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Molecular Mechanism of Integrin αvβ6 in Liver Metastasis of Colon Cancer Based on SDF-1/CXCR4

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ABSTRACT

With the changes in people's dietary life, the incidence and mortality of colon cancer have risen sharply. Invasive metastasis of colon cancer is the main reason affecting the prognosis. Therefore, it is very important to study the molecular mechanism of SDF-1/CXCR4 and integrin αvβ6 in liver metastasis of colon cancer. Floating cells were used to detect the appearance of β6, and the relationship between SDF-1/CXCR4 and the molecular mechanism of colon cancer redirection was analyzed. Use immunohistochemistry to detect the appearance of SDF-1/CXCR4 and αvβ6 protein, and combine the data with clinical-pathological data for statistical analysis. Experimental data showed that the positive expression rates of αvβ6 protein in well-differentiated and poorly differentiated colon cancer tissues were 21.4% and 30.6%, respectively, and the difference was statistically significant (P<0.01). The results show that the appearance of SDF-1/CXCR4 in colon cancer cells (CCC) has nothing to do with the type of cancer cells, and increases with the decrease of cell differentiation. This has a great relationship with the classification of the clinical TNM disease stage. The later the disease stage, the higher the expression level. The αvβ6 has a strong correlation with the invasion and metastasis of colon cancer and can be used as a criterion for judging liver metastasis and prognosis.

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Introduction

The incidence of colon cancer is increasing year by year. About 25% of colon cancer patients have undergone surgery, followed by liver metastasis, which is closely related to mortality (1, 2). Progressive factors are formed by the secretion or secretion of various tumor cells and related inflammatory cells, which can participate in the formation of blood vessels, affect the immune response of tumors, and change the microenvironment of tumor cells (3). Tumor cells use this system to obtain the expression of receptor factor receptors and respond to specific repressor concentration gradients, thereby inducing tumor cell invasion and direction changes (4, 5). Integrin αvβ6 is a special type of conformation, which is only expressed in embryonic and damaged tissue repair and epithelial malignancies, but not in the epithelium of healthy adults (6, 7). Therefore, it is essential to find molecular markers of colon cancer growth, invasion and metastasis, and block its growth and migration pathways at the molecular level.

SDF-1 is a progressive protein, and CXCR4 has a high affinity. Chen et al. believe that adenosine A2A receptor activation can inhibit the SDF-1/CXCR4 interaction (8, 9). However, whether the activation of A2A receptors is related to the SDF-1/CXCR4 signaling pathway, which regulates brain metastasis, is unclear (10, 11). Their research uses western blot to detect protein levels. They used cell invasion and migration experiments to evaluate the metastatic ability of PC-9 cells. Lactate dehydrogenase and cell proliferation tests showed cell viability (12). The results of their in vitro experiments were further confirmed in nude mice. A2A receptor stimulation can protect the function of the blood-brain barrier (BBB) (13-15).

Integrins recognize the extracellular matrix and cytoskeleton, mediate the bonding reaction between cells and the extracellular matrix, and send and

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receive specific signals that regulate the biological characteristics of cells. Zhu et al believe that the positive connection between continuous cell movement and defective tight junction (TJ) barriers allows increased antigen penetration (16). The collective migration of cells involves the adhesion between cells and the extracellular matrix (ECM) (17, 18). They used IL-4 to treat well-differentiated hence, and studied the effect of IL-4 on cell migration through genetic and pharmacological methods, live-cell imaging, apex models, and immunostaining (19-21). Driven by the reorganization of the actin cytoskeleton, it also induces the collective migration of epithelial cells and changes in cell morphology (22, 23). In addition, after airway epithelial injury in patients with allergic rhinitis, β5 and β6 integrins are both enriched in basal cells. Their results indicate that αvβ5 and αvβ6 are the key mechanism receptors for IL-4 to induce the collective migration of HNEC through the FAK signaling pathway (24, 25).

This article mainly studies the molecular mechanism of integrin αvβ6 in colon cancer liver metastasis based on SDF-1/CXCR4, explores the role of integrin αvβ6 in SDF-1/CXCR4 mediating colon cancer liver metastasis, and further studies the interaction and potential molecular mechanism in the process of liver metastasis of colon cancer, especially the theoretical basis for the new type of colon cancer metastasis-oriented liver metastasis, and effectively prevent liver metastasis of colon cancer.

Materials and methods

Cell line

The colon cancer cell line is HT-29 and Caco-2, and the rectal cancer cell line is Lovo, SW480 cells. The integrin β6 gene (wild type β6) lacking the ERK2 binding site was ligated to plasmid pcDNA1neo and transfected into SW480 cells.

Main reagents

The main reagents used in the experiment are shown in Table 1.

Instruments and equipment

The equipment used in the experiment is shown in Table 2.

Table 1. Main reagents; serial number (SN)

SN	Reagent name	Manufacturer
1	Integrin αvβ6 function blocking antibody 10D5	American Chemicon
2	Goat anti-integrin β6 primary antibody SC-6632	American Sant Cruz
3	Mouse anti-integrin αvβ6 primary antibody E7P6	American Chemicon
4	PE-labeled anti-human CXCR4 loss primary antibody	American BioLegend
5	RT-PCR primers for integrin β6	Shanghai Boshang Biological Company
6	CXCR4 specific inhibitor AMD3100	American Sant Cruz

Table 2. Equipment; serial number (SN)

SN	Equipment name	Manufacturer
1	Histology automatic staining machine	German Leica
2	Optical microscope	Olympus Corporation Of Japan
3	Cell CO_2 incubator	German Heraeus
4	PCR Amplifier	German Biometra
5	Micro oscillator	Jiangsu Jintan Zhengji Company
6	Constant temperature oscillator	China Jintan Scientific Instrument Factory

Preparation of Reagents

Cell culture medium: First dissolve a kind of F12K medium powder in 950ml steam water, add 3.5g NaHCO3, the solute is completely dissolved, add two kinds of steam water to 1000ml. In addition, use 1.5 mol/L HCl to adjust the pH to 7.1~7.4. Finally, sterilize the syringe filter and store them at 5°C. Take out the PET bottle filter and collect 5.5 ml of sterile medium. The concentration of pre-fetal bovine serum in the medium is 9.5%, that is, the volume ratio of the medium to the fetal bovine serum is 8:1.

MEM medium: The standard product of PD98059 purchased is 4.5 mg, 1.5 ml DMSO is added, the dissolved concentration is 5 mg/ml, and the molecular weight of PD98059 is 276.54 g/mol. If 200ml DMEM medium containing 10μMPD98059 is prepared, 121.7μl of 6mg/ml PD98059 mother liquor must be added to 200 ml DMEM medium.

Cell culture

Remove the cryotube cells from the liquid freezer, and then immediately put them in a 37°C to 40°C

water bath to preheat them until they are completely thawed. Place the cell suspension in a bottle, then place it in a supplemental medium at 37°C and grow in a 5% CO2 aging growth box. On the next day, observe the cells with an inverted microscope and continue to culture after the liquid exchange.

Cell adhesion

Collect SW480 wild-type β6 cells, SW480 mutant β6 cells and SW480 mock cells in good growth conditions with PBS solution, and gently blow to form a single cell suspension. It was washed twice with the cell adhesion buffer prepared in advance. cell counts. There are 50,000 cells in each well of a 96-well plate, adding cells at 37°C, the process of cell mucosal formation and even protein; fix the remaining adherent cells with 1% glutaraldehyde solution. The cells were treated with 1% SDS solution, and the absorbance value of the 600n/n well was read on the enzyme scale for data processing.

2.3.3 Transwell migration experiment

Preparation of single-cell suspension: the log phase cells were starved in serum-free medium for 24 hours, sucked dry with a sterile pipette, and trypsinized with 0.25% EDTA, and washed with PBS buffer solution at 1000rpm after digestion.

Add IL-8 containing 10ng/ml as a chemokine to the lower layer of the small chamber. Then put it into the cell incubator and continue to incubate for 24 hours; remove the Transwell incubator, wipe the upper cells and Matrigel gel with a cotton swab, and wash 3 times with pre-warmed PBS. After fixing with 4% polyformic acid for 10 minutes, dye with 0.1% crystal violet for 20 minutes. The cells penetrating the Matrigel gel were stained purple, and the average value was counted and repeated 3 times.

Staining: After fixing the cells with 4% polyoxymethylene, aspirate the fixative, air dry, stain with 0.1% crystal violet for 20 minutes, and store at room temperature away from light.

Flow cytometry to detect β6 expression. The cells in each group were trypsinized, collected in a flow cytometry tube, and centrifuged at 1000 r/min for 6 min. The supernatant was discarded, 500 μL of blocking solution was added to each tube, and the tube was gently blown and allowed to stand for 10 minutes. The rat anti-human β6 monoclonal antibody E7P6 was added at 1:20 and left at room temperature for 25 minutes. After centrifugation at 1000r/min for 6 minutes, the supernatant was discarded, 1ml PBS was added, the mixture was gently blown, and then washed twice with shaking for 10 minutes each.

Statistical Methods

All data were analyzed using SPSS 19.0 statistical software. Use the t-test to compare the average value (ratio) of two samples, and use one-way dispersion analysis to compare the average value of multiple samples. The relationship between the findings of αvβ6, SDF-1/CXCR4 and clinicopathological characteristics was studied by using Kay square test and Fisher. Spearman analyzed the correlations found in the three gastric cancer tissues. $P<0.05$ found a significant difference.

Results and discussion

Expression of integrin αvβ6 in colon cancer and adjacent tissues

Table 3 shows the performance results of integrin αvβ6 in tissues with different depths of invasion. The positive rate of αvβ6 in the non-infiltrated serosa group was 58.8%, and the positive rate of αvβ6 in serosa infiltration and other groups was 51.5%. The positive rates of integrin αvβ6 in highly-medium differentiated and poorly differentiated colon cancer tissues are 21.4% and 30.6%, respectively.

Table3. Integrin αvβ6 expression results in tissues of different depths of invasion; Clinicopathological factors (A), Number of cases (B), αvβ6 positive rate (C)

A		B	C	P
Tumor size	<5cm	17	10(58.8%)	0.722
	≥5cm	33	17(51.5%)	
Depth of infiltration	Less than serosa	14	3(21.4%)	0.121
	Serosa or beyond	36	11(30.6%)	
Tumor differentiation	High school differentiation	15	4(26.7%)	0.221
	Poorly differentiated	35	21(60.0%)	
Tumor stage	I+II	27	11(40.7%)	0.025
	III+IV	23	17(73.9%)	

There is a significant difference between the two (P <0.01). The positive rate of αvβ6 in the lymph node metastasis group was 60.0%, which was significantly higher than the 26.7% in the non-metastatic group. A significant difference was observed (P<0.01). The positive rates of αvβ6 in stage I+II and III+IV colon cancer tissues were 40.7% and 73.9%, respectively (P<0.01). From the above results, the appearance of αvβ6 is related to the infiltration depth of colon cancer, the degree of tumor differentiation, lymph node metastasis and TNM staging, and has nothing to do with the size of the tumor.

The migration and metastasis of tumor cells depend on their ability to regulate proteolytic enzymes and degrade periplasm. The endopeptidase matrix metalloproteinase (MMP), which relies on a single zinc ion, is closely related to the extracellular matrix template and its degradation under physiological and pathological conditions. Under the conditions of high cell density in colon cancer, it tends to other β subunits, depending on the PKC signaling pathway, and selectively enhances the expression of αvβ6. When the expression of αvβ6 increases, the secretion of MMP-9 is promoted, the pericellular matrix is degraded, and the decrease of cell density feedback increases PKC activity, and the expression of αvβ6 increases again, which promotes tumor cell growth and promotes cell growth, thereby forming a high-density state. Therefore, it forms a closed feedback loop and continuously promotes the growth and migration of tumor cells.

Role of Integrin αvβ 6 in SDF-1/CXCR4 Mediated Directional Migration of CCC

The effect of integrin αvβ6 on the directional migration of CCC mediated by SDF-1/CXCR4 is shown in Figure 1. Invasion and metastasis of CCC through the SDF-1/CXCR4 biological axis is a multi-step complex process that requires multiple elements and molecules to complete. First, the so-called "navigation system" must provide directions for the migration of CCC to specific organs.

Ketones are a super family of polypeptide cytokines that promote an inflammatory response. They selectively attract and activate different types of cells. They participate in ketones under many pathological and physiological conditions, such as inflammation, tissue damage, allergies, and infections. The changes of total RNA purity under the interference of different concentrations of isotope αvβ6 are shown in Figure 2. The appearance of CXCR4 is related to colon cancer lymph node metastasis but has nothing to do with other clinical factors, which indirectly proves the correlation between CXCR4 and colon cancer lymph node metastasis. The neutralizing antibody against SDF-1 inhibited the proliferation of XG-1 and XG-2 cells caused by exogenous IL-6. In addition, in the presence of relatively high concentrations of IL-6, the inhibitory effect of anti-SDF-1 antibodies on XG-1 and XG-2 cells is also greatly reduced.

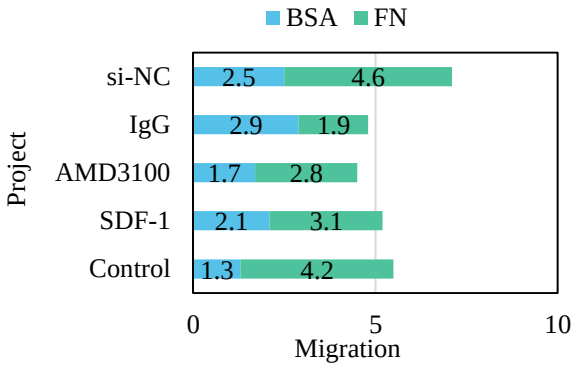

Figure1. Effect of integrin αvβ6 on SDF-1/CXCR4-mediated directional migration of CCC

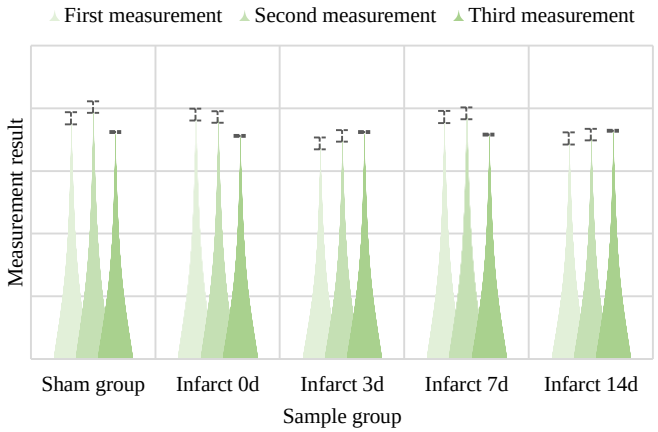

Figure 2. Total RNA purity under the interference of different concentrations of integrin αvβ6

Effect of αvβ6 on directional migration of cancer cell line caco-2

The accelerated endocytosis of integrin αvβ6 in high cell density cultures is shown in Figure 3. In the highly metastatic colorectal cancer cell lines HT-29 and WiDr, β6 subunit and CXCR4 mRNA expression levels are high; in the non-metastatic cell line Caco-2,

β6 subunit and CXCR4 mRNA expression levels are low. As a result of western blotting, the protein expression levels of β6 subunit and CXCR4 were also high in HT-29 and WiDr, but low in Caco-2. In addition, integrins αvβ6 and CXCR4 are closely related to liver metastasis of colorectal cancer. HT-29 and WiDr cells with high liver metastasis potential express high levels of αvβ6 and CXCR4, while non-metastatic cell lines Caco-2 express low levels The αvβ6 and CXCR4 are consistent with the results of clinical statistical analysis.

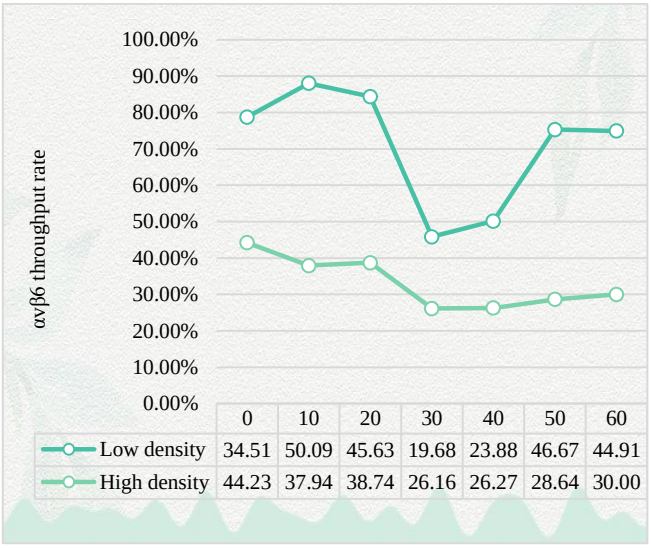

Figure 3. High cell density culture accelerates the endocytosis of integrin αvβ6

Analysis of SDF-1/CXCR4 axis and colorectal cancer invasion and metastasis

CXCR4 has a high incidence in colorectal cancer tissues, and it is weakly positive for normal tissues and benign tumors around cancer. In addition, the discovery of CXCR4 is closely related to lymph node metastasis and remote metastasis, and measuring the appearance of CXCR4 in colon cancer tissue may have a certain value in predicting the progression of the disease. The correlation between SDF-1/CXCR4 and αvβ6 is shown in Figure 4. The appearance of CXCR4 in clinical non-small cell colon cancer tissues was determined according to immunohistochemistry, and the results showed that the appearance of CXCR4 was the same as other tumor tissues, and was restricted to the cell membrane and cytoplasm of colon cancer tissues. Compared with adjacent and normal intestinal tissues, the incidence of CXCR4 reached 85.0%, which further supports the association between CXCR4 and colon cancer.

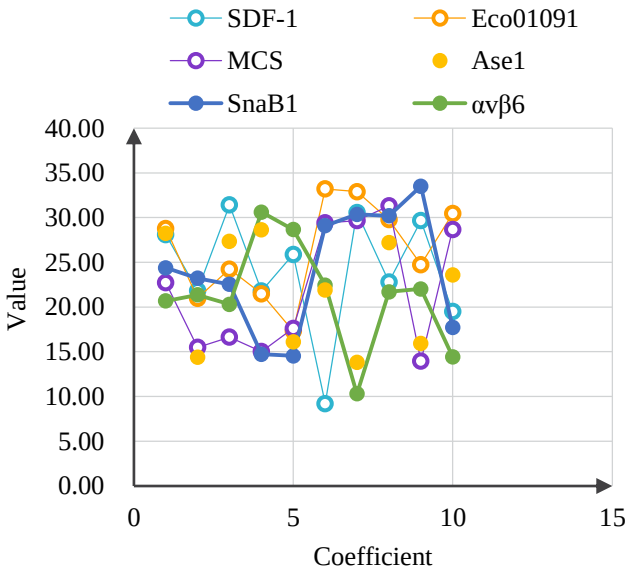

Figure 4. Correlation expression of SDF-1/CXCR4 and αvβ6

Integrin αvβ6 can directly participate in the movement of cells by rapidly carrying out the circulation process of endocytosis between the cell membrane and the cytoplasm. The overall αvβ6 plays an important role in the directional movement of CCC mediated by SDF-1/CXCR4. That is to say, SDF-1/CXCR4 may promote the directional movement of CCC through integrin αvβ6.

Lifestyle factors and aging cause many colorectal cancers and a small number are due to inherited genetic disorders (26). Risk factors include diet, obesity, smoking, and lack of adequate physical activity. Diet-related factors that increase the risk of this disease include consumption of red meat and processed meats and excessive consumption of alcohol (27). Another risk factor is inflammatory bowel disease, which includes Crohn's disease and ulcerative colitis. Some inherited conditions that cause colon cancer include hereditary familial adenomatous polyps and inherited non-polyposis colon cancer, but these account for less than five percent of cases (26). The disease usually begins with a benign tumor that develops into cancer and metastasizes over time (27).

More than 90% of cancer deaths occur due to metastasis (28). Primary tumors can be treated well with surgery or complementary chemical treatments,

but metastasized cancers are resistant to treatment (29, 30). The nature of resistance indicates the high incidence of death among individuals with metastases (31). The multi-stage nature of the metastasis process means a specific and complex program (32). Identifying the essential genes and proteins involved and showing their relationship to each other and the disease is the key to identifying and treating invasive cancers (31). To cause metastasis, the genetic conditions of the tumor cells and the micro-environmental conditions of the target organ must be favorable (28). The existence of unacceptable conditions in any of the stages of this process can cause it to stop and, as a result, create a state of latency. By examining the critical factors in the metastasis process recently identified, new relevant patterns can be drawn that can provide practical solutions for future research and achievements (31, 32). Understanding the genes, proteins, and micro-environmental conditions affecting the metastasis process in the form of different stages can help to understand better this deadly process and, therefore, its proper treatment (29).

The SDF-1/CXCR4 axis activates lung cancer cells and initiates the process of lung cancer infiltration and metastasis through calcium influx, MAPK/ERK, PI3K/AKT signal transmission pathways, and VEGF has a certain synergistic effect on tumor metastasis-related genes MMP, lung cancer infiltration and metastasis.

Colon cancer is currently the tumor with the highest morbidity and mortality among all malignant tumors. The high migration activity of tumor cells is related to clinical treatment. SDF-1/CXCR4 increases the incidence of colon cancer cell αvβ6 through the ERK/ETs-1 pathway and directly participates in cell migration through diet. SDF-1/CXCR4 interacts with Integrin αvβ6 to promote the metastasis of CCC to the liver.

As an important receptor of tumor cells, integrin αvβ6 makes the extracellular matrix and intracellular skeletal protein crosslink and participates in tumor progression, including tumor survival, basement membrane and extracellular matrix adhesion and penetration. Integrin αvβ6 mediated resistance to colorectal cancer chemotherapy depends to a large extent on the ERK/MAPK signal transduction pathway, especially on the direct connection between the internal stage of β6 cells and ERK2.

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None.

Interest conflict

The authors declare no conflict of interest.

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