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Mini Review

A defense system against multiple diseases via biological garbage clearance mediated by soluble scavenger proteins

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The circulating protein apoptosis inhibitor of macrophage (AIM) is incorporated into normal hepatocytes and inhibits lipid storage within them, thereby decreasing liver steatosis. In contrast, AIM accumulates on the surface of hepatocellular carcinoma (HCC) cells and induces elimination of the cells, thereby preventing HCC tumor development. Based on these findings, we hypothesize the presence of a set of circulating proteins that specifically mark biological garbage, such as cancer cells, dead cell debris, or degenerated proteins/cells, and promote efficient elimination of such undesired substances, thereby preventing progression to multiple diseases. We propose designating these marker proteins as "soluble scavenger proteins" (SSPs), and their potential therapeutic application to various refractory diseases.

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Abbreviations:
AIM, apoptosis inhibitor of macrophage; HCC, hepatocellular carcinoma; FASN, fatty acid synthase; HFD, high-fat diet; RCA, regulators of complement activation; SSP, soluble scavenger protein; DAMP, damage-associated molecular patterns; AKI, acute kidney injury

Key words soluble scavenger protein (SSP), apoptosis inhibitor of macrophage (AIM), hepatocellular carcinoma, phagocytosis

Introduction

A variety of biological garbage, such as cancer cells, apoptotic or necrotic cells, and degenerated cells/proteins, develop constitutively in our body due to both physiological and pathological events. Such undesired substances are usually eliminated quickly, and tissues are regenerated through the proliferation of neighboring healthy cells and/or maturation of immature precursor cells. When the elimination system is impaired, biological garbage accumulates and leads to the development of diseases; cancer cells proliferate and form tumors, and the persistence of dead cells or degenerated cells/proteins is not only toxic to surrounding normal tissue cells, but also causes secondary inflammation particularly through the release of damage-associated molecular patterns (called DAMPs) by necrotic cell bodies[1, 2], which leads to progressive fibrosis and abnormal tissue remodeling. Thus, the biological garbage scavenging response is essential for preventing disease and maintaining the body in homeostasis (Fig. 1). One important garbage-elimination mechanism is the specific recognition of waste by effector scavenging cells, namely, phagocytes. Although our knowledge of scavenging cells and their scavenging manner has increased, particularly though identification of multiple scavenger receptors[3, 4], the mechanism through which unwanted garbage is distinguished from surrounding normal cells and specifically eliminated remains largely unknown. To date, only phosphatidylserine has been identified as an “eat-me signal”[5]. Phosphatidylserine and its receptors/adaptors participate in the identification and engulfment of apoptotic cells by phagocytes, but the means through which different types of biological garbage are recognized are not currently understood. However, the phosphatidylserine system suggests that specific proteins might accumulate on biological garbage to act as “marking molecules” and promote garbage elimination. In recent years, we have obtained interesting results suggesting that apoptosis inhibitor of macrophage (AIM), which we identified as an apoptosis inhibitor a decade ago[6], is a candidate marking molecule required for efficient garbage elimination. In this review, we present the concept of the garbage elimination system and its potential role in disease prevention, and discuss its possible therapeutic application with reference to recent progress in AIM research.

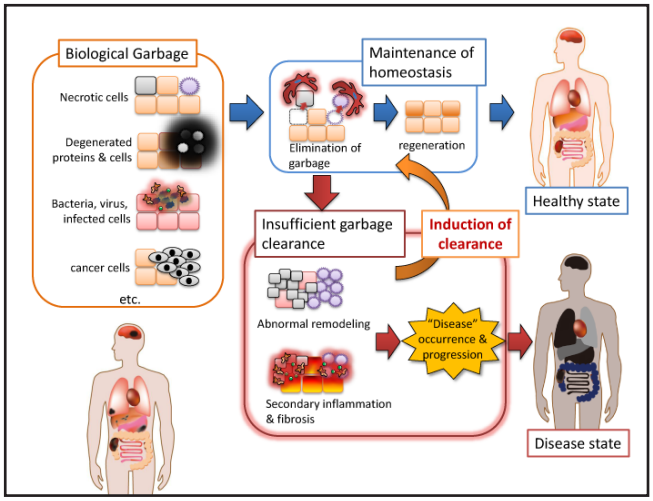

Fig. 1 Clearance of biological garbage and diseases

A variety of biological garbage such as necrotic cells, degenerated cells/proteins, infectious pathogens, or cancer cells, develop constitutively in our body. Such undesired substances are eliminated quickly, followed by the regeneratin of tissues, to maintain the homeostasis of the body. When the eliminating process is impaired, the biological garbage accumulates, resulting in the development of diseases. Such disease state will be recovered by inducing garbage clearance, which might be a new theraputic strategy for various refractory diseases.

Characteristics of AIM

AIM, also known as CD5-like antigen (CD5L), is a circulating protein that was initially identified as a supporter of macrophage survival. Serum AIM levels are relatively high (approximately 5 μg/mL) in humans and mice[7, 8]. AIM belongs to the scavenger receptor cysteine-rich superfamily, which all share a highly conserved cysteine-rich domain of approximately 100 amino acids[6]. The AIM protein sequence is well conserved between humans and mice with 78% amino acid homology but variations in the glycosylation state[6, 7, 9]. As far as we are aware, human and mouse AIM are functionally equivalent[9]. AIM is solely produced by tissue macrophages and is transcriptionally regulated by nuclear receptor liver X receptor/retinoid X receptor (LXR/RXR) heterodimers[10-12]. Hamada et al. have reported that the transcription factor MafB is also involved in the regulation of *AIM* mRNA expression[13]. Recently, we analyzed serum AIM levels in more than 10,000 healthy human individuals[8]. AIM levels are high in young women (teens to 20s) and gradually decrease with age until approximately 50 years of age, after which they are fairly steady. In contrast, AIM levels in men are consistent throughout life, and are similar to those seen in women greater than 50 years of age. Interestingly, AIM associates with IgM pentamers in the blood, which protects AIM from renal excretion and serves to maintain high levels

of circulating AIM[14, 15]. As such, serum AIM and IgM levels are strongly correlated in humans and mice[15]. However, AIM itself does not appear to influence IgM levels; this is supported by the normal IgM levels observed in AIM-deficient (*AIM*$^{-/-}$) mice[15].

Effects of AIM on normal cells

Unlike many other soluble proteins, AIM does not mediate signal transduction in target cells, but is incorporated through scavenger receptor-mediated endocytosis[7]. Typically, AIM is endocytosed into adipocytes and hepatocytes through CD36, where it binds to and inactivates cytoplasmic fatty acid synthase (FASN)[7, 16]. This leads to a reduction in lipid droplet-coating proteins, such as fat-specific protein 27 and perilipin, and decreases triacylglycerol deposition within the cells[17]. This action by AIM prevents obesity and fatty liver progression[7, 16]. In *AIM*$^{-/-}$ mice fed a high-fat diet (HFD), bodyweight gain is significantly higher than in wild-type (*AIM*$^{+/+}$) mice; *AIM*$^{-/-}$ mice show a remarkable increase in visceral adipose tissue mass[7]. This hyper-obese phenotype is abrogated by the administration of recombinant AIM (rAIM) to *AIM*$^{-/-}$ mice[7]. Similarly, *AIM*$^{-/-}$ mice fed a HFD show more advanced liver steatosis than *AIM*$^{+/+}$ mice, with increased liver mass and liver triacylglycerol content[16]. Since IgM does not co-localize with AIM incorporated into the cytoplasm of adipocytes and hepatocytes, it seems likely that AIM-IgM dissociation occurs as AIM is incorporated into cells. In humans, we have observed significant negative correlations between circulating AIM levels and body mass index, abdominal circumference, and body fat percentage[8]. Thus, in normal adipocytes and hepatocytes, circulating AIM serves to regulate cellular fat deposition, thereby preventing obesity and fatty liver.

AIM as a marker of HCC cells

As discussed above, circulating AIM is incorporated into normal hepatocytes. However, once hepatocytes have undergone malignant transformation to hepatocellular carcinoma (HCC) cells, AIM is no longer endocytosed, but accumulates on the cell surface[16]. Defective endocytosis is a common characteristic of many different types of cancer cell[18, 19]. In HCC cells, this results in the accumulation of AIM on the cell surface after AIM-CD36 binding, with insufficient AIM cellular incorporation. As such, AIM distinguishes HCC cells from normal hepatocytes. Furthermore, cell surface AIM specifically stimulates HCC cell death by necrosis, thereby preventing tumor development. Indeed, in contrast to *AIM*$^{+/+}$ mice, in which HCC tumors essentially do not develop after HFD-induced severe steatosis, all *AIM*$^{-/-}$ mice bear multiple HCC tumors when fed a HFD for a year[16]. Similarly, when subjected to the strong carcinogen diethylnitrosamine, the number and size of the HCC masses that develop after several months are significantly greater in *AIM*$^{-/-}$ mice than in *AIM*$^{+/+}$ mice[16]. This HCC tumor prevention appears to be specifically due to AIM-induced cell death; *AIM*$^{-/-}$ and *AIM*$^{+/+}$ mice show comparable grades of HFD-induced inflammation and fibrosis in the liver, which is currently recognized as an important basis for liver carcinogenesis[16]. Furthermore, rAIM injection can prevent HCC tumor development in *AIM*$^{-/-}$ mice fed a HFD. These findings suggest that AIM does not inhibit the hepatocyte carcinogenesis caused by advanced liver steatosis, similar to that observed in human non-alcoholic steatohepatitis[20], but prevents HCC tumor development through the elimination of cancer cells.

The killing mechanism of cell surface AIM

AIM is not a signaling molecule and thus extracellular AIM does not provoke signaling cascades. But how does the accumulation of AIM on the cell surface induce HCC cellular necrosis? Surprisingly, we found that the complement cascade is diverted to kill cancer cells instead of invading pathogens. In contrast to invading bacteria, mammalian cells are protected from the complement cascade by expressing of multiple regulators of complement activation (RCAs)[21, 22], such as CD55, complement receptor 1-related gene/protein-y, complement factor H, and CD59, on their cell surface. Cell surface AIM binds directly to RCAs and inactivates the complement-inhibiting effect of RCAs. This is reminiscent of the AIM-mediated reduction in FASN in normal hepatocytes and adipocytes. The precise mechanism through which AIM-binding decreases RCA activity remains unknown and requires further investigation. It should be emphasized, however, that normal hepatocytes are resistant to the complement cascade because they endocytose AIM, thus preventing AIM from binding to RCAs on the cell surface.

AIM may promote the clearance of dead cell debris

The adequate removal of cell debris is important in maintaining tissue homeostasis; there are several reports which indicate that insufficient clearance of dead

cells disturbs recovery from injury in various tissues, including the lung, heart, mammary gland, and liver[23-27]. This is thought to occur primarily by the debris causing further inflammation. Thus, in to order to avoid secondary inflammation and to promote tissue recovery, necrotic HCC cells need to be rapidly removed from the liver. In fact, the accumulation of AIM on the surface of necrotic HCC cells appears to serve this purpose. We have found that an AIM coating markedly increases the preference of phagocytes, including macrophages, to necrotic cell debris (unpublished results). When we induced the expression of cell surface-bound AIM in Hepa1.6 mouse HCC cells after these cells are transplanted into the liver of *AIM*$^{-/-}$ mice, a massive increase in necrosis, caused by activation of the of the complement system, and rapid efficient clearance of dead HCC cell debris by infiltrating Kupffer macrophages was observed[16]. This AIM-stimulated clearance of cell debris is most probably involved in the injury recovery of different tissues. We found that there is impaired AIM-mediated cell debris clearance in the kidney of *AIM*$^{-/-}$ mice, which impairs their recovery from kidney damage (unpublished results). It is perhaps noteworthy that the clearance of dead cells is usually performed by "professional phagocytes", such as macrophages. However, tissue epithelial cells have recently been termed "semi-professional" phagocytes due to their role as clearers of dead cells. For instance, bronchial epithelial cells are able engulf apoptotic cells and secrete anti-inflammatory cytokines in order to suppress airway inflammation[24]. Furthermore, mammary tissue homeostasis and future lactation in the post-partum mammary gland is influenced by epithelial cell-mediated dead cell clearance[26]. Similar findings have been reported in kidney diseases[28]. It would be interesting to address by which phagocytes and in which diseases the effect of AIM on debris engulfment is functional.

Soluble scavenger proteins

We hypothesize that a number of different proteins, other than AIM, might be also involved in garbage clearance, and that each protein may possess specific preferences for different types of garbage. We propose designating these proteins "soluble scavenger proteins" (SSPs). It is possible that some secreted proteins belonging to the scavenger receptor cysteine-rich super family[29, 30], like AIM, may be the candidates of SSP. On the basis of the features of AIM, we have defined the properties of SSPs as follows (Fig. 2). Firstly, SSPs should be circulating proteins which

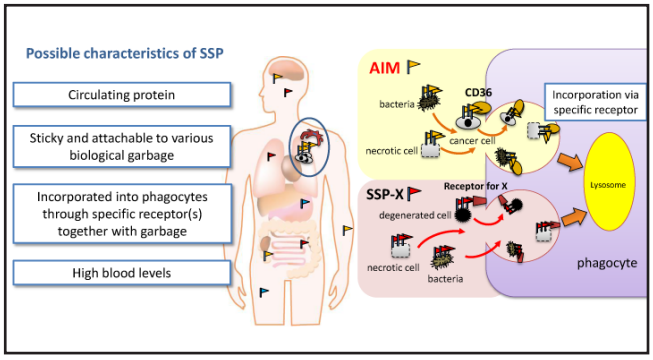

Fig. 2 A scheme for possible characteristics and action of SSP

The SSP-X that possesses AIM-like characteristics may eliminate biological garbage as AIM does. A number of SSPs may exist in the blood and contribute to maintain the body in homeostasis.

can be delivered throughout the body. Secondly, SSPs should possess a "sticky" nature, which enables them to efficiently accumulate on the surface of garbage. Lozano's group reported that AIM attaches to kind of bacteria and induce their coagulation[31]. Thirdly, SSPs should also bind to the specific receptors that mediate garbage engulfment through incorporation of the SSP. Lastly, SSP blood levels should be high, similar to AIM blood levels, distinctive to those of soluble signaling proteins (which are far lower). The identification of new SSPs and their garbage clearance functions should shed light on unknown mechanisms of disease development and provide new avenues to defend against disease progression.

Furthermore, it may be possible to reclassify some diseases based on SSPs; a disease group might be classified according to a common etiology, namely, the accumulation of biological garbage due to insufficient activity of a specific SSP. If SSP-based disease classification is possible, then a common therapeutic strategy of SSP administration, could be applied to many diseases which had previously been considered as quite independent diseases. As we have demonstrated, AIM administration successfully ameliorates HCC[16] and prevents kidney injury in *AIM*$^{-/-}$ mice (unpublished results). This strategy could be applied to patients who possess insufficient amounts of the SSP or who suffer diseases in which the endogenous SSP cannot access the accumulating biological garbage due to vascularity issues or other biological barriers.

Perspectives

The overall goal of disease biology researchers is to improve the prognosis of patients with a variety of life-

threatening diseases. There are two principal strategies employed to achieve this difficult objective; elucidation of the molecular mechanisms of disease development and the targeting of existing disease in order to eliminate it. The latter strategy, disease elimination, can be achieved by surgical resection of the diseased regions (where possible) and the administration of small therapeutic compounds. Many of these small compounds are effective, at least in slowing disease progression, but some serious side-effects can accompany their use; this is particularly true of cancer therapeutics. Furthermore, for a number of diseases, no viable therapeutic strategies currently exist. For instance, although numerous therapeutic strategies have been proposed and/or tested for acute kidney injury (AKI), none have reached the clinic[32-34]. However, it is noteworthy that humans have survived incurable diseases (i.e. cancer and AKI) for more than a million years, and for most of that period we have been without specific therapeutic tools. Therefore, we hypothesize that our endogenous systems, such as SSP-mediated garbage clearance, which are the first line of defense in preventing undesirable substances from establishing disease, must be highly effective. As such, we should try to exploit these systems to temper diseases which are difficult to cure using modern medicine.

In this review, we have proposed a new concept: the garbage clearance system mediated by SSPs. We have discussed its potential therapeutic application to various refractory diseases with reference to new findings from AIM research. Although a huge amount of work is still required in order to determine whether SSPs can be used as a novel therapeutic concept and therapeutic tool, we believe that SSPs could form the basis of next-generation therapeutic strategies for various diseases in future.

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Conflict of interests

None

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