Permanent Abstract Number: 731

A minigene platform to validate novel immunogenic peptides arising from somatic mutations as therapeutic cancer vaccines

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Abstract

Cancer vaccines have shown promise in treating late stage cancers, which have failed traditional therapies. Somatic mutations provide a rich source of potential cancer vaccines with minimal T cell tolerance. We built a proprietary cancer vaccine prediction platform, OncoPept*VAC* using a combination of features that include TCR binding, human leukocyte antigen (HLA) binding, gene expression and proteasomal processing. By applying OncoPept*VAC* on a set of patient-derived somatic mutations, we prioritized potential immunogenic peptides for validation. To validate the immunogenicity of the mutant peptides, we developed a robust CD8$^+$ T cell – dendritic cell co-culture assay to examine T cell activation in the presence of added synthetic peptides. Our screening assay revealed that non-mutated wild-type peptides in many instances induced detectable T cell activation, although their HLA binding affinity was weak. We suspect that addition of high concentration of the peptides from outside may overcome their weak HLA-binding property, allowing them to bind HLA and activate T cells. To reduce the bias of artificial HLA presentation, we have developed a minigene platform to screen wild-type and mutant peptide pairs to test their immunogenicity. Predicted mutant immunogenic peptides were cloned as minigenes and expressed in HLA restricted antigen presenting cells (APCs). The minigene expressing APCs were co-cultured with autologous CD8$^+$ T cells and T cell activation was monitored by flow cytometry. Our assay reveals that mutant peptides expressed as both short 9-mers peptide flanked by proteasomal cleavage sites as well as 25-mers with native cleavage sites triggered the generation of cytotoxic T lymphocytes. In conclusion, our analysis demonstrates that the two approaches for investigating immunogenicity of peptides - minigene approach and external addition of peptide approach - have differential utilities for testing and validating the immunogenicity of somatic mutations derived from tumors.

MedGenome's proprietary OncoPept*VAC* pipeline to predict cancer vaccines from somatic mutations

Fig 1A.

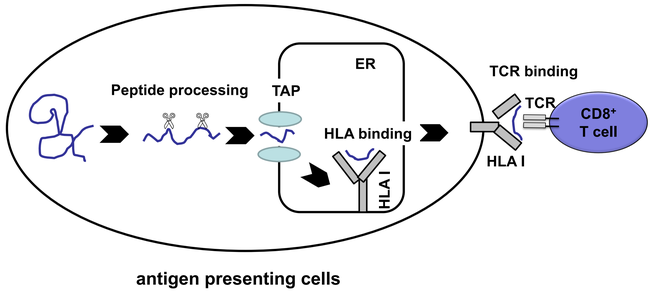

Fig 1B. OncoPept*VAC* pipeline for the prediction of immunogenic peptides from somatic mutations

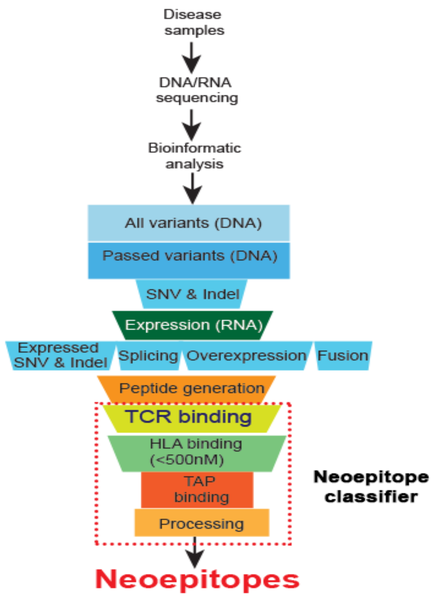

Figure 1. MedGenome's proprietary OncoPept*VAC* pipeline predicts cancer vaccines with high accuracy.

A. Biological process of antigen presentation by human leukocyte antigen (HLA) class I molecules. Proteins are processed by either proteasome or other proteases to generate small peptides. Transporter associated with antigen processing (TAP), a protein that spans the membrane of the endoplasmic reticulum (ER), transports the peptides into the ER. HLA I binds with the peptides and transports them to the cell surface. Immunogenic peptide - HLA I complex induces CD8$^+$ T cell activation.

B. Workflow of OncoPept*VAC* pipeline. Whole exome sequencing and RNA-seq are performed using the genomic DNA and RNA from the patient's tumor. All mutant peptides are passed through the OncoPept*VAC* pipeline. The potential immunogenic peptides are predicted based on a series of prioritization steps including peptide processing, peptide TAP binding, HLA binding and TCR binding.

Development of a dendritic cell - T cell assay with synthetic peptides added from outside

Fig 2A.

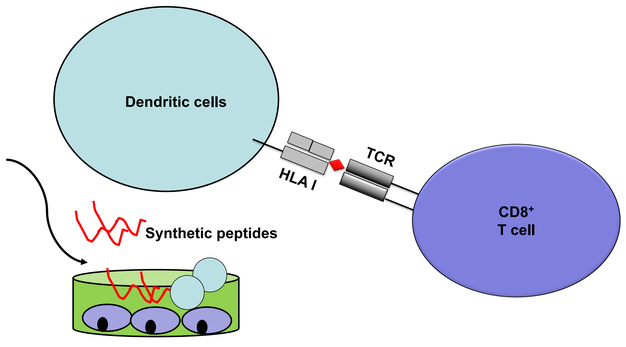

Fig 2B.

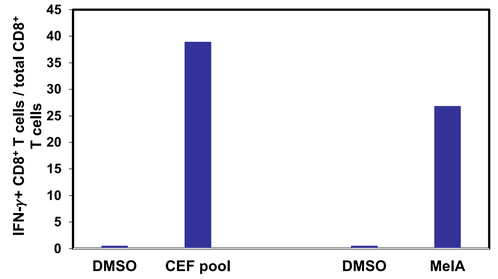

Fig 2C.

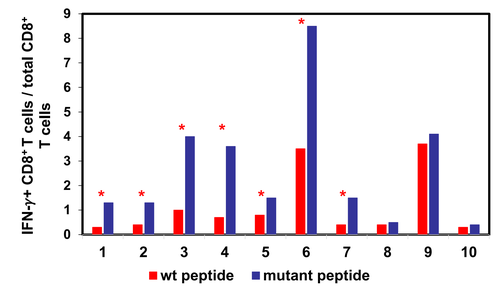

Figure 2. Development of a dendritic cell - T cell assay to examine the immunogenicity of peptides by addition of the synthetic peptides from outside.

A. Illustration of the dendritic cell - T cell co-culture using synthetic peptides. Monocytes isolated from human peripheral blood mononuclear cells (PBMCs) are differentiated into mature dendritic cells (DCs). Mature DCs are incubated with synthetic peptides and then co-cultured with autologous CD8$^+$ T cells isolated from the same human PBMC. [1]

B. Summarized flow cytometry data showing that the known immunogenic peptides trigger an increased production of IFN-γ^+ CD8$^+$ T cells compared to the DMSO control in the synthetic peptide DC - T cell assay.

C. 10 different neo-epitopes predicted by OncoPept*VAC* pipeline are tested in the synthetic peptide DC - T cell assay using their wild-type counterparts as control. 7 out of 10 neo-epitopes induce T cell activation as indicated by asterisks.

Caveats of synthetic peptide DC - T cell assay:

- Neo-epitopes which trigger T cell activation may not be processed and presented inside dendritic cells, when peptides are added from outside.
- Wild-type peptides may non-specifically stimulate T cell activation as they are added in bulk.
- Peptide synthesis can be expensive and time-consuming.

References

1) Wolfl and Greenberg. 2014. Antigen-specific activation and cytokine-facilitated expansion of naive, human CD8+ T cells. *Nat Protoc.* 9(4): 950–966.
2) Lu et al. 2014. Efficient identification of mutated cancer antigens recognized by T cells associated with durable tumor regressions. *Clin Can Res.* 20(13):3401-10.
3) Aurisicchio et al. 2014. A novel minigene scaffold for therapeutic cancer vaccines. *OncoImmunology.* 195:3724–3733.

Development of a minigene dendritic cell - T cell assay platform

Fig 3A.

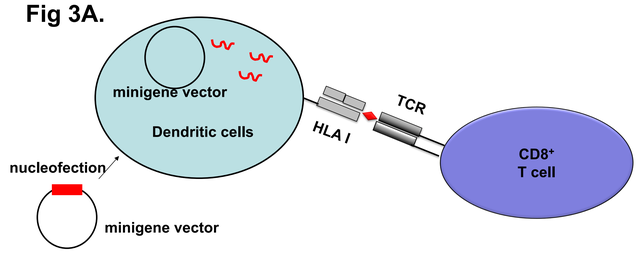

Fig 3B.

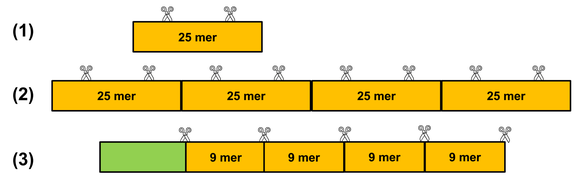

Fig 3C.

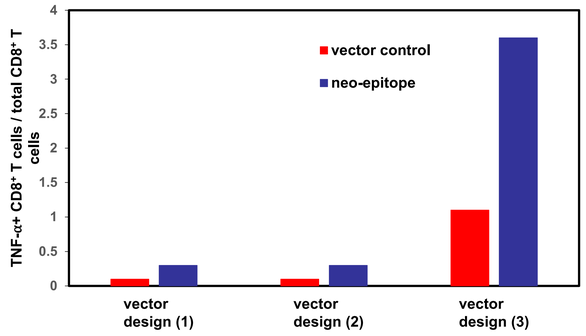

Figure 3. Validation of the immunogenicity of the neo-epitopes by a minigene dendritic cell - T cell assay.

A. Illustration of the minigene dendritic cell - T cell co-culture assay. Monocytes are extracted from human PBMCs and matured into DCs. Vectors expressing short peptides as minigenes are nucleofected into DCs. Autologous CD8$^+$ T cells from the same PBMCs are incubated with nucleofected DCs.

B. Different designs of minigenes encoding either 9-mers neo-epitope flanked by the proteasomal cleavage sites or 25-mers neo-epitope with its native cleavage sites.[2,3]

C. Representative data show neo-epitope encoded by different forms of minigene vectors induce more cytotoxic CD8$^+$ T cells' production compared to the empty vector control as determined by flow cytometry.

Conclusions & Future Directions

- Testing of two different assays - minigene platform and external addition of synthetic peptides - are successfully developed to evaluate the immunogenicity of mutant peptides derived from somatic mutations from patients' tumors.
- A "high-throughput" minigene assay is under development to meet the needs for clinical applications.