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Pyroptosis: An inflammatory cell death implicates in atherosclerosis

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ABSTRACT

Cell death and inflammation are the fundamental biological processes in both normal physiology and pathology. Apoptosis is the most well-studied process of cell death, but there are also many other forms of cell death such as necrosis, autophagy and pyroptosis. Cell death could be observed throughout atherosclerosis and plays an important role in determining the fate of atherosclerotic lesion. Inflammation, the primary response of innate immunity, is considered essential in initiating and driving atherosclerosis. Apoptosis and autophagy had been reported in atherosclerosis, however, the mechanism of cell death involved in atherosclerosis still remain largely unknown. Cell death and inflammation are inextricably linked with their effectors modulating the process of atherosclerosis. Therefore, we proposed hypothesis that pyroptosis, an inflammatory form of cell death, may be implicated in atherosclerosis and play an important role in lesion instability.

Introduction

Cell death and inflammation are two important pathophysiological mechanisms in many diseases. In atherosclerotic heart disease, an increased number of cell deaths occur in human atherosclerotic plaques, especially in advanced plaques. Death of smooth muscle cells (SMCs), macrophages, and other types of vascular cells are abundantly observed in advanced lesion. SMCs death in atherosclerosis may decrease lesion cellularity, weaken the fibrous cap of the plaque, and promote lesion instability [1]. Although death of macrophage in early atherosclerotic lesion was reported to be beneficial because decreasing the number of these cells in the plaque could attenuate the inflammatory response and lessening the synthesis of matrix metalloproteinases (MMPs), deaths of macrophages in advanced lesion promote necrotic core formation and atherosclerosis instability [2,3]. Mechanisms of macrophages death in atherosclerotic lesion involved in attenuating the lesion cellularity, as well as promoting inflammation. Dying macrophage in atherosclerotic lesions leads to the release of growth factors, cytokines, proteases, and intracellular lipid into the extracellular spaces, which, in turn, initiate inflammation, promote plaque disruption and arterial thrombosis, the proximate causes of acute cardiovascular events [2–4].

The mechanisms of cell death and inflammation have become a focus of research in atherosclerosis. A number of groups have been devoted to unravel the link between cell death and inflammation in advanced lesions and several groups proposed that apoptosis and defective efferocytosis, known as post-apoptotic necrosis, was the major reason for lesion cell death and inflammation [5]. Indeed, there is evidence of apoptosis and defective efferocytosis in advanced lesion. However, the data from studies was not convinced. Results from electron microscopy studies showed that the large majority of dying cells in human atherosclerotic plaques had a typical ultrastructure cell lysis, but not apoptosis [6–8]. Caspase-3, the executor caspase of apoptosis, was rarely activated in animals or human advanced lesions [9,10]. More importantly, targeted deletion of caspase-3 or p53 (p53 induces apoptosis) in plaque macrophages has been shown to inhibit lesion apoptosis and increase macrophages abundance, inflammation and atherosclerotic lesion [11]. From a logical point of view, if cells within atherosclerotic lesions died in a post-apoptotic way, blocking these apoptotic mediators should theoretically decrease atherosclerosis and inflammation but atherosclerosis and inflammation actually did not regress. More importantly, TUNEL positive reaction, which was previously reported as a criterion for apoptosis, has been observed in other type of cell death, such as necrosis and pyroptosis [12,13]. Thus, these results accumulatively raised a question whether non-apoptotic cell death is involved in lesion macrophage death.

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Hypothesis

We hypothesize that pyroptosis, a caspase-1 dependent cell death, may be involved in macrophage cell death in atherosclerosis. This inflammatory cell death may be a mechanism for linking cell death and inflammation in advanced atherosclerotic lesion and hence play an important role in lesion instability.

Discussion

Cell death is typically discussed dichotomously as either apoptosis or necrosis. Apoptosis is described as an active, programmed process of autonomous cellular dismantling that avoids eliciting inflammation. It is characterized by activation of the apoptotic caspases (caspase-3 and caspase-9), as well as distinctive DNA cleavage. During apoptosis, dying cells do not release their cytoplasmic content to extracellular space [13,14]. Thus, apoptosis does not induce an inflammatory response and is considered to be immunologically silent. Necrosis has been characterized as passive, accidental cell death resulting from environmental perturbations with uncontrolled release of inflammatory cellular contents [13,14]. Despite the widespread use of the apoptosis-versus-necrosis paradigm, many other forms exist, including autophagy, oncosis, necroptosis and pyroptosis.

Pyroptosis is a novel form of cell death which is uniquely dependent on caspase-1 [15]. Caspase-1 is not involved in apoptosis and caspase-1-deficient cells respond normally to most apoptotic signals [14,16]. An important function of caspase-1 is to process the precursor of the inflammatory cytokines, IL-1β and IL-18, to their active forms [16]. Caspase-1 activation in macrophages infected with *Salmonella* or *Shigella* resulted in processing of these cytokines and death of the host cell [12,17]. The mechanism and outcome of this form of cell death are distinctly different from apoptosis, which actively inhibits inflammation. The morphology of pyroptosis seems to be a combination of both apoptotic and necrotic cells death. Pyroptotic cells lose their cell membrane potential and undergo DNA fragmentation, and like apoptotic cells death, it also shows positive terminal deoxynucleotidyl transferase–mediated dUTP nick end-labeling (TUNEL) staining [13,18]. As an inflammatory form of cell death, pyroptosis now has been described in monocyte, macrophage, dendritic cells and other cell types, stimulated either by bacterial agents or by non-bacterial stimuli [18,19]. The caspase-1 activation and dependence cell death had been confirmed to play a significant role in the immune and central nervous systems [18,20,21]. Now, there is increasing evidence supporting the hypothesis that pyroptosis may be involved in atherosclerosis, especially in advanced atherosclerotic lesions.

The most prominent biochemical character of pyroptosis is of caspase-1 dependence. Many studies had described that caspase-1 abundantly expressed and activated in animal or human atherosclerosis and was more closely correlated with signs of atherosclerotic plaque destabilization [6,22]. Ruptured plaques exhibited strong immunoreactivity to caspase-1 but weak to apoptotic executor, caspase-3 [10]. The mechanism of caspase-1 involvement in advanced lesion had been correlated with caspase-1 mediated inflammation. IL-1β and IL-18, two major substrates of caspase-1, were reported to be mainly responsible for caspase-1 mediated inflammation in atherosclerotic [23]. They lead to the release of chemokines, MMPs, and protease, which propagate inflammation, promote plaque disruption and arterial thrombosis. Target loss of IL-1β with monoclonal antibodies reduce biomarkers of atherosclerosis, including IL-6, IL-8, MCP-1 and TNF-α *in vitro* and inhibit atherosclerotic plaque formation in Apolipoprotein E-deficient mice [24]. Similarly loss of function of IL-18 *in vitro* cultures could decrease the production of IL-6 by endothelial cells, macrophages and smooth muscle cells, as well as IL-8, MMPs, and IFN- by smooth muscle cells [25,26].

The presence of caspase-1 in atherosclerotic lesion was, also, linked with signs of cell death, the TUNEL positive reaction. TUNEL positive had been initially served as a criterion of apoptosis, however, recent searches confirmed TUNEL positive merely means degradation of DNA and its detection in dead cells does not specifically indicate the underlying mechanism of death. The TUNEL positivity of *Salmonella* and *Shigella* infected macrophages initially led to the assumption that the cell death induced by these bacteria was apoptotic, but further investigation uncovered the cell death was not apoptosis but pyroptosis by caspase-1 dependence [12,17]. Studies had shown that TUNEL positive cells within ruptured plaques exhibit a strong immunoreactivity to caspase-1. Moreover, pharmacological blocking of caspase-1 with Ac-YVAD-CHO in animal model of atherosclerosis dramatically decreased DNA fragmentation [27]. Thus, it is reasonable to speculate whether the same situation occurs in lesion cell death.

Furthermore, mechanistic studies on pyroptosis add supports to this hypothesis. Caspase-1 activation during pyroptosis required a protein platform called inflammasome [28]. It is composed of members of the cytosolic Nod-like receptors (NLRs) family, procapase-1 and/or apoptosis-associated speck-like proteins containing a CARD (ASC) adaptor. Four inflammasomes have been characterized till date, based on a different NLR, namely NLRP1, NLRP3, NLRC4, and the non-NLR absent in melanoma-2 (AIM2) [28]. Among these inflammasomes, NLRP3 inflammasome is mostly understood to be activated by a range of pathogen-associated molecular patterns (PAMPs) such as LPS, peptidoglycan (PGN), and silica crystal [29,30]. A recent study reported that oxidized low-density lipoprotein and cholesterol crystal which are the most abundant existence in necrosis lesion, could activate NLRP3 inflammasome and caspase-1, and lead to release of IL-1β and IL-18 in murine macrophages [31]. Although cell death was not measured in this research, there are large number of other studies had indicated cytotoxity of oxidized low-density lipoprotein and cholesterol crystal in atherosclerotic lesion, and it is easy to envisage the consequence of pyroptotic macrophages in atherosclerotic lesion. Serial pyroptosis occurs in response to cholesterol crystals or oxidized low-density lipoprotein, where one macrophage fails to digest the crystal, dies via pyroptosis, and then another macrophage phagocytoses the same crystal and dies via pyroptosis repeatedly. This cycle could diminish lesion cellularity, cause and exacerbate significant inflammation by releasing inflammatory mediators from the pyroptotic cells, and more importantly, promote lesion instability. The contribution of inflammation and cell death to lesion instability had vastly described in numbers of studies.

In conclusion, here we provide evidence to verify hypothesis that a novel cell death, pyroptosis may involve in atherosclerosis and may be as a link of cell death and inflammation. Data from various animal and human experiments persuasively support the hypothesis. Our hypothesis would add a new understanding of the mechanism of cell death and inflammation in atherosclerosis and provide new perspective to manipulate atherosclerosis. Certainly, further investigation about whether and how pyroptosis occurs in atherosclerosis should be elucidated.

Conflict of interest statement

None of the authors has any potential financial conflict of interest related to this manuscript.

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