Identifying drug therapies for plexiform neurofibromas in Neurofibromatosis Type 1 through chemo-genomic analysis

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Background: Plexiform neurofibromas (pNFs) impact ~40% of people with Neurofibromatosis Type 1 (NF1), a common neurogenetic syndrome resulting in tumors throughout the nervous system. pNF may cause disfigurement, neurologic morbidity and malignant transformation to sarcoma. As there are no standard therapies for these tumors, there is an urgent need for new therapeutic agents. This work seeks to characterize the genomic and transcriptomic profile of a panel of human tumor-derived pNF cell lines to explore how genetic background interacts with drug response in NF1 pNF to inform mutation specific drug discovery.

Diverse set of Plexiform NF cell lines

To compare the effect of NF1 loss in culture, we collected data from a panel of human cell lines including NF1 WT Schwann Cells (indicated in red below), non-tumor nerve from NF1 patients (indicated in green below) and plexiform neurfibromas from NF1 patients (blue). Cells were immortalized via the viral delivery of human TERT and murine Cdk4.[1]

Table 1: Description of cell lines included in this study. Cell lines comprise NF1 +/+ cells (in red), NF1 +/- cells (in green) and NF1 -/- cells (in blue).

Sample Name	NF1 Genotype	Cell Line
whole blood	++	
ipn02.3	++	Immortalized
pn02.3	++	Primary
ipn02.8	++	Immortalized
ipnNF95.11c	+-	Immortalized
pnNF95.11c	+-	Primary
pNF95.11bC	—	Primary
ipNF95.11bC	--	Immortalized
pNF05.5	—	Primary
ipNF05.5 (single clone)	--	Immortalized
ipNF05.5 (mixed clone)	--	Immortalized
pNF95.6	--	Primary
ipNF95.6	--	Immortalized
ipNF06.2A	--	Immortalized

Molecular characterization of NF1

We assessed copy number changes in the pNF cell line panel using Illumina OMNI arrays. SNP arrays were segmented to identify regions using DNACopy from Bioconductor.[2] Log ratio values surrounding the NF1 gene region are depicted in Figure 1. Loss of heterozygosity was confirmed in the two primary cultures, pNF05.5 and pNF05.5 and their immortalized counterparts. Each of the remaining set of -/- cell lines were independently confirmed to have an NF1 point mutation.

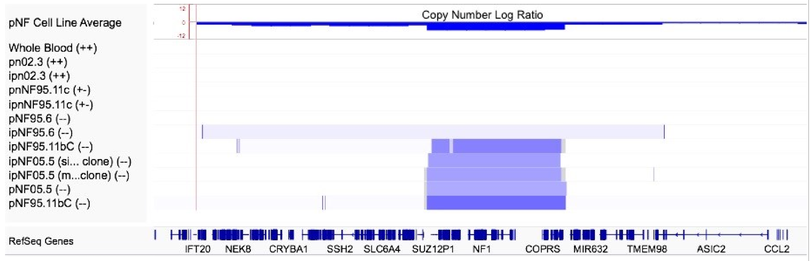


Figure 1: Log R Ratios of cell line panel in genomic region surrounding NF1 gene locus. The more blue in the region, the more loss measured in that region.

Paired-end RNA-seq was carried out in the cell lines on the Illumina HiSeq. Reads were quantified with Kallisto[3] aligned to Gencode[4] v24 gene annotations.

Total transcripts per million (TPM) of each *NF1* gene are shown in Figure 2. Primary cell cultures with homozygous loss of *NF1* exhibit lower expression of *NF1* compared to those cell lines with the *NF1* gene. However, in both the *NF1*$^{+/-}$ cell lines (pNF9511.c) and the *NF1*$^{+/+}$ cell lines (pn02.3), *NF1* expression is lower in the immortalized cells.

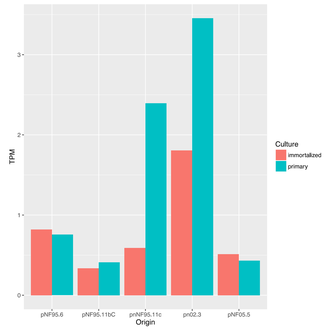


Figure 2: Transcripts per million (TPM) of NF1 across the cell lines. Primary culture is in teal while immortalized cells are in pink.

Differential Expression Analysis

We assessed genes differentially expressed between *NF1 -/-* cells and the other cell lines using Sleuth[5], the differential expression tool designed to work with Kallisto transcript quantities.

When we compared primary *NF1*$^{-/-}$ cell lines to the other cell lines (*NF1*$^{+/+}$ and *NF1*$^{+/-}$) we found 390 differentially expressed transcripts (q<0.1) of which 294 encode transcripts and map to 242 unique protein-coding genes.

A volcano plot of the genes is depicted in Figure 3 with the differentially expressed genes in red and all others in black.

NF1 was identified as down-regulated in the *NF1*$^{-/-}$ cells alongside AMIGO2, a gene that regulates Akt signaling via interaction with PDK1[6] and PLXDC2, one of the two cell-surface receptors for PEDF, a neurotrophic and anti-angiogenic factor[7].

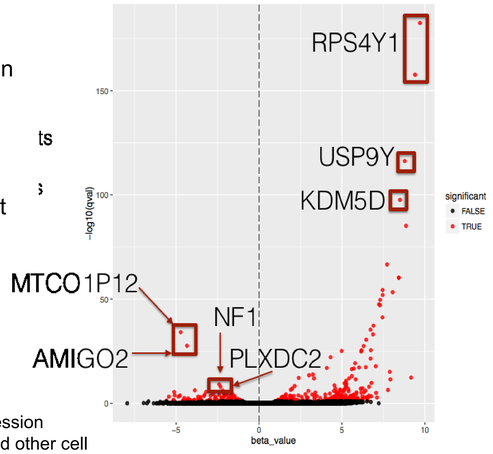


Figure 3: Volcano plot of gene expression changes between primary NF1-/- and other cell lines. X-Axis represents Beta values derived from Sleuth and Y-axis is the –log10(q-value).

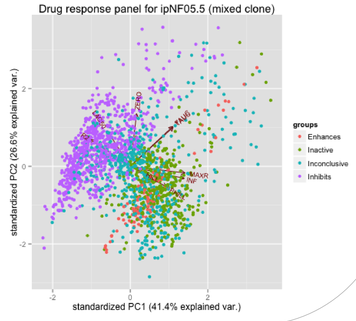
High-throughput drug screening characterizes pNF drug response

The immortalized pNF cell lines were screened in a high-throughput dose-response screening platform comprised of 1912 single-agent compounds representing diverse mechanisms of action[8].

Dose-response curves were fit using the curve response algorithm[9] to derive metrics for each drug and each cell. Area under the curve (AUC) was calculated from both the fitted curve and raw data points as well as the % activity at the maximum concentration (MAXR) and the log AC_{50} value that measures the concentration at which cell activity is reduced by 50%.

Each metric represents a distinct aspect of the cellular response to each drug. Figure 4 represents each drug mapped to two dimensions in a principal component graph, with each metric represented as red arrows. Lack of correlation between curve metrics underscores distinct role of each.

Figure 4: Relationship between curve metrics reduced to two dimensions via principal component analysis (PCA). 2D representation of drug response in this cell line is represented by points on plot with color indicating predicted impact of drug. Arrows represent individual parameters from curve-fitting process.



Chemo-genetic analysis

To compare the behavior of drugs across cell lines of different genotypes, we used a basic linear regression for the identification of those drugs with distinct behavior. Figure 5 depicts the drugs with distinct maximum response rates (MAXR) (p<0.001) between *NF1*$^{+/+}$ cell lines and the others.

Interestingly, many drugs, particularly those that target TUBB or ERBB2, are less effective in the *NF1*$^{-/-}$ cells. Pathway analysis based on MAXR revealed that drugs inhibiting BTK, HSP90, and MAP2K1 targets exhibited significantly more killing in the *NF1*$^{-/-}$ cells.

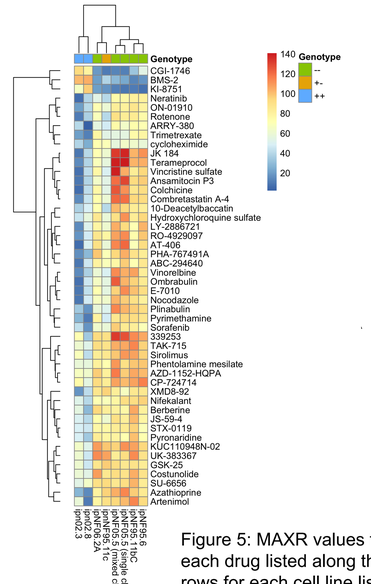


Figure 5: MAXR values for each drug listed along the rows for each cell line listed across the columns. Cell line genotype is indicated by colors along the top.

Next steps: from chemo-genetic to chemo-genomic

As we measure basal transcript levels from each of the pNF cell lines we can identify specific transcript levels that correlate with drug response. Figure 6 depicts how levels of *NF1* correlate with the MAXR values of the Btk inhibitor CGI-1746. This drug was identified as active as it was both the top correlated drug and identified through the linear model above.

We are in the process of identifying all genetic variants in the pNF cell lines via exome-Seq. In doing so we will be able to identify specific tumor variants that give rise to altered drug sensitivity. We will also be able to put the pNF cell lines in the context of other cancer cell lines available in the Cancer Cell Line Encyclopedia[7].

All complete data will be freely available at: http://www.synapse.org/pnfCellLines to serve as a resource for the research community.

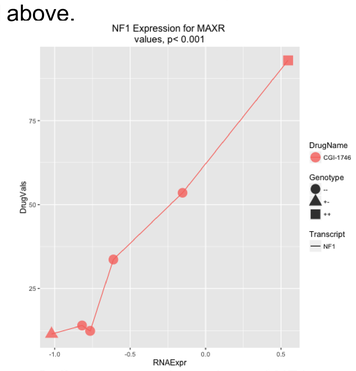


Figure 6: Gene expression values of NF1 (x-axis) alongside MAXR values of CGI-1746, the drug found to be most correlated with NF1 expression.

References

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