p53 mutant cell line as a model for a neoantigen discovery pipeline

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Introduction

Currently, there is great interest in the potential of personalized cancer therapies. Use of a patient's MHC peptides to reawaken killing mechanisms has gained renewed interest due to availability of patient specific genomes. We used WT and p53 knock out mutant cells as a way to easily generate mutated peptides as surrogates for neoantigens. These cell lines were profiled by shotgun proteomics using two different separations online with an Orbitrap: 1) a standard nanoLC packed column and 2) a pillar-arrayed-column (µPAC). The data was used to develop an informatic proteogenomic pipeline, which could be applied to neoantigen discovery, and separation performance of the two methods compared for ability to detect mutated peptides.

Materials and methods

A nanoRSLC UltiMate 3000 coupled to an Orbitrap Q-Exactive Biopharma was used. Sample was loaded onto the 300µmIDx 5mm PepMap C18 trap column and separated either on a µPAC (Pillar-Arrayed-Column, PharmaFluidics) cartridge with 2µm interpillar distance and 2 m separation path operated or on a PepMap C18 (2µm, 100Å; 75µm ID x 50cm).

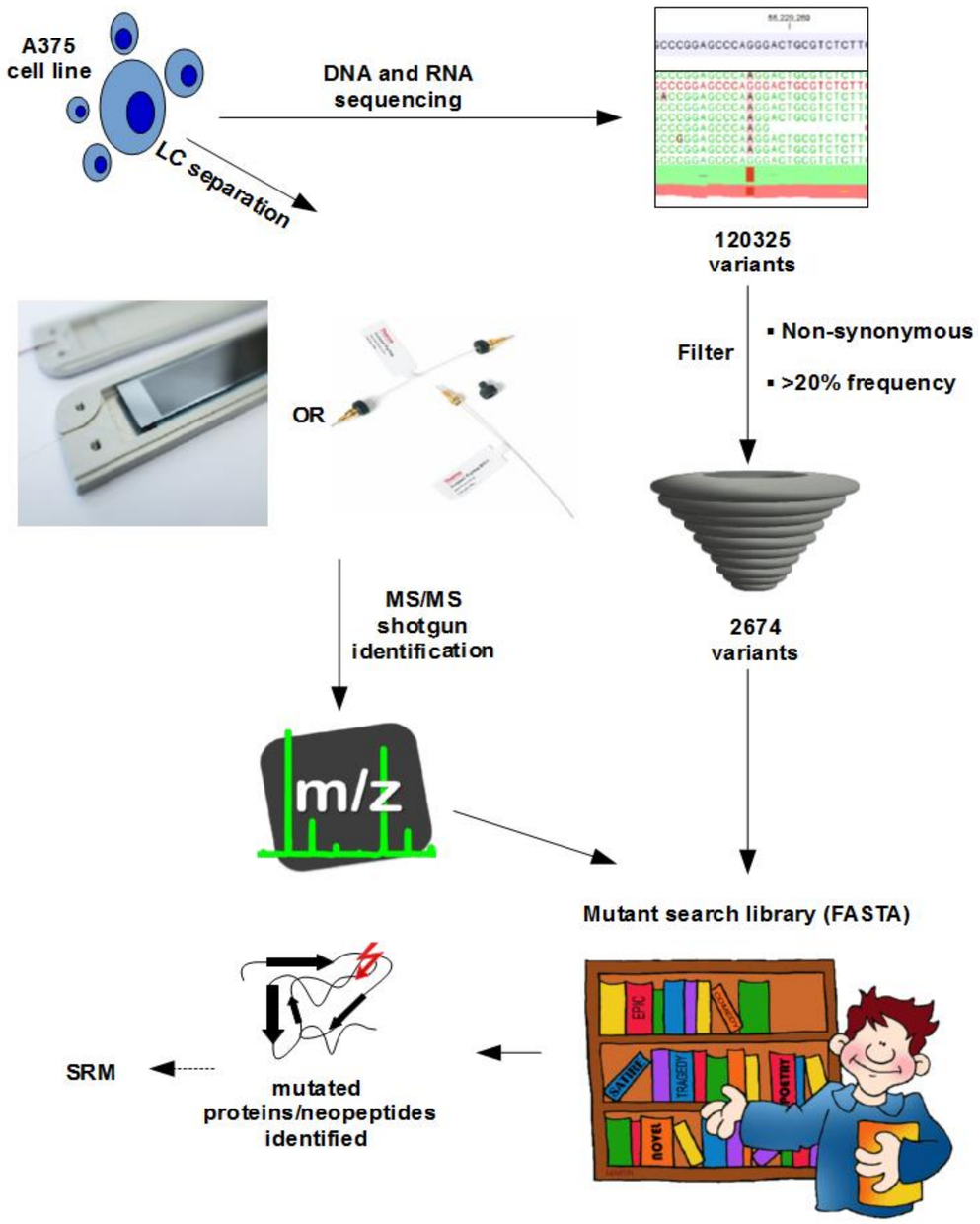

Fig.1 A proteogenomic platform to identify mutations in protein sequences.

Results

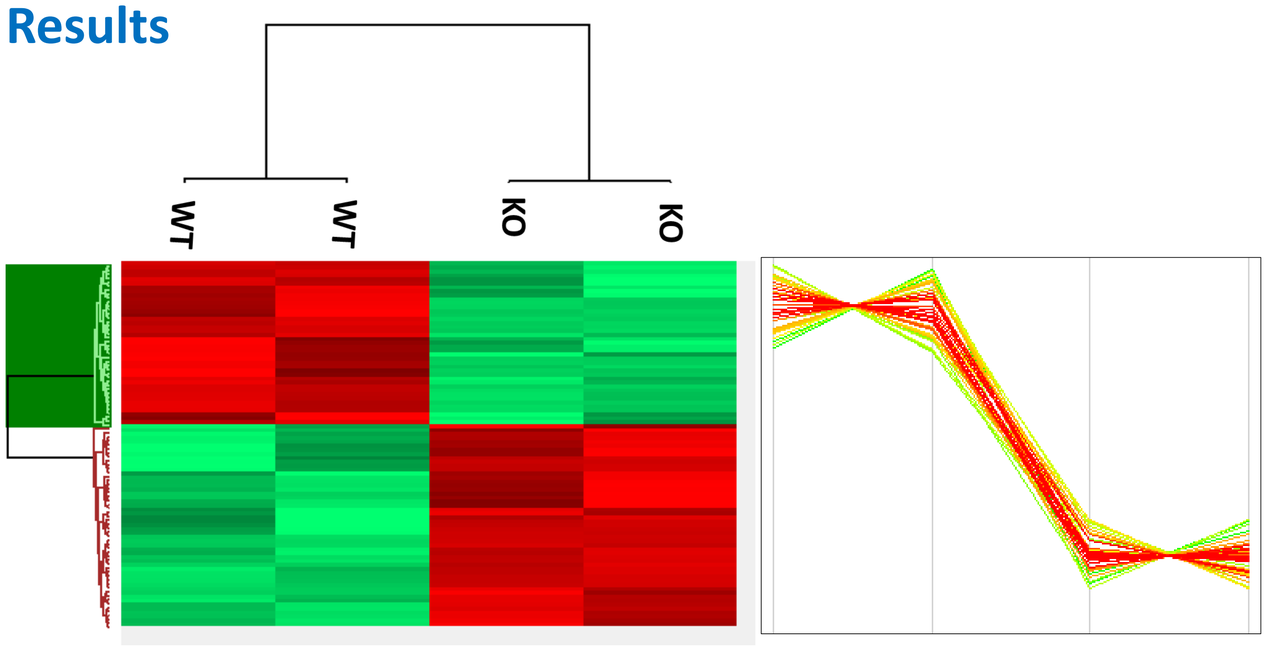

Fig.2 Hierarchical clustering of Z-scored intensities for highly significant proteins ($P < 0.05$) resulted in WT and p53 knock out mutant cells.

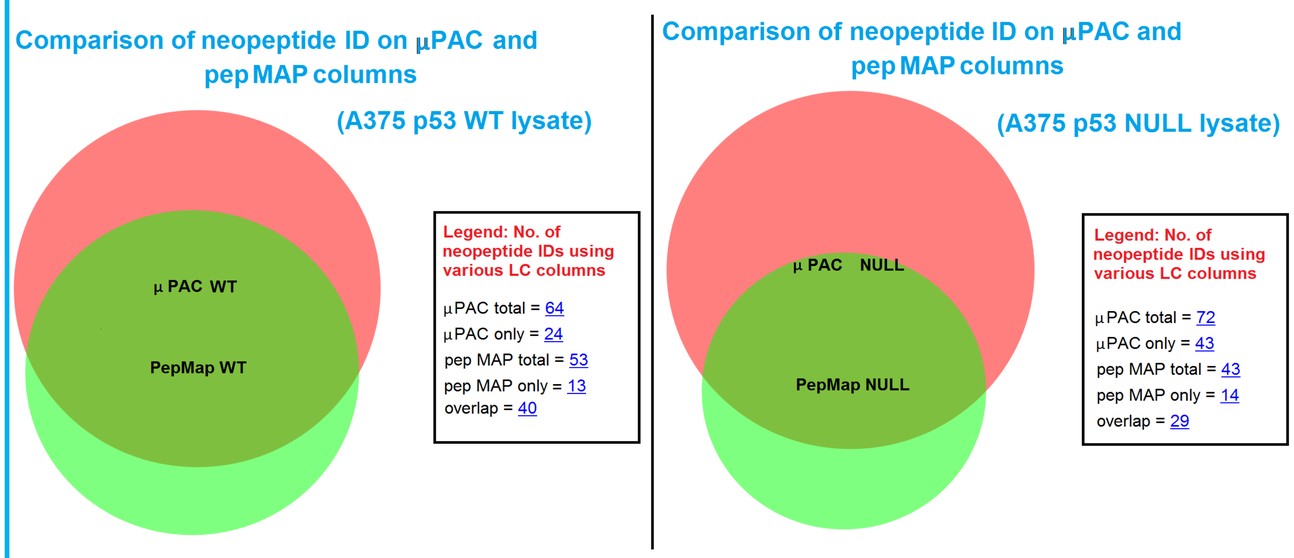

Fig.3 A comparison of neopeptide ID on uPAC and PepMap columns in A375 cell line with WT p53 and CRISPR on p53.

Conclusion

p53 mutant cell lines allows to identified differentially express and significance proteins which may have role in the progression of cancer. Pillar-arrayed-column outperformed traditional NanoLC column for mutant peptide detection. The µPAC column showed considerable lower backpressure and higher peak capacity with excellent reproducibility in comparison to the PepMap column. This proteogenomic pipeline will benefit from the better separation of the µPAC column tha t will provide better coverage of the mutational landscape in tumor tissues and will be adopted to neoantigen discovery.

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