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Intestinal epithelial cells necroptosis and its association with intestinal inflammation

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Abstract

This review focuses on the role of necroptosis, an alternative mode of cell death, in the pathogenesis of diseases associated with intestinal inflammation whose prevalence has been significantly increased for last decades, which substantiates the relevance of this issue. Necroptosis is a programmed necrosis accompanied by the activation of RIPK3 and MLKL kinases. The article covers molecular mechanisms of necroptosis, the role of necroptosis of epithelial intestinal cells in the regulation of intestinal homeostasis, its potential triggers, as well as features of necroptosis during the development of intestinal inflammation. The current review suggests that the development and use of medicines that may target necroptosis-associated kinases seem to be a promising therapeutic strategy.

Keywords: necroptosis, cell death, intestinal epithelial cells, inflammatory bowel disease, intestinal inflammation

ІШЕК ЭПИТЕЛИОЦИТТЕРІНІҢ НЕКРОПТОЗЫ ЖӘНЕ ОНЫҢ ІШЕКТІК ҚАБЫНУ КЕЗІНДЕГІ РӨЛІ

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ТҰЖЫРЫМДАМА

Бұл шолуда некроптоздың – жақында ашылған жасушалық өлім түрінің – ішектің қабынба ауруларының патогенезіндегі рөлі қарастырылады, ол соңғы он жылдықтарда кеңінен таралып отыр және сол себептен зерттеліп отырған мәселе өзекті болып табылады. Некроптоз RIPK3 және MLKL киназдарының белсендірілуімен жанама әсерленген бағдарламаланған некроз болып табылады. Мақалада некроптоздың молекулярлық механизмдері, ішектің эпителий жасушалары некроптозының ішектегі гомеостазды реттеудегі рөлі, процестің әлеуетті триггерлері, сондай-ақ асқазан-ішек жолдарындағы қабыну процестерінің дамуы кезінде ішек эпителиоциттерінің некроптозы ерекшеліктері сипатталған. Бұл әдебиеттерді талдау нысанасы некроптозды жүзеге асыруға жұмсалған киназдар болып табылатын дәрілік құралдарды әзірлеу және қолдану келешегі туралы түйін жасауға мүмкіндік береді.

Негізгі сөздер: некроптоз, жасушалық өлім, ішектің эпителий жасушалары, ішектің созылмалы қабынба аурулары, ішектің қабынуы

НЕКРОПТОЗ ЭПИТЕЛИОЦИТОВ КИШЕЧНИКА И ЕГО СВЯЗЬ С ИНТЕСТИНАЛЬНЫМ ВОСПАЛЕНИЕМ

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РЕЗЮМЕ

В данном обзоре рассматривается роль некроптоза - недавно открытого вида клеточной смерти - в патогенезе воспалительных заболеваний кишечника, распространенность которых значительно увеличилась за последние десятилетия, что обуславливает актуальность изучаемой проблемы. Некроптоз представляет собой запрограммированный некроз, опосредуемый активацией киназ RIPK3 и MLKL. В статье описаны молекулярные механизмы некроптоза, роль некроптоза эпителиальных клеток кишечника в регуляции гомеостаза в кишечнике, потенциальные триггеры процесса, а также особенности некроптоза эпителиоцитов кишечника при развитии воспалительных процессов в желудочно-кишечном тракте. Анализ данных литературы позволяет сделать вывод о перспективности разработки и применения лекарственных препаратов, мишенями которых являются киназы, вовлеченные в реализацию некроптоза.

Ключевые слова: некроптоз, клеточная смерть, эпителиальные клетки кишечника, хронические воспалительные заболевания кишечника, воспаление кишечника

Introduction

Chronic inflammation in the gut has been drawing much attention of both researchers and clinicians for last decades. In particular, numerous studies focus on the etiology and pathogenesis of inflammatory bowel disease (IBD), which is characterized by chronic intestinal inflammation [1]. There are two forms of IBD: Crohn's disease (CD) and ulcerative colitis (UC). The former is associated with transmural inflammation and affects all parts of the gut, while the latter is characterized by mucosal superficial colonic inflammation [2]. The highest prevalence of both forms of IBD is observed in Europe (Norway, Germany). The prevalence of CD is 1 in 310 individuals, whereas it reaches 1 in approximately 200 individuals for UC in Europe [3].

IBD is considered a polygenic and multifaceted disease whose development is associated with certain features of intestinal microbiome, abnormal immune response to intestinal bacteria, some dietary features (e.g. consumption of certain food additives), defects in intestinal barrier, etc. [2, 4]. Thus, the disease is a result of the complex interaction between various genetic, immunological and environmental factors.

It has been reported that IBD is accompanied by the increased rate of apoptosis and necrosis of intestinal epitheliocytes, which may be involved in intestinal damage control in IBD patients [5, 6]. Thus, the study of cell death modes of intestinal cells may significantly contribute to elucidation of pathogenetic mechanisms that underlie the development of IBD. In addition to apoptosis and necrosis, the role of recently described alternative cell death modes (necroptosis, ferroptosis, and pyroptosis) in IBD is of huge interest. Diverse and converging lines of evidence indicate that necroptosis is involved in the pathogenesis of inflammatory diseases, including IBD. This review article focuses on the features of necroptosis of intestinal epithelial cells (IECs) and its role in intestinal inflammation.

The aim of this paper was to review researches that focus on necroptosis of IECs and its contribution to the development and progression of intestinal inflammation.

Necroptosis and its molecular mechanisms

Necroptosis is a recently discovered form of cell death [7-9]. Morphologically, it resembles necrosis, i.e. it is characterized by the loss of membrane integrity, release of intracellular contents into the extracellular environment and the subsequent pro-inflammatory response due to exposure of damage-associated molecular patterns (DAMPs), since the recognition of DAMPs by pattern-recognition receptors (PRRs) triggers inflammation [10]. However, in contrast to necrosis, necroptosis is a mode of programmed cell death whose activation can be induced by either intrinsic or extrinsic factors, including lipopolysaccharides, DNA damage, TNF signaling, toll-like receptor (TLR) ligands, as a result of Fas and TNF-related apoptosis-inducing ligand (TRAIL) action, reactive oxygen species (ROS), etc. [6-8]. It has been reported that necroptosis is associated with the activation of receptor-interacting protein kinase 3 (RIPK3) and mixed lineage kinase domain-like protein (MLKL) [11]. The former participates in the formation of necrosome required for necroptotic cell death, whereas the latter acts as a downstream effector of RIPK3 and upon being phosphorylated it forms oligomers and is translocated to the cell membrane [11, 12]. Thus, its downstream activation by RIPK3 is required for membrane rupture observed during necroptosis [12].

Recent researches have also described the role of caspase-8 in necroptosis. This proteolytic enzyme is involved in co-regulation of necroptosis and apoptosis acting as a decision-maker or a switch between necrosis and apoptosis [13]. There is overwhelming evidence that caspase-8 interacts with cFLIPL (cellular FLICE-like inhibitory protein) forming a complex where the enzyme becomes inactive. It leads to inhibition of apoptosis. However, when caspase-8 is inhibited, receptor-interacting protein kinase 1 (RIPK1) and RIPK3 are active and cell death occurs via necroptosis, since caspase-8 inactivates these kinases [13-15]. Thus, caspase-8-mediated degradation of RIPK1 and RIPK3 blocks necroptosis and promotes apoptosis. It is also worth emphasizing that necroptosis is a caspase-independent mode of cell death observed when caspases are either inhibited or fail to be activated. It occurs in order to assure cell death when apoptosis cannot be fulfilled due to improperly acting caspases [14].

Role of IECs

Intestinal epithelium is characterized by an extremely fast renewal [16]. In particular, the daily shedding rate of IECs is 10 [5]. Thus, it is obvious that the balance between cell survival and death is tightly regulated. It has been reported that both apoptosis and necroptosis occur in intestinal lining under physiological conditions [17]. However, data on the role of necroptosis in the maintenance of intestinal homeostasis are limited. However, it is known that pro-apoptotic and pro-necroptotic signals are counterbalanced by survival and proliferation signals determining a fate of IECs [16]. Such signals may also come from intestinal environment, including gut bacteria or products of their metabolism, since IECs are involved in the crosstalk between intestinal microbiota and the host immune system [18], substantiating the relevance of studying the role of intestinal bacteria in regulation of necroptosis of IECs.

Immunogenic role of necroptosis

Necroptotic cell death is characterized by immunogenic properties, which are associated with the liberation of DAMPs from the cells, underwent necroptosis [19]. The list of necroptosis-associated DAMPs includes high mobility group box 1 protein (HMGB1), IL-33, S100 proteins, mitochondrial DNA, ATP molecules, etc. [20-22]. However, the number of recognized and described necroptosis-associated DAMPs is lower than the necrosis-associated ones. In addition, their immunogenic properties are hardly characterized and described. Their in-depth study may help to better understand the immunomodulatory role of necroptosis and its participation in inflammatory processes. Despite scarce data on immunogenic effects of DAMPs released from necroptotic cells, there is strong evidence that DAMPs released from necroptotic cancer cells promote maturation of dendritic cells and synthesis of IFN-γ [21]. It has been also reported that necroptosis-associated DAMPs are recognized by receptors located on the surface of immune cells with their activation and secretion of chemokines and cytokines. Chemokines such as CXCL1, CXCL2 and cytokines such as IL-1 and IL-6 are known to be released by activated immune cells in response to the exposure to necroptosis-associated DAMPs [22]. Their action contributes to the intensification of inflammation.

However, the pro-inflammatory role of necroptosis is not limited to the passive release of DAMPs through the ruptured membrane. Zhu et al (2018) stated that cytokine genes (e.g. TNFα target genes) are overexpressed in various types of necroptotic cells [23]. Such findings indicate the activation of TNFα-mediated inflammation by necroptosis. Taking into account the role of TNFα in the development of IBD, necroptosis may

be linked with the intestinal inflammation via the mechanism mentioned above.

Thus, it is interesting to note that due to its immunogenicity necroptosis can be considered an inducer of anti-tumor immunity. There are some lines of evidence that necroptotic cell death stimulates immune response against tumor cells. In particular, Aaes et al. (2016) believe that the abovementioned ability of necroptotic cancer cells to activate dendritic cells and to stimulate IFN-γ generation may be considered as an adaptive immune response [21], which makes necroptosis a promising concept for the development of novel anti-cancer medicines [19, 24]

Necroptosis in intestinal inflammation

It has been shown that necroptosis is activated in intestinal crypts in Crohn's disease in response to TNF-α if caspase-8 is compromised [13]. It is worth noting that under normal conditions caspase-8 participates in the maintenance of gut integrity preventing flow of luminal bacteria through the intestinal wall via blocking necroptosis of IECs and their shedding by the mechanism described above, namely cleavage of RIPK1 and RIPK3 [15]. The role of caspase-8 in intestinal necroptosis is supported by the fact that epitheliocytes undergo necroptosis with the spontaneous development of colitis in caspase-8-deficient mice [25, 26]. In addition, Pierdomenico et al. (2014) demonstrated that caspase-8 is downregulated against the background of RIPK3 overactivation in inflamed intestinal tissue in IBD [27]. Findings of Negroni et al. (2017) showed during an in vitro and ex vivo experiment using an intestinal cell line and samples of intestinal tissue collected from patients with IBD that necroptosis of intestinal cells exacerbates intestinal inflammation promoting synthesis of cytokines and affecting gut integrity [11]. It has been demonstrated that the necroptosis-mediated loss of the intestinal barrier integrity is induced by TNF-α [5]. It is interesting to note that besides cytokines the rate of IEC necroptosis can be regulated by dietary exogenous factors. Xiao (2018) reported that a protein- and fat-rich diet promotes necroptosis of enterocytes via mTOR upregulation in mice in an in vivo experiment [27].

Numerous authors have demonstrated that intestinal inflammation, including IBD, is accompanied by pro-inflammatory cytokine TNF-α and inflammation-associated transcription factor NF-κB upregulation [29, 30]. Both of them are known to activate p53 upregulated modulator of apoptosis (PUMA), a pro-apoptotic BCL-2 family protein. There is strong evidence that it is induced by NF-κB, enhanced under the influence of TNF-α. The action of PUMA is associated with the enhanced necroptosis rates observed in intestinal inflammation due to its involvement in MLKL and RIPK3 phosphorylation via PUMA-mediated release of mitochondrial DNA and its interaction with the corresponding DNA sensors [31]. Thus, PUMA-mediated amplification of necroptosis signaling contributes to cell death of IECs by necroptosis in intestinal inflammation.

We strongly believe that new approaches targeting necroptosis and necroptosis-associated kinases RIPK1, RIPK3, and MLKL may be developed for IBD treatment. The role of caspase-8 in the regulation of both apoptosis and necroptosis in the intestine also makes it a potential target for new therapeutics provided for patients with IBD [11, 32]. In addition, inhibition of PUMA may also seem to be beneficial to relieve intestinal inflammation.

Attempts to target necroptosis for the treatment of IBD

Some drugs have already been demonstrated to downregulate necroptosis in animal models with intestinal inflammation. In particular, necrosulfonamide (NSA) shows protective effects blocking necroptosis of IECs in mice with TNBS-induced colitis [33]. Such effects of NSA are mediated by its ability to bind necroptosis-associated MLKL and inhibit this kinase.

Liu et al. (2015) demonstrated that Necrostatin-1 administration shows anti-inflammatory effects in mice with DSS-induced colitis. Necrostatin-1 is an inhibitor of necroptosis-associated proteins RIP-1 and RIP-3 and its administration resulted in their downregulation, upregulation of caspase-8 and a decrease in the production of pro-inflammatory cytokines in DSS-induced colitis [34].

The results of pioneering studies of necroptosis-blocking drugs confirm that this therapeutic strategy seems to be effective and may be used to alleviate the symptoms of CD and UC.

Conclusion

Intestinal epithelial cells necroptosis might be involved in the regulation of intestinal inflammation. Inhibition of this strongly pro-inflammatory mode of cell death by anti-necroptotic medicines seems to be a promising therapeutic strategy whose efficacy requires further consideration. Some drugs that inhibit necroptosis of IECs such as NSA or Necrostatin-1 have been already shown to be effective in animal experiments.

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