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GRP75 of CHO Cells Responds to Ribosylation

Dear Editor,

Recently, type 2 diabetic patients were clinically observed to suffer from dysfunction not only in glucose metabolism, but also ribose [1–2]. Ribose is rapidly reacted with protein [3–4], producing cytotoxic ribosylated products [5], whose progress is much faster than glucose [6–7]. A 75 ku glucose regulated protein (GRP75) is generally recognized as a member of the heat shock protein 70 (HSP70) class of proteins, which is induced under conditions of low glucose and other nutritional and environmental stresses [8–9]. Administration of ribose induces a decrease in blood glucose [10], and thus changes in GRP75 should be studied when cells are exposed to ribose.

In order to investigate whether GRP75 is involved in the ribosylation of cellular protein, we added 50 mmol/L ribose to the medium in which CHO cells were cultured. Cell lysates were taken for measurements of the ribosylated products using Western blotting at different time intervals (Figure 1a). It was observed that a protein band around 72 ku is ribosylated with time (Figure 1b). Then, we incubated CHO cells with different concentrations of ribose and detected protein levels of GRP75 with a monoclonal antibody against GRP75 (CST, USA) (Figure 1c). As shown in Figure 1d, the level of GRP75 was promoted with the increase of ribose. The increase in GRP75 levels are in a ribose concentration dependent manner. This suggests that GRP75 may be one of major components in the ~72 ku ribosylated protein band on the gel. To demonstrate that GRP75 is a major ribosylated protein, we did co-immunoprecipitation experiments (Figure 2). We used anti-AGE antibody (Wako, Japan) as the "bait" to fish for the ribosylated

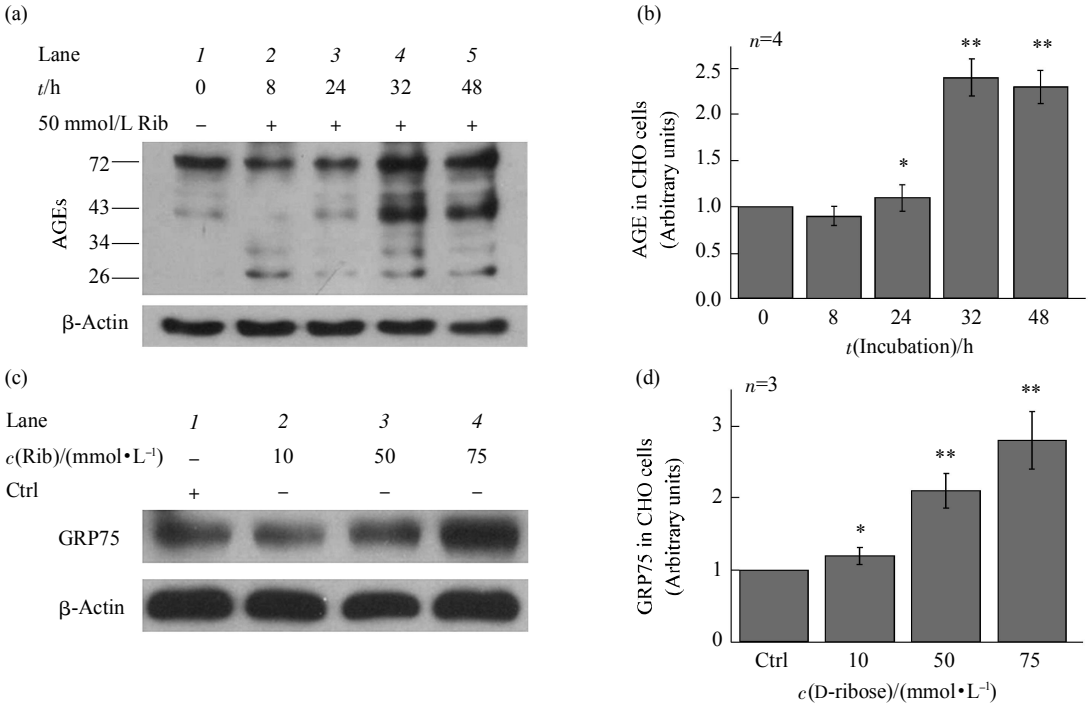
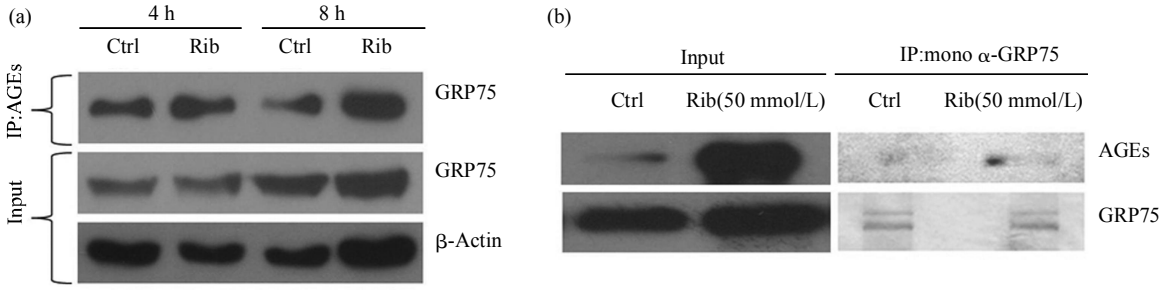


Fig. 1 Increase in ribosylated products and GRP75 of CHO cells in the presence of ribose

Chinese hamster ovary (CHO) cells were incubated with 50 mmol/L ribose and ribosylated products (AGEs) were taken for measurements with Western blotting (a) and gray density (b) at different time intervals. GRP75 was also detected with Western blotting (c) and gray density (d). Rib, ribose; AGEs, advanced glycation end products; *t*, time; *c*, concentration and Ctrl, control.

products (advanced glycation end products, AGEs) that interacted with the target GRP75 in CHO cells. As shown in Figure 2a, AGEs are bound to GRP75 in the cell lysates during the incubation at 4 and 8 h. Sequentially, we employed anti-GRP75 as a bait to fish GRP75 and found that the ribosylated products (AGEs) were co-immunoprecipitated with GRP75 under the experimental conditions (Figure 2b). The co-immunoprecipitated protein with anti-AGE was analyzed by mass spectrum (Thermo-Finnigan, USA), demonstrating GRP75 as a major precipitated protein (Figure 2c). These results indicate that GRP75 is a major component in the ribosylated protein and its level is positively correlated with the concentration of ribose. Whether GRP75 acts as a factor to protect cells in a stress or as a response to ribosylation in diabetes needs further investigation.



(c) Mass spectrum analysis of AGEs products around 72 ku

No.	Reference	Score	MW	Accession	Peptide (hits)
	Scan(s)	XC	Sp	RSp	Ions
1	gi|298351697|sp|P86233.1|GRP75_MESAU RecName: Full=Stress-70 protein, mitochondrial	40.19	48377.2	298351697	4(40000)
2	gi|298351706|sp|P86246.1|K1C19_MESAU RecName: Full=Keratin, type Ⅰ cytoskeletal 19; AltName:	20.18	18109.5	298351706	2(20000)
3	gi|298351707|sp|P86247.1|K2C8_MESAU RecName: Full=Keratin, type Ⅱ cytoskeletal 8; AltName:	20.12	18110.5	298351707	2(2000)

Fig. 2 Co-immunoprecipitation to verify interaction between GRP75 and AGEs as well as mass spectrum analysis of the products

CHO cells were incubated with 50 mmol/L ribose and aliquots were taken for co-immunoprecipitatioin at different time intervals. GPR75 was co-immunoprecipitated with ribosylated products by using the antibody of anti-AGE (a). In contract, monoclonal antibody against α-GRP75 can fish not only GRP75 but also ribosylated products (b). The mass spectrum analysis shows a major component of the Co-IP with anti-AGE is GRP75 protein (c).

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