Proteogenomics Identifies Common Drugabble Pathways in Undifferentiated Pleomorphic Sarcoma

Marcos Y Mayordomo[1,2]; Georges Bedran[1,2]; Nathan a Grimes[1]; Larry Hayward[1]; Jakub Factor[1]; Rob O'Neill [3]; Borek Vojtesek[4]; Helen Creedon[1]; Satya Saxena[5]; Katy Teo[1]; Val Brunton[1]; Donald Salter[1]; Ted Hupp[1,2]; Javier A Alfaro [1,2]

[1]University of Edinburgh, Edinburgh, United Kingdom; [2]University of Gdansk, Gdansk, Poland; [3]University of Cambridge, Cambridge, United Kingdom; [4]Masaryk Memorial Cancer Institute, Oncology, Czech Republic; [5]University of Baltimore, Baltimore, MD

INTRODUCTION

Recent advances in mass spectrometry have provided extensive analysis of cancer proteomes. Combined with the profiling of somatic alterations by next-generation sequencing, integrated proteogenomic analysis have become one of the best methods for therapeutically approach cancer diseases.

Undifferentiated pleomorphic sarcomas (UPS) are the most common form of adult sarcoma. As with most rare cancers, there are limited systemic treatment options. To seek novel therapeutic approaches we characterized the proteogenomic landscape of UPS.

Oesophageal adenocarcinoma (OAC) is another type of cancer where proteogenomics can be an useful tool. The main treatment for OAC patients is high-risk surgery and only benefits patients with localize tumors, making necessary the discovery of new treatment and early detection methods.

METHODOLOGY

Next generation sequencing (NGS) of 20 UPS exomes, 12 OAC whole genomes and their matched normal tissue defined the somatic mutational landscape of single nucleotide variants (SNVs) and copy number variants (CNVs).

Additionally we analyzed the transcriptomes and the proteomes of the matched patient samples using RNA sequencing and combined DDA and SWATH mass spectrometry.

RESULTS

Copy number variation analysis identified that co-mutation or deletion in RB1 and p53 loci in 15/20 patients highlighting loss of key cell-cycle checkpoint pathways as an important signature of UPS (**Figure 1**).

However, single nucleotide variations were largely patient-specific with very few common mutations identified between patients. A combination of DDA and SWATH-mass spectrometry identified commonly over-produced pathways contrasting with the SNV heterogeneity between patients.

Nevertheless, common intra-tumour variants in T-cell receptor variable regions identified by deep sequencing suggest an oligoclonal immune infiltrate in the range associated with other cancers responsive to immunotherapy.

Another example of proteogenomic integration that we have performed is on a dataset of 12 OAC patients. Combined variant calling of DNA and RNA sequencing were used to build custom libraries to scan for possible mutations detected on protein level by mass spectrometry.

In figure 2 we can see an example of one patients of the OAC cohort. Mutated genes detected in RNA-sequencing were represented against detected genes in mass spectrometry. The libraries used for this experiment were a combination of the detected SNV in genomic level, common OAC biomarkers and a list of common mutated genes from the Catalogue of Somatic Mutations in Cancer (COSMIC).

The mutations present on these libraries were highlighted on the figure. In order to obtain the evidence of the variant nucleotide in the RNA level, a further examination of the mutations was performed by exploring the mapped sequenced reads covering the specific regions.

To validate the tumour-specific mutation on the protein level the evidence peptide was also explored to determine the number of reporter ions for the mutated peptides.

The combination of NGS and mass spectrometry have provided a valuable insight of the mutated proteome inside tumour cells, granting a unique opportunity to develop personalized cancer treatments.

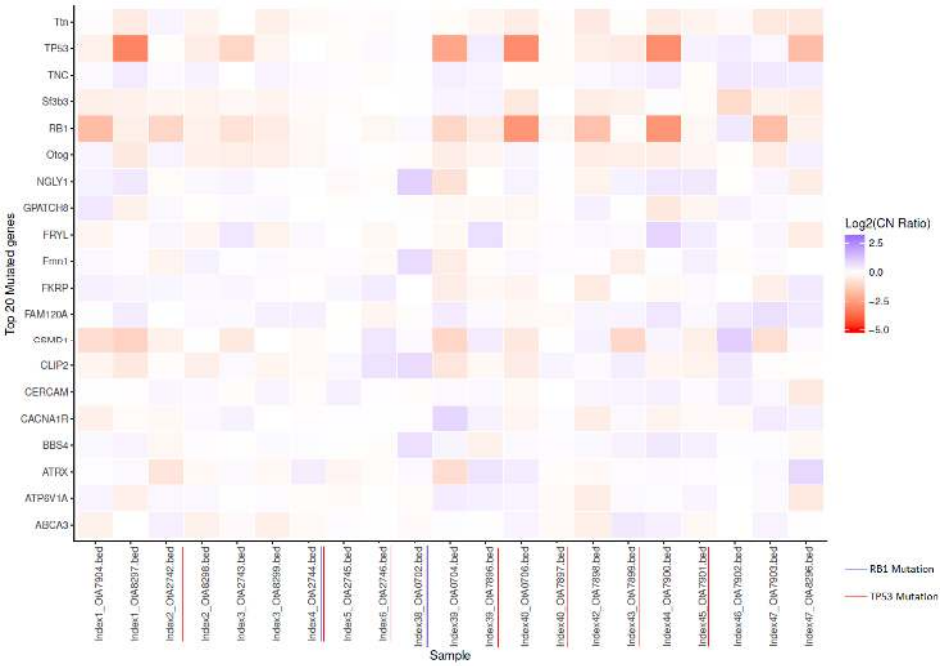

Figure 1. Heat map of the log2 copy number ratio from the top 20 mutated genes in 20 patients of undifferentiated pleomorphic sarcoma. 15 of 20 patients show an alteration in the TP53 and RB1 locus

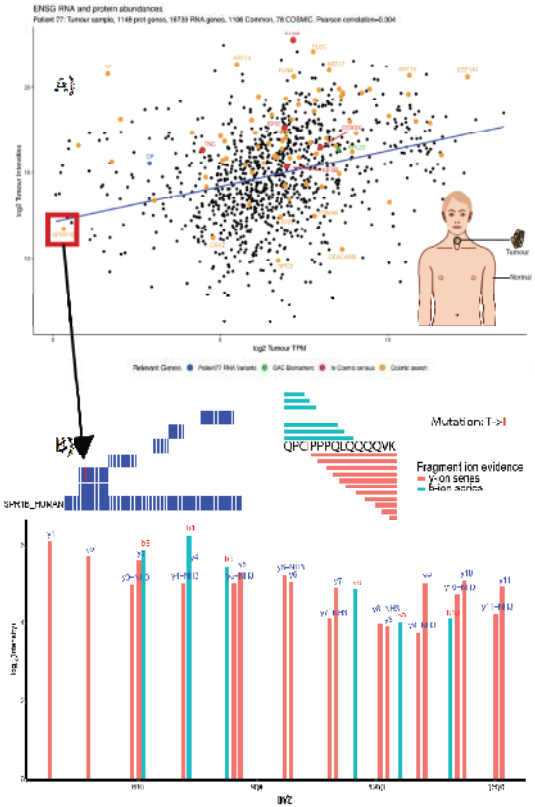

Figure 2. **A)** Example of RNA (x-axis) to protein (y-axis) abundance correlation in a single Oesophageal cancer patient. Potential mutated proteins identified by searching genomic data or public databases are highlighted in orange. **B)** Evidence for a specific mutation identified by proteomics is highlighted.

CONCLUSION

Our data demonstrate that the UPS cancer proteome is non-trivially influenced genomic, transcriptomic and post-transcriptional dysregulation.

These data suggest that cancer-specific neopeptide vaccines might be the most realistic, common option for developing therapeutics that target the population of patients with UPS and that the modelling of neoantigen flux in UPS needs to be performed within the background of p53 and RB loss.

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