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Abrogation of HSP27-Mediated Chemo- and Radio-Resistance by HSP27 Binding PKCδ V5 Mimetic Heptapeptide

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1. Introduction

Small heat shock protein (sHSP) has been suggested to protect cells from apoptotic cell death triggered by hyperthermia, ionizing radiation, oxidative stress, Fas ligand, and cytotoxic drugs [1,2]. Furthermore, HSP25 was found to directly bind to the V5 region of PKCδ and inhibit the PKCδ activity, resulting in a dual form of HSP25-mediated cytoprotection and blocking apoptosis. Overexpression of HSP27 may predict poor response to chemotherapy and radio-therapy, and hence poor prognosis in general [3].

In the present study, we demonstrated that amino acid residues 668 and 674 of V5 region of PKCδ was necessary for HSP27 binding[4]. Based on this information, we prepared heptapeptide containing the region required for HSP27 binding, and demonstrated that heptapeptide had chemo- and radio-sensitizing properties and neutralized endogenous HSP27 in human lung cancer cells which frequently overexpressed HSP27.

2. Methods

Cell Culture.

Human non-small cell lung cancer cell lines, H460 and H1299 cells, were grown in RPMI 1640 supplemented with 10% FBS, glutamine, HEPES, and antibiotics at 37 °C in a 5% CO_2 humidified incubator. L929 (Murine fibroblast)cell was cultured in Dulbecco's minimal essential medium (DMEM) (GIBCO, Gaithersburg, MD) supplemented with 10% FBS, glutamine, HEPES, and antibiotics at 37 °C in a 5% CO_2 humidified incubator.

Tumor xenografts in nude mice

A single cell suspension ($3x10^6$ cells) with a viability of 95% was subcutaneously injected into the hindleg of 5 week old BALB/c athymic nude mice (Charles River Japan): Volume injected was 50μl per mouse to avoid leakage, and different site was used for each injection. When tumor reached a minimal volume of 200 mm^3, radiation (12 Gy) with local regional application were started. Each group had 3 mice, and tumor volumes were determined according to the formula $(Lxl^2)/2$ by measuring tumor length (L) and width (l) with a caliper.

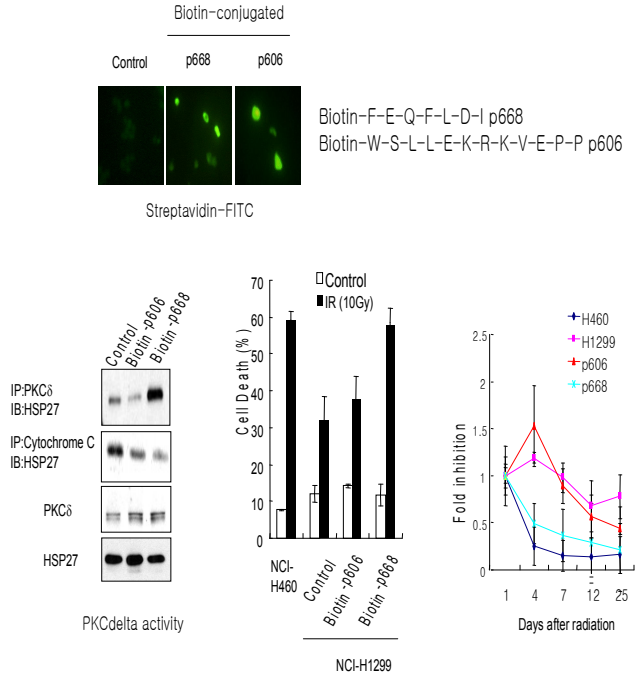
Polyacrylamide gel electrophoresis and Western blot.

For polyacrylamide gel electrophoresis (PAGE) and Western blot, cells were solubilized with lysis buffer [120 mM NaCl, 40 mM Tris (pH 8.0), 0.1% NP40], the samples were boiled for 5 min, and equal amount of protein (40μg/well) was analyzed on 10% SDS-PAGE. After electrophoresis, proteins were transferred onto a nitrocellulose membrane and processed for immunoblotting. Blots were further incubated with horseradish peroxidase-conjugated secondary antibody diluted at 1:5,000, and specific bands were visualized by chemiluminescence (ECL, AmershamBiosciences, Uppsala, Sweden International). Autoradiographs were recorded onto X-Omat AR films (Eastman Kodak Co. Rochester, NY, USA).

3. Results

Heat shock protein (HSP) 27 was overexpressed in lung tumor tissues and treatment of NCI-H1299 cells which show high expression of HSP27 with small interference RNA (si-RNA) targeted for HSP27 abolished the resistance of radiation or cisplatin, suggesting that HSP27 is responsible for radio- and chemo-resistance in lung cancer cells. Furthermore, PKCδ activation is involved in sensitization of cancer cells, and carboxy terminus of PKCδ V5 region, interacted directly with HSP27, resulting in inhibition of PKCδ activity and PKCδ-mediated cell death. Therefore, we prepared various deletion mutants of V5 region and found that peptide sequence spanning residues 668-674 of V5 region was the binding site that interacted with HSP27. Treatment of NCI-H1299 cells with biotin labeled heptapeptide of residues p668-674 (E-F-Q-F-L-D-I) efficiently interacted with HSP27 and dramatically increased radiation induced cell death, while PKCδ activity inhibited by HSP27 overexpression was restored. In vivo nude mice grafting data also suggested

that NCI-H1299 cells were sensitized by this heptapeptide treatment.



4. Conclusion

We demonstrate that heptapeptide of PKCδ-V5 region sensitized human cancer cells induced by variety of death stimuli through it's interaction with HSP27, thereby sequestering HSP27, and delineate a novel strategy for the selective neutralization of HSP27.

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References

[1] Lee YJ, Lee DH, Cho CK, et al. HSP25 inhibits radiation-induced apoptosis through reduction of PKCdelta-mediated ROS production. Oncogene 2005;24:23:3715-25.

[2] Bruey JM, Ducasse C, Bonniaud P, et al. Hsp27 negatively regulates cell death by interacting with cytocheome c. Nature Cell Biol 2000;2: 645-52.

[3] Storm FK, Mahvi DM, Gilchrist KW. Heat shock protein 27 overexpression in breast cancer lymph node metastasis. Ann Surg Oncol 1996;3:6:570-3.

[4] Lee YJ, Lee DH, Cho CK, et al. HSP25 inhibits protein kinase C delta-mediated cell death through direct interaction. J Biol Chem 2005;280:18:18108-19.