NSC-87877 Inhibits Cdc25A and Cdc25B

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The protein phosphorylation level inside cells is controlled by the balance between activities of protein kinases and phosphatases. Protein phosphorylations are involved in the regulation of diverse processes, such as cellular metabolism, proliferation, differentiation, growth, migration, and invasion of normal and malignant cells.1,2 The protein tyrosine phosphatase (PTP) superfamily comprises over 100 proteins. Based on the amino acid sequences of their catalytic sites, PTPs are divided into the classical dual-specificity protein tyrosine phosphatases (DUSPs), tyrosine-specific low molecular weight phosphatases, the Cdc25 family, and eyes absent (EyA) protein.3

Recent studies indicate that modulation of PTP enzymatic activity could have a role in regulating a large spectrum of cellular functions and disease susceptibility.4 Thus, chemical compounds that regulate the activity of PTPs have been extensively screened to be used as potent therapeutic reagents.

Cell division cycle 25 (Cdc25) phosphatases are members of PTP subfamily, which dephosphorylate a phosphorylated-threonine or -tyrosine residue of the same protein substrate and are essential regulators of the cell cycle. The three human Cdc25 isoforms (A, B, and C) have been reported to control different phases of the cell cycle. The overexpression of Cdc25A and Cdc25B has been reported in numerous human tumors and linked to oncogenic transformation and human cancers in a variety of ways.5,6,7 Cdc25 phosphatases are inactivated by various mechanisms, including degradation and inhibition.9

8-Hydroxy-7-(6-sulfonaphthalen-2-yl)diazemyl-quinoline-5-sulfonic acid (NSC-87877) was originally identified as a potent inhibitor of Src homology region 2 (SH2) domain-containing phosphatase 1 (SHP-1) and SHP-2 PTPs (Fig. 1).10 They contain two SH2 N-terminal domains and a C-terminal protein tyrosine phosphatase domain. SHP-2 is involved in the function of several growth factors and metabolic pathways and acts in disease pathways such as leukemia, diabetes, neurodegeneration, and cancer.11,12 Since there might be more phosphatases that can be targets of NSC-87877, we screened 15 PTPs by in vitro phosphatase assays to identify PTPs that are inhibited by NSC-87877. An inhibition curve was plotted for each PTP and the inhibitory concentration 50 (IC50) values were calculated. As shown in Table 1, Cdc25A and Cdc25B were inhibited with IC50 value of 0.6 ± 0.004 µM and 1.29 ± 0.35 µM, respectively. The IC50 values for other PTPs were higher than 50 µM, suggesting that NSC-87877 has low inhibitory effect on these phosphatases.

We examined the inhibitory effects of NSC-87877 on Cdc25A and Cdc25B. When both phosphatases were treated with various concentrations of NSC-87877, phosphatase activities were decreased by the inhibitor in a dose-dependent manner (Fig. 2A and B). Molecular modeling and site-directed mutagenesis studies suggested that NSC-87877 binds to the catalytic cleft of SHP-2.10 Kinetic studies with NSC-87877 and Cdc25s revealed competitive inhibitions as shown by the Lineweaver-Burk plot, suggesting that NSC-87877 binds to the catalytic cleft of Cdc25A and Cdc25B in the same manner as SHP-2 (Fig. 3A and B). The Ki values of both Cdc25A and Cdc25B for OMFP were 66 µM and 13 µM, respectively. The Ki values for Cdc25A and Cdc25B were 0.59 µM and 1.28 µM, respectively.

In the present study, we screened PTPs to search for targets of NSC-87877 by performing in vitro PTP activity assays. We

Table 1. Inhibition of PTPs by NSC-87877. Inhibitory IC50 of enzyme activity was determined for the various PTPs including PTPRO, PTPRE, PTPN7, Cdc25A and Cdc25B. Data are presented as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Protein tyrosine phosphatase</th>
<th>IC50 (µM)</th>
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<tbody>
<tr>
<td>PTPRO</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>PTPRE</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>PTPN7</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>cdc25A</td>
<td>0.60 ± 0.004 (n=3)</td>
</tr>
<tr>
<td>cdc25B</td>
<td>1.29 ± 0.35 (n=3)</td>
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found that NSC-87877 inhibits activity of Cdc25A and Cdc25B in a dose-dependent manner and is a potent competitive inhibitor of Cdc25A and Cdc25B. The overexpression of Cdc25A and Cdc25B has been reported in various cancers, such as breast cancer, prostate cancer, pancreatic ductal adenocarcinoma, non-Hodgkin’s lymphoma, and non-small-cell lung cancer. It has recently been suggested that Cdc25 inhibitors were able to reduce the growth of pancreatic cell lines that expressed high levels of Cdc25B. For these reasons, we suggest that inhibition of the Cdc25A and Cdc25B using NSC-87877 may be a novel therapeutic approach for the treatment of cancer.

Experimental Section

Recombinant PTP proteins. Plasmids for expression of 6x His-tagged proteins of human PTPRO, PRPRE and PTPN7 were constructed in pET-28a (+) and transformed into BL21(DE3)-RIL E.coli. Plasmids for expression of glutathione S-transferase (GST)-PTP fusion proteins of human Cdc25A and Cdc25B were constructed in pGEX and transformed into BL21(DE3)-RIL E.coli. Expression of recombinant protein was induced with 1mM isopropyl-β-D-thiogalactopyranoside at 37 °C or 30 °C for 8 ~ 10 h. Cells were harvested and then lysed by sonication in 50 mM Tris-HCl (pH 8), 300 mM NaCl, 1% Tergitol-type NP-40, 1 mM phenylmethylsulphonyl fluoride (PMSF). The lysate was clarified at 4000 rpm for 30 min at 4 °C. The supernatant for His-tagged protein was used to reduce the growth of pancreatic cell lines that expressed high levels of Cdc25B. For these reasons, we suggest that inhibition of the Cdc25A and Cdc25B using NSC-87877 may be a novel therapeutic approach for the treatment of cancer.

In vitro PTP activity assay and kinetic analysis. The activity of phosphatases was measured using the substrate 3-O-methylfluorescein phosphate (OMFP; Sigma) at concentrations varying with the K_m of each enzyme in a 96-well microtiter plate assay based on methods described previously. The NSC-87877 (Calbiochem) and OMFP were solubilized in H_2O and DMSO, respectively. All reactions were performed at a final concentration of 1% DMSO. The final incubation mixture (150 µL) was optimized for enzyme activity and comprised of 30 mM Tris-HCl (pH 7), 75 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.4 mM dithiothreitol (DTT), 0.33% bovine serum albumin (BSA) and 100 nM of PTPs. Reactions were initiated by the addition of OMFP and the incubation time was 30 min at 37 °C. Fluorescence emission from the reaction product was measured with
a multi-well plate reader (GENios Pro; excitation filter, 485 nm; emission filter, 535 nm). The reaction was linear over the time period of the experiment and was directly proportional to both enzyme and substrate concentration. Half-maximal inhibition constant \( (IC_{50}) \) was defined as the concentration of an inhibitor that caused a 50% decrease in the PTP activity. Half-maximal inhibition constants and best curve fit for Lineweaver-Burk plots were determined by using the curve fitting program Prism 3.0 (GraphPad Software). All experiments were performed in triplicate and were repeated at least three times.

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