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Research Article

The Role of NLRC3 Protein in the Proliferation and Apoptosis of Cancer Cells

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Abstract

A large number of experimental studies at home and abroad have demonstrated that NLRC3 protein plays a significant regulatory role in the proliferation and apoptosis of cancer cells.The NLRC3 protein plays an important role in the proliferation and apoptosis of cancer cells. High expression of NLRC3 inhibited the proliferation of tumors, and apoptosis decreased after NLRC3 knockout. NLRC3 is a negative regulator of the phosphatidylinositol 3-kinase/protein kinase B/ mammalian target of rapamycin (PI3K-Akt-MTOR) pathway, which inhibits mTOR activation and reduces proliferation-related mRNA translation and protein synthesis. The structure and protein localization of NLRC3 can easily interact with apaf-1, an engine protein in the apoptotic body, which may be involved in the release of cytochrome c from the mitochondria, leading to enhanced apoptosis of cancer cells. Parsing NLRC3 protein structure, explore NLRC3 proteins in cancer cell proliferation and apoptosis effect and its molecular mechanism in the process of determining how NLRC3 through the inhibition of cell proliferation and promote apoptosis inhibiting tumor, explain the molecular mechanism of cell proliferation, apoptosis signaling pathways, designed for better suppress the occurrence of cancer and the theoretical basis for drugs to treat cancer.

Keywords: Apoptosis; Cancer; Cell proliferation; NLRC3.

Introduction

Cancer is one of malignant diseases, and the research on anticancer target proteins has never stopped. NLRC3 (Nod like receptor C3) is a newly discovered NOd-like receptor that can regulate a variety of biological processes, such as cell proliferation, inflammatory response and apoptosis, etc. to reduce the occurrence of tumors.

NLRC3 is composed of n-terminal Caspase Recruitment domain (CARD), central nucleotide binding domain (NBD) and C-terminal leucine-rich repeat (LRR). Studies have shown that NLRC3 is a negative regulator of the PI3K-MTOR signaling pathway, and NLRC3 inhibits the activation of PI3K-dependent kinase AKT. Down-regulation of mTOR signaling pathway and reduction of cell proliferation mediate the protection of colorectal cancer [1-3]. And NLRC3 inhibition of pro -, the cutting of caspase 1 interference NALP3 inflammatory corpuscle complex assembly and activity, make Ⅲ stage of colorectal cancer patients with good prognosis [4-6]. The expression and activation of the apoptotic effector's caspase-8, Caspase-3 and Caspase-7 in NLRC3-/- mice were significantly decreased, and cell apoptosis was decreased [3]. Flow cytometry detection showed that the apoptosis of NLRC3 cells was significantly reduced after knockout [7]. NLRC3 may inhibit tumorigenesis by participating in the regulation of apoptotic signaling pathways. In-depth analysis of NLRC3's role in promoting cell apoptosis and inhibiting cell proliferation, and interpretation of its molecular mechanism, so as to lay a theoretical foundation for better revealing how NLRC3 inhibits the occurrence of colorectal tumors.

Results

NLRC3 structure and function

Nucleotide-binding domain receptors (NLRs), also known as nod-like binding domains (Leucine-rich repeat containing rs), can identify pathogen-related and damage-related molecules and mediate the response of the innate immune system to various pathogens, tissue damage and other cellular stresses [8]. NLRC3, also known as NOD4, is a NOd-like receptor with the structural domain of caspases - Recruitment domain. It has three structural domains: N-terminal Caspase-Recruitment domain. CARD, nucleotide binding domain (NBD) in the center, and C-terminal leucine-rich repeat (LRR). These domains play roles in ligand induction, automatic regulation and inflammatory response regulation in signal cascades [9, 10]. The three-dimensional model of human NLRC3 was established by homology modeling, and the electrostatic potential surface of NLRC3 LRR showed a continuous positive charge, which was composed of 43 lysine or arginine residues [11-13]. The positive charge surface accounts for 74% of the NLRC3 LRR solvent exposure area, and this increased positive charge promotes the interaction between NLRC3 and DNA. Pull-down and surface plasmon resonance experiments confirmed LRR binding to virus dsDNA. The NBD region contains conserved Walker A and B ATPase(WA/WB) motivs that structurally affect DNA binding. The WA/WB motif reduces the LRR binding virus dsDNA and affects the ATPase activity. The binding of viral DNA ligands to NLRC3 increases its ATPase activity, promotes the release of interferon gene-activated protein STING, and thus weakens the type I interferon response [14]. Moreover, NLRC3 is a negative regulator of innate immune signals induced by DNA sensor STING, and NLRC3 interacts with STING to block the interaction between STING and Tank-bound kinase 1 (TBK1) and the production of downstream type I interferon [15]. NLRC3 can regulate the formation of card-like proteins, activate the maturation of Caspase1 and interleukin-1BATE, and inhibit the occurrence of inflammatory responses [5]. NLRC3 interferes with the assembly and activity of NALP3 inflammalome complex by interacting with the subunit of inflammalome complex, and negatively regulates inflammatory response. It is speculated that NLRC3 assists the body in timely stopping inflammation and preventing the chronic development of inflammation, thus reducing the occurrence of tumors. And NLRC3 inhibit systemic inflammation Ⅲ stage of colorectal cancer patients with good prognosis. Hints at NLRC3's therapeutic potential in preventing and treating cancer.

The effect of NLRC3 on cancer cell proliferation

Analysis of NOD like receptor expression profiles in colon cancer tissues in 10 databases showed that NLRC3 expression was significantly reduced in colorectal cancer tissues compared with healthy colon tissues, suggesting that reduced NLRC3 expression is a risk factor for promoting the occurrence of tumors [16]. Compared with wild-type mice, NLRC3-/- mice showed more significant proliferation of colon epithelial cells when cultured in vitro. In contrast, when overexpressing NLRC3 in human colon cell line HCT116, the proliferation of HCT116 cells overexpressing NLRC3 was significantly reduced compared with control cells [2]. Ma et al. knocked down NLRC3 expression in HepG2 and hep3B by small RNA interference, and the results showed that proliferation of HepG2 and hep3B cells increased significantly compared with control cells [7]. It suggests that the normally expressed NLRC3 plays an important role in maintaining cell proliferation homeostasis and preventing tumorigenesis. In 2017, Rajendra Karki et al. published an article in Nature to clarify the relationship between NLRC3 and the occurrence and development of tumors. The reduced EXPRESSION of NLRC3 can promote the generation of tumors, while the high expression of NLRC3 can inhibit the occurrence and development of tumors, confirming that NLRC3 is a negative regulator of the phosphoinothiol 3-kinase/protein kinase mammal target of rapamycin (PI3K-Akt-MTOR) pathway [3]. NLRC3 can inhibit inflammatory response, regulate cell proliferation, and inhibit tumor occurrence by inhibiting the activation of this pathway [17, 18].

Mammalian target of rapamycin (mTOR) belongs to serine threonine protein kinase, which is involved in the regulation of protein synthesis, cell cycle, cell energy metabolism and other pathways, and plays an indispensable role in cell proliferation, differentiation and survival. MTOR activation enhances the phosphorylation level of its target protein ribosome S6 protein kinase 1(S61) and eukaryotic translation initiation factor 4E binding to albumin 1(4E-BP1), which can promote mRNA translation and protein synthesis related to cell proliferation.

Studies have shown that NLRC3 can not only directly bind and inhibit mTOR activation, but also bind to TRAF6 and lead to TRAF6 ubiquitination to inhibit mTOR activation [19, 20]. In addition, NLRC3 can inhibit the activation of P85 subunit by interfering the p85 subunit of PI3K and the mutual binding of catalytic subunit P110a, resulting in the phosphorylation level of its downstream molecules of 3-phosphoinositio-dependent protein kinase 1 (PDK1), AKT and mTOR, and the reduction of cell proliferation. NLRC3 is a negative regulator of PI3K-MTOR signaling pathway. Intestinal epithelial cells lacking NLRC3 cannot control cell proliferation. As the key role of NLRC3 as an inhibitor of mTOR pathway, NLRC3 mediates the protection of colorectal cancer, regulates cell proliferation and then inhibits the occurrence and development of tumors [3].

Effects of NLRC3 on apoptosis of cancer cells

Apoptosis is a common biological phenomenon in eukaryotes. It refers to the genetically controlled and automatically ordered cell death in order to maintain the stability of the internal environment under certain physiological or pathological conditions. Apoptosis plays an important role in regulating the formation and differentiation of organs and tissues and maintaining homeostasis. The occurrence of apoptosis is strictly regulated by the organism,

and apoptosis disorder may lead to the occurrence of malignant tumors. Apoptosis studies have proved the existence of intrinsic cell death pathway, also known as mitochondrial signaling pathway. Studies on mammalian cell apoptosis have clarified the basic process of this signal transduction pathway: Various cell apoptosis signal stimulation caused in the outside of the mitochondrial membrane from mitochondrial cytochrome c release of cytoplasm, into the cytoplasm of cytochrome c and apoptotic protease activating factor Apaf - 1 (apoptosis protease activating factor 1) c - the WD - 40 structure domain, Apaf - 1 / Cyt c complexes and ATP/dATP stimulate the formation of the combination of the heptamer, at the same time, Apaf-1 exposes its Caspase recruitment domain (CARD), recruits Procaspase-9, and combines with procaspase-9, which also has the CARD domain, to form a macromolecular complex composed of cytochrome C, Apaf-1 and Procaspase-9, namely Apoptosome. The binding procaspase-9 self-clews and is activated as Caspase-9, and caspase-9 cuts and activates the downstream effect caspase-3, which leads to the occurrence of many apoptotic morphological and biochemical events [21-23].

Studies have shown that the expression and activation of the apoptotic effector's caspase-8, Caspase-3 and Caspase-7 in NLRC3-/- mice significantly decreased, and cell apoptosis decreased [3]. In addition, Ma et al. detected the effect of knockdown NLRC3 on cell proliferation by flow cytometry and found that, compared with the control group, the apoptosis of cells after low-expression NLRC3 was significantly reduced [7]. It is suggested that NLRC3 plays a role in maintaining the normal expression and function of Caspases. However, due to the lack of studies on NLRC3 regulating the expression of the above caspases, the mechanism of how NLRC3 affects the expression of caspases is still unclear. Also, due to the lack of studies on the interaction mechanism between NLRC3 and Caspases, it is still unclear how NLRC3 inhibits tumor genesis through promoting apoptosis of caspases.

CONCLUSION

Studies on THE NLR receptor family found that NOD1 and NOD2 activated the NF-KB pathway and activated the production of IFN-I. NLRP4, NLRC5, NLRP6, and NLRP12 reduce nF-KB pathway activation and IFN-I production [15]. NLRP1, NLRP3, and NLRP6 cause inflammation. NLRX1 inhibits the production of RNA viruses and lPS-induced cytokines. NLRC3 negatively regulates inflammatory response, inhibits the production of IFN-I, inhibits cell proliferation, and promotes cell apoptosis. However, the mechanism by which NLRC3 promotes apoptosis to inhibit tumorigenesis remains unclear. Studies on apoptosis in various mammalian cells have shown that in the pathway of Cyt C-mediated apoptosis signal transduction, under the stimulation of apoptosis signal, Cyt C is released from mitochondria into cytoplasm, where Cyt C and apAF-1 and caspase-9 precursors form apoptotic bodies, leading to the activation of caspase-9 and the subsequent activation of Caspase-3. The components of apoptotic bodies have been isolated and purified by protein chromatography, immunoprecipitation and other techniques, and their three-dimensional structure has also been analyzed, and the function of apoptotic pathways has been determined [24]. Studies have shown that NLRC3 is composed of n-terminal CARD domain, central NBD domain and C-terminal LRR domain, which can easily interact with apAF-1, an engine protein in apoptotic body. Moreover, NLRC3 protein is in the mitochondria and may be involved in the release of Cyt C from the mitochondria. The analysis of the structure of NLRC3 protein and the in-depth analysis of the conserved binding sites are conducive to the interpretation of the molecular mechanism between the interacting proteins, laying the foundation for the diagnosis and prediction of markers, and providing favorable support for drug research and development.

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Conflict of Interests:

None

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