

INTERVIEW about

HSP110 and antigen presenting cells

with

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What is HSP110?

Manjili and Subject: HSP110 is a large molecular weight heat shock protein highly capable of chaperoning large proteins. While HSP110 is related in sequence to HSP70, it does not fall into the HSP70 “family” and indeed represents one additional gene family. It is recognized today that this “superfamily” of sequences, the HSP70 “family” and HSP110 “family”, comprise two distinguishable stress protein families of eukaryotic cells that share a common evolutionary ancestor (1). In addition, there is a third family of stress proteins that also maps to this HSP70 superfamily. This group is called the GRP170 family. We will stick to HSP110 here, but come back to GRP170 at the end. All members of this superfamily have the ability to bind to and stabilize partially denatured protein and then facilitate the folding of these proteins with help from other stress proteins, a process called “chaperoning”. HSP110 has mole-

cular chaperoning properties that, while similar to HSP70/HSC70 are also more robust (HSC70 represents the constitutively expressed HSP70 family member, i.e. the HSP70 family itself has multiple members). A major function of HSC70 is to interact with partially unfolded protein substrates at 37°C, which are expected to occur in countless cellular pathways. Using known approaches to HSC70/HSP70 function, it was shown that HSP110 also binds to and stabilizes full-length heat denatured proteins *in vitro*, but does so more efficiently than HSC70 or HSP70. These studies show that HSP110 is approximately four fold more efficient in binding to and stabilizing denatured protein substrates when compared to HSC70 or HSP70 (2,3). It is this very strong ability to “hold” or bind protein antigen by HSP110 that makes it particularly important as a “danger signal” compared to other HSPs/stress proteins.

Can HSP110 induce a “danger signal” to alert the immune system to its associated antigen?

Manjili and Subject: For many years, HSPs have been considered to be exclusively intracellular proteins with intracellular functions and their appearance outside of the cell to be artifacts, e.g. due to cell lysis. Recent views have changed. Cell damage is no longer considered an artifact, but to have essential functions in alarming the host to damaged or diseased tissues. In addition, HSPs are reported to be expressed on the cell surface. Although the mechanism for surface expression of HSPs remains to be determined, chaperoning function of HSPs

suggest that they may bind to transmembrane proteins or their receptors inside the cell upon stressful conditions and move to the cell surface as it is the case for surface IL-15. HSPs released from cells could be a crucial signal that is able to activate the immune system to recognize “dangerous” physiological situations (such as viral-induced tissue lysis or necrosis). This suggests that HSPs, which are clearly intracellular proteins of all living cells, may have developed a natural extracellular function related to the early evolution of the immune response (4).

When HSP110 is used as an extracellular protein in vaccine formulation (i.e. when HSP110 is purified from a tumor or is prepared using recombinant proteins expressed in the laboratory), an immune response can be induced against its associated antigen (3,5,6). Such immunoadjuvant properties of HSP110 stem from its dual functions while interacting with

Are DCs important for immunoadjuvant function of HSP110?

Manjili and Subject: Tumor cells mostly express self proteins to which high affinity T cells were deleted during negative selection while low affinity T cells were selected through positive selection in the thymus. The repertoire of T cell receptors recognizing tumor antigens exist at such a low affinity that could not bind to the antigen long enough and strong enough to receive activation signals. Therefore direct priming of T cells by tumor cells will not occur. On the other hand, DCs as professional antigen presenting cells, express co-stimulatory molecules upon their activation, thereby enhancing avidity of T cells against antigens pre-

immune system, i.e. induction of both innate and adaptive immune responses. HSP110 can not only interact with antigen presenting cells and result in the induction of secretion of proinflammatory cytokines, it can also up-regulate expression of MHC class II, CD40, and co-stimulatory molecules thereby enhancing antigen presentation capacity of dendritic cells (DCs) (7). Importantly, we have recently shown that HSP110 can also induce or enhance secretion of TNF- α and IL-12. These proinflammatory responses could act as “danger signals” for recruitment of lymphocytes and induction of immune responses. In addition, HSP110 can induce immune responses against its associated antigen by mechanisms that are under investigation.

sented within their MHC class I or II molecules at high levels. This phenomenon, termed cross priming, has been utilized to activate T cells against tumor antigens. Activated T cells will then kill tumor cells when they see the antigens to which they had been sensitized. DCs are therefore important for immunoadjuvant functions of HSP110. However, the latest findings that HSP110 can also enhance secretion of pro-inflammatory cytokines in tumor cells opens up an avenue where tumor-released HSP110 may also facilitate T cell activation via induction of secretion of immune stimulatory cytokines.

Are there HSP110-specific receptors on antigen presenting cells or tumor cells?

Manjili and Subject: HSP110, like other HSPs, is a molecular chaperone that can bind unfolded proteins. We have shown that HSP110 binds reporter proteins four-fold more efficiently than does HSP70 (3). This would suggest that HSPs are different in their affinities for binding to other proteins and this property may also be important in binding to cell surface receptors. Indeed, recent studies of HSP110 and its homolog in the endoplasmic

reticulum, GRP170, show that chaperoning appears to be responsible for binding to macrophages in a receptor like manner (8). Additionally, while HSP110 and GRP170 bind to APCs in a receptor-like fashion (7,9), it is expected that binding occurs with more than a single receptor. We are presently attempting to identify the individual receptors involved (Facciponte et al, manuscript in preparation).

What is new about HSP110?

Manjili and Subject: What's new about HSP110 is its "sister" protein resident in the endoplasmic reticulum (ER). Just like HSP70 and HSP90, that also have "sister" proteins in the ER called GRP78 (or BiP) and GRP94 (or gp96), HSP110 has GRP170. It is very important to understand that both sets of stress proteins, HSPs and GRPs, are independently regulated by different stress environments. While HSPs are induced by oxidative conditions, reperfusion injury as occurs after stroke or heart attack, or heat shock (including mild thermal exposures in the febrile range), GRPs are induced by chronic hypoxia, low pH and glucose starvation, similar to conditions that may arise during tissue injury and during the process of growth of some tumors. Thus each of these stress protein sets and specifically HSP110/GRP170, are independently regulated by very important physiological conditions related to tissue damage and disease states. This undoubtedly has important implications that are not yet understood with respect the role of such stress environments on the immune system. Like HSP110, GRP170 is a very strong chaperone (or "holder") of protein antigen and therefore a very good antigen carrier. Like HSP110, it also is a danger signal to the immune system. We have recently shown that GRP170 binds DCs in a receptor-mediated fashion and induces DCs to up-regulate the expression of MHC class II, CD86, CD40 molecules and to secrete pro-inflammatory cytokines (9). Thus, GRP170 is another major molecular chaperone of mammalian (and indeed all eukaryotic) cells that, like HSP110, has received little attention. A great deal of work is needed to determine how HSP110 and GRP170 fit into the larger scheme of these different and important stress responses that are induced each under different physiological states of direct relevance to various disease states.

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