RESEARCH HIGHLIGHT

ATP-triggered unconventional secretion of GAPDH from macrophages: Its putative role as a modulator of inflammation

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Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a key glycolytic enzyme that is predominantly localized in the cytoplasm. However, emerging evidence indicates that GAPDH is secreted from mammalian cells and performs some of its biological functions in the extracellular space. In particular, it has been reported that high levels of GAPDH are secreted from macrophages that play important roles in the innate immune system. Despite these findings, since GAPDH is a leaderless protein, the mechanisms by which it reaches the extracellular environment remain unclear. In this regard, we have recently reported that extracellular ATP is able to trigger the secretion of GAPDH from mouse microglial cells, the resident macrophages in the brain. In addition, the activation of microglial cells by lipopolysaccharide (LPS) clearly facilitated ATP-induced GAPDH secretion. Importantly, exosome secretion mediated by the P2X7 receptor (P2X7R), an ATP-gated cation channel, was shown to play a critical role in the induction of unconventional GAPDH secretion from LPS-primed microglial cells. We also found that secreted GAPDH affects the LPS-induced phosphorylation of p38 mitogen-activated protein kinase in microglial cells. Furthermore, our preliminary data demonstrated that the ATP-induced secretion of GAPDH occurs in macrophage cell lines derived from other mouse peripheral tissues, such as the liver. Together, our findings suggest that GAPDH is generated extracellularly through the stimulation of activated macrophages by ATP, and the secreted GAPDH acts as a mediator of macrophage-related inflammation in an autocrine/paracrine fashion.

Keywords: Glyceraldehyde-3-phosphate dehydrogenase; Macrophages; P2X7 receptor; Unconventional secretion

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Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a key enzyme in glycolysis, a metabolic process in which glucose is turned into energy (ATP). In addition to glycolysis, cytosolic GAPDH has been reported to be involved in numerous other cellular processes in mammalian cells, such as DNA repair, intracellular membrane trafficking, cytoskeletal dynamics, oxidative stress response, and apoptosis [1-3]. These functions of GAPDH seem to be regulated by its oligomerization, post-translational modification, and subcellular localization [2,3]. Based on its

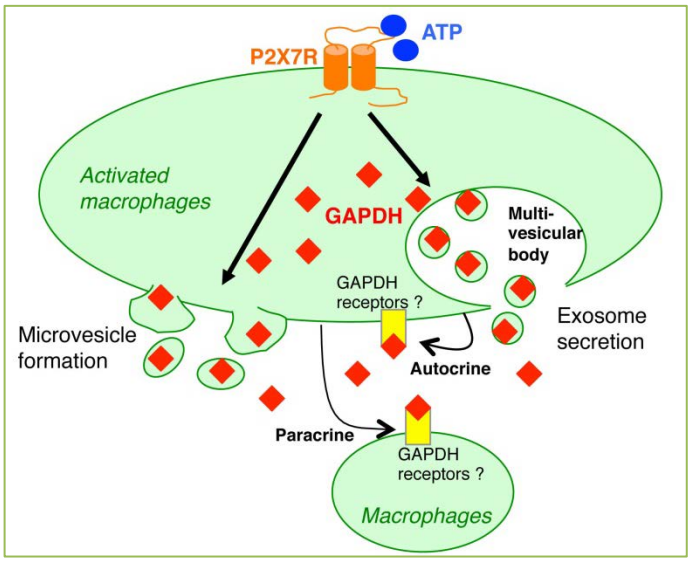

Figure 1. ATP-triggered P2X7R-mediated unconventional secretion of GAPDH from macrophages. P2X7R activation by extracellular ATP (in blue) triggers the robust secretion of GAPDH (in red) from activated macrophages. P2X7R-mediated microvesicle formation and/or exosome secretion are probably associated with ATP-induced GAPDH secretion. The secreted GAPDH might contribute to the regulation of inflammatory responses related to macrophages in an autocrine/paracrine manner. Unidentified GAPDH receptors (in yellow) that mediate these functions might be expressed in macrophages.

multifunctional properties, GAPDH has been extensively examined as an intriguing example of a moonlighting protein; i.e., proteins that exhibit activities that are distinct from their classically identified functions [1,2].

Most GAPDH is located inside cells because it lacks a signaling sequence and cannot be transported via conventional secretion pathways. However, recent studies have demonstrated that GAPDH is also found outside of cells and carries out some of its biological functions in the extracellular space. Extracellular GAPDH was detected in conditioned medium derived from various mammalian cell lines, and it was suggested that it modulates cell-cell and/or cell-matrix interactions [4]. It has also been reported that GAPDH is expressed on the surfaces of macrophages and functions as a transferrin or lactoferrin receptor in these cells [5,6]. Notably, macrophages have been demonstrated to secrete high levels of GAPDH [7], indicating that secreted GAPDH could act as a regulatory factor during macrophage-mediated inflammation. However, the mechanisms underlying the secretion of GAPDH from macrophages have not been fully elucidated.

Accumulating evidence suggests that exogenous ATP induces the unconventional secretion of leaderless proteins from macrophages [8]. The involvement of the P2X7 receptor (P2X7R), an ATP-gated cation channel that is highly expressed by monocyte/macrophage lineage cells, in this process has been well characterized [8]. The activation of P2X7R by ATP seems to be critical for the induction of the unconventional secretion of several intracellular proteins including cytokines and alarmins, such as interleukin-1β (IL-1β) and high mobility group box protein 1 (HMGB1), from macrophages [8-10]. Based on these previous findings, our recent study revealed new evidence that exogenous ATP triggers the unconventional secretion of GAPDH from mouse microglial cells, the resident macrophages in the brain [11]. In a secretome analysis, we also confirmed the presence of GAPDH in culture supernatants derived from ATP-stimulated microglial cells (unpublished observations).

Initially, we noticed that lipopolysaccharide (LPS) priming markedly enhances the ATP-induced secretion of GAPDH from microglial cells [11], suggesting that microglia activation facilitates the induction of GAPDH secretion, and thus, secreted GAPDH might contribute to inflammatory processes. We also found that ATP-induced P2X7R-mediated microvesicle formation and exosome secretion are associated with the unconventional secretion of GAPDH from LPS-primed microglial cells (Figure 1) [11]. Given the functional significance of macrophage-derived exosomes in inflammation [12], the finding that GAPDH is enriched within secreted exosomes is probably relevant to its putative role in macrophage-mediated inflammation. We further demonstrated that exogenous GAPDH modulates the LPS-induced activation of p38 mitogen-activated protein (MAP) kinase in microglial cells [11]. This finding supports the suggestion that GAPDH contributes to the regulation of microglia/macrophage-related inflammation in an autocrine/paracrine fashion (Figure 1).

Regarding macrophages derived from peripheral tissues, we have developed a simple and efficient procedure for obtaining macrophage-like cells from mixed primary cultures of mouse liver or kidney cells [13,14]. Using this system, we have already established tissue-derived clonal mouse macrophage cell lines that have been immortalized via the retroviral transduction of the human *c-myc* gene [13,14]. Among them, we have detected ATP-induced GAPDH secretion in the liver-derived mouse macrophage cell line KUP-5 [13] (unpublished observations). Therefore, it raises the possibility that the ATP/P2X7R signaling pathway is required for the induction of robust GAPDH secretion in various types of tissue-derived macrophages.

GAPDH is highly conserved across various species. In some pathogenic microorganisms, GAPDH is expressed on the outer surface of the microorganism or is detected as a secretory product [15-18]. Extracellular GAPDH derived from certain pathogenic microorganisms is considered to function

as a virulence factor that modulates the host immune system, which protects such organisms from host defense mechanisms [16,18-20]. Given their strong homology, mammalian GAPDH molecules might also act to modulate the mammalian immune system. It is thus conceivable that the GAPDH secreted from macrophages contributes to innate immune system-associated inflammatory reactions in mammals.

Recently, Takaoka *et al.* reported the intriguing finding that intraperitoneal preinjection with GAPDH protein ameliorates LPS-induced, sepsis-related severe acute lung injury (ALI) in mice [21]. They suggested that GAPDH exhibits anti-inflammatory activity and so has potential as a therapy for sepsis-related ALI [21]. On the other hand, we demonstrated the facilitative effect of GAPDH on LPS-induced p38 MAP kinase phosphorylation in microglial cells [11]. Since p38 MAP kinase plays an essential role in macrophage-mediated inflammation [22], our study suggests that exogenous GAPDH has proinflammatory properties. This might be supported by the concept of alarmins, which are endogenous molecules that alert the innate immune system and trigger defensive immune responses [23]; i.e., GAPDH might act as an alarmin. Further studies will be required to address the discrepancies between the findings of the *in vivo* study performed by Takaoka *et al.* and our *in vitro* study with regard to the innate immune functions of GAPDH. However, these investigations shed light on the extracellular role of secreted GAPDH as a mediator of macrophage-related inflammation that acts in an autocrine/paracrine manner.

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