MDR1 Gene Overexpression Confers Resistance to C-MET Inhibitor in Multidrug Resistant Cancer Cell Line

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Abstract

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MDR1/MDR2/MDR3 treatment. Each cell line was treated with PHA665752 (Sigma-Aldrich) at 0.5 µM and/or verapamil (Sigma-Aldrich) at indicated concentration for 48 h. Cell viability of both cell line was assessed by MTT assay. Protein expression level of both cell line were assessed by western blot.

Western blot. Total protein extracts (50 µg) from each cell line were analyzed by Western blotting with antibodies of MDR1 (Santa Cruz Biotechnology Inc., Santa Cruz, CA), actin- and PARP (Chemicon, Temecula, CA). A gel loading buffer was used to adjust for loading differences, the optical density of each protein was normalized to that of the housekeeping protein.

Quantitative real-time PCR (qPCR). Total RNA (2 µg) was prepared from each cell line and reverse transcribed to cDNA. The cDNA (0.5 µl) was performed with qPCR Master Mix kit (Ampliqon, A)}

Materials and Methods

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Capsee 3′/5′ activity assay. Approximately 1×10⁶ MDR5-DX5-C5 and DX5-C5 cells on 6-well plates were treated with indicated dosage of PHA665752 or transfected with the indicated concentration of pc-Met shRNA plasmid for 48 h. At the end of the incubation, 100 µl Capsee-Glo 3′/7′ Reagent (Promega) was added to the treated cells in each well. After 30 mins of incubation at room temperature, the relative luminescence unit (RLU) was measured by GLOMAX 2020 Luminoimeter (Promega) and as an indication of Capsee 3′/7′ apopotic activity.

shRNA transfection. The pc-Met shRNA transfected cells were plated at a density of 100 cells/100 cm² dish and grown for 7 days until the discrete colonies could be visualized. The colonies were stained with 0.5% crystal violet and counted. In addition, cell number estimated by dissolving the crystal violet in 70% ethanol (1 µl/ml) and then optical density (OD) values measured at 562 nm. In each group, the levels of colony formation were normalized against control pcDNA6 plasmid transfected MDR5-DX5-C5 cells.

Figure 1. Characterization of MDR1 function of Ds-C5 and MCT-7/ADR cells. (A) MDR protein expression level in MDS/Dx5 and MCF-7/ADR cells. (B) Actin- and PARP (Chemicon, Temecula, CA) of the indicated concentration of MDR1 gene knockdown. (C) MCT-7 (filled histogram) and MCT-7/ADR cells (open histogram).

Figure 2. The hepatocyte growth factor (HGF)/MET signal pathway was involved in resistant cell proliferation.

Figure 3. The MDR cells were resistant to PHA665752 toxicity. The Ds-C5 cells (b) and MCT-7/ADR (D) were incubated with the indicated concentrations of PHA665752 for 48 h. At the end of treatment, cells were harvested for measurement of viability using the MTT assay. *P < 0.05, **P < 0.01, ***P < 0.001, the viability are expressed as the mean ± S.D. n = 3.

Figure 4. The MDR cells were resistance to PHA665752 induced-apoptosis. The MDR cells were treated with the indicated concentrations of PHA665752 for 48 h. At the end of treatment, cells were harvested for measurement of Annexin-V stained cells. (A) MES-SA (Sigma-Aldrich) at 0.5 µM and/or verapamil (Sigma-Aldrich) at indicated concentration for 48 h. Cell viability of both cell line was assessed by MTT assay. Protein expression level of both cell line were assessed by western blot.

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Figure 5. Resistance of MDR cells to PHA665752 is reversed by the verapamil. The Ds-C5 cells (A) and MCT-7/ADR (B) were incubated with the indicated concentrations of PHA665752 (A) and/or 2 µM verapamil (Vera) for 48 h. At the end of treatment, cells were harvested for measurement of viability using the MTT assay. *P < 0.05, **P < 0.01, ***P < 0.001, the viability are expressed as the mean ± S.D. n = 3.

Figure 6. Reduction of cell viability in transient c-Met shRNA knockdown MDR cells. The cells were transfected with the indicated concentrations of pc-Met shRNA plasmid for 48 h. At the end of treatment, cells were harvested for measurement of viability using the MTT assay. *P < 0.05, **P < 0.01, ***P < 0.001, the viability are expressed as the mean ± S.D. n = 3.

Summary

1. Compared to parental MDR cells, the Ds-C5 cells with high P-gp were more resistant to c-Met inhibitor PHA665752. The resistance was reversed by co-treatment of MDR inhibitor verapamil and PHA665752. The viability was decreased in the c-Met shRNA knockdown Ds-C5 cell line in a dose dependent manner in vitro and in vivo. We demonstrated that existence of P-cytoplasmic in Ds-C5 cell line would attenuate c-Met inhibitor PHA665752 induced death.