The endoplasmic reticulum (ER) chaperone GRP78 is secreted during ER Stress and alleviates endothelial cell inflammation

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Introduction: Glucose-Regulated Protein 78kD (GRP78) is a chaperone and the main regulator of the ER-stress response which is triggered by a variety of conditions that disturb folding of proteins in the ER. Upon ER Stress, GRP78 activates the unfolded protein response (UPR), which aims to clear unfolded proteins and restore ER homeostasis. Recently, extracellular secretion of GRP78 was described. However, the pathophysiological relevance of secreted GRP78 in atherosclerosis and endothelial cell inflammation remains to be elucidated.

Aim of this study is to investigate the role of GRP78 secretion in endothelial cells.

Methods and Results: First, we sought to investigate if vascular cells secrete GRP78 during ER Stress. Human coronary artery endothelial cells (HCAEC) were treated with the ER stress inductor tunicamycin for up to 48h. After ER Stress induction, Western Blot and ELISA experiments detected an increased intracellular GRP78 expression. Intriguingly, prolonged ER Stress also promoted extracellular secretion of GRP78. Unbiased proteomic analysis of the HCAEC secretome confirmed that after ER-Stress induction, GRP78 is one of the most highly upregulated extracellular proteins (2.43-fold). Co-incubation with Brefeldin A, an inhibitor of ER-Golgi protein transport, abolished extracellular secretion (Fig.1). Hence, ER-Stress-induced GRP78 secretion is an actively regulated process.

Next, the effect of GRP78 containing conditioned medium (CM) on HCAEC was analyzed. For control, HCAEC were co-incubated with BFA, or GRP78-knockdown was performed by siRNA.

Treatment with GRP78 containing CM decreased GRP78 mRNA expression in target cells (0.35-fold vs. control [+BFA], p<0.0001). Furthermore, it increased viability ( BFA: 93.0 % vs. 83%, p=0.002; GRP78-siRNA: 94% vs. 85%, p=0.01) and decreased the rate of apoptosis (e.g. BFA: 1.7-fold vs. 5.1, p=0.02). Moreover, expression of markers of vascular inflammation and ER Stress (e.g., NF-κB and CHOP) were decreased when compared to CM without GRP78 (Fig.2). Furthermore, increased H2O2-generation was measured (BFA: 1.4-fold, p>0.001, GRP78-siRNA:1.3-fold, p=0.004) (Fig. 2).

To confirm that the beneficial effects of the CM are in fact caused by GRP78, we treated HCAEC with recombinant GRP78 and observed an increased cell viability in a dose-dependent manner (1000 ng/ml: p=0.02 vs. control).

CRIPTO is a membrane-bound co-receptor for growth factors and was previously linked to GRP78-signalling. siRNA-Knockdown of CRIPTO reduces viability and leads to cell apoptosis, irrespective of GRP78-treatment. Thus, GRP78-mediated effects may be mediated by CRIPTO.

Conclusion: Endothelial ER Stress promotes GRP78 secretion. Presence of GRP78 in conditioned medium ameliorates subsequent ER Stress and endothelial inflammation, which play a critical role in atherogenesis. Further investigations are important to shed light on the mechanism of release and the effect on target cells.
Figure 1

Figure 2