Abstract

Bcl-2-associated athanogene 3 (BAG3) belongs to the family of co-chaperone proteins that interact with the heat shock protein 70 (Hsp70) and is involved in a number of cellular processes including proliferation and apoptosis. BAG3 contains the BAG domain which interacts with the ATPase domain of Hsp70. BAG3 is also characterized by the presence of a WW domain, two conserved Ile-Pro-Val (IPV) motifs and a proline-rich (PXXP) repeat that mediate the binding to partners different from Hsp70. These diverse and multiple interactions underlie the ability of BAG3 to modulate major biological processes such as development, cytoskeleton organization and autophagy. In our laboratory, BAG3 has been recently found in a soluble or membrane-associated form and it has been detected in the serum obtained from patients with pancreatic cancer or heart failure. Moreover, we found that BAG3 is able to bind the cell surface of macrophages and activate the production of inflammatory associated components, such as Nitric Oxide (NO) and Interleukin (IL) -6. To identify novel interacting partners of BAG3 an affinity chromatography on nickel-charged resin was performed, in J774A.1 cells, using recombinant BAG3 (rBAG3) followed by mass spectrometry analysis of the rBAG3-containing complexes. Among these, Interferon- Inducible TransMembrane (IFITM) -2 and Neuropilin (NRP) -1 were the only transmembrane proteins and therefore represented good candidates as receptors for BAG3. Our results show that NRP-1 and IFITM-2 are both essential for the binding of rBAG3 to the cell surface of macrophages and its activation for IL-6 release.

We then investigated if BAG3 binding activates some of the signaling pathways known to be involved in macrophage activation. In particular we focused on the phosphatidylinositol 3-kinase (PI3K) and on the p38 pathway that are both involved in Cox-2, iNOS and IL-6 induction in macrophages. We demonstrated that BAG3 signaling is mediated by the receptor complex we identified, since IFITM-2 and/or NRP-1 silencing abrogates BAG3- induced phosphorylation of AKT and p38.

We than focus our study on human monocytes, rBAG3 binds the cell surface and induces the release of many pro-inflammatory cytokines and chemokines. Furthermore, we have shown that rBAG3 can bind T lymphocytes cells surface after lipopolysaccharide (LPS) stimulus.

All together these findings suggest a role for extracellular BAG3 in immune response.