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Editorial

Heat Shock Protein gp96 as an Immune Chaperone of Inflammation and Cancer

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Heat shock protein gp96 [1], also known as grp94 [2], endoplasmin [3], ERp99 [4],and HSP90b1 [5]; is an endoplasmic reticulum (ER) paralogue of heat shock protein 90 (HSP90). However, the structure and function of gp96 are distinct from other cytosolic HSP90. gp96 is induced by the accumulation of misfolded proteins in the cells [6]; it binds to and hydrolizes ATP [7]. gp96 is the most abundant and a ubiquitous glycoprotein in the ER lumen. Accumulating evidence including work from my own laboratory reveals that gp96 serves as a unique and an important chaperone for inflammation and cancer. The key evidence is summarized in this editorial.

gp96 as a molecular chaperone for Toll-like receptors (TLRs) was first discovered in 2001. This study showed that a murine pre-B-cell line (70Z/3) that was rendered deficient of gp96 is unresponsive to ligands for TLR2 and TLR4 [8]. Using classic gene knockout strategies, subsequent studies conclusively demonstrated that gp96 is an obligate master chaperone for folding most of TLRs (except TLR3), integrins, platelet glycoprotein Ib-IX-V complex, and the Wnt co-receptor low-density lipoprotein receptor-related protein 6 (LRP6) [9-15].

TLRs are germline-encoded pattern recognition receptors (PRR) that function in recognizing pathogen-associated molecular patterns (PAMP) such as lipopolysaccharide (LPS), dsRNA, unmethylated CpG DNA and flagellin for initiation of the innate immunity and subsequent activation of the adaptive immunity [16,17]. Yang *et al* found that gp96-deficient macrophages fail to respond to ligands for both surface and intracellular TLRs except TLR3. In the absence of gp96, TLRs fail to translocate to the cell surface or endosome, and are retained in the ER. Furthermore, macrophage-specific gp96-deficient mice are highly susceptible to the acute infection by *Listeria monocytogenes* (LM), indicating the functions of macrophages in the clearance of this organism are regulated by TLR signaling [9]. More recently, Morales *et al* showed that macrophage-specific gp96-deficient mice are more resistant to chemical-induced colitis. Macrophage-specific gp96-deficient mice have significantly less inflammation in the colon and lower percentages of Th17 and Th1 cells in colonic lamina propria compared with wild type littermates. Intriguingly, by using a standard colon cancer model induced by azoxymethane (AOM) and DSS, they found that macrophage-specific gp96-deficient mice developed fewer and smaller tumors, indicating that deletion of gp96 in macrophages renders mice resistant against colitis-associated colon cancer [18]. This study demonstrates the critical role of gp96 and its clientele (such as TLRs) on myeloid cells in conferring intestinal inflammation, inducing genetic instability in the colonic epithelium, and promoting inflammation-associated colon tumorigenesis.

Historically, one of the most well-known clients of gp96 is the immunoglobulin (Ig) heavy chain [19] and several studies have suggested the roles of gp96 in B cell function. For example, gp96 is induced more than 10 fold during B cell activation [4]; it has been shown to participate in the assembly of B cell receptor (BCR) complexes through its association with Igα molecules [20]. To answer the direct roles of gp96 in B cell biology *in vivo*, Liu and Li generated a B cell specific gp96 deficient mouse [10]. They found that B cells are unable to proliferate in response to multiple TLR ligands and are defective in the expression/function of selective integrins. In this study, they also demonstrated that gp96 null conventional B cells do not accumulate efficiently in the lymph nodes and innate-like B cells (B1 cells and marginal zone B cells) fail to compartmentize properly which is most likely due to selective integrin defect. Moreover, both proliferation and Ig production by the gp96 null B cells are attenuated even though a robust proximal BCR signaling was preserved. Despite these multiple defects, B cell selective gp96-deficient mice are able to mount robust antibody response against both T cell-dependent and T cell-independent antigen, indicating gp96 plays more selective roles in the function of B cells *in vivo* by chaperoning a limited set of client proteins in B cell biology.

gp96 is a key downstream chaperone in the ER to mediate unfolded protein response (UPR) [21]. UPR is an evolutionally conserved mechanism to maintain protein quality control in the secretory pathway. Although under the steady state conditions, gp96 is not required for B cell activation, germinal center formation, plasma cell differentiation, and class-switching or affinity maturation [10]. It is unclear if gp96 is required for plasma cell biology and for the development of multiple myeloma (MM) during chronic ER stress conditions. MM is an incurable plasma cell neoplasm whose pathogenesis is closely linked to dys-regulated unfolded protein response (UPR) in the endoplasmic reticulum (ER). Constitutive activation of UPR in mice causes myeloma, as demonstrated by transgenic expression of a master UPR transcription factor XBP1s (XBP1s Tg mice) [22]. To address this question, Hua *et al* generated a mouse model with over-expression of XBP1s and deletion of gp96 in B cell compartment simultaneously; it was found that the persistence of plasma cells as well as the development of myeloma is critically dependent on gp96 [15]. Furthermore, a recent study by Liu *et al.* demonstrated that gp96 is a chaperone for Wnt co-receptor LRP6 and

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Wnt signaling is decreased in the absence of gp96 [13]. gp96 knock down causes severe compromise in MM cell growth which can be significantly rescued by GSK3β inhibitors. In the absence of gp96, MM cells undergo mitotic catastrophe and apoptosis which correlated with decreased expression of survivin a Wnt target molecule. This study indicates that myeloma is uniquely dependent on gp96 for survival, which is mediated in part by a LRP6-Wnt-survivin pathway. This finding suggests that gp96 is a novel therapeutic target for multiple myeloma.

Currently, the number of identified protein substrates that are dependent on gp96 for folding is still limited. Further identification of more gp96 client proteins and understanding the functional implications of gp96-client network in inflammation and cancer may catalyze the development of novel gp96-targeted therapeutics for these conditions.

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