Developing a MICB-based vaccine to overcome immune-system evasion by cancer cells

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Introduction

- Tumors have developed several mechanisms of immune system evasion. Ligand engagement of NKG2D by cellular stress ligand MICA/B typically induces cytokine production & T-cell proliferation. Tumor cells have evaded immune stimulation by cleaving MICA/B from the surface disabling recognition by NKG2D and causing internalization of existing NKG2D receptors, compromising immunological competence.

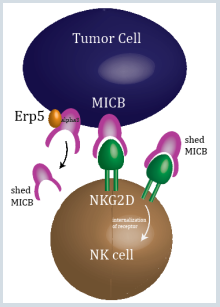

Figure 1: Shedding of MICB via Erp5 interaction and subsequent effects of shed MICB on the MICB-NKG2D synapse

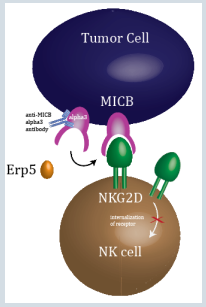

Figure 2: Proposed therapeutic strategy to stabilize MICB protein via production of an antibody against the MICB alpha-3 domain

- Ferritin is a 24 subunit protein that has been efficient in antigen presentation and immune-stimulation. Hence, a ferritin nanoparticle vaccine is used to generate antibodies against the MICB alpha3 domain.

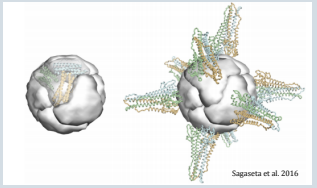

Figure 3: Ferritin nanoparticle design as described by Kanekiyo et al, 2013 where the HA antigens are presented externally on each subunit of the 24 subunits of the ferritin nanoparticle vaccine.

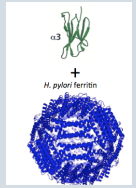

Figure 4: Application for antigenic presentation of the MICB alpha3 protein.

- Mesoporous Silica Rods (MSRs) spontaneously assemble in-vivo to create a cellular microenvironment where host immune cells are recruited. The scaffold engages in a sustained release of inflammatory signals and adjuvants.

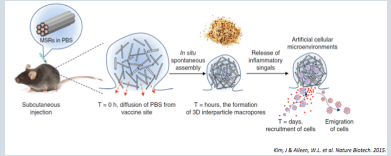

Figure 5: Function of MSRs from injection, spontaneously assembly to recruitment of host immune cells.

Methods

MICB005:02-phage-Zsgreen Cloning

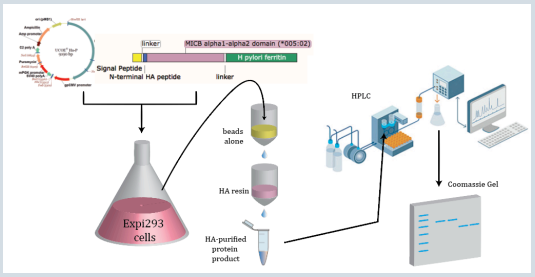

Figure 6: MICB005-ferritin phage2sgreen construct is inserted into the UCOE vector and transfected into Expi293 cells. The cell supernatant is run over a pre-clear column and then immunoprecipitated with anti-HA affinity column. The protein is eluted from the column and purified further using Superose 6 size exclusion column. The purified protein is analysed by running a Coomassie gel.

Doxycycline Inducible System

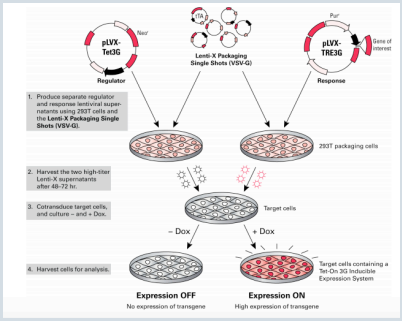

Figure 7: Regulator (stably expresses Tet0n3G) and response (TRE3G promoter and MICB gene) lentiviral vectors are co-transduced into target cells. The doxycycline controlled-transactivator domain specifically binds to the tetracycline response element, turning on expression of MICB in the presence of Dox.

MICB Transgenic Mouse Model

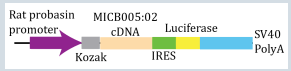

Figure 8: The transgene insert contains a probasin promoter which ensures organ specificity, Kozak sequence and IRES for translation, the MICB protein, and Luciferase for detection in-vivo. The transgenic DNA is cloned similarly to as shown above.

Results

HPLC Data

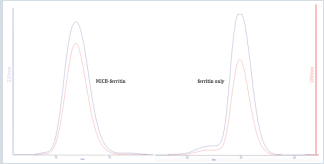

Figure 9: HPLC data shows fraction created by MICB-ferritin protein at 12 minutes and the fraction created by the ferritin protein at 15 minutes using a Superose 6 size exclusion column

Protein purification confirmed with Coomassie gel

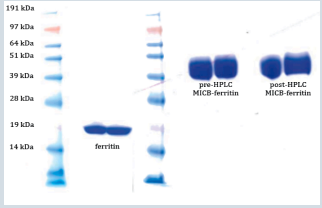

Figure 10: Coomassie stain to confirm successful protein purification. The left band is the unreduced protein while the right band shows the reduced protein. Ferritin is approximately 450kDa, with 24 subunits so each subunit is approximately 18.75kDa as shown in the Coomassie gel. MICB ferritin is approximately 39kDa.

Flow cytometry results show induction of MICB expression with doxycycline

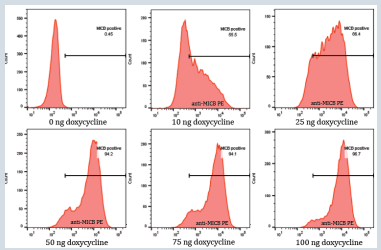

Figure 11: Following staining with antiMICB PE, flow cytometry results show that for sorted B16F10 cells with high MICB expression, there is an increase in MICB expression associated with an increase in doxycycline dosage.

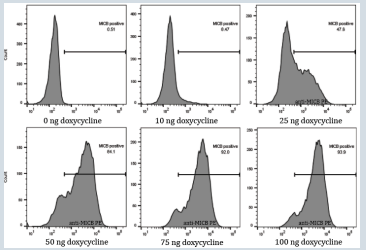

Figure 12: Following staining with antiMICB PE, flow cytometry results show that for sorted B16F10 cells with low MICB expression, there is an increase in MICB expression associated with an increase in doxycycline dosage.

MICB binds to mouse NKG2D

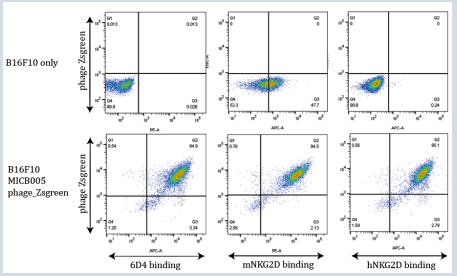

Figure 12: FACS results show that the MICB005_phageZsGreen B16F10 cells successfully bound to 6D4, mouse NKG2D as well as human NKG2D while B16F10 only does not.

Identification of MICB transgenic insert by PCR

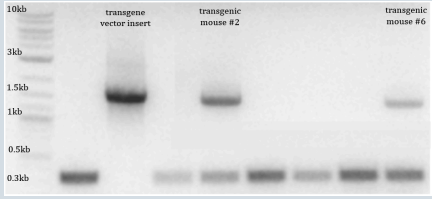

Figure 13: DNA isolated from the tails of transgenic mice show the presence of the transgenic insert in two of the six mice in the litter.

Conclusions and Future Work

- The MICB-ferritin construct was successfully cloned and protein was produced using mammalian expression system. Purified MICB-ferritin will be loaded onto MSRs and the efficacy of the vaccine will be tested using murine melanoma tumor models.
- 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.
- The transgenic insert was successfully detected in 33% of mice in-vivo. This transgenic strain will be important to break tolerance and ensure the produced prophylactic response seen by the MICB-ferritin vaccine is not just an innate immune response to non-self antigen.

Acknowledgements

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