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# PU-H71 effectively induces degradation of IκB kinase β in the presence of TNF-α

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**Abstract** This study is to determine if PU-H71, a heat shock protein inhibitor, induces killing of malignant breast cells together with treatment of tumor necrosis factor-α (TNF-α). The related molecular mechanisms were also studied. A primary mammary epithelial cell line HMEC2595 cells and the highly metastatic breast cell line MDA-MB-231, the HER2-positive BT-474 cells, and the ER-positive MCF7 cells were treated with PU-H71 in the presence or absence of TNF-α. The effects of PU-H71 and TNF-α treatments on cells viabilities and on intracellular signaling pathway proteins were determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, apoptosis assays, immunoblot assays, and luciferase assays. It was found that TNF-α enhances the toxic effects of PU-H71 on tumor cells but not normal cells. PU-H71 treatments lead to degradation of IKKβ. Moreover, PU-H71 down-regulates the NF-κB transcriptional activity induced by TNF-α treatment. The experimental results indicated PU-H71 effectively induces cell killing of malignant breast cells in the presence of TNF-α, possibly through a mechanism related to degradation of IKKβ. It is suggested that combination of PU-H71 and TNF-α treatments might be an effective therapeutic strategy of breast malignancies.

**Keywords** PU-H71 · IKKβ · TNF-α · NF-κB pathway

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## Introduction

The heat shock protein (Hsp90) is a chaperone protein responsible for the correct folding and functionalities of some proteins [1–4]. Such kinds of proteins are usually termed as client proteins of Hsp90. It has been reported that many of the Hsp90 client proteins are involved in key oncogenic pathways including proliferation, cell cycle progression, inhibition of apoptosis, and metastasis [5]. In the presence of Hsp90 inhibitors, the chaperoning function of Hsp90 is blocked and the client proteins are misfolded, and then ubiquitinated and targeted for proteasomal degradation [6–8]. Such kinds of Hsp90 inhibitors have been found to be able to induce killing of many kinds of malignant cells [6]. PU-H71 is a novel purine-scaffold Hsp90 inhibitor [6], which was developed in the laboratory of Dr. Gabriela Chiosis at Memorial Sloan Kettering Cancer Center and was licensed to Samus Therapeutics, USA. PU-H71 is recently shown to have therapeutic effects on some malignancies [9–12]. However, it needs to continue to study if PU-H71 can be applied in combination with other agents to generate a better method for treating breast malignancies.

It has been reported that tumor necrosis factor-α (TNF-α) plays important roles in cell proliferation, survival, differentiation, and apoptosis [13]. Because TNF-α may induce apoptosis of tumor cells, it may be used to induce killing of the tumor cells. However, TNF-α also induces activation of several other cellular pathways such as cell proliferation and survival processes, which may facilitate tumor development [14]. Therefore, other chemotherapy may be needed to increase the apoptosis induction of TNF-α, but decreases its activation function in the cell proliferation and survival processes. Therefore, novel reagents are necessary to be used in combination with TNF-α for treating malignancies.

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