Cytostatic Activity of Adenosine Triphosphate-Competitive Kinase Inhibitors in \textit{BRAF} Mutant Thyroid Carcinoma Cells

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\textbf{ABSTRACT}

\textbf{Context:} The V600E mutation accounts for the vast majority of thyroid carcinoma-associated BRAF mutations.

\textbf{Objective:} The aim was to study the effects of the two BRAF V600E ATP-competitive kinase inhibitors, PLX4032 and PLX4720, in thyroid carcinoma cell lines.

\textbf{Experimental Design:} We examined the activity of PLX4032 and PLX4720 in thyroid carcinoma cell lines harboring BRAF V600E (8505C, BCPAP, SW1736, BHT101), NRAS Q61R (HTH7), KRAS G12R (CAL62), HRAS G13R (C643), or RET/PTC1 (TPC-1) oncogenes. Normal thyrocytes (PC Cl 3) were used as control.

\textbf{Results:} Both compounds inhibited the proliferation of BRAF mutant cell lines, but not normal thyrocytes, with a half maximal effective concentration ($EC_{50}$) ranging from 78-113 nM for PLX4720 and from 29-97 nM for PLX4032. Doses equal to or higher than 500 nM were required to achieve a similar effect in BRAF wild-type cancer cells. Phosphorylation of ERK 1/2 and MAPK kinase (MEK) 1/2 decreased upon PLX4032 and PLX4720 treatment in BRAF mutant thyroid carcinoma cells but not in normal thyroid cells or in cell lines harboring mutations of RAS or RET/PTC1 rearrangements. PLX4032 and PLX4720 treatment induced a G1 block and altered expression of genes involved in the control of G1-S cell-cycle transition. 8505C cell tumor xenografts were smaller in nude mice treated with PLX4032 than in control mice. This inhibition was associated with reduction of phospho-ERK and phospho-MEK levels.

\textbf{Conclusions:} This study provides additional evidence of the promising nature of mutant BRAF as a molecular target for thyroid carcinoma cells.

The Glucocorticoid-Induced Leucine Zipper Gene (GILZ) Expression Decreases after Successful Treatment of Patients with Endogenous Cushing’s Syndrome and May Play a Role in Glucocorticoid-Induced Osteoporosis

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\textbf{ABSTRACT}

\textbf{Context:} Glucocorticoid-induced bone loss is a serious complication in patients with endogenous Cushing’s syndrome. However, the mechanism(s) by which excess glucocorticoids influence bone metabolism is not completely understood.

\textbf{Objective:} The aim of the study was to investigate the functional role of glucocorticoid-induced leucine zipper (GILZ) in bone remodeling with special focus on glucocorticoid-induced osteoporosis (GIO).

\textbf{Patients:} Nine patients with endogenous Cushing’s syndrome participated in the study.

\textbf{Research Design and Methods:} We analyzed bone biopsies from Cushing’s patients before and after treatment to screen for expression candidate genes with putative roles in GIO. Microarray analysis combined with real-time RT-PCR revealed that the gene encoding GILZ ranked among the topmost regulated genes and was selected for functional characterization \textit{in vitro}.

\textbf{Results:} GILZ mRNA was expressed by human fetal osteoblasts (hFOB), human mesenchymal stem cells (hMSC), osteoblasts differentiated from hMSC, and osteoclasts. GILZ was increased by dexamethasone in a time- and dose-dependent manner in hFOB. Inhibition of GILZ in hFOB cells by small interfering RNA decreased typical osteoblast-related genes, suggesting a physiological role in promoting osteoblast maturation. Our data further support a functional role for GILZ in normal bone remodeling by modulating expression of TNF-(ligand) receptor superfamily/osteoprotegerin in favor of increased ratio in hFOB. Finally, osteoclasts exposed to conditioned media from GILZ-silenced hFOB indicated effects on osteoclast activity.

\textbf{Conclusion:} Taken together, these results implicate the transcription factor GILZ in the pathophysiology of GIO by regulating osteoblast maturation and bone turnover.