Chemotherapy induces expression and release of heparanase leading to changes associated with an aggressive tumor phenotype

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Abstract

High heparanase expression is associated with enhanced tumor growth, angiogenesis, and metastasis in many types of cancer. However, the mechanisms driving high heparanase expression are not fully understood. In the present study, we discovered that drugs used in the treatment of myeloma upregulate heparanase expression. Frontline anti-myeloma drugs, bortezomib and carfilzomib activate the nuclear factor-kappa B (NF-κB) pathway to trigger heparanase expression in tumor cells. Blocking the NF-κB pathway diminished this chemotherapy-induced upregulation of heparanase expression. Activated NF-κB signaling was also found to drive high heparanase expression in drug resistant myeloma cell lines. In addition to enhancing heparanase expression, chemotherapy also caused release of heparanase by tumor cells into the conditioned medium. This soluble heparanase was taken up by macrophages and triggered an increase in TNF-α production. Heparanase is also taken up by tumor cells where it induced expression of HGF, VEGF and MMP-9 and activated ERK and Akt signaling pathways. These changes induced by heparanase are known to be associated with the promotion of an aggressive tumor phenotype. Importantly, the heparanase inhibitor Roneparstat diminished the uptake and the downstream effects of soluble heparanase. Together, these discoveries reveal a novel mechanism whereby chemotherapy upregulates heparanase, a known promoter of myeloma growth, and suggest that therapeutic targeting of heparanase during anti-cancer therapy may improve patient outcome.

Introduction

Heparanase, an endo-β-d-glucuronidase that trims the heparan sulfate chains of proteoglycans, is elevated in cancers and drives different cellular events to fuel tumor progression [1–5]. In the hematological malignancy multiple myeloma, data from our lab and others has demonstrated that heparanase drives angiogenesis, tumor growth, and metastasis [6–12], establishing it as a master regulator of aggressive tumor behavior. Soluble heparanase is present in the peripheral blood and plasma from bone marrow aspirates of myeloma patients, and high heparanase activity in the bone marrow strongly correlates with high microvessel density, consistent with the known role of heparanase in angiogenesis [9]. Despite clear evidence for high heparanase expression in cancers, molecular mechanisms that elevate heparanase expression or generate soluble heparanase in cancer patients are not fully understood.

Chemotherapy is the most widely used form of anti-cancer therapy [13]. In spite of several

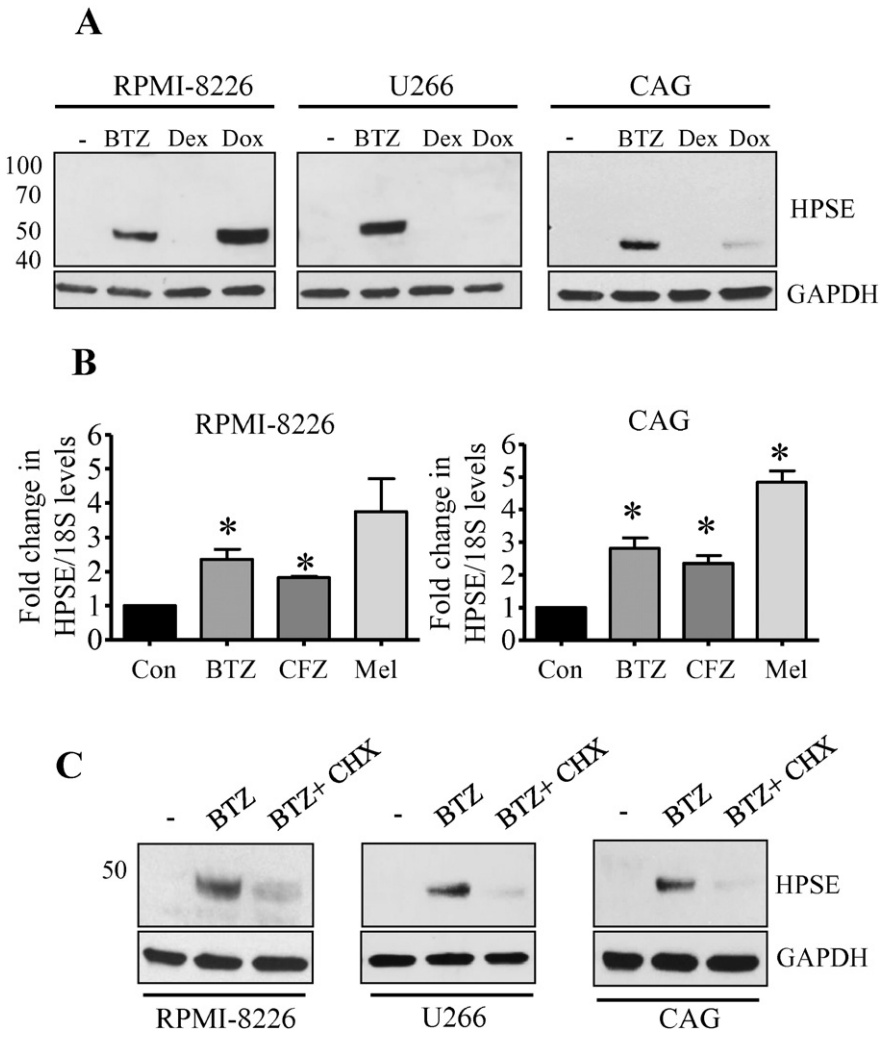

Fig. 1. Anti-myeloma therapy elevates heparanase expression. (A) Heparanase protein was assessed by western blotting of total cell extracts from different myeloma cell lines after 14 h treatment with BTZ (25 nM), Dex (10 μM) or doxorubicin (Dox, 5 μM). At these concentrations the drugs inhibited cell proliferation to a similar extent. Untreated cells served as controls. GAPDH is the loading control. (B) Heparanase transcript level in myeloma cells was assessed by real time PCR after 8 h treatment with BTZ (50 nM), CFZ (100 nM), Mel (20 μM). Transcript levels in vehicle treated cells served as control, $*p < 0.05$ versus vehicle treated cells. (C) HPSE protein levels as revealed by western blotting after treatment with BTZ (25 nM) alone for 14 h or with BTZ after 2 h pretreatment with cycloheximide (CHX, 30 μg/ml).

advances in the clinical use of chemotherapy, often it doesn't eradicate cancer. Especially in an aggressive cancer like multiple myeloma, even after several rounds of chemotherapy involving concurrent administration of different drugs, tumor cells persist and regrow leading to relapse. In addition, chemotherapy often has undesirable side effects and in some cases chemotherapy has been shown to actually lead to enhanced tumor growth and resistance [14,15]. We recently demonstrated that chemotherapy induces syndecan-1 shedding from tumor cells [16]. We also recently demonstrated that in myeloma patients, tumor cells that survive past chemotherapy had dramatically elevated levels of heparanase expression compared to cells harvested prior to therapy [17]. The above findings led us to speculate that treatment of myeloma cells with chemotherapeutic drugs may have the undesirable effect of stimulating the expression of heparanase. In the present work we find that anti-myeloma drugs activate the NF-κB pathway thereby elevating heparanase expression in tumor cells. In addition, chemotherapy leads to release of a soluble form of heparanase from tumor cells. Both macrophages and tumor cells endocytose the therapy-induced soluble heparanase in a heparan sulfate dependent manner. Uptake of therapy-induced soluble heparanase elevates TNF-α secretion from macrophages and stimulates the expression of pro-tumorigenic genes (HGF, MMP-9, and VEGF) and activates ERK