Understanding the Mechanism of Action of PG545 Heparanase Inhibitor in Lymphoma Models

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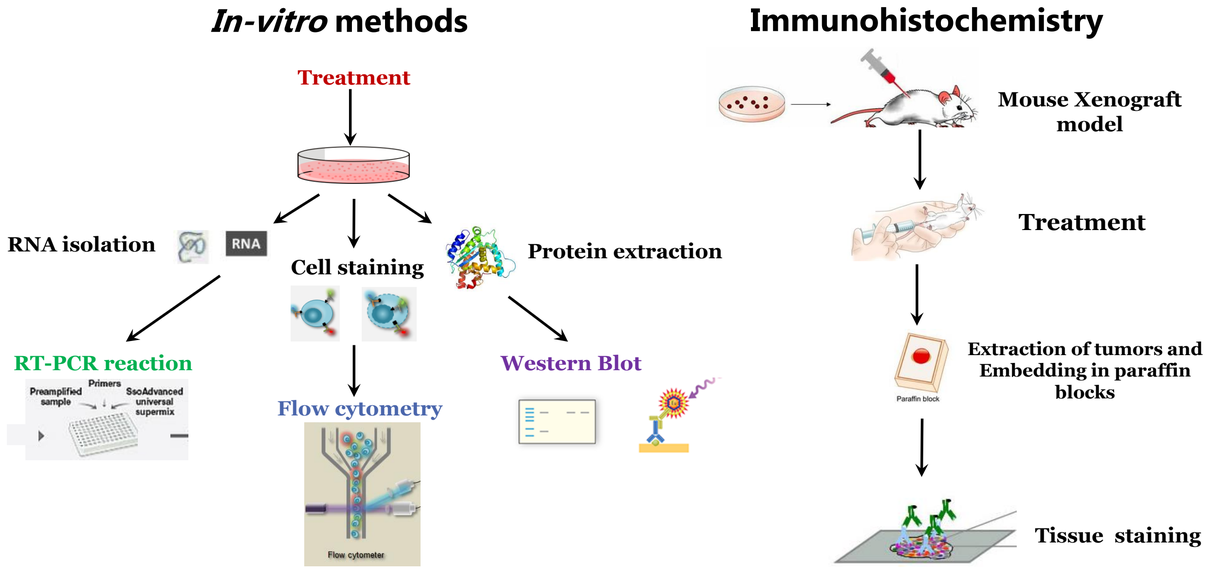
Introduction

Mutations in the DNA can lead to uncontrolled proliferation of cells resulting in development of cancer. The fifth most common cancer in North America is lymphoma (In the US - 80,500 cases per year) (1). Lymphoma is a type of blood cancer, and tumors can be formed in lymph nodes, spleen, bone marrow, blood, or other organs. Heparanase is an enzyme that cleaves heparan sulfate side chains on proteoglycans in the extracellular matrix (ECM). This cleavage results in remodeling of the ECM and release of ECM-bound molecules (i.e. growth factors) leading to increased cell proliferation and metastasis (2). In the recent years, many heparanase inhibitors were developed. One of them – the PG545 is currently being tested in a Phase I clinical trial (3). It was found that heparanase expression is increased in lymphoma biopsies and lymphoma cells are highly sensitive to PG545 treatment (4).

The aim of our study was to investigate the mechanism of action of PG545 in lymphomas

Methods



Results

Chemotherapy Increases Heparanase Expression in Lymphoma Cells

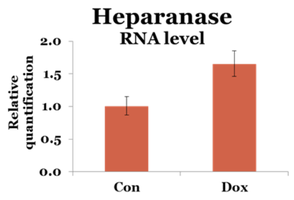


Figure 1. Ramos lymphoma cells were treated with 2.5µg/ml of doxorubicin for 24 hours. Real Time PCR (RT-PCR) was performed on cDNA with primers to heparanase.

Tumors Treated with PG545 Heparanase Inhibitor Are Highly Apoptotic

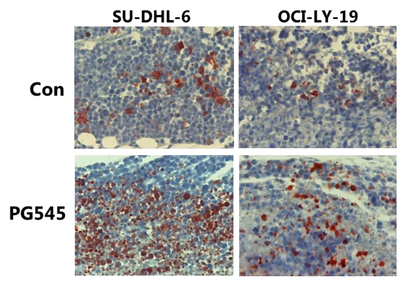


Figure 2. Mice were injected with SU-DHL-6 and OCI-LY-19 lymphoma cells and treated with PG545 heparanase inhibitor. After 4 weeks mice were sacrificed, and tumors were excised and embedded in paraffin blocks. Sections of the tumors were stained for cleaved caspase-3, a marker of apoptosis

PG545 Decreases Cell Proliferation Rate *in-vitro*

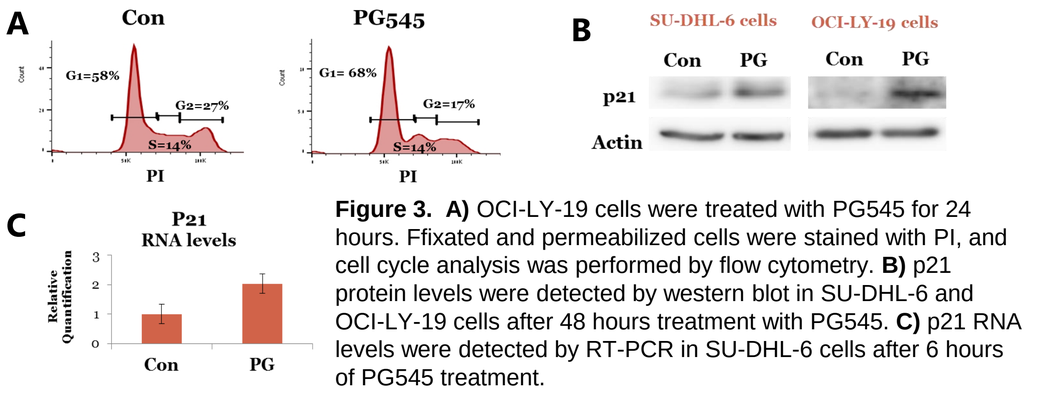


Figure 3. A) OCI-LY-19 cells were treated with PG545 for 24 hours. Ffixated and permeabilized cells were stained with PI, and cell cycle analysis was performed by flow cytometry. **B)** p21 protein levels were detected by western blot in SU-DHL-6 and OCI-LY-19 cells after 48 hours treatment with PG545. **C)** p21 RNA levels were detected by RT-PCR in SU-DHL-6 cells after 6 hours of PG545 treatment.

PG545 Increases Apoptosis in lymphoma cells in-vitro

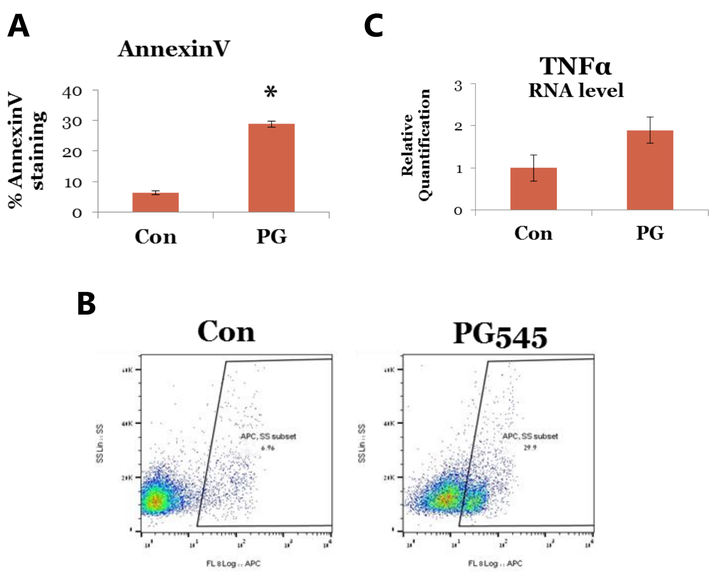


Figure 4. A&B) Flow cytometry analysis of AnnexinV staining in Daudi cells after 5 hours treatment with PG545. **C)** RT-PCR analysis of TNFα levels in SU-DHL-6 cells after 6 hours treatment with PG545.

PG545 activates ER stress and NFκB pathways

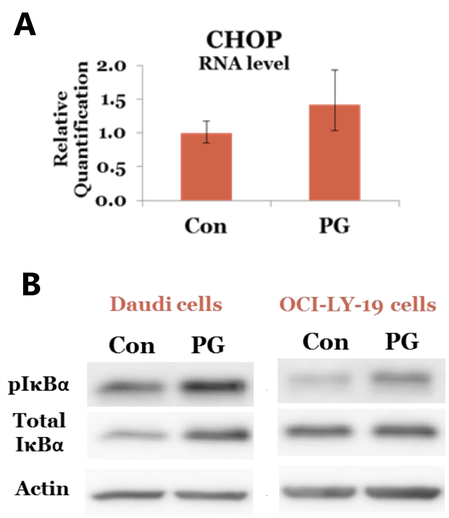


Figure 5. A) RT-PCR analysis of CHOP levels in Daudi cells after 6 hours treatment with PG545. **B)** Daudi and OCI-LY-19 cells were treated with PG545 for 1 hour and cell lysates were run on western blot for detection of phosphorilated form of IκBα.

Inhibition of ER Stress Reduces Cell Apoptosis and Prevents the Activation of NFκB

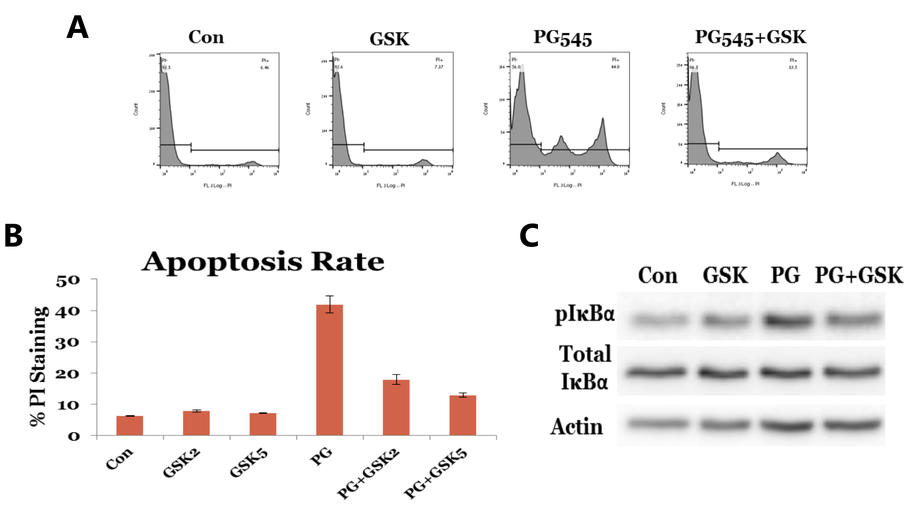
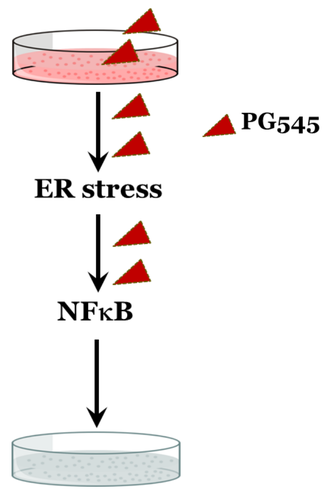


Figure 6. A&B) Raji cells were preincubated with either 2µM or 5µM of GSK, an inhibitor of ER stress pathway, for 2 hours prior to treatment with PG545. Cells were then analyzed by flow cytometry for PI staining. **C)** After 2 hours preincubation with 5µM of GSK, Daudi cells were treated with PG545 for 1 hour and cell lysates were run on western blot for detection of phosphorylated IκBα.

Discussion and Conclusions

Our results show that chemotherapy treatment increases heparanase expression in lymphoma cells. These results emphasize the need of combination of heparanase inhibitors together with conventional lymphoma treatments. In a mouse xenograft model of lymphoma, PG545 heparanase inhibitor decreased tumor size and induced apoptosis in the tumor tissue. Since no direct connection was found between heparanase inhibition and apoptosis we aimed to understand this effect of PG545 on lymphoma cells. We found that i*n-vitro* PG545 treatment of lymphoma cells results in decreased cell proliferation and increased cell apoptosis. In an attempt to further reveal the mechanism by which apoptosis in lymphoma cells occurs, we looked for activation of signaling pathways in the cells, and found out that treatment with PG545 resulted in activation of two pathways – the NFκB pathway, and the ER stress pathway. Using an inhibitor of ER stress pathway, we established that the mechanism of action of PG545 is activation of ER stress, followed by activation of NFκB pathway, leading to cell apoptosis. These findings will assist in directing PG545 to specific indication (i.e., lymphoma) as it enters advanced phase II and III clinical trials.



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Acknowledgments

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