Keynote Lecture III
New Approaches to Cancer Immunotherapy

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Dr Xuetao Cao was born in 1964 and received his PhD in immunology from the Shanghai Second Military Medical University (SMMU) in 1990. From 1992, he became Professor of the Department of Immunology at the SMMU. From 1999, he became the Director of the Institute of Immunology at SMMU. He is now the Professor and Director of the Institute of Immunology at Tsinghua University, Beijing. His field is the tumor immunology and immunotherapy. He published more than 90 papers in the international journals (SCI) including Nature Immunology, Blood, J Immunol, Cancer Res, J Biol Chem.

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Efficient antitumor immunity requires recognition of tumor antigens by the host immune system presentation of tumor antigens by antigen-presenting cells (APC) and the production of potent effector immune cells. Tumor cells evade host immunosurveillance primarily by downregulating surface expression of major histocompatibility complex I (MHC-I) and costimulatory molecules, secreting inhibitory cytokines or expressing apoptosis-inducing molecules on the cell surface. Immunological manipulation of either tumor cells or APC, in particular dendritic cells (DC), which play crucial roles in the initiation of innate and adaptive immunity, can cause regression of established, invasive cancers. HSPs are a family of chaperones involved in protein folding and translocation which are crucial regulators of diverse cellular events such as cell proliferation, differentiation, survival and apoptosis. Tumor-derived HSPs can induce protective immunity against their tumors of origin when used as vaccines, due to their incorporation of the chaperoned tumor-derived peptides and their role as a danger signal to the immune system. These adjuvant effects activate APC, including DC, leading to more efficient uptake, processing and presentation of HSP-chaperoned peptides. Therefore, based on the above observations, we designed a new kind of artificial antigen-presenting vesicles containing HSPs and chemokines, which can be used as potent cancer vaccine through chemoattracting DC and activating T cell immunity.

HSP70L1 is a novel HSP we cloned from human DC cDNA library. HSP70L1 shares high homology with the members of HSP70s, has heat inducible characteristics and typical motifs of HSP70 subfamily. Recombinant HSP70L1 can bind to and be internalized by DC. Interaction of recombinant HSP70L1 with DC can promote DC maturation and stimulate secretion of proinflammatory cytokines IL-12, IL-1β, TNF-α and chemokines IP-10, MIP-1α, MIP-1β, RANTES. HSP70L1 is functionally different from HSP70 because the specific binding of HSP-DC to DC cannot be blocked by HSP70, and HSP70L1 can induce DC to secrete IP-10 but HSP70 cannot. Moreover, HSP70L1 is more potent than HSP70 in stimulation of IL-12, CC-chemokines, CCR7 and CXCR4 expression by DC. Immunization with the HSP70L1-OVA257-264 peptide hybrid induces OVA257-264-specific Th1 response and CTL, resulting in a significant growth inhibition of E.G7-OVA tumor. Therefore, HSP70L1 can activate DC and acts as a new potent adjuvant for peptide immunization. HSP70L1 antigen peptide hybrid, as a kind of effective vaccine, may be useful in controlling cancer or infectious diseases.

As introduced above, HSP can activate DC and induce chemokine production. We went further to demonstrate that heat shock could induce tumor cells to secrete chemokines. Considering that tumor cell-derived exosomes can be used as a tumor vaccine to induce antitumor responses, we prepared a novel form of exosomes from heat shocked tumor cells that contain HSPs, chemokines and are capable of effectively eliciting therapeutic

antitumor immunity. Exosomes derived from the murine Lewis lung carcinoma cell line 3LL, following heat shock induction at 42oC for 1h (HEXO), contain higher levels of MHC-I, MHC-II, B7.2, B7.1, ICAM-1, HSP70, HSC70 and HSP60 than exosomes derived from non-heat-shocked tumor cells (EXO). Importantly, HEXO contain chemokines, including β chemokines MIP-1α, MIP-1β, MIP-3β, MCP-1, and TECK, and the alpha chemokines SDF-1α and IP-10. In vitro, HEXO can chemoattract and adhere to dendritic cells (DC), as well as activate T cells directly, in a DC-independent manner, through both the MHC-I and MHC-II pathways. In vivo, systemic administration of HEXO induces significant regression of pre-established tumors, while intratumoral injection of HEXO results in their complete eradication. Although immunization with both HEXO and EXO can induce specific CTL responses, HEXO are more effective. In vivo depletion of T cell subsets and NK cells using specific antibodies reveals that systemic HEXO-induced antitumor immunity is mediated primarily by CD4+ and CD8+ T cells. Therefore, HEXO appear to act as artificial antigen-presenting vesicles, and have potential for application as a novel, efficient vaccine for cancer immunotherapy.

We went further to show that immunization of HLA-A2.1/Kb transgenic (Tg) mice with low dosage of HS-Exo, derived from carcinoembryonic antigen (CEA)-positive tumor cells and containing CEA proteins, are more efficient in priming CEA-specific CTL than with same amounts of Exo, which can be adoptively transferred to SW480 (HLA-A2+CEA+)-bearing nude mice and in turn result in inhibited tumor progression and prolonged survival. Moreover, in vitro incubation of lymphocytes, derived from HLA-A*0201+ healthy individuals and HLA-A*0201+ CEA+ cancer patients, with autologous DC pulsed with HS-Exo can induce CEA-specific and HLA-A*0201-restricted CTL. Our studies suggest that HS-Exo derived from CEA+ tumor cells exhibit superior immunogenicity than Exo, and that HS-Exo can potentially be a new approach to directly activate the immune system against CEA-positive tumors.