Small Molecule Modulator of p53 Signaling Pathway: Application for Radiosensitizing or Radioprotection Agents

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1. Introduction

The tumor suppressor p53 is key molecule to protect the cell against genotoxic stress and the most frequently mutated protein in cancer cells [1, 2]. Lack of functional p53 is accompanied by high rate of genomic instability, rapid tumor progression, resistance to anticancer therapy, and increased angiogenesis [3-6]. In response to DNA damage, p53 protein rapidly accumulated through attenuated proteolysis and is also activated as transcription factor [7-10]. Activated p53 up-regulates target genes involved in cell cycle arrest and/or apoptosis and then lead to suppression of malignant transformation and the maintenance of genomic integrity.

Chemical genetics is a new technology to uncover the signaling networks that regulated biological phenotype using exogenous reagents such as small molecules. Analogous to classical forward genetic screens in model organism, this approach makes use of high throughput, phenotypic assay to identify small molecules that disrupt gene product function in a way that alters a phenotype of interest. Recently, interesting small molecules were identified from cell based high throughput screening and its target protein or mechanism of action were identified by various methods including affinity chromatography, protein array profiling, mRNA or phage display, transcription profiling, and RNA interference.

2. Methods

2.1 Cell Culture

HepG2, RKO, RKO-E6, IMR90 and WI-38 were maintained in DMEM supplemented with 10% FBS, 120 g/ml penicillin, and 200 g/ml streptomycin. SW480 was cultured in Leibovitz's L-15 supplemented with 10% FBS, 120 g/ml penicillin and 200 g/ml streptomycin. HCT-116 and HT-29 were maintained in Macoy's 5a supplemented with 10%FBS, 120 g/ml pen penicillin, and 200 g/ml streptomycin. HCT-15 was cultured in RPMI 1640 supplemented with 10%FBS, 120 g/ml pen penicillin, and 200 g/ml streptomycin

2.2 High Throughput Screening system

The RKO reporter cell line was established by selecting RKO cells transfected with the plasmid expressing neomycin resistance gene using media containing G418 (700 μg/ml). The cells were inoculated into 384 well plates at 5000 cells/well using automatic dispenser (Multidrop 384, Labsystems). After 12-16h, Signal activator was added and then chemicals were treated to wells at a final concentration of 10 μM of test compounds. Luciferase assays were performed using Dual luciferase assay kit .

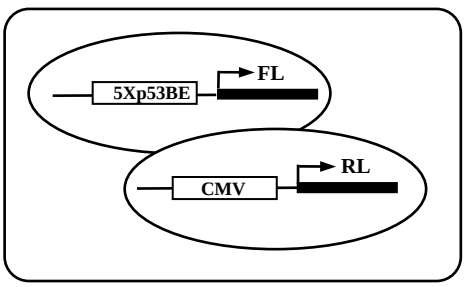
2.3 Western Blot

Proteins were separated 4-12% gradient SDS-PAGE (Invitrogen) and transferred to nitrocellulose membrane (Bio-Rad). The membranes were blocked with 5% nonfat milk and probed with anti-p53 and anti-Mdm2 antibodies (Oncogene). Membranes were then incubated with horseradish peroxidase conjugated anti-mouse IgG (Amersham Biosciences) and visualized using the ECL system (Amersham Biosciences).

3. Results

3.1 Development of HTS targeting p53 pathway

To establish reporter cell lines, we transfected reporter construct containing Firefly luciferase gene to RKO cells. Next, we examined whether signal activator activated reporter gene, reporter cells were incubated with signal activator. Compared to control (without signal activator), luciferase activity was dramatically increased by addition of signal activator. We also established a control cell line, which contains Ranilla luciferase genes under the control of CMV promoter. We used these cell lines for high throughput screening to identify the chemical, which inhibited the cellular signaling. RKO reporter and control cells were mixed and cultured with signal activator for 18 hr in the presence of chemicals at a concentration of 10 uM in 384 well plate formats. Under this condition, a dominant lead compound from this screen was identified.



RKO cell

Figure 1. A schematic diagram of screening system

3.2 The effect of small molecule on p53 protein

To determine whether small molecule identified in the p53 screen inhibits the stabilization of endogenous p53 protein, RKO cells were treated with small molecule and p53 protein levels were examined by western blot. We found that p53 protein level was dramatically reduced by various concentrations of small molecule in a dose-dependent manner. We also observed the small molecule-mediated reduction of p53 protein levels in other cells including HCT116. Thus, consistent with screening results in our developed assays for p53 activity, small molecule clearly induced p53 protein levels in the cells.

4. Conclusions

In this study, we identified small molecules from cell based high throughput screening. Small molecules modulated p53-dependent transcription, intracellular p53 level and expression of p53 target gene. These small molecules can be developed into radiosensitizing or radioprotection agents.

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