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Inhibition of PLK Induces Growth Arrest and Apoptosis in Tumorigenic OS Cells

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Background: Osteosarcoma (OS) is the most common non-hematologic primary tumor of bone in children and adults. Despite intensive efforts to improve both chemotherapeutics and surgical management, 40% of OS patients still succumb to this disease. High-dose cytotoxic chemotherapy and surgical resection have improved prognosis, with long-term survival for non-metastatic disease approaching 70%. However, most OS tumors are high grade and tend to rapidly develop pulmonary metastases. Toward development of treatments with greater therapeutic efficacy, our group has been investigating the nature of intratumoral heterogeneity in OS. We found that subpopulations of cells in culture from primary OS biopsies were selectively capable of activating an exogenous reporter construct comprised of the human Oct4 promoter linked to the coding sequence for Green Fluorescent Protein (GFP) (hOct4/GFP). The Oct4/GFP+ cells proved to be >100 times more tumorigenic than GFP- cells from within the same tumor when injected into NOD/SCID mice. In the present study, we used the Oct4/GFP reporter to identify drugs that can selectively inhibit oncogenes that promote cell cycle progression and increased proliferation in OS cells. We targeted Polo-Like Kinase 1 (PLK1), a serine/threonine-specific kinase that plays an important role in mitosis and the maintenance of genomic stability. While PLK inhibition has previously been shown to reduce tumor growth in OS cell lines, differential effects *within* OS tumor cell populations have not been investigated.

Objective: To Investigate PLK as a potential target for inhibition of tumor cell growth in OS tumor initiating cells.

Methods: Human OS cell lines were established from patient biopsies and transfected with the hOct4/GFP reporter. Subcutaneous tumors were generated in NOD/SCID mice by injection of Oct4/GFP+ cells isolated by Fluorescence Activating Cell Sorting (FACS). Those tumors were able to generate two populations, Oct4/GFP+ and GFP- cells that were subsequently separated by FACS and analyzed for mRNA expression using Affymetrix microarrays. To investigate the effects of PLK inhibition, FACS separated GFP+ and GFP- populations were cultured in monolayer and treated with the inhibitors BL2536 and GW843682X. Tumorigenic assays of proliferation, clonability and migration were evaluated. Cell cycle analysis, apoptosis and cell viability were determined by Flow Cytometry. To assess whether drug treatment affect tumor growth *in vivo*, GFP+ cells were pretreated with 10 nM of the inhibitors and injected in NOD/SCID mice. IC50s were calculated using GraphPad Prism.

Results: Microarray analysis revealed an upregulation of PLK1 of more than 1.7–fold in the Oct4/GFP+ cells relative to the GFP- population in all three patient derived OS cell lines, OS156, OS521 and OS28. We next investigated the effects of PLK inhibitors, BL2536 or GW843682X, to determine the sensitivity of drug inhibition between each OS cell line and among subpopulations within the same tumor. IC50s showed that OS20, OS156 and DCS0313 OS cell lines were more resistant to the inhibitors than OS521 and D2713. Strikingly, all the Oct4/GFP+ populations of the tumors were more sensitive to drug treatment compared to GFP- cells. Cell cycle analysis showed that treated cells with high doses (>0.5 μM) of either of the inhibitors were mainly arrested in the G2/M phase whereas small doses (<10 nM) induced G1 arrest, suggesting increased senescent cell populations. Immunofluorescence and western blotting analysis confirmed that the administration of BL2536 or GW843682X led to significant decrease of PLK1. Within tumors, proliferation, clonability and migration were reduced in the GFP+ cell population relative to GFP- cells at doses of 10 nM. Consistent with these *in vitro* findings, tumor cell growth of Oct4/GFP+ pretreated cells injected in NOD/SCID mice was inhibited at a low dose of 10 nM. These results confirm that PLK inhibition can have a potential therapeutic usage in OS *and* that selective dosage can affect the cell fate in OS tumors.

Conclusions: These findings offer evidence of the differential sensitivity of the Oct4/GFP+ and GFP- populations from the same tumor, confirming that the intratumoral heterogeneity is a major obstacle for the treatment of OS, as the presence of minor populations that are insensitive to therapy can lead to disease relapse.