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The Significance of Cytoskeleton System in Tumor Cell Infiltration

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ABSTRACT

Cytoskeleton system is mainly composed of three kinds of fibrils: microtubules, microfilaments and intermediate filaments. They are a complex network of protein filaments in the cytoplasm of eukaryotic cells. They not only act as scaffolds in cells, but also play an important role in maintaining the movement of cells, participating in the material transport and signal transmission in cells. It is found that the whole cytoskeleton system is closely related to tumor invasion and growth. Therefore, this article reviews the overview of the cytoskeleton system and its significance for tumor cell invasion and growth.

1. Overview of the Cytoskeleton System

The cytoskeleton system refers to the protein fiber network framework in eukaryotic cells. It is a three-part system consisting of microtubules (MT), microfilament (MF) and intermediate filaments (IF) composition. The three are highly coordinated and distributed, and are connected with the nucleus, cytoplasmic membrane, and organelles to form a cell morphology skeleton and movement coordination system to maintain the shape of the cell and maintain the function of cell movement, and have important significance for signal transmission. The cytoskeleton system, the genetic system within the cell, and the biofilm system are collectively called the "three intracellular systems".

2. The Structure and Function of Microtubules and Tumor Cell Infiltration

Microtubules are hollow tubular structures with a diameter of 24-27 nm and an inner diameter of about 15 nm. They are distributed in the cytoplasm and nucleus of many cells. The tube wall is surrounded by 10-13.5 nm protofilaments. The tube length varies from a few microns to a few centimeters. Microtubules can be assembled into single tube, double tube and triple tube, which are found in structures such as cilia, centrioles and spindles, respectively. Microtubules have functions related to cell support, movement and cell division. In addition, it also participates in the transport of intracellular substances. Microtubules constitute the reticular scaffold of cells to maintain cell morphology; participate in cell contraction and pseudopodia movement; participate in the displacement of organelles, especially the division and displacement of chromosomes, which require the help of microtubules. It may also participate in the transportation of substances in the cell, and may play a role in the microcirculation system of transporting macromolecular particles in the cell. The infiltration and metastasis of tumor cells is one of the biological characteristics of malignant tumors. The active mobility of tumor cells is an important factor in infiltration and growth. Microtubules, one of the components of

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the cytoskeleton system, are dispersed in the cytoplasm and move in normal cells. And it plays an important role in the activities of tumor invasion.

In traditional concepts, microtubules determine the shape of cells and play a role in mitosis and organelle transport. However, recent studies by Gomez et al. showed that although the structure of microtubules has an impact on cell morphology, changes in cell shape have a greater impact on the structure of microtubules. They used Drosophila embryonic cells as target cells, treated the cells as nearly elliptical, and used the cell aspect ratio, eccentricity, and microtubule standard deviation and microtubule deviation as indicators to compare cell shape changes and microtubule structure changes. They first knocked out the embryonic cell's blastocyst contraction factor, so that the cells could not perform blastocyst contraction, and found that the aspect ratio of the embryonic cell during the same period dropped by 43%, the eccentricity dropped by 10%, and the microtubule standard deviation increased by 45%. Microtubule deviation increased by 141%. They also transfected the Gal4 promoter into the cells to promote apoptosis and change the morphology of the cells. They found that the aspect ratio of the cells decreased by 26%, the eccentricity decreased by 4%, and the standard deviation of microtubules increased by 14%. The above changes are the changes that occur only after the cell morphology changes, not the changes brought about by gene mutations. Subsequently, they completely destroyed the intracellular microtubule structure by making the cell express the microtubule shearing protein spastin, and found that the morphology of the cell did change (P=0 02), but the degree is lighter (the aspect ratio is reduced by 25%, and the eccentricity is reduced by 1%).

The structure of microtubules in the cell is constantly undergoing the conversion of depolymerization and repolymerization. This process is called microtubule dynamics. Depolymerization is the transformation of microtubules from growth to shortening, and repolymerization is the transformation from shortening to growth. Previous studies believe that microtubules are constantly damaged during the aging process and are more prone to disaggregation. Recent studies [6] have shown that depolymerization is easy to occur at the positive end of the microtubules that are initially formed. However, once the positive end begins to extend, the ability to disaggregate rapidly declines. The process of depolymerization and repolymerization of microtubules is affected by microtubule-associated proteins (MAPs) and motor proteins. MAPs include microtubule structure-related proteins, microtubule positive end tracer proteins and so on. Microtubule structure related proteins include MAP1, MAP2, MAP4 family and Tau protein. They are called structurally related proteins because they do not exist.

The enzyme activity is only bound to the surface of the microtubules, thereby enhancing the stability of the microtubules. The positive end of the microtubule tracer protein specifically acts on the growth end of the microtubule and regulates the growth of the microtubule. Early research believed that microtubule structure-related proteins are mainly distributed in the nervous system and perform physical functions. MAP1 is divided into three subtypes: MAP1A, MAP1B, and MAP1S. MAP1A and MAP1B are mainly distributed in neurons and play a role in guiding the formation of axons; MAP1S is widely expressed in cells and regulates cell division and autophagy. MAP2 is the highest content of neurotubule-associated protein. It exists in the entire neuron in the early developmental stage of the neuron. Then MAP2 in the axon disappears and only exists in the dendrites. MAP4 is distributed in a variety of cells and also plays a role in stabilizing the structure of microtubules. Tau protein can promote the polar formation of neurons.

With the elongation of axons, the abnormality of Tau protein can lead to many kinds of neurodegeneration Lesions. In recent years, it has been discovered that in addition to affecting microtubule motility, microtubule structure-related proteins may also be related to the occurrence and development of tumors. Tessema et al. screened the methylation level of gene promoters on 117 frozen case specimens of non-small cell lung cancer, 5 human bronchial epithelial cell lines, 5 human small airway epithelial cell lines, and 23 non-small cell lines. It was found that the CpG island of the promoter of the MAP1B gene has a significantly higher methylation ratio in tumor tissues, and the methylation ratio is higher in the case of COPD (combined 68%, not 37%), and the tumor gene map project. The information in the database matches. However, there is no difference in the expression level of MAP1B in tumor tissues and non-tumor tissues. Therefore, the expression of MAP1B is related to tumor transformation, but the specific mechanism remains to be further studied. Bauer et al. collected pathological specimens of breast cancer patients who underwent surgical treatment after paclitaxel adjuvant chemotherapy, extracted total RNA from 14 cases and performed gene chip analysis, and found that in the cases of complete pathological remission, the expression of MAP2 gene was higher than that of incomplete remission. The number of cases is 4 times higher. They collected MAP2 mRNA in 5 breast cancer cell lines and found that the expression of MAP2 was highly correlated with paclitaxel sensitivity in vitro (correlation coefficient R2>0 99). Later, they used two groups of breast cancer

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cell lines MCF-7 and MDA-MB-468 to overexpress MAP2, and the two groups of cell lines were treated with paclitaxel and found that the number of cells decreased by 53 7% and 46 4%, respectively. In addition, in 47 needle biopsy cases, patients with high MAP2 expression had a higher percentage of complete remission. Therefore, MAP2 can play a synergistic effect with paclitaxel and can be used as a biomarker of paclitaxel sensitivity. This may be related to the effect of both MAP2 and paclitaxel on microtubules, which promotes cell cycle arrest in G2/M phase. Yang et al. used Westernblot and real-time quantitative polymerase chain reaction to detect the Tau protein in three prostate cancer cell lines, and found that two of them had expression. Subsequently, they cultured the two cell lines into docetaxel-resistant cell lines and found that the expression of Tau increased, and the expression of Tau was positively correlated with the PI3K/Akt/mTOR pathway. In addition, silencing the wild-type and drug-resistant strains of Tau inhibited the growth of tumor cells and increased the sensitivity to docetaxel. Therefore, Tau can play an antagonistic effect with paclitaxel and can be used as a biomarker of docetaxel sensitivity, which may be related to the competition between Tau and docetaxel for the binding site of microtubules. The above results all suggest that microtubules are related to the occurrence, development and treatment of tumors, but further research is still needed to reveal the relationship between the two.

3. The Structure and Function of Microfilaments and Tumor Cell Infiltration

Microfilaments are solid filamentous structures with a diameter of 5-7nm, which are distributed in the cytoplasm and nucleus of most cells, but myofilaments in the cytoplasm of the cells are the most developed. Long and short filaments are connected to each other and surround all organelles. Microfilaments can exist in the form of monofilaments, or form a network, or they can exist in bundles. Actin is the main component of microfilaments, and it exists in two forms in the body: actin monomer (G-actin, also known as globular actin) and fibrous muscle assembled from actin monomers F-actin. Among them, the actin monomer is a globular protein with a molecular weight of about 43kDa, which is divided into three types: α, β, and γ according to the isoelectric point [1]. The complex intracellular cytoskeleton network composed of microfilaments and their related regulatory proteins participates in most of the biological behaviors in life. In malignantly transformed cells, cells often show the destruction of cytoskeleton and abnormal aggregation of microfilaments. The infiltration and metastasis of tumor cells are related to the changes in the expression of microfilaments and related proteins. The abnormal aggregation of microfilaments can enhance the mobility of tumor cells.

In order to clarify the functions of various actins, researchers have established different types of knockout mice. Kumar et al. tried to establish α-cardiomyocyte type knockout mouse models. The mice died during embryonic or perinatal period, and the myocardial fibers were severely disordered, suggesting that α-cardiomyocytes are closely related to the formation of cardiomyocytes. Crawford et al. established the α-skeletal muscle type gene knockout mouse model and found that their skeletal muscle function is very weak, and they all died on the 9th day after birth, suggesting that the α-skeletal muscle type is necessary for muscle contraction. Schildmeyer et al. established an α-smooth muscle type gene knockout mouse model and found that the expression of α-skeletal muscle type was increased compensatoryly, although the cardiovascular system of mice developed normally. However, the vasoconstriction ability becomes weaker, the blood pressure is lower than that of normal mice, and the blood flow rate becomes slower, suggesting that α-smooth muscle type regulates vasoconstriction, which is closely related to arterial tension and the activity of myofibroblasts. Kumar et al. overexpressed the γ-smooth muscle type gene in α-cardiomyocyte type knockout mice, and some mice survived, but when they became adults, symptoms such as cardiac insufficiency and myocardial hypertrophy appeared, suggesting that γ-smooth muscle type could be partially replaced. The role of α-cardiomyocytes in cardiomyocytes. Shawlot et al. tried to establish a β-cytoplasmic sub-equivalent gene mouse model, all of which died of non-specific exhaustion early in life, suggesting that the β-cytoplasmic type is necessary for cell survival. Belyantseva et al. established a γ-cytoplasmic knockout mouse model and found that these mice are thinner than wild-type or heterozygous types in the early developmental stage, and some can survive to adulthood and are fertile, but a large part of them are due to development. Delayed death, and some random deaths in adulthood, suggesting that γ-cytoplasmic type is related to growth and development. In the cell, actin is divided into two forms: monomericactin (G-actin) white monomer and filamentous actin (F-actin) polymer. F-actin is a long-chain fiber composed of multiple G actins, and two F-actins are combined in anti-parallel to form a spiral chain, which exerts physiological functions. The nucleotide binding site of each molecule of G-actin binds to one molecule of ATP and connects with one Mg^{2+} or Ca^{2+} to form an actin ATP-divalent cation complex. When G-actin is combined with F-actin, ATP is hydrolyzed to ADP, which provides energy for the process.

Actin is widespread in eukaryotic cells. In most cells,

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β-cytoplasmic type:γ-cytoplasmic type is about 2:1. However, in different cells, this ratio will change. For example, the mouse testis is 1:1, the liver is 25:1, and the aorta is 6:1. The content of actin also changes with changes in pathological processes, and the β-cytoplasmic type is often highly expressed in aggressive tumors, such as aggressive colorectal cancer and murine sarcoma virus-transfected MDCK cells.

Cells or melanoma T1C1 cells. Simiczyjew et al. [2] overexpressed β-cytoplasmic and γ-cytoplasmic type in human colorectal adenocarcinoma LS174T cell line and found that the ratio of F-actin to G-actin increased, indicating the degree of actin polymerization increased, and observed under a phase-contrast fluorescence microscope, the vesicles of the cell membrane grow actively. The above results all suggest that the increase of actin content can enhance the exercise ability of tumor cells.

Cell movement is closely related to the formation of cell membrane protrusions. Moving cells will protrude two kinds of pseudopods at the front, namely filopodia and lamellopods. The formation of pseudopodia relies on the formation of actin microfilament skeletons under the cell membrane. In lamellipodia, G-actin aggregates into F-actin, and F-actin forms a cross branch network; in filament pseudopodia, F-actin forms a parallel bundle structure. Cell movement is relying on pseudopodia to crawl. The formation of pseudopodia is mediated by Rho family GTPase. The formation of lamellipodia is mediated by Rac1 protein, and the formation of filopodia is mediated by Cdc42 protein. Chen et al. analyzed the expression of Rac1 in 150 cases of lung cancer tissues and 30 cases of adjacent lung tissues, and the expression of Cdc42 in 110 cases of lung cancer tissues and 30 cases of adjacent lung tissues. The results showed that Rac1 was expressed in 94/150 (62 67%) of lung cancer tissues, and almost not expressed in lung tissues adjacent to cancer; Cdc42 was expressed in 80/110 (72 73%) of lung cancer tissues, and almost expressed in lung tissues adjacent to cancer.

Almost not expressing. The expression levels of Rac1 and Cdc42 are significantly positively correlated with lymph node metastasis, TMN staging and pathological differentiation. The five-year survival rate of Rac1 negative patients is 32 14%, and the five-year survival rate of positive patients is 17 02%; the five-year survival rate of Cdc42 negative patients is 36 67%. The positive rate is 13 75%, indicating that the occurrence, development and prognosis of Rac1 and Cdc42 tumors are closely related. In cell experiments, they used scratch experiments and invasion tests to confirm that the expression levels of Rac1 and Cdc42 are positively correlated with the motility of cells. After being stimulated by epidermal growth factor, Rac1.

Darby grows more lamellipodia than Rac1 silenced cells, and this process is effected through the Rac1-Pak1 pathway; Cdc42 expresses more filopodia than Cdc42 silenced cells. When the expression levels of Rac1 and Cdc42 are down-regulated, tumor cells are less resistant to anti-tumor drugs such as nedaplatin and curcumin.

Increased sensitivity. The above results all indicate that the expression level and distribution of actin filaments affect the migration and invasion of tumor cells, and the regulatory factors related to filaments may be a new target for tumor treatment.

4. The Structure and Function of Intermediate Filaments and Tumor Cell Infiltration

Intermediate filament (IF) exists in the cell cytoplasm and is a tubular structure with a diameter of about 8-11 nm. Because its diameter is between microfilament and microtubule, it is called intermediate filament. The composition of the intermediate filament is more complicated than that of microtubules and microfilaments. Intermediate filaments are composed of intermediate filament proteins. According to different biochemical characteristics such as immunological and electrophoretic properties, intermediate filament proteins can be divided into five types: cytokeratin, flexible paper-like protein, intermyolin, and glial fibril fibrils. Acidic protein, nerve filament [2]. The expression of intermediate filament protein in tumor cells is closely related to the degree of differentiation of tumor cells.

5. The Importance of the Cytoskeleton System to the Infiltration and Growth of Tumor Cells

Human malignant tumor cells, especially tumor cells that are located around the tumor and infiltrate the surrounding tissues, have pseudopod-like cytoplasmic protrusions, well-developed microfilament meshes inside, and increased filamentous actin aggregates of microfilaments. It is easy to interact with myosin and cause contraction, which is of great significance to the movement and invasive growth of cancer cells. In the process of tumor cell movement, microfilaments are the most important structural skeleton that constitutes the lamellar pseudopodia of the motor cells, and the adhesion bands and adhesion plaques are the physical connections between the extracellular matrix and the stress fibers formed by the microfilaments in the cells. The coordinated aggregation of multiple microfilaments can generate a prominent force on the cell surface, drive the extension of the plasma membrane at the front edge of the cell to form pseudopodia, and promote the movement of tumor cells [3]. In breast cancer, the

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actin-related protein ARP2 and WAVE2 are co-expressed, which affects the structure of microfilaments, increases pseudopodia, and enhances cell motility. It is closely related to the invasiveness of breast infiltrating ductal carcinoma cells, so it can be used as one of the prognostic indicators of invasive breast cancer [4]. After the fascin is inhibited, it can block the production of filopodia [5], reduce the nuclear movement and deformability of tumor cells [6], thereby inhibiting the migration and invasion of tumor cells. Zhang Hongying, Yang Guanghua et al. [7] studied the relationship between the cytoskeleton and three different human rhabdomyosarcoma cell lines with different metastatic potential and found that the number of microfilaments and microtubule skeletons in rhabdomyosarcoma cells is reduced and dysplasia. The potential is negatively correlated ($P<0.05$); the frequency of actin bodies is positively correlated with the metastatic potential of rhabdomyosarcoma ($P<0.05$); there is no obvious abnormality in the structure of intermediate filaments in rhabdomyosarcoma, and there is no difference in fluorescence intensity between the two significance ($P>0.05$), it is concluded that the abnormality of the cytoskeleton of different degrees may be related to the different infiltration and metastasis potential of rhabdomyosarcoma, and it may become one of the indicators for judging the malignancy and prognosis of rhabdomyosarcoma. Lu Rui, Ke Yang [8] and other studies on human gastric cancer cell line BGC-823 showed that the microfilament skeleton assembly state in tumor cells is negatively correlated with the ability of infiltration and metastasis.

6. Summary

The current research has a relatively systematic and clear understanding of the cytoskeleton system. The growth and infiltration of tumor cells is a multi-stage process and is closely related to the complete microtubule system in the cell. We still lack effective treatments to inhibit the infiltration and growth of tumor cells. The close connection between the cytoskeleton system and the infiltration and growth of tumor cells can provide new ideas for the treatment of tumors.

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