SHARPIN is a Component of the Linear Ubiquitin Chain Assembly Complex (LUBAC) Regulating Multiple Cellular Signaling Pathways

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Abstract

SHARPIN is a newly identified component of the linear ubiquitin chain assembly complex (LUBAC) demonstrating critical roles in multiple cellular signaling and physiological events. Recent biomedical studies have revealed that SHARPIN plays important roles in IL-1 and/or TNF-α induced activation of NFκB, apoptosis and β1-integrin signaling. Loss of function of SHARPIN leads to multi-organ defects characterized by eosinophilic inflammation and deregulation of immune system. Expression of SHARPIN was up regulated also in tumors of different tissue origin, which implicated oncogene features. Herein we briefly introduced the gene structure, biological function and network, and animal models of disease related to SHARPIN.

DESCRIPTION

Chronic Proliferative Dermatitis Mutant cpdm as a spontaneous mutant mouse model of human chronic proliferative dermatitis is firstly reported by HogenEsch et al. in 1993 [1]. Cpdm mice is characterized by skin erythema, numerous white scales, hair loss and severe itch, accompanied with deficiency in Payer’s patch, and structural abnormalities in the development of immune organs such as spleen and lymph nodes. T/B cell immune function may be involved in disorder development. Multi organ eosinophilic inflammation in skin, lungs, bronchus, gastrointestinal tract, liver and spleen, or other kinds of damage including hepatosplenomegaly, spine and joint deformity, exophthalmos, atresia vulvae, decreased activity and weight loss can also be observed. Finally, cpdm mice die mainly due to immune dysfunction and dry chapped skin. In 2007, Seymour et al. Reported that Sharpin deficiency is the genetic basis of the cpdm mutant mice, which provided the molecular basis and experimental tools for studying the biological functions of SHARPIN protein and clarifying the pathogenesis of chronic proliferative dermatitis mice [2]. In recent years, a large number of studies found that SHARPIN plays an important role in cell apoptosis, immune and inflammatory reaction as well as tumor.

Structure and expression of SHARPIN gene

SHARPIN is a SHANK-associated RH domain-interacting protein [3]. Human Sharpin gene is located on chromosome 8 (Chr8q24.3), consisting of 5605 base pairs and 9 exons [4]. The full-length of cDNA sequence includes 1816 bases encoding a 45KDa protein of 380 amino acids. Mouse homologue Sharpin gene is on chromosome 15 (m.Chr15.D3) [4], sharing 95% homogenous cDNA sequence of human SHARPIN. The sequence structure of SHARPIN protein, highly conserved in human, mouse, cattle, dogs and rabbits and other species, mainly includes HOILL-N region with ubiquitin proteasome activity, AA172-305 SHANK binding region and the C terminal zinc finger region [5,6].

SHANK family includes SHANK1, SHANK2, SHANK3, which highly expressed in nervous system. SHANK contains multiple domains such as ankyrin repeats, SH3 domain, PDZ domain and SAM domain. SHANK can directly interact with guanylate kinase-associated protein (GKAP) and Homer protein, and play a role of molecular scaffold in the postsynaptic dense body. In 2001, Lim et al. found that SHARPIN directly interacts with the ankyrin repeats of SHANK and forms a complex with SHANK in the postsynaptic density of excitatory synapses in brain so as to regulate different spliceosomes of SHANK family, related protein networks and brain development [3]. Initially, investigators took the opinions that SHARPIN just co-localizes with SHANK in excitatory synapses in mice brain and combined with SHANK forming a complex to play a role of molecular scaffold. However, the subsequent experiments found that SHARPIN are widely expressed in a variety of tissues including skin, kidney, liver, heart, lung, muscle and testis, suggesting that SHARPIN is an
important protein involved in the physiological activities of the human body [3].

**SHARPIN is a component of linear ubiquitin chain assembly complex**

Ubiquitin-proteasome pathway is the main pathway of protein degradation in eukaryotic cells. Ubiquitination regulates a variety of cell activities including cell cycle, apoptosis, DNA damage and repair, transcription regulation, cancer, immune and inflammatory reaction. Recent protein studies have focused on SHARPIN. Three Nature papers have verified that SHARPIN together with HOIL-1L (longer isoform of hem-oxidized iron-regulatory protein 2 ubiquitin ligase-1) and HOIP (HOIL-1L interacting protein) forms the linear ubiquitin chain assembly complex and regulates the activation of NFκB (nuclear factor kappa B) pathway [7-9]. Sieber et al analyzed gene structures of Hoip, Hoil-1l and Sharpin in mice and finally found the gene structure of Sharpin resembles that of Hoil-1l including Ubl (ubiquitin-like) domain and NPL4 zinc-finger (NZF) domain. SHARPIN combines with HOIP by NZF domain to form linear ubiquitin chain assembly complex which ubiquitinates NEMO (NFκB essential modulator) and RIP (Ribosome inactivating protein), and therefore activates NFκB pathway. [10] So the deficiency of SHARPIN function not only impedes the successful assembly of linear ubiquitin complex, but also leads to the imbalance of NFκB and apoptosis pathway, resulting in immune system disorders and multiorgan inflammation [7].

**SHARPIN regulates cell apoptosis**

Studies suggested that the function deficiency of SHARPIN results in the increases of epidermal keratinocytes apoptosis [11]. The cell apoptosis pathways are endogenous and exogenous. The exogenous pathway is characterized by the combination of extracellular stimulating factors and cell surface receptors activating caspase-8 and thus leading to cell apoptosis. While the endogenous pathway of apoptosis also named mitochondrial pathway is characterized by the loss of mitochondrial membrane potential, contents such as cytochrome C releasing from mitochondria, the activation of caspase-9, the further activation of downstream caspase-3 and finally cell apoptosis. Liang et al found some important apoptotic keratinocytes in the skin of mutant mice due to Sharpin deficiency [12]. The molecular and biochemical changes were featured by disappearance of mitochondria microtubules, highly condensed materials within mitochondria, the loss of mitochondrial membrane potential, contents released from mitochondria, overexpression of caspase-9 and -3, caspase-3 phosphorylation have been validated, implicating a mitochondria-dependent intrinsic apoptosis pathway [12].

Some other studies found that SHARPIN plays a role of anti-apoptotic signaling inhibiting hepatocyte apoptosis. TNF-α stimulates both pro- and anti-apoptotic signaling in hepatocyte. Anti-apoptotic signaling depends on a cascade of ubiquitination steps leading to NF-κB activation [10]. On one hand, SHARPIN is a part of linear ubiquitin assembly complex and loss of SHARPIN function blocks the ubiquitination cascade reaction and activation of NF-κB pathway. On the other hand, the loss of SHARPIN function reducing or delaying the phosphorylation and degradation of NF-κB greatly decreases the activation of NF-κB pathway. Both the blocking and decreasing the activation of NF-κB pathway hinder anti-apoptotic signaling resulting in the weaken anti-apoptosis ability of cells. In addition, loss of SHARPIN function leads to increased hepatocellular sensitivity of apoptosis induced by TNF-α. Injection of TNFα-inducing lipopolysaccharides in vivo leads to hepatocyte apoptosis. Using this principle, Sieber et al compared the degree of mice liver damage in the Sharpin-deficiency group and the control group through the injection of LPS inducing TNF-α release in vivo and verified that the loss of SHARPIN function increased the sensitivity of cells to apoptosis signaling induced by TNF-α, leading to premature apoptosis of liver cells and serious liver damage. Therefore, SHARPIN plays a role of anti-apoptosis and inhibiting liver inflammation.

In addition, Gerlach et al. proved that SHARPIN interferes apoptosis induced by TNF-α [9]. Ikeda et al also confirmed the sensitivity induced by TNF-α to apoptosis increases and cells die rapidly by the way of FADD (Fas-associated death domain) and caspase-8, due to the deficiency of SHARPIN function [7]. Therefore, SHARPIN regulates the activation of NF-κB pathway and cell apoptosis in different aspects.

**SHARPIN regulates toll-like receptor 2 (TLR2) pathways**

TLR2 is an important receptor in the activation of immune defense and inflammation. TLR2 involves in multiple cellular physiological processes including the activation of NF-κB transcription factors through signal transduction mechanism and regulation of TNF-α, IFN-γ and IL-12. Zak et al. found that the effects of SHARPIN deficiency on the TLR2-induced transcriptome are strikingly correlated with the effects of the hypomorphic L153p/pnfr2 point mutation in NEMO, suggesting the interaction between SHARPIN and NEMO [13]. SHARPIN deficiency leads to inhibition of IL-12 production after the activation of TLR2 in macrophage. Studies have also shown that exogenous IL-12 could alleviate chronic proliferative dermatitis due to Sharpin deficiency in CPDM mice [14]. So there is a close relationship between SHARPIN and TLR2 signaling and SHARPIN plays an important role in production of cytokines, immune defense and inflammatory reaction [15,16].

**SHARPIN inhibits β1-integrin activation**

Integrin is a major family of cell surface receptors and mainly mediates adhesion, migration between cells, cells and extracellular matrix, and maintains the dynamic balance of tissue in vivo. Rantala et al. found that SHARPIN is an endogenous inhibitor of β1-integrin [17]. The expression and activity of integrin increase in keratinocytes, fibroblasts and leukocytes of Sharpin mutant mice. SHARPIN functions as an inhibitor of β1-integrin activity in human cancer cell and primary leukocytes. Loss of SHARPIN correlates with increased β1-integrin activity in vivo. SHARPIN inhibits β1-integrin activation in the way of inhibiting recruitment of Talin and Kindlin to β1-subunits [17].

**SHARPIN is associated with tumor**

Jinyoung Jung et al. confirmed high expression of SHARPIN in malignant tumor tissue of some organs such as liver, kidney, ovary and pancreas by phenotypic analysis and immunohistochemical method in 2010 [18]. Researchers also confirmed that the
cultured CHO-K7 cells with over expression of SHARPIN grew more significantly by the method of cultivating tumor cells in vitro. Therefore, SHARPIN may play an active role in cell growth and proliferation, and have important roles in tumor development based on the confirmation that the expression of SHARPIN was positively correlated with the growth of tumor indicated by in vitro experiments.

Animal model of SHARPIN: chronic proliferative dermatitis mutant mice (cpdm)

In 1993, HogenEsch et al. found a spontaneous mutation in multiple organs in C57BL/KaLawRij mice and named it as chronic proliferative dermatitis characterized by erythema, papules, scales and lichenified plaques accompanied with severe itch, scratch and rhabagades [1]. Mutant mice developed lesions at the age of 3 to 4 weeks. The lesions occurred on the skin characterized by erythema, papules on the basis of erythema and hair loss, accompanied by itch, scratch and exudation. That was the acute stage. When the mice were at the age of 5 weeks at the subacute stage, the lesions turned into enormous scales, fusion of plaques, increase or decrease in chromatosis. When the mice were at the age of 6 to 10 weeks, the condition became chronic. Thick and hardened island confertus plaques, increased silver white scales and skin lichenification could be observed, which resulted in more serious itch, increased scratches, rhabagades and oozing of blood. The activities of mice reduced. The Th1/Th2 was imbalanced. The expression of Th2 type cytokines such as IL-4, IL-5, and IL-13, was increased. Eosinophils in peripheral blood also increased significantly. Histopathology showed hyperproliferation of keratinocytes, increased epidermal thickness and eosinophil infiltration in dermis. In 2007, Seymour et al. found that clinical phenotype of the mutant mice were linked to a 376Kb region on chromosome 15 (mChr.15) by positional cloning technology [2]. Further mutation screening, gene expression analysis, and gene trapping experiments validated the loss of SHARPIN in C57BL/KaLawRij and CBy.OcB3/Dem strains of mice. The mutations of Sharpin gene did not result in an amino acid change, but disrupted the reading frame and resulted in an early stop codon beginning at position 624 near the 3’-end of exon 1 and position 552 near the 5’-end of exon 1, leading to the loss of SHARPIN [2].

Liang et al. found that loss of SHARPIN function resulted in the weight loss of experiment mice, reduced behavior activities, and increased epidermis thickness, the increasing of leukocytosis in the peripheral blood, local infiltration of eosinophil and the increased apoptotic keratinocytes in epidermis [11]. Mice treated with anti-IL5 had a 90% decreasing of circulating eosinophils and 50% in cutaneous eosinophils, but did not relieve hypereosinophilic dermatitis due to the loss of SHARPIN, suggesting that eosinophilia was not the trigger factors of the disease [19]. Skin phenotype in SHARPIN mutant mouse can be maintained after experimental full-thickness skin graft, suggesting that skin lesions are not a secondary outcome of Sharpin mutation. In order to study the biological function of SHARPIN in the skin, Liang et al. found that excessive activation of NF-κB pathway induced by IL-1 family after the loss of SHARPIN function by skin gene expression chip [20]. The knockout of membrane IL-1 receptor IL1RAP (interleukin 1 receptor accessory protein) can block the inflammatory signals stimulated by IL-1 in cells. When the activation of NF-κB pathway is further inhibited by Bortezomib, the skin phenotype caused by loss of Sharpin function is almost completely reverted. In order to study the interaction mechanism between Sharpin and NF-κB pathway, Liang et al. found TRAF2, the critical protein linked the interaction between Sharpin and NF-κB pathway, using immunoprecipitation and immunoblotting, clarifying that Sharpin inhibited the activation of NF-KB pathway mediated by TRAF2 [21]. The skin lesions of Sharpin mutant mice are caused by IL-1 inflammatory stimuli and excessive activation of NF-κB pathway mediated by TRAF2. Bortezomib may be the new therapeutic drug for these inflammatory skin diseases.

PROSPECT

SHARPIN is an important component of linear ubiquitin assembly complex, involved in a variety of physiological events including cell proliferation/apoptosis, cell adhesion, cytokine release, immune defense, and inflammatory reaction. SHARPIN is closely associated with inflammation, immune response, and cancer. However, there are still no direct evidences about the specific function of human SHARPIN. No papers about the relationship between human SHARPIN and eosinophilic diseases such as atop dermatitis, tumor, and autoimmune diseases, have been reported. More further and comprehensive translational studies are needed to solve clinical problems using laboratory findings.

REFERENCES

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