ImmunoTools *special* Award 2015

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Identification of tumor specific antigens associated with RET/PTC3 expression

Relocated in transformation/papillary thyroid carcinoma, RET/PTC3 (RP3) is a fusion oncogene that causes a form of papillary thyroid cancer (PTC).[1] In addition to driving transformation, the constitutively active kinase precociously phosphorylates itself as well as other intracellular proteins, thereby providing tumor-specific targets for the adaptive immune system. Inappropriate and constitutive phosphorylation that drives transformation can also drive neoantigen formation.[2]

This project focuses on the ability of RP3 to produce neoantigens via phosphorylation, a modification that is easily detected by T lymphocytes. Specifically, we hypothesize that the autophosphorylating activity of RP3 is responsible for previously documented RP3 antigenicity and, further, that aberrant RP3 activity produces additional "downstream" phosphopeptide-based tumor-specific targets.

It has been demonstrated by mass spectrometry sequencing that CD4 T cells can readily discriminate between an MHC class II-bound phosphopeptide and its unphosphorylated counterpart.[3] This discriminative ability gives credence to the proposition that therapeutic immunization with phosphopeptides in order to enhance tumor-specific immunity need not lead to autoimmune manifestations.[4] This further justifies interest in identifying phosphopeptides in RP3-expressing cells. This proposal focuses on MHC-II-restricted presentation of RP3-derived peptides. Thus far, we have detected only MHC-II-restricted responses to RP3-expressing cells. Indeed, we already have demonstrated a CD4 T-cell response to the kinase domain of RP3.[5] CD4 T-cell participation is increasingly appreciated as being critical for effective and long lasting anti-tumor immunity.[6] The kinase activity of RP3 could thus lead to the generation of far more immunogenic phosphopeptides which can be used in future vaccination strategies against RP3 mediated tumor.

In order to identify peptide sequences that are immunogenic on the basis of their phosphorylations, we will obtain pairs of overlapping phosphopeptides for each of the 16 potential phosphorylation sites. This phosphopeptide library will also be tested in ELISpot assay using splenocytes coming from mice immunized with $MHCII^{+}RP3^{+}$ cells or $MHCII^{+}RP3^{-}$ cells as control.

Moreover, we will evaluate the role of RP3 oncogene in suppressing the immune response. As above, we will immunize two group of C57BL/6 mice with $MHCII^{+}RP3^{+}$ or $MHCII^{+}RP3^{-}$ cells, after 15 days mice will be scarify, spleens collected and splenocyes will be pulled together. Thanks to ImmunoTools anti-mouse antibodies, we will test by flow cytometry the level of myeloid-derived suppressor cells (MDSCs), dendritic cells, NK cells and the functionality of $CD4^{+}$ and $CD8^{+}$ T cells.

In conclusion, cancer immunotherapy continues to be an attractive but unpredictable treatment option. In contrast to infectious agents, most malignancies rarely display abundant antigenicity and/or the inflammatory signals that are required to induce an effective adaptive response. This constitutes a major roadblock for cancer immunotherapy. An exception may be papillary thyroid carcinoma which appears to provide both signals in substantial quantities. Exploring the immunogenic properties of such a neoplasm could enhance general principles of cancer immunotherapy.

References:

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ImmunoTools *special* AWARD for **Laura Sponton** includes 25 reagents

FITC - conjugated anti-mouse CD3e, CD4, CD8a, CD11a, CD11b, CD80, Gr-1, a/b TCR, isotype control IgG2b

PE - conjugated anti-mouse CD3e, CD4, CD8a, CD11a, CD11b, CD80, Gr-1, NK cells, g/d TCR, isotype control IgG2b

APC - conjugated anti-mouse CD4, CD8a, CD11a, CD11b, Gr-1, isotype control IgG2b