Proteasome Inhibitors Induce Differentiation, Cell Cycle Arrest And Apoptosis In Osteosarcoma

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Disclosures:

Introduction: Osteosarcoma (OS) is the most common primary bone tumor of childhood and adolescence. Despite numerous treatment approaches, OS remained difficult to treat and often reoccurs after primary treatment. This may be related to heterogeneity of the cells in response to commonly used treatments like Doxorubicin. Our group works on intratumoral heterogeneity and previously we could fractionate two cell populations based on ability of the cells to activate exogenous human Oct4/GFP reporter. We found that tumorigenic potential is almost entirely restricted to the GFP+ population. Differential analysis of global gene expression and functional assays indicate that in addition to differences in tumorigenecity, the GFP+ and GFP- cell populations may also exhibit differences in their sensitivity to cytotoxic drugs. In OS156, we found up regulation of CCNB2, ATF4 previously demonstrated for multiple myeloma and lymphoma cell lines, suggesting that this pathway might be a good candidate to target for treatment. Proteasome Inhibitors (PI) are a group of FDA approved drugs targeting the proteasome degradation pathway. The aim of this study was to investigate the effects of the proteasome inhibitor Bortezomib on proliferation, tumorigenesis, Differentiation and metastatic ability of OS156 osteosarcoma cells.

Methods: Human osteosarcoma cell lines were generated from patient biopsies and transfected with phOct4/GFP plasmid as described before. Xenograft tumors were generated by sub-cutaneous transplantation of GFP+ cells into NOD-SCID mice and fractionated into GFP and GFP+ populations by FACS as described previously. Cultures of lung metastases were generated by the in vitro expansion of pulmonary nodules harvested from tumor-bearing mice in standard culture media supplemented with G418. GFP+, GFP- and Lung metastases were incubated with Doxorubicin and Bortezomib for 72 hours and Protein and mRNA were collected. IC50 was calculated using Graphpad Prism®. Cell cycle analysis and cell viability was determined by PI staining and flow cytometric analysis for DNA content and dye exclusion, respectively. mRNA was used in gene expression analyses via real-time PCR based targeted microarrays (RT² Profiler PCR Array Qiagen®) or real-time PCR using primers specific for human Osterix, RUNX2, and Osteocalcin; human beta actin was used as an internal control. Western blots were done using CCNB2, ATF4 and GAPDH antibodies. PicoSirius Staining was used to measure ECM collagen levels before and after treatment. Cell migration was assessed using a wound healing method with or without treatment by IC50 of drugs.

Results: Doxorubicin Doxorubicin is 187± 0.05nM for GFP- cells, 68.5±0.07nM for GFP+ cells and 95±0.12nM for Lung metastases. Bortezomib IC50 is 5.8±0.02nM for GFP- cells, 11.8±0.02nM for GFP+ cells and 7.7±0.01nM for Lung metastases. In doses from 1 to 5 nM, Bortezomib up-regulates the transcription of major regulators of osteoblast differentiation, such as, Osterix. PicoSirius staining results confirm this data. In 5 to 20 nM, Bortezomib induced cell cycle arrest at G2/M by Stabilization of Cyclin B2 protein as indicated by Western blot, while the transcriptional level of Cyclin B2 reduced via GADD45 activation. In higher doses, Bortezomib induced apoptosis and cell death. Proteasome inhibition reduced cell migration to a greater extent than Doxorubicin treatment and the difference was statistically significant (p=0.0001, 1way ANOVA test).

Discussion: Our data show that tumor cells are heterogenic in terms of response to treatment and some cells are almost three times more resistant to Doxorubicin. Although we have a variation in Bortezomib sensitivity among cell populations, Bortezomib shows a range of favorable dose dependent anti-cancer effects on osteosarcoma including differentiation, cell cycle arrest and apoptosis. Also the Doxorubicin resistant population is the most sensitive one to Bortezomib. Bortezomib can inhibit cell migration as a surrogate measure of metastasis in compared to Doxorubicin. Interestingly, the Bortezomib IC50 in osteosarcoma cell lines is similar to the IC50 previously demonstrated for multiple myeloma and lymphoma cell lines, suggesting that Bortezomib can be potentially used in osteosarcoma in the same doses as lymphoma and multiple myeloma. Doxorubicin clinical safety margin is limited because of life-threatening Doxorubicin induced cardiotoxicity on the other hand the reported side effects for Bortezomib is limited to GI upset and peripheral neuropathy . All these data show that PIs are good candidates for treatment of osteosarcoma but further in vivo assessment necessary to validate these findings.

Significance:

Acknowledgments:

References: