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LETTER TO THE EDITOR

Geldanamycin induces CHOP expression through a 4-(2-aminoethyl)-benzenesulfonyl fluoride-responsive serine protease

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Dear Editor:

Geldanamycin is a benzoquinone ansamycin, which was originally described as a tyrosine kinase inhibitor. However, subsequent studies have revealed that geldanamycin binds to and inhibits heat-shock protein 90 (Hsp90) activity [1]. Hsp90 is a molecular chaperone involved in the conformational maturation of proteins such as mutated p53, Raf-1, Akt, Bcr-Abl, and ErbB2. It is suggested that agents inhibiting Hsp90 have anti-cancer properties, although the precise molecular mechanisms underlying the anti-cancer effects of geldanamycin are not well understood.

Increasing evidence has suggested that diabetes and neurodegenerative disorders such as Parkinson's and Alzheimer's diseases are related to the disruption of endoplasmic reticulum (ER) function. In response to ER stress, unfolded proteins accumulate and aggregate in the ER, which will trigger many rescuer responses, including the unfolded protein response (UPR) and ER-associated degradation. Interestingly, geldanamycin has been shown to upregulate ER chaperones and the expression of CHOP [2]. Moreover, Hsp90 associates with PERK and IRE1α, ER-resident trans-membrane protein kinases involved in ER stress response [3]. These observations suggest that Hsp90 is involved in ER stress response and may play an important role in ER stress-related diseases. Therefore, we investigated the potential mechanism by which geldanamycin affects ER stress using the L929 fibroblastoma cell line.

The UPR is characterized by activation of the signal transduction pathway triggered by IRE1. Activation of IRE1 induces X-box binding protein (XBP-1) mRNA splicing [4]. XBP-1 protein produced from the spliced mRNA then functions as a transcription factor to induce ER-stress genes [4]. The XBP-1 splicing was observed in geldanamycin-treated cells in our experiments, indicating that geldanamycin induces the ER stress response pathway (data not shown). We further investigated whether geldanamycin induces CHOP expression in L929 cells. As assessed by RT-PCR (see Supplement 1 for Materials and Methods), we observed an increase in the CHOP transcript in geldanamycin-treated cells (10 μM, 2 h) (Figure 1D and 1E). The CHOP protein level was also significantly induced at 6 h after geldanamycin treatment (Figure 1F and 1G).

We next investigated intracellular mechanisms of geldanamycin-induced CHOP expression (see Supplement 1 for Materials and Methods). The PERK-eIF2α pathway has been reported to be involved in ER stress-induced CHOP expression [5]. Thus, we investigated whether geldanamycin can induce eIF2α phosphorylation. As a positive control, the ER stress-inducing reagent Tg (3 μM, 2 h) caused an increase in eIF2α phosphorylation in L929 cells (Figure 1A). However, geldanamycin did not increase eIF2α phosphorylation at all examined time points (Figure 1B and 1C). Geldanamycin suppresses the binding of 3-phosphoinositide-dependent protein kinase-1 (PDK1) to Hsp90 and inhibits the activation of Akt [6]. As shown in Figure 1B, Akt phosphorylation was inhibited in geldana-

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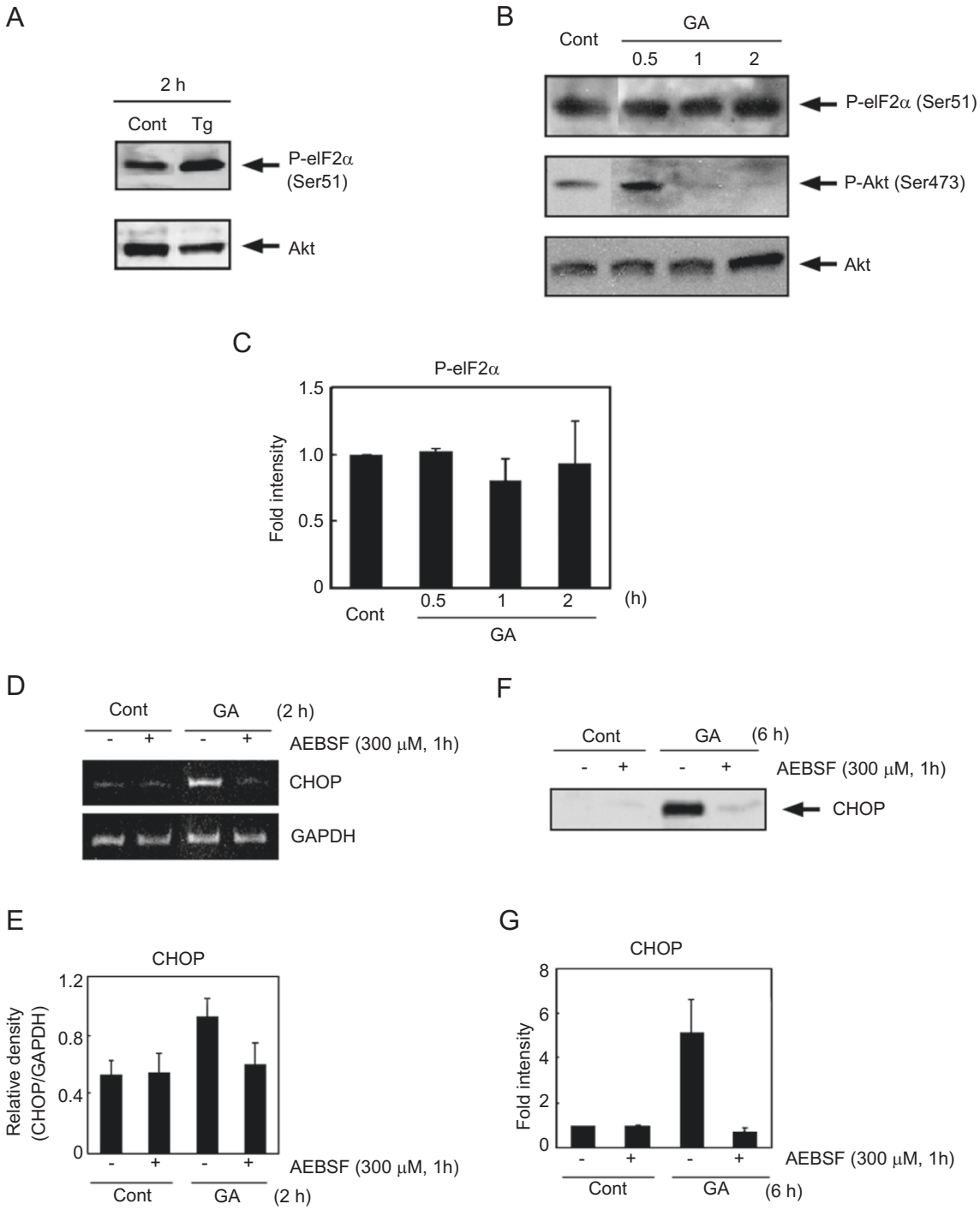

Figure 1 (A) L929 cells were treated with thapsigargin (Tg: 3 μM) for 2 h and Western blotting was performed using antibodies against phospho-eIF2α (Ser51) and Akt. **(B)** Cells were treated with geldanamycin (GA, 10 μM) for the indicated time periods. Phospho-eIF2α (Ser51), phospho-Akt (Ser473), and Akt were detected by Western blotting. **(C)** The expression levels are expressed as the fold intensity compared with the control group. **(D)** Cells were pretreated with AEBSF (300 μM) for 1 h and then stimulated with geldanamycin (GA, 10 μM) for 2 h. RT-PCR was performed using specific primers for each mRNAs. **(E)** The levels of CHOP mRNA were quantified by densitometric analysis and normalized to that of the corresponding GAPDH internal control. **(F)** Cells were pretreated with AEBSF (300 μM) for 1 h and then stimulated with geldanamycin (GA, 10 μM) for 6 h. CHOP protein induction was detected by Western blotting. **(G)** The levels of CHOP protein were quantified by densitometric analysis and are presented as the fold intensity compared with the control group.

mycin-treated cells, indicating that geldanamycin functions effectively under our experimental conditions. These results suggest that eIF2α is not involved in geldanamycin-induced CHOP expression.

Serine protease has been suggested to be involved in ER stress-related diseases [7, 8]. Thus, we investigated the effect of 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF) on geldanamycin-induced CHOP expression. AEBSF is a potent serine protease inhibitor, which acts by sulfonylation of the serine residue at the active site [9]. As shown in Figure 1D and 1E, geldanamycin-induced CHOP transcription was completely inhibited by AEBSF. The inhibitory effect of AEBSF on geldanamycin-induced CHOP expression was also confirmed at the protein level (Figure 1F and 1G). These results suggest that a serine protease is involved in geldanamycin-induced CHOP expression. ER stress has been shown to lead to the activation of activating transcription factor 6 (ATF6), and the process involves the cleavage of ATF6 by a serine protease called site-1 protease (S1P) [8]. The cytoplasmic domain of ATF6 has been reported to induce CHOP expression [10]. Moreover, AEBSF has been reported to inhibit the activation of ATF6 through inhibiting S1P [7]. Thus, it seems likely that the inhibitory effect of AEBSF on geldanamycin-induced CHOP expression is mediated by inhibition of ATF6 activation.

The present results raise the possibility that one of the mechanisms of the anti-cancer effect of geldanamycin may be mediated through CHOP induction. Our results also suggest that AEBSF is a unique and interesting compound with respect to ER stress response, and its detailed pharmacological role awaits to be investigated in the future. Nevertheless, the present study provides new insight into the molecular mechanism of geldanamycin-induced cell dysfunction, which could be important for understanding cancer and/or ER stress-related diseases.

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(Supplementary Information is linked to the online version of the paper on the Cell Research website.)