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Sulfated polymannuroguluronate, a novel anti-AIDS drug candidate, inhibits HIV-1 Tat-induced angiogenesis in Kaposi's sarcoma cells

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ABSTRACT

Kaposi's sarcoma (KS), a neoplasm often associated with iatrogenic and acquired immunosuppression, is characterized by prominent angiogenesis. Angiogenic factors released from KS and host cells and HIV viral products—the protein Tat are reported to be involved in angiogenesis. Mounting evidence further suggests that multiple angiogenic activities of Tat contribute to AIDS-associated Kaposi's sarcoma (AIDS-KS). Herein, we report that sulfated polymannuroguluronate (SPMG), a novel anti-AIDS drug candidate now undergoing phase II clinical trial, significantly eliminated Tat-induced angiogenesis in SLK cells both in vitro and in vivo. SPMG significantly and dose-dependently inhibits proliferation, migration, and tube formation by SLK cells. SPMG also dramatically arrested Tat-driven KDR phosphorylation and blocked the interaction between Tat and integrin β1, thus inhibiting the phosphorylation of the downstream kinases of FAK, paxillin and MAPKs. In addition, SPMG was noted to block the release of bFGF and VEGF from ECM. All these collectively favor an issue that SPMG functions as a promising therapeutic against Tat-induced angiogenesis and pathologic events relevant to AIDS-KS, which adds novel mechanistic profiling to the anti-AIDS action of SPMG.

1. Introduction

Kaposi's sarcoma (KS), an angioproliferation disease, is a form of malignancy which occurs in elderly or immunosuppressed patients, and is often associated with acquired immune deficiency syndrome (AIDS). KS generally arises on the skin of the extremities as multiple patches, plaques, or nodular lesions, but it can also involve mucosa and visceral organs [1].

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Abbreviations: AIDS, acquired immune deficiency syndrome; AIDS-KS, AIDS-associated Kaposi's sarcoma; bFGF, basic fibroblast growth factor; ECM, extracellular matrix; ERK, extracellular-signal regulated kinase; FAK, focal adhesion kinase; GAGs, glycosaminoglycans; GST-Tat, glutathione S-transferase-Tat; HIV-1, human immunodeficiency virus type 1; JNK, c-jun amino-terminal kinase; KS, Kaposi's sarcoma; MAPK, mitogen-activated protein kinase; PBS, phosphate-buffered saline; RGD, arginine-glycine-aspartic acid; SPMG, sulfated polymannuroguluronate; Tat, transactivator of transcription; VEGF, vascular endothelial growth factor; KDR, kinase insert domain-containing receptor.

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Characteristic histological features of KS include the proliferation of spindle-shaped cells (KS cells, KSC) of vascular origin considered to be the tumor cells of KS, and of normal endothelial cells forming blood vessels (angiogenesis), inflammatory cell infiltration, and edema [2]. KS is much more frequent and aggressive in the setting of human immunodeficiency virus type 1 (HIV-1) infection, suggesting that HIV itself or molecules produced during HIV infection might play important roles in KS development and progress [3].

Tat (the HIV-1 transactivator of transcription) is a small regulatory polypeptide of 86–102 amino acids released by HIV-1-infected cells, which recently has been pointed out as a key progression factor of KS due to its involvement in all the biological steps of angiogenesis and progression [4,5]. In addition to the transactivation of HIV-1 gene expression, Tat can modulate the expression of many cellular genes including those for cytokines, adhesion molecules, major histocompatibility complex class I proteins and oncogenes, as well as cellular functions such as cell survival, growth and angiogenesis [6]. In its extracellular form, Tat stimulates proliferation, adhesion, migration and invasion of KSC and normal endothelial cells activated by inflammatory cytokines, and promotes the tube formation in vitro and angiogenesis in vivo as well [7,8]. Therefore, the inhibition of Tat production or prevention of its activity could be a useful way to inhibit the development and progression of KS in AIDS patients [9,10].

Glycosaminoglycans (GAGs), such as heparin, have been critically identified as pathological chaperons in Tat-mediated biological response in targeted cells [11]. And heparan sulfate proteoglycans (HSPGs) have been shown to act as Tat receptors. Conversely, free heparin inhibits the uptake of intracellular Tat and affects the cell surface interaction mediated by Tat [12]. Growing evidences show that heparin and heparin analogs represent potent extracellular Tat antagonists of possible therapeutic value [13].

Sulfated polymannuroguluronate (SPMG), a new heparin-like sulfated polysaccharide extracted from brown alga, is rich in 1,4-linked β-D-mannuronate, with an average of 1.5 sulfates and 1.0 carboxyl groups per sugar residue and an average molecular weight of 10 kDa. SPMG has entered phase II clinical trial in China, making it the first marine sulfated polysaccharide with the potential of becoming an anti-AIDS drug. Our previous in vitro and in vivo studies demonstrated that SPMG inhibits HIV replication and may exert this effect by interfering with HIV entry into host T lymphocytes [14,15]. We further found that SPMG was able to interact with Tat via binding to its basic domain. Of particular note was the highly selective affinity of SPMG for Tat ($K_D = 8.69 \times 10^{-10}$ M), as compared with heparin ($K_D = 1.86 \times 10^{-9}$ M) but not other GAGs including heparan sulfate (HS), dermatan sulfate C, chondroitin sulfates A and hyaluronic acid. This preferentially allows SPMG to serve as a specific scaffold for physically blocking the Tat basic domain-mediated actions facilitated by cell surface heparin/HS, conferring Tat-involved pathological events. In fact, our more recent data have showed that SPMG blocks HIV-1-associated neuroinflammatory signaling by targeting the Tat protein in THP-1 cells [16].

The aim of the present study is to unravel the possible involvement of SPMG in Tat-mediated KS-associated angiogenesis, and to explore its possible mechanisms, in an additional hope to further our understanding of the unidentified roles of SPMG in AIDS-driven KS-associated angiogenesis.

2. Materials and methods

2.1. Reagents

SPMG, a new kind of sulfated polysaccharide extracted from brown algae, depolymerized by acidic hydrolysis, and then sulfatation ($M_W \sim 10{,}000$ Da), was provided by the Marine Drug and Food Institute (Ocean University of China, Qingdao, China). One hundred millimolar of SPMG was prepared by dissolving the powder in serum-free medium (SFM) and stored at 4 °C until use. Human bFGF/VEGF Quantikine immunoassay kits were obtained from R&D (R&D Systems, Minneapolis, MN). Flk-1/KDR (C-1158), integrin β1 (M-106), GST(Z-5), phosphorylated tyrosine (PY350), paxillin kinase (H-114) antibodies and protein A–agarose were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA); phospho-FAK (Tyr397) and FAK antibodies were obtained from Sigma Chemical Co. (St Louis, MO); phospho-ERK1/2 (Thr202/Tyr204), ERK1/2, phospho-JNK (Thr183/Tyr185) and the HRP-linked anti-rabbit IgG were all obtained from Cell Signaling Technology (Beverly, MA). JNK antibody was purchased from Abcam (Cambridge, UK). CD31 (bs-0195R) and IgG-FITC (bsF-0295G) antibodies were purchased from Beijing Biosynthesis Biotechnology (Beijing, China).

2.2. Cells culture

Human Kaposi's sarcoma cell line (SLK), kindly provided by Dr. Jay A. Levy and Dr. Sophie Leventon-Kriss (The NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH), was maintained in RPMI 1640 (Gibco Laboratories, Grand Island, New York, USA) supplemented with 10% foetal calf serum (FCS; Gibco), 2 mM L-glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a humidified incubator with 5% CO_2.

2.3. Purification of recombinant GST-Tat

GST-Tat was prepared as described previously [11]. Briefly, Plasmid pGEX-2T (Pharmacia, Uppsala, Sweden) and pGST-Tat were kindly provided by Profs. M. Giacca and M. Prestai (International Centre for Genetic Engineering and Biotechnology, Trieste, Italy). Recombinant GST-Tat was expressed in *E. coli* as fusion proteins, the cells were lysed, and the lysates were mixed with 1 ml of 50% (v/v) slurry of glutathione-cross-linked agarose beads (Sigma, St. Louis, MO). The fusion protein was allowed to bind to the beads at 4 °C on a rotating wheel for 1 h. The fusion proteins were freed from nucleic acid contamination using a high-salt wash (3 M NaCl), and the purified fusion protein was eluted with 100 mM Tris containing 2 mM DTT and 20 mM reduced glutathione (Sigma, St. Louis, MO). Protein purity and integrity were routinely checked by SDS-PAGE and Coomassie blue staining. The bioactivity of recombinant proteins was evaluated using surface plasmon resonance (SPR), flow cytometry, confocal microscopy and western blotting, and the proteins were stored at −70 °C until use [16].