HSP25 Inhibits PKCδ-Mediated Apoptosis through Direct Interaction

Yoon-Jin Lee[1#], Dae-Hoon Lee[1#], Chul-Koo Cho[1], Sangwoo Bae[1], Gil-Ja Jhon[2], Su-Jae Lee[1], Jae-Won Soh[3] and Yun-Sil Lee[1,*]

[1]Laboratory of Radiation Effect, Korea Institute of Radiological and Medical Sciences, Seoul 139-706, Korea, [2]Division of Molecular Life Science, Ewha Woman's University, Seoul 120-750, Korea [3]Laboratory of Signal Transduction, Department of Chemistry, Inha University, Incheon, 402-751 Seoul, Korea

1. Introduction

Small heat shock protein (sHSP) has been suggested to protect cells against apoptotic cell death triggered by a variety of stimuli, including hyperthermia, ionizing radiation, oxidative stress, Fas ligand and cytotoxic drugs. Several mechanisms have been proposed to account for the HSP27 mediated negative regulation of apoptosis. HSP27 specifically interacts with cytochrome c when released from the mitochondria to the cytosol, thus preventing the formation of the apoptosome. Another mechanism that contributes to cell protection from stressful stimuli through the elimination of unfolded proteins is the extralysosomal, energy dependent, ubiquitin-proteasome degradation pathway. HSP27 binds to polyubiquitin chains as well as 26S proteasome, and the ubiquitin-proteasome pathway is involved in the activation of transcription factor NF-kB by degrading its main inhibitor, I-kBα.

In the present study, we for the first time observed that HSP25 bound directly to V5 region of kinase active PKC□, which resulted in dual function of HSP25 mediated cytoprotection: one is a inhibition of PKCδ activity, therefore blocking of apoptosis and the other is HSP25 phosphorylation to increase cytoprotective effect of HSP25. This dual function facilitated HSP25 mediated antiapoptotic effects triggered by radiation or oxidative stress.

2. Results and Discussion

The coordinated interaction of kinases, phosphatases, and other regulatory molecules with scaffolding proteins is emerging as a major theme in intracellular signaling networks. There are now an increasing number of PKC-binding proteins believed to play a role in directing the location and function of individual PKC isoforms to particular subcellular locations. In this study, we have found that HSP25 is a PKC□-binding protein. sHSP is a pleiotropic inhibitor of cell death whose physiological protective effects are observed mainly in stressed cells. Several mechanisms have been proposed to account for this anti-apoptotic activity. HSP25 could increase the antioxidant defenses of cell by glutathione content and MnSOD enzyme activity. HSP27 also binds to activated protein Akt, a protein that generates a survival signal in response to growth factor stimulation and inhibits cell death by phosphorylating and inactivating procaspase-9 or by preventing the release of cytochrome c from the mitochondria. HSP25 also inhibited cell growth which relates to inhibition of PKC□-mediated ERK1/2 activation. The present study identifies yet another mechanisms by which HSP25 interferes with apoptotic pathways. This antiapoptotic activity is potentiated by dual functions: One is that HSP25 interacts with V5 region of PKC□ catalytic domain, thereby preventing PKC□-mediated apoptosis. The other function is that interaction of kinase active PKC□ with HSP25 induces HSP25 phosphorylation at Ser15 and Ser86, which potentiates cytoprotection of HSP25.

Physiological importance of binding capacity of PKCδ-HSP25 interaction in correlation with radioresistance in lung carcinoma cell lines implicates that HSP27 overexpression which showed radioresistance (Fig. 10), in part, relates to HSP25/27-PKCδ interaction. Since HSP27 expression in lung carcinoma cells are well correlated with radioresistance, therapeutically, V5 region of PKC□ might be useful for inhibition radioresistance by HSP27.

PKC□ activity plays essential role in apoptosis of cells and sHSP is constitutively expressed in many cancer cells to regulate negatively in induction of apoptosis. Interacting sHSP with PKC□ could represent an important aspect of the physiological role of this small HSP. This property might account for the protective effect of this protein when induced in response to stress such as radiation and oxidative stress.

Acknowledgement

This study was supported by Ministry of Science & Technology (MOST), Korean government, through its National Nuclear Technology Program.

Fig. Hypothetical scheme

Fig. Hypothetical scheme