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Directed evolution for improved secretion of cancer–testis antigen NY-ESO-1 from yeast

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Abstract

NY-ESO-1 is a highly immunogenic tumor antigen and a promising vaccine candidate in cancer immunotherapy. Access to purified protein both for vaccine formulations and for monitoring antigen-specific immune responses is vital to vaccine development. Currently available recombinant *Escherichia coli*-derived NY-ESO-1 is isolated from inclusion bodies as a complex protein mixture and efforts to improve the purity of this antigen are required, especially for later-stage clinical trials. Using yeast cell surface display and fluorescence activated cell sorting techniques, we have engineered an NY-ESO-1 variant (NY-ESO-L5; $C^{75}A$ $C^{76}A$ $C^{78}A$ $L^{153}H$) with a 100× improved display level on yeast compared to the wild-type protein. This mutant can be effectively produced as an Aga2p-fusion and purified in soluble form directly from the yeast cell wall. In the process, we have identified the epitope recognized by anti-NY-ESO-1 mAb E978 (79–87, GARGPESRL). The availability of an alternative expression host for this important antigen will help avoid artifactual false positive tests of patient immune response due to reaction against expression-host-specific contaminants.
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Cancer–testis (CT)[2] antigens are a class of tumor-associated antigens with expression normally restricted to germ cells in the testis, ovaries or trophoblast cells, and not in adult somatic tissues [1–3]. Although the biological function of these proteins remains unclear, their gene regulation in cancer patients is disrupted, leading to the aberrant expression of CT antigens in a wide variety of tumors. The first CT antigen, MAGE-1, was identified in the early 1990s by T-cell epitope cloning [4,5]. Since then, serological expression cloning techniques (SEREX) [6], recombinant antigen expression on yeast surface (RAYS) [7] and differential mRNA expression analysis [8] have led to the identification of approximately 90 CT antigens, and their number is expected to grow in the coming years. The immunogenicity of some CT antigens in cancer patients makes them an ideal target for the development of tumor vaccines [2]. One of the most promising and widely studied CT antigens in cancer immunotherapy is NY-ESO-1 [9]. This 180 amino acid long protein was first identified by SEREX in an esophageal squamous cell carcinoma in the late 1990s at the

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[1] These authors contributed equally to this work.
[2] *Abbreviations used:* CT, cancer–testis; RAYS, recombinant antigen expression on yeast surface; HLA, human leukocyte; DTH, delayed type hypersensitivity; YSD, yeast cell surface display; FACS, fluorescence activated cell sorting; HRV 3C, human rhinovirus 3C.

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