Development of Heat Shock Proteins with Controlled Distribution Properties and Their Application to Vaccine Delivery

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Effective delivery of antigen to antigen presenting cells is a key issue for developing vaccines for viral infections and cancers. Controlling the *in vivo* distribution of antigens that are administered as peptide or DNA forms will be effective in increasing antigen-specific immune responses including the induction of cytotoxic T lymphocytes. Heat shock protein 70 (Hsp70), a family of highly conserved molecules that are induced under stress conditions and plays essential roles as a molecular chaperon, can present a broad repertoire of tumor antigens to dendritic cells and elicit innate immunity. Based on the structure-distribution correlation obtained using many macromolecular compounds with diverse physicochemical properties, we have developed Hsp70-antgen conjugates with controlled tissue and intracellular distribution properties. A peptide from ovalbumin was selected as a model antigen and was conjugated with Hsp70 to obtain Hsp70-peptide fusion proteins (Hsp70-pep). The fusion proteins or plasmid DNA expressing them were used to induce the peptide-specific immune response. Poly-histidines, which is reported to have an ability to accelerate the release of endocytosed compounds from endosomes to the cytosol, was coupled to Hsp70-pep to facilitate its delivery to the cytosol where the interaction of the peptide with MHC class I takes place. On the other hand, the delivery of intracellular antigens of tumor cells was challenged by using cell-penetrating peptide (CPP)-coupled Hsp70. The escape of the Hsp70-CPP fusion protein from tumor cells was first confirmed, and its application to anti-tumor therapy was examined in tumor-bearing mice.