Cancer Biomark 28:329–340. https://doi.org/10.3233/cbm-201554
7. Tong H, Li T, Qiu W, Zhu Z (2019) Claudin-1 silencing increases sensitivity of liver cancer HepG2 cells to 5-fluorouracil by inhibiting autophagy. Oncol Lett 18:5709–5716. https://doi.org/10.3892/ol.2019.10967
8. Wu J, Gao F, Xu T, Li J, Hu Z, Wang C, Long Y, He X, Deng X, Ren D, Zhou B, Dai T (2020) CLDN1 induces autophagy to promote proliferation and metastasis of esophageal squamous carcinoma through AMPK/STAT1/ULK1 signaling. J Cell Physiol 235:2245–2259. https://doi.org/10.1002/jcp.29133
9. Song P, Li Y, Dong Y, Liang Y, Qu H, Qi D, Lu Y, Jin X, Guo Y, Jia Y, Wang X, Xu W, Quan C (2019) Estrogen receptor β inhibits breast cancer cells migration and invasion through CLDN6-mediated autophagy. J Exp Clin Cancer Res 38:354. https://doi.org/10.1186/s13046-019-1359-9
10. Visco ZR, Sfakianos G, Boudreau MH, Simpson S, Rodriguez I, Whitaker R, Yao DY, Berchuck A, Murphy SK, Huang Z (2021) Epigenetic regulation of Claudin-1 in the development of ovarian cancer recurrence and drug resistance. Front Oncol 11:620873. https://doi.org/10.3389/fonc.2021.620873
11. Dhawan P, Singh AB, Deane NG, No Y, Shiu SR, Schmidt C, Neff J, Washington MK, Beauchamp RD (2005) Claudin-1 regulates cellular transformation and metastatic behavior in colon cancer. J Clin Invest 115:1765–1776. https://doi.org/10.1172/jci24543
12. Luan N, Chen Y, Li Q, Mu Y, Zhou Q, Ye X, Deng Q, Ling L, Wang J, Wang J (2021) TRF-20-MONK5Y93 suppresses the metastasis of colon cancer cells by impairing the epithelial-to-mesenchymal transition targeting Claudin-1. Am J Transl Res 13:124–142
13. Resnick MB, Konkin T, Routhier J, Sabo E, Pricolo VE (2005) Claudin-1 is a strong prognostic indicator in stage II colonic cancer: a tissue microarray study. Mod Pathol 18:511–518. https://doi.org/10.1038/modpathol.3800301
14. Ahmad R, Kumar B, Chen Z, Chen X, Müller D, Lele SM, Washington MK, Batra SK, Dhawan P, Singh AB (2017) Loss of claudin-3 expression induces IL6/gp130/Stat3 signaling to promote colon cancer malignancy by hyperactivating Wnt/β-catenin signaling. Oncogene 36:6592–6604. https://doi.org/10.1038/onc.2017.259
15. Oshima T, Kunisaki C, Yoshikara K, Yamada R, Yamamoto N, Sato T, Makino H, Yamagishi S, Nagano Y, Fujii S, Shiozawa M, Akaime M, Wada N, Rino Y, Masuda M, Tanaka K, Imada T (2008) Reduced expression of the claudin-7 gene correlates with venous invasion and liver metastasis in colorectal cancer. Oncol Rep 19:953–959
16. Wöss K, Simonovic N, Strobl B, Macho-Maschler S, Müller M (2019) TYK2: an upstream kinase of STATs in cancer. Cancers (Basel). https://doi.org/10.3390/cancers11111728
17. Yu H, Lee H, Herrmann A, Buettner R, Jove R (2014) Revisiting STAT3 signalling in cancer: new and unexpected biological functions. Nat Rev Cancer 14:736–746. https://doi.org/10.1038/nrc3818
18. Avalle L, Camporeale A, Camperi A, Poli V (2017) STAT3 in cancer: a double edged sword. Cytokine 98:42–50. https://doi.org/10.1016/j.cyto.2017.03.018
19. Lin D, Guo Y, Li Y, Ruan Y, Zhang M, Jin X, Yang M, Lu Y, Song P, Zhao S, Dong B, Xie Y, Dang Q, Quan C (2017) Bioinformatic analysis reveals potential properties of human Claudin-6 regulation and functions. Oncol Rep 38:875–885. https://doi.org/10.3892/or.2017.5756
20. Sugimoto K, Ichikawa-Tomikawa N, Kashiwagi K, Endo C, Tanaka S, Sawada N, Watabe T, Higashi T, Chiba H (2019) Cell adhesion signals regulate the nuclear receptor activity. Proc Natl Acad Sci USA 116:24600–24609. https://doi.org/10.1073/pnas.1913346116

21. Yang M, Li Y, Ruan Y, Lu Y, Lin D, Xie Y, Dong B, Dang Q, Quan C (2018) CLDN6 enhances chemoresistance to ADM via AF-6/ERKs pathway in TNBC cell line MDAMB231. Mol Cell Biochem 443:169–180. https://doi.org/10.1007/s11010-017-3221-8

22. Guo Y, Lin D, Zhang M, Zhang X, Li Y, Yang R, Lu Y, Jin X, Yang M, Wang M, Zhao S, Quan C (2016) CLDN6-induced apoptosis via regulatingASK1-p38/JNK signaling in breast cancer MCF-7 cells. Int J Oncol 48:2435–2444. https://doi.org/10.3892/ijo.2016.3469

23. Yu S, Zhang Y, Li Q, Zhang Z, Zhao G, Xu J (2019) CLDN6 promotes tumor progression through the YAP1-sna11 axis in gastric cancer. Cell Death Dis 10:949. https://doi.org/10.1038/s41419-019-2168-y

24. Cao X, He GZ (2018) Knockdown of CLDN6 inhibits cell proliferation and migration via PI3K/AKT/mTOR signaling pathway in endometrial carcinoma cell line HEC-1-B. OncoTargets Ther 11:6351–6360. https://doi.org/10.2147/ott.S174618

25. Lin Z, Zhang XW, Liu ZZ, Liu QH, Wang LP, Lu Y, Liu YY, Wang M, Yang ML, Jin XS, Quan CS (2013) The distinct expression patterns of claudin-2, -6, and -11 between human gastric neoplasms and adjacent non-neoplastic tissues. Diagn Pathol. https://doi.org/10.1186/1746-1596-8-133

26. Wu Q, Liu Y, Ren Y, Xu X, Lu Y, Li Y, Quan C (2010) Tight junction protein, claudin-6, downregulates the malignant phenotype of breast carcinoma. Eur J Cancer Prev 19:186–194. https://doi.org/10.1097/CEJ.0b013e328337210e

27. Kohmoto T, Masuda K, Shoda K, Takahashi R, Ujiro S, Tange H, Wu Q, Liu Y, Ren Y, Xu X, Lu Y, Li Y, Quan C (2010) Tight junction protein, claudin-6, downregulates the malignant phenotype of breast carcinoma. Eur J Cancer Prev 19:186–194. https://doi.org/10.1097/CEJ.0b013e328337210e

28. Micke P, Mattsson JS, Edlund K, Lohr M, Jirström K, Berglund O'Shea JJ, Schwartz DM, Villarino AV, Gadina M, McInnes IB, Laurence A (2015) The JAK-STAT pathway: impact on human disease and therapeutic intervention. Annu Rev Med 66:311–328. https://doi.org/10.1146/annurev-med-051113-024537

29. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144:646–674. https://doi.org/10.1016/j.cell.2011.02.013

30. Ubel C, Mousset S, Trufa D, Sirbu H, Finotto S (2013) Establishing the role of tyrosine kinase 2 in cancer. Oncoimmunology 2:e22840. https://doi.org/10.4161/onci.22840

31. Kong FE, Li GM, Tang YQ, Chen Y, Tatarek J, Kelliher MA, Neuberg DS, Levine RL, Moriggl R, Kenner L, Friedrich K, Haan C, Petersen I, Heimel T, Kramer OH (2014) SIAH2 antagonizes TYK2-STAT3 signaling in lung carcinoma cells. Oncotarget 5:3184–3196. https://doi.org/10.18632/oncotarget.1899

32. Herrmann A, Laub M, Nagao T, Song CY, Chan WC, Lee H, Yue C, Look T, Mullforth R, Li R, Jenkins K, Williams J, Budde LE, Forman S, Kwak L, Blankenstein T, Yu H (2017) CTLA4 promotes Tyk2-STAT3-dependent B-cell oncogenicity. Cancer Res 77:5118–5128. https://doi.org/10.1158/0008-5472.CAN-16-0342

33. Liu H, Wang M, Liang N, Guan L (2019) Claudin-9 enhances the metastatic potential of hepatocytes via Tyk2/Stat3 signaling. Turk J Gastroenterol 30:722–731. https://doi.org/10.4152/tjg.2018.1513

34. Liu H, Wang M, Liang N, Guan L (2019) Claudin-9 enhances the metastatic potential of hepatocytes via Tyk2/Stat3 signaling and is associated with poor prognosis in patients with hepatocellular carcinoma. Diagn Pathol 13:72. https://doi.org/10.1186/s13046-018-0749-1

35. Bekki H, Kohashi K, Yamada Y, Iura K, Ishii T, Maekawa T, Cheng W, Zhu WJ, Mo JQ, Gong YF, Tang H, Zhao Y, Zhang VN, McNatt SA, Foreman NK, Handler MH (2010) Claudin 6 antibody-drug conjugate. Sci Transl Med. https://doi.org/10.1186/2159-8290.CD-12-0504
46. De Vos J, Jourdan M, Tarte K, Jasmin C, Klein B (2000) JAK2 tyrosine kinase inhibitor tyrphostin AG490 downregulates the mitogen-activated protein kinase (MAPK) and signal transducer and activator of transcription (STAT) pathways and induces apoptosis in myeloma cells. Br J Haematol 109:823–828. https://doi.org/10.1046/j.1365-2141.2000.02127.x

47. Zhang J, Liu C, You G (2018) AG490, a JAK2-specific inhibitor, downregulates the expression and activity of organic anion transporter-3. J Pharmacol Sci 136:142–148. https://doi.org/10.1016/j.jphs.2018.01.006

48. Fan Z, Zhang W, Cao Q, Zou L, Fan X, Qi C, Yan Y, Song B, Wu B (2022) JAK2/STAT3 pathway regulates microglia polarization involved in hippocampal inflammatory damage due to acute paraquat exposure. Ecotoxicol Environ Saf 234:113372. https://doi.org/10.1016/j.ecoenv.2022.113372

49. Tait Wojno ED, Hunter CA, Stumhofer JS (2019) the immunobiology of the interleukin-12 family: room for discovery. Immunity 50:851–870. https://doi.org/10.1016/j.immuni.2019.03.011

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.