Developing a MICB-Based Vaccine to overcome immuno-evasive cancer cells

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Background:

The MICA alpha-3 domain and NKG2D: Tumors have developed several mechanisms of evading the immune response. In a typical immune response, ligand engagement of NKG2D causes a co-stimulation of cytokine production, T-cell proliferation and NK-cell activation that allows for rejection of tumor cells. However, in immune-evasive tumors, there is considerable NKG2D downregulation, leading to impaired NK and CD8+ T-cell function. MICA/B was found to induce internalization and degradation of NKG2D receptors. MICA/B is a ligand that is expressed on the cell surface during cellular stress whose expression and interaction with NKG2D can be affected by the disulphide isomerase, Erp5. Erp5 proteolytically cleaves MICA at the alpha3 domain, causing it to be shed from the tumor cell surface. The cleavage of the MICA domain from the surface and presence of unbound, shed MICA in the sera allows tumor cells to avoid detection by NKG2D-expressing immune cells as well as reduce overall immune competence since the shed MICA can cause internalization and degradation of NKG2D on NK-cell surfaces. Thus, if the Erp5-MICA/B interaction can be inhibited and MICA/B can be stabilized on the surface, NK cells should effectively be able to find and eradicate tumor cells expressing MICA/B. This investigation will strive to stabilize MICA/B on the surface via an antibody specific to the alpha3 domain of the MICA/B receptor as shown in Figure 2 below.

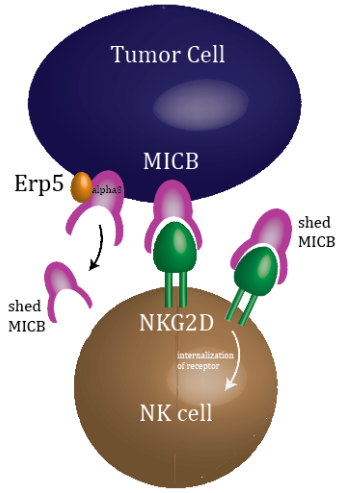


Figure 1: Shedding of MICB via Erp5 interaction and subsequent effects of shed MICB on the MICB-NKG2D synapse

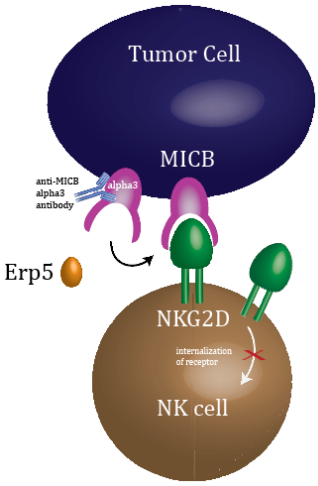


Figure 2: Proposed therapeutic strategy to stabilize MICB protein via production of an antibody against the MICB alpha-3 domain

MICB-ferritin nanoparticle vaccines: The antibodies themselves are generated using a MICB based-ferritin nanoparticle vaccine described by Kanekiyo et al in 2013. Ferritin is a protein that naturally forms nanoparticles composed of 24 identical polypeptides and conjugation of ferritin with the antigen of interest produces a nanoparticle vaccine that externally presents the antigen on each of the 24 subunits. This structure-based, self-assembling synthetic nanoparticle vaccine has been experimentally shown to improve the potency of the produced antibody titer (4). To further enhance the immune response against MICB, the nanoparticle vaccines will be injected alongside with mesoporous silica rods (MSRs) which spontaneously assemble in-vivo to create a cellular microenvironment to recruit host immune cells and sustain an immune response to further strengthen the immune response generated against the alpha3 domain of MICB.

Results:

(1) Successful MICA/B ferritin nanoparticle vaccine cloning, purification and isolation

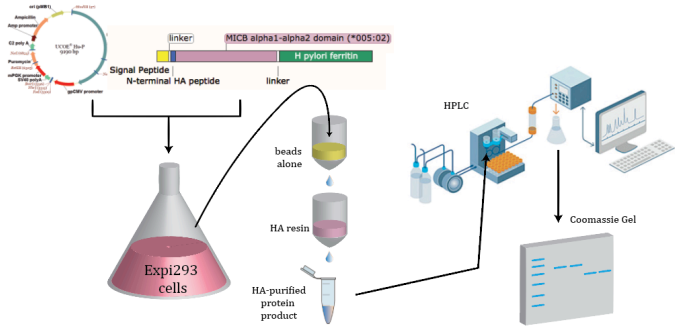


Figure 3: The MICB005-ferritin phageZsgreen construct is inserted into the UCOE vector and transfected into Expi293 cells. The cell supernatant is run over a pre-clear column and purified further using Superose 6 size exclusion column on the HPLC (Figure4). The purified protein is analysed by running a Coomassie gel (Figure 5).

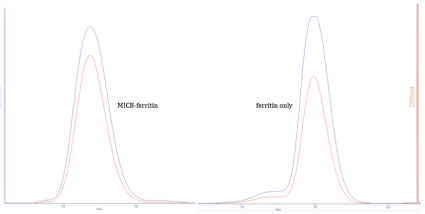


Figure 4: HPLC data shows fraction created by MICB-ferritin protein at 12 minutes and the fraction created by the ferritin protein at 15 minutes

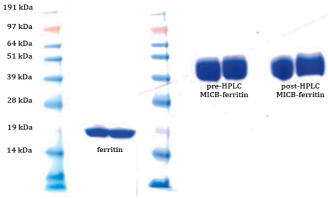


Figure 5: Coomassie stain to confirm successful protein purification.

(2) MICB005 phage Zsgreen B16F10 successfully binds to mouse and human NKG2D:

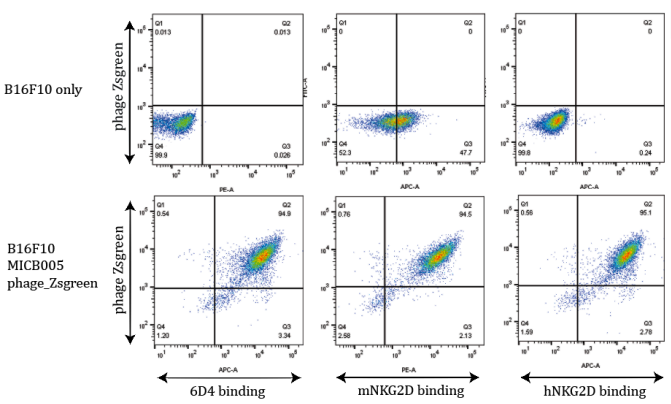
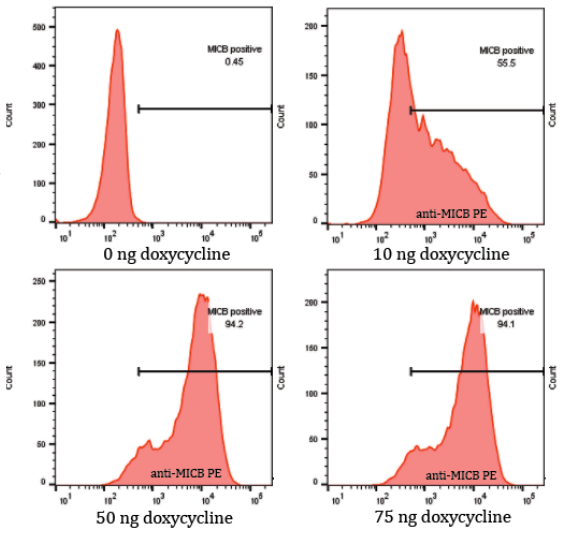


Figure 6 (left): FACS results show that the MICB005_phageZsGreen B16F10 cells successfully bound to 6D4, mouse NKG2D and human NKG2D while B16F10 only does not. This demonstrates that not only do MICB transfected B16F10 cells interact with NKG2D, but also that the MICB construct used reacts similarly with mouse NKG2D as human NKG2D which is informative for future mouse-model studies with the vaccine.

Figure 7 (below): *Regulator (stably expresses TetOn3G) and response (TRE3G promoter and MICB gene) lentiviral vectors are co-transduced into target cells. The doxycycline controlled-transactivator domain specifically binds to the tetracycline response element, turning on expression of MICB in the presence of Dox. Following staining with antiMICB PE, flow cytometry results show that for sorted B16F10 cells with high MICB expression, there is an increase in MICB expression associated with an increase in doxycycline dosage.*

(3) Creation of successful doxycycline inducible MICB expression in-vitro



Conclusions:

(1) The MICB-ferritin construct was successfully produced using a mammalian expression system and will be tested using murine melanoma tumor models.
(2) Successful binding of the MICB-phageZsGreen construct B16F10 cells to mNKG2D and hNKG2D demonstrates that the construct will work as expected in mouse models and in human clinical trials in binding to NK cells via NKG2D.
(3) MICB expression was effectively induced in-vitro with increasing concentrations of doxycycline. The doxycycline inducible system will be used in a therapeutic setting to determine if pre-existing tumors can be regressed in-vivo.
These conclusions are promising for the development of a MICB-based vaccine that will both help prevent immune-evasive tumors and potentially regress the formation of existing tumors.