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Commentary Open Access

PTD-J-X: To Elicit Humoral and Cellular Immunity as Will

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Commentary

Concept proving papers upon a PTD-J-X antigen presenting system were reported recently. The X antigen segment of the recombinant protein could be simply built up to elicit humoral and/or cellular immunity, specifically and effectively.

It is conventionally pictured that extracellular antigens are engulfed by antigen presenting cells (APCs) via endocytosis. Then the antigens are digested in lysoendosome to produce peptide epitopes which are loaded to class II major histocompatibility complex (MHC-II) before recycling back onto cell membrane. On the other hand, intracellular antigens are processed by proteasome and the peptide epitope products are transported into endoplasmic reticulum where these epitopes are assembled into *de novo* synthesized MHC-I molecules (Figure 1). Recently, a PTD-J-X antigen presenting model which was composed of a protein transduction domain (PTD), the cell penetrating peptide of HIV Tat protein, a J-domain of Hsp40 and an X polypeptide was introduced. The X polypeptide was an assortment of either Th2 epitopes (MHC-II associating peptides) and B epitopes, which could associate with B cell receptor, or Th1 epitopes (MHC-II associating peptides) plus Tc epitopes MHC-I associating peptides [1].

At first, the combination of the Th2 epitopes and B epitopes of FMDV VP1 was tested. Humoral immunity was elicited as expected. Hsp72, the inducible member of Hsp70 family encoded by HspA1 [2], which is able of associating with the J-domain [3] and stimulating innate immunity through Toll-like receptor 2 and 4 (TLR2/4) could enhance the FMDV VP1 specific IgG titer for more than one order [4]. Besides the linear B epitopes used in the above instructive case, the head domain (amino acid 361 to 543) of canine adenovirus 1 (CAdv1) capsid fiber protein was examined as a conformational epitope. High neutralization titers (1024 to 4096) against CAdv1 were detected in the canine sera after boost. The membrane penetrating activity of PTD-J-DsRed recombinant protein was about 3 folds higher than that of PTD-DsRed. There were not any detectable membrane penetrating activities for the DsRed and J-DsRed controls. Nearly 7% of PTD-J-DsRed recombinant protein could be transduced into Huh-7 cells [5], therefore, it was speculated that PTD-J could be utilized to present antigen inside a cell. An hTERTepi polypeptide which was composed of human telomerase reverse transcriptase (hTERT) Th and Tc epitopes was designed and PTD-J-hTERTepi fusion protein was prepared. Unlike CT26 colon cancer cells which could colonize in congenic BALB/c mice to form toumrs, CT26(hTERT#10), a CT26 cell line constitutivrly expressing full length hTERT, was rejected. Rest T effector cells isolated from the spleen cells of BALB/c mice which had been immunized with CT26(hTERT #10) could be reactivated by PTD-J-hTERTepi as demonstrated by the expression of IFNγ. It is interested to note that much more cells were activated to express IFNγ when Hsp72 was supplemented.

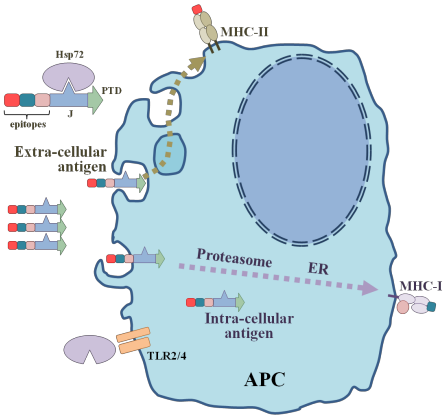

Figure 1: The extracellular and intracelluar routes of the PTD-J-X fusion protein, the PTD-J-epitopes fusion proteins can act as extracellular antigens which are processed in APC via endocytosis route to present the Th epitopes on MHC-II molecules. By using the cell penetrating activity of the PTD, the PTD-J-epitopes fusion proteins can act as intracellular antigens, too. Cellular immunity can be elicited with the cooperation between Th1 cells which are activated by MHC-II-epitope complex and Tc cells which are activated by MHC-I-epitope complex. The cellular immunity can be further enhanced by Hsp72 protein which was found to elicit innate immunity by way of TLR2 and 4 and to stimulate IFNγ expression.

Furthermore, mice pre-treated or post-treated with PTD-J-hTERTepi could diminish the transplanted CT26 (hTERT#10) tumor more quickly than the untreated control. Hsp72 could enhance this effect significantly in the post-treatment cases [6]. It is curious how could Hsp72 augment both humoral and cellular immunity? Besides hyperthermia, cells were induced to express Hsp72 under stresses, such as infection or tumorigenesis. Hsp72 could be secreted and the extracellular Hsp72 acted as a danger signal [7] to alarm surrounding cells and induced TLR2/4 mediated inflammation through Siglec receptors [8]. It was also found that Hsp72 could stimulate dendritic cell maturation [9,10] so as to elevate the antigen presenting capability. IFNγ secreted by the effector cells, predominantly by Th cells [11], could stimulate cancer cells to release Hsp72 [12]. Therefore, it could be imaged that the addition of Hsp72 recombinant protein was involved in the activation and maturation more APCs to augment immunity. Post-translational modifications are the other aspects on the effects of Hsp72. It is worth to mention that methylation of the lysine residue K561 in the lid domain of Hsp72 would change the localization of Hsp72 protein from cytosol to nucleus. The methylated Hsp72 was found associated with chromosomes and interacted with Aurora B

kinase to promote cell proliferation [13,14]. Taken together, these studies constructively suggested that the PTD-J-X system is a versatile platform to present recombinant protein antigens for both humoral as well as cellular immunization. And Hsp72 is a fundamental adjuvant for this system.

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References

1. Chuang CK, Su YS, Fan CT, Chen MY, Lee WC (2009) A dual-functional E. coli vector for expressing recombinant protein with high solubility and antigen presentation ability. Protein Expr Purif 65: 51-56.
2. Kampinga HH, Hageman J, Vos MJ, Kubota H, Tanguay RM, et al. (2009) Guidelines for the nomenclature of the human heat shock proteins. Cell Stress Chaperones 14: 105-111.
3. Kampinga HH, Craig EA (2010) The HSP70 chaperone machinery: J proteins as drivers of functional specificity. Nat Rev Mol Cell Biol 11: 579-592.
4. Lin TY, Su YS, Lin TY, Hung LY, Chuang CK (2013) Hsp70 Protein Can Enhance the Immunogenicity of FMDV VP1 Epitopes Fused with PTD-J Domain. Scholarly J Biol Sci 2: 39-48.
5. Lin TY, Su YH, Lee KH, Chuang CK (2015) The J-domain of heat shock protein 40 can enhance the transduction efficiency of arginine-rich cell-penetrating peptides. Biomed Res Int 2015: 698067.
6. Lin TY, Su YH, Chuang CK (2015) PTD-J Domain acts as an antigen carrier for cell mediated immunity. J Immunotherapy Application 2: 1.
7. Campisi J, Leem TH, Fleshner M (2003) Stress-induced extracellular Hsp72 is a functionally significant danger signal to the immune system. Cell Stress Chaperones 8: 272-286.
8. Fong JJ, Sreedhara K, Deng L, Varki NM, Angata T, et al. (2015) Immunomodulatory activity of extracellular Hsp70 mediated via paired receptors Siglec-5 and Siglec-14. EMBO J 34: 2775-2788.
9. Redzovic A, Gulic T, Laskarin G, Eminovic S, Haller H, et al. (2015) Heat-Shock Proteins 70 Induce Pro-Inflammatory Maturation Program in Decidual CD1a(+) Dendritic Cells. Am J Reprod Immunol 74: 38-53.
10. Valentinis B, Capobianco A, Esposito F, Bianchi A, Rovere-Querini P, et al. (2008) Human recombinant heat shock protein 70 affects the maturation pathways of dendritic cells in vitro and has an in vivo adjuvant activity. J Leukoc Biol 84: 199-206.
11. Young HA, Hardy KJ (1990) Interferon-gamma: producer cells, activation stimuli, and molecular genetic regulation. Pharmacol Ther 45: 137-151.
12. Bausero MA, Gastpar R, Multhoff G, Asea A (2005) Alternative mechanism by which IFN-gamma enhances tumor recognition: active release of heat shock protein 72. J Immunol 175: 2900-2912
13. Cho HS, Shimazu T, Toyokawa G, Daigo Y, Maehara Y, et al. (2012) Enhanced HSP70 lysine methylation promotes proliferation of cancer cells through activation of Aurora kinase B. Nat Commun 3: 1072.
14. Jakobsson ME, Moen A, Bousset L, Egge-Jacobsen W, Kernstock S, et al. (2013) Identification and characterization of a novel human methyltransferase modulating Hsp70 protein function through lysine methylation. J Biol Chem 288: 27752-27763.