BI2536 Mediated Polo-Like Kinase 1 Inhibition Reduces Osteosarcoma Cell Proliferation, Induces Apoptosis, and Decreases Tumor Growth in Mice Xenografts

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INTRODUCTION
Osteosarcoma is the most common primary bone malignancy in children and young adolescents. Treatment options for osteosarcoma patients with localized disease may include surgery combined with high dose pre- and post-operative chemotherapy. Unfortunately, many patients do eventually relapse. Upon recurrence of the tumor locally or distantly, they have limited treatment options that are usually unsuccessful. Our prior studies on screening lentiviral shRNA libraries have already identified the serine/threonine-specific Polo-like kinase 1 (PLK1) is a kinase which plays an important role in mitosis and the maintenance of genomic stability. PLK1 also has been reported to be highly expressed in a variety of cancer cell lines. Recently, BI2536, a novel and specific inhibitor of PLK1 showed low micromolar (IC50 of 0.83nM) anti-proliferative activity towards cancer cells. BI2536 binds with PLK1, resulting in mitotic arrest and apoptosis in susceptible tumor cells. In this study, we show that inhibition of PLK1 by BI2536 blocks cell proliferation and tumor growth, induces mitotic arrest and finally leads to apoptosis of osteosarcoma cell lines.

METHODS:

Cell cultures
The human osteosarcoma cell lines, U-2OS and KHOS were cultured in RPMI 1640 supplemented with 10% FBS, 100-units/ml penicillin and 100μg/ml streptomycin at 37°C in 5% CO2 -95% air atmosphere.

3 Dimensional (3D) cultures
Each well of glass chamber slides was covered evenly by 2mm Growth Factor Reduced Matrigel formed basement membrane. A single cell suspension was seeded on the solidified basement membrane. Cells were grown in an Assay Medium plus 5ng/ml epidermal growth factor and 2% Matrigel.

Flow cytometry
KHOS and U-2OS were synchronized by culturing in RPMI 1640 without FBS for 18 hours. BI 2536 was added to each flask and, 72 hours later, cells were collected and resuspended in cold PBS and fixed by adding absolute ethanol. Samples were sent to Flow Cytometry Core Facility Center for Regenerative Medicine for analysis.

Western blotting
Protein lysates were harvested from osteosarcoma cells and xenograft tumor tissues by using 1×RIPA Lysis Buffer (Upstate Biotechnology) and the concentrations were determined using Protein Assay Reagent. Western blotting analysis was performed as previously described.

Immunofluorescence
Osteosarcoma cells were seeded in each well of eight-well glass chamber slides. After 24 hours of culturing, BI 2536 was added with different concentrations to each well and incubated for 72 hours. Immunofluorescence was performed as previously described.

Apoptosis assay
Quantification of apoptosis was evaluated by using the Apo-ONE Homogeneous Caspase-3/7 Assay kit from Promega according to manufacturer’s instructions (Madison, WI).

Animal studies
The protocol for animal use in this project has been approved by the Massachusetts General Hospital Subcommittee on Research Animal Care (SRAC) under the protocol number 2009N000229. KHOS cells were inoculated subcutaneously with matrigel into the right flank of 4-week-old Crl:SHO-PrkdcScid/Hrhr nude female mice. Seven days after injection, 200μl 30mg/kg BI 2536, which was dissolved in DMSO and diluted with 0.9% NaCl and 0.1N HCl, was injected once i.v. into the tail vein. The health of the mice and evidence of tumor growth were daily examined and tumor volume was measured once every three days with a digital caliper. Tumor volume was calculated as (W3 x L) / 2 (W as width and L as length).

RESULTS:

BI2536 mediated PLK1 inhibition reduces growth and viability of osteosarcoma cell lines
The minimal effective concentrations (MEC) of BI 2536 on KHOS and U-2OS were established as 20nM and 40nM. Treatment of cells with BI2536 decreased cell proliferation in both osteosarcoma cell lines, especially at or greater than the minimal effective concentrations. BI2536 inhibits the PLK1 expression in dose- and time-dependent manner
In both osteosarcoma cell lines the expression of PLK1 was inhibited by BI 2536 in a dose- and time-dependent manner.

BI2536 induces mitotic arrest and apoptosis in osteosarcoma cell lines
Majority of the cells treated with BI 2536 for 24 and 48 hours arrested in G2/M phase. The addition of BI2536 in MEC resulted in greater levels of apoptosis in both KHOS and U-2OS. Significant decrease of ant apoptotic and increase of proapoptotic protein expression levels were also observed in dose- and time-dependent manners.

BI2536 inhibits osteosarcoma cell proliferation in 3D culture and a mouse model
The growth of osteosarcoma cells was obviously inhibited in 3D culture with approximately the same concentrations of BI2536 as the 2D’s. For animal studies, BI2536 or the vehicle control was injected once i.v. into the tail vein of Crl:SHO-PrkdcScid/Hrhr nude female mice with 150 mm3 of KHOS xenograft tumors. BI2536 treated mice had significantly smaller tumors compared with control mice (P<0.001). The expression of PLK1 at protein level was much lower in tumor tissues of the mice treated with BI2536 than with vehicle control.

DISCUSSION:
Recently, PLK1 has been identified as a crucial kinase for human osteosarcoma cell growth and survival. Targeting PLK1 with siRNA or shRNA resulted in tumor cell growth inhibition and induced apoptosis. Similar results have been reported in rhabdomyosarcoma. In this study, we further analyzed the effects of PLK1 depletion with the novel inhibitor BI2536. We observed antitumor activities of BI 2536 both in vitro and in animal tumor models of osteosarcoma. Firstly, we observed that BI 2536 could obviously inhibit cell growth and viability in 3D and 2D culture models of KHOS and U-2OS cells. It became evident that expression of PLK1 could be inhibited by BI 2536 in dose- and time-dependent manners. Secondly, Inhibition of PLK1 by BI 2536 induced mitotic arrest and cell apoptosis in KHOS and U-2OS cells. Finally, in mice xenograft models BI 2536 showed the ability to block the growth of human osteosarcoma.

We validated the inhibition effects of BI 2536 on PLK1 expression and established the minimal effective concentration in osteosarcoma cell lines. Concentrations as low as 20 to 40nM of BI 2536 were sufficient to inhibit PLK1 expression and cause mitotic arrest, growth pattern changes and eventually proceed to apoptosis and cell death. These effects, which were hallmarks of PLK1 inhibition, suggest that the inhibitor was exerting its effects in osteosarcoma cells by blocking PLK1. Importantly, the expression of PLK1 was significantly higher in sarcoma cell lines and osteosarcoma tissues than in human osteoblast cells. Osteoblast cells proved to be less sensitive to PLK1 inhibition by siRNA or shRNA, implying that PLK1 has a unique function in osteoblast cells. According to xenograft mouse models, BI 2536 significantly decreased the tumor volume and its PLK1 expression level, which also suggests the possibility of directing against PLK1 as a therapeutic target in the treatment of osteosarcoma.

In conclusion, our findings suggest that PLK1 can be exploited as a potential target in the treatment of osteosarcoma, and its inhibitor BI 2536 could be examined in clinical trials of human cancer therapy in the future.