European Journal of Medicinal Chemistry 121 (2016) 209–220

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Mini-review

Recent advances in the discovery of heparanase inhibitors as anti-cancer agents

Li Jia, Shutao Ma*

Department of Medicinal Chemistry Key Laboratory of Chemical Biology (Ministry of Education) School of Pharmaceutical Sciences, Shandong University, 44 West Culture Road, Jinan, 250012, PR China

ARTICLE INFO

Article history:
Received 16 March 2016
Received in revised form
9 May 2016
Accepted 22 May 2016
Available online 24 May 2016

Keywords:
Heparanase
Heparan sulfate
Inhibitors
Anti-Cancer agents

ABSTRACT

Heparanase, an only endo-β-D-glucuronidase capable of cleaving heparan sulfate (HS) side chains at specific sites, contributes to remodeling of the extracellular matrix (ECM) and releasing of HS-linked growth factors, cytokines and signaling proteins. In addition, heparanase also plays an indispensable role in tumor angiogenesis, invasion and metastasis, indicating that it is a promising target for the development of antitumor drugs. Recent progress leads to three classes of heparanase inhibitors, including active analogs of endogenous substance, synthetic small molecule compounds and natural products. In this review, following an outline on the heparanase structure and function, an overview of the advancement of heparanase inhibitors as novel and potent anti-cancer agents will be given, especially introducing various existing heparanase inhibitors, as well as their inhibitory activities and mechanisms of action.

1. Introduction

Cancer, also known as malignant tumors, is a generic term for a large group of diseases that can affect any part of the body. The proliferation and metastasis of tumor cells have two essential processes including the generation of new blood vessels and the tumor cells across the barriers which are composed of the extracellular matrix (ECM) and the vascular basement membrane (BM) [1]. The heparan sulfate (HS) glycosaminoglycan side chains are covalently linked to a core protein to form heparan sulfate proteoglycan (HSPG), which is an important component of the cell surfaces and ECM [2]. HS modulates signal transduction to tumor cells by interacting with various HS-bound growth factors such as fibroblast growth factor-2 (FGF-2) [3], vascular epidermal growth factor (VEGF) [4], as well as heparin-binding epidermal growth factor-like growth factor (HB-EGF) [5]. The HSPG plays an indispensable effect in many pathophysiological processes including tumor angiogenesis, cell invasion and metastasis through the degradation of HS.

Heparanase was discovered as an endo-β-D-glucuronidase capable of cleaving HS glycosaminoglycan side chains at sites of low sulfation, yielding HS fragments with appreciable size (4–7 kDa) [6]. Thus the heparanase acts as an important part in tumor angiogenesis, cell invasion and metastasis. Overexpression of heparanase has been found in numerous human tumors and several studies have confirmed a correlation between the heparanase expression and the reduced survival as well [7]. Moreover, heparanase is identified as the single HS-degrading enzyme in human cancer, indicating that it is a promising target for the development of antitumor drugs. Several heparanase inhibitors discovered on the basis of heparanase, including active analogs of endogenous substance, synthetic small molecule compounds and natural products, have been reported. For example, PI-88, found as a lead compound, has been progressed into clinical phase III trials in postresection hepatocellular carcinoma recently [8]. In this review, we summarize the recent advances of heparanase inhibitors as novel and potent anti-cancer agents, emphasizing various existing heparanase inhibitors, inhibitory activities and mechanisms of action.

2. Structure and function of heparanase

2.1. Heparanase structure

To date, only one gene (HPSE-1) of the human heparanase genes encodes a protein with the activity of heparanase, mapping to

* Corresponding author.
E-mail address: mashutao@sdu.edu.cn (S. Ma).

http://dx.doi.org/10.1016/j.ejmech.2016.05.052
0223-5234/

chromosome 4 at band 4q21.3 [9]. HPSE-1 is expressed as two mRNA transcripts by alternative splicing, HSPE-1a (5 kb) and HSPE-1b (1.7 kb), having the same open reading frame and encoding the same polypeptide of 543 amino acids with a theoretical molecular weight of 61.2 kD [9,10]. Then, the polypeptide is posttranslationally cleaved into a 8 kDa N-terminal subunit (Gln^{36}-Glu^{109}) and a 50 kDa C-terminal subunit (Lys^{158}-Ile^{543}) that non-covalently associate to form the active heterodimer [11,12], which is essential for heparanase enzymatic activity [13]. Cellular processing of the latent proenzyme into its active 8 + 50 kDa heterodimer comprises the removal of the linker segment (Ser^{110}-Gln^{157}) of 6 kDa, which is inhibited by a specific inhibitor of cathepsin L [14]. Furthermore, multiple site-directed mutagenesis, cathepsin L gene silencing and knockout experiments strongly suggest that cathepsin L is the key protease responsible for posttranslational activation of proheparanase [15].

The predicted three-dimensional modelled structure of heparanase consists of a triose phosphate isomerase TIM-barrel fold sheltering the active site of heparanase and a C-terminus domain that is crucial for heparanase secretion and signaling function [16]. There are conserved glutamic acidic residues, a putative proton donor at Glu^{225} and a nucleophile at Glu^{343}, involving in the catalytic mechanism of heparanase according to the site-directed mutagenesis [17]. Moreover, the C-domain that contains eight β-strands organized in two sheets connected by a loop is required for the heparanase enzymatic activity. The domain is also the mediator of the heparanase non-enzymatic activity [18].

A heparanase homolog identified as heparanase 2 is alternatively spliced into three mRNA transcripts [19]. It appears to regulate levels of heparanase expression, but it has not yet been shown to have enzymatic activity [20]. Clearly, the function of heparanase 2 and its value as a tumor marker will be worthy of further study in the future.

2.2. Heparanase function in cancer

Heparanase produced by tumor cells is a predominant enzyme in the metastasis of malignant tumors. It can mediate the degradation of HS that is a major barrier to the invasion and spread of tumor cells in the ECM and BM. It has been verified a direct correlation between the levels of heparanase expression and the rates of tumor invasion and metastasis. An evidence has indicated that T-lymphoma and melanoma cells from a non-metastatic to metastatic phenotype follow stable transfection and overexpression of heparanase [10]. Overexpression of heparanase in cancer cells is partially explained by the fact that HPSE-1 gene expression is repressed by the tumor suppressor transcription factor p53, which is mutated in 50% of tumors [21]. Furthermore, heparanase expression levels correlate with tumor vascularity in cancer patients, suggesting that heparanase plays a significant role in tumor angiogenesis [22]. Heparanase enables the release and activation of HS-binding factors such as FGFs and VEGF, which can promote endothelial cells migration and sprout toward the angiogenic stimulus [23,24]. All of those imply that heparanase functions are not only limited to tumor metastasis but also engaged in accelerated growth of the primary lesion. Notably, cancer patients exhibiting high expression levels of heparanase have a remarkably shorter postoperative survival time than patients whose tumors contain low expression levels of heparanase [22], further manifesting the importance of heparanase as a master regulator of cancer progression and metastasis. Moreover, heparanase has been revealed as an important regulator of blood coagulation recently. Its overexpression levels in human tumors, together with the prothrombotic state of most neoplasms, shows potential clinical significance in the procoagulant function [25].

Chronic inflammatory conditions are presented in the microenvironment of most tumors [26] and have been shown to contribute to cancer progression [27] through mobilization of tumor-supporting immunocyte populations, such as tumor-associated macrophages (TAMs) and neutrophils, which supply bioactive molecules that facilitate tumor cells survival, angiogenesis, invasion, and metastasis [26,28]. Remarkably, heparanase has been reported to induce inflammation-driven tumorigenesis in many conditions, such as Barrett's esophagus [29], hepatitis C infection [30], chronic pancreatitis [31] and ulcerative colitis [32]. It appears that heparanase creates a tumorigenic microenvironment characterized by enhanced NFkB and STAT3 signaling, elevated levels of cyclooxygenase 2 and increased vascularization by sustaining continuous activation of macrophages that supply cancer-promoting cytokines, including TNF-α, interleukin-1 and interleukin-6 [32].

3. Heparanase inhibitors

3.1. Active analogs of endogenous substance

As one of the closest mimics of HS, heparin is a natural choice as a heparanase inhibitor. However, it is limited as an anti-cancer drug due to its potent anticoagulant activity [33]. Considerable efforts have thus been expended in the development of modified heparins and related polysulfated compounds with reduced anticoagulant activity. In particular, modified heparins or sulphated oligosaccharides, such as PI-88, PG545, SST0001 etc., have been developed as potent heparanase inhibitors, which will be introduced in detail as follows.

3.1.1. Oligosaccharide mimetics

PI-88 (compound **1**), a highly sulfated phosphosulfomannan, has been progressed into clinical phase III trials in postresection hepatocellular carcinoma recently [8]. PI-88 is a complex mixture of chemically sulfated oligosaccharides, ranging from di-to hexasaccharide, with the major components of pentasaccharide (60%) and tetrasaccharide (30%). Besides acting as a heparanase inhibitor [34], PI-88 inhibits angiogenesis (IC_{50} = 0.98 μM) [34] directly by antagonizing the interactions of angiogenic growth factors such as FGF-2 and VEGF and their receptors with HS [35]. Furthermore, Hsulf-1 and Hsulf-2, two human extracellular endoglucosamine 6-sulfatases, which serve important roles in angiogenesis and growth of cancer cells, are upregulated in a number of cancers [36]. They are efficiently inhibited by PI-88 in a concentration dependent manner. PI-88 is associated with decreased cell proliferation, increased apoptosis, inhibition of angiogenesis and a significant reduction in the number of invasive carcinomas [37]. After the clinical studies, PI-88 has been demonstrated to have good safety and tolerability profiles, which are clinical benefit to patients with various cancers [37].

A series of synthesized polysulfated glycosides of α(1 → 3)/(1 → 2)-linked mannopenta- and -tetrasaccharides as PI-88 analogs were evaluated for their ability to inhibit angiogenesis [38]. Among them, compound **2** inhibited heparanase activity with an IC_{50} of 1.7 μM, and in particular, showed improved pharmacokinetics in a rat model due to its lipophilic octyl group [34]. Compound **3** (Fig. 1), compared to **2**, had a similar inhibitory activity and improved pharmacokinetics of a slightly short half-life and a high plasma clearance [34,38].

Suramin (compound **4**, R = Me), a polysulfonated naphthyl urea, inhibited heparanase with an IC_{50} of 48 μM [39]. On the one hand, Suramin inhibited cell growth by blocking the growth factors binding to cell surface receptors. On the other hand, it exerted anti-angiogenic and anti-metastatic effects through blocking the activity