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A NETWORK-BASED APPROACH TO INVESTIGATE THE DIFFERENT RESPONSE TO VEMURAFENIB IN BRAF V600E MUTANT CANCERS

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Abstract

One of the best known oncogene is the BRAF gene, implicated in cell growth, differentiation and survival processes. The most frequent mutation for the BRAF gene is the mutation BRAF V600E which appears in many tumors (melanoma, thyroid cancer, colon cancer, lung cancer, etc.) and it is often associated with a poor prognosis. The agent commonly used to treat cancer patients with BRAF V600E mutation is vemurafenib. However, numerous studies have shown that different BRAF V600E mutant cancers do not respond uniformly to the vemurafenib inhibitor.

In order to investigate the reason for this phenomenon, we applied SWIM, a software able to identify putative regulatory (switch) genes involved in drastic changes to the cell phenotype, to gene expression profiles data of different BRAF V600E mutant cancers and their normal counterparts publicly available on the TCGA (The Cancer Genome Atlas). This analysis led to hypothesize that the heterogeneity in the response to vemurafenib may be due to a different number of kinases with a sequence homologous to BRAF among the switch genes of each analyzed tumor.

Key words: BRAF V600E cancers, vemurafenib, network analysis

1. Introduction

One of the best known driver genes is the BRAF oncogene, which encodes for a member of the RAF kinase family. The most frequent mutation for the BRAF gene is the point mutation c.1799T> A which involves the replacement of the amino acid valine (V) with glutamic acid (E) in the activation segment of the BRAF kinase (V600E).

BRAF V600E mutation occurs in almost 100% of hairy cell leukemias (HCL), in approximately 50% of cutaneous melanomas (SKcm), in 37-50% of papillary thyroid cancers (THca), in 5-8% of metastatic colorectal cancers (CRC), and in 1-2% of lung adenocancarcinomas (LUad) [1] and, in some of these cancers, this mutation is associated with poor prognosis due to an aggressive disease phenotype.

So far, selective small-molecule BRAF inhibitors are approved by the FDA for the treatment of patients with BRAF V600E such as vemurafenib. However, several studies showed that different histotypes of BRAF V600E mutant tumors do not respond uniformly to BRAF inhibitor vemurafenib: high response rates in hair cell leukemia and melanoma, intermediate responses in thyroid and non small cell lung cancer (NSCL), low responses in colorectal cancer (Table 1).

In the present work, we sought to elucidate why different tumors harboring BRAF V600E mutation show heterogeneity in response to vemurafenib through a network-based approach implanted by SWIM bioinformatics tool.

Cancer	Overall response rate
HCL	96-100%
SKcm	51%
THca	38.5%
NSCL	33%
CRC	4.8%

Table 1. Response to vemurafenib in BRAF V600E mutant tumours

2. Material and Methods

Dataset

We analyzed gene expression profiles from high-throughput RNA-sequencing of different cancers harbouring the BRAF V600E mutation downloaded from the TCGA data portal on 6 December 2014. High-throughput sequencing data correspond to level 3 data (i.e. normalized expression data) given in terms of FPKM (i.e. fragments per kilobase of exon per million fragments mapped). We examined: 294 tumor samples and 59 normal samples for THca, 36 tumor samples and 50 normal samples for CRC, and 9 tumor samples and 58 normal samples for LUad.

SWIM software

SWitchMiner (SWIM) is a software with a user-friendly graphical user interface, developed in MATLAB and downloadable from the supplementary material included in the reference [2]. SWIM combines topological properties of co-expression networks with genome-wide analysis and is able to identify a small pool of regulatory genes, called switch genes, which have been shown to be critically associated with drastic changes in cell phenotypes.

3. Results

By running SWIM tool, we compared data of BRAF V600E mutant thyroid, colorectal, and lung cancers with their corresponding normal samples in order to unveil the switch genes that could potentially explain the heterogeneity of tumour response to targeted agents.

SWIM identified 227 switch genes out of 2167 DEGs in the thyroid cancer versus normal thyroid comparison; 183 switch genes out of 1895 DEGs between colorectal cancer and its normal tissue; and 298 switch genes out of 1738 DEGs between lung adenocarcinoma and normal lung.
We found that switch genes included protein-coding genes as well as long non-coding RNAs and pseudogenes and only seven switch genes (CDH3, PLEKHN1, LEMD1, SPTBN2, ETV4, C1orf170, C8orf73) are shared among all cancers (Figure 2).

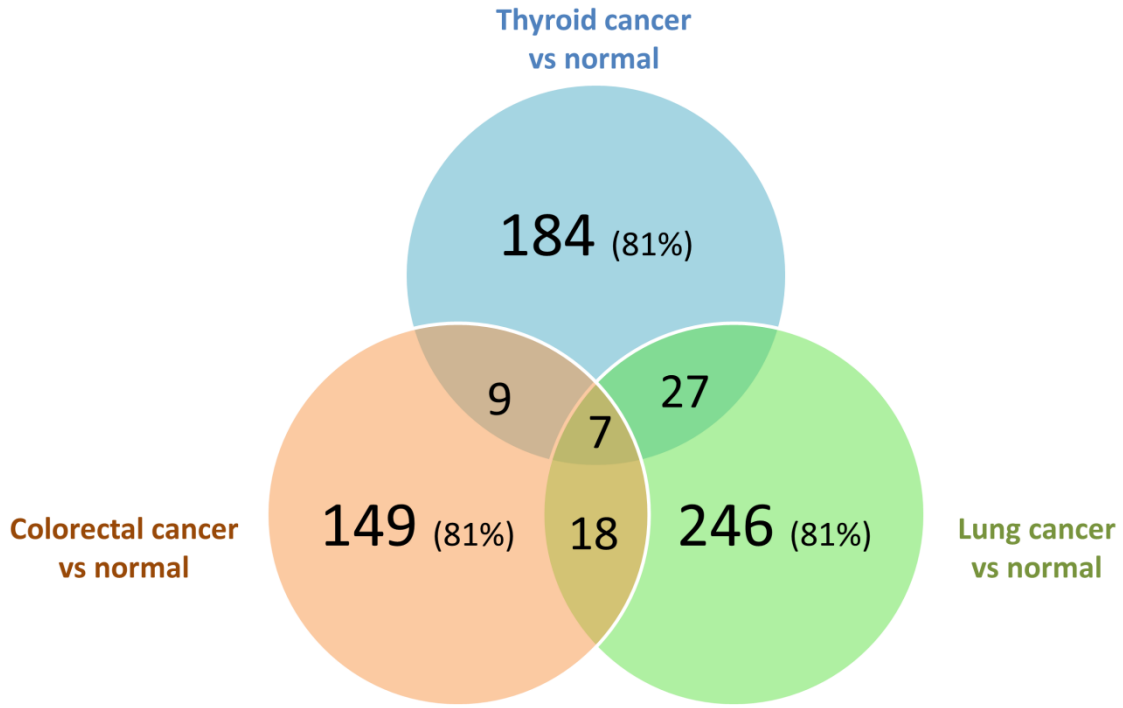

Figure 1. Venn diagram of switch genes for each BRAF V600E cancer compared to its normal tissue.

We hypothesized that different tumours with a mutation in the same driver gene *(BRAF)* do not respond uniformly to the same inhibitor (vemurafenib) because other kinases in addition to the known ones may be targeted by vemurafenib. Therefore, we first identified the switch genes that encode for kinases and then, we employed Geneious R11 desktop platform

((https://www.geneious.com) to identify those kinases with the maximum identity score to kinases reported as known targets of vemurafenib.
We found 14 kinases in LUad, 7 in THca, and 3 in CRC (Table 2). None of these switch genes were shared among the different BRAF mutant cancers analysed

LUad	THca	CRC
BUB1	DAPK2	SPHK1
CDK1	EPHB3	TRIB3
EFNA3	ERBB3	TYRO3
EFNA4	MAP3K6	
EFNA5	MET	
ITPKA	PFKFB2	
MAST1	TGFBR1	
MELK		
NME1		
SGK2		
STK32A		
STK39		
STYK1		
TK1		

Table 2. Switch genes kinases in BRAF V600E mutant cancers.

In order to predict which of these kinases are preferential targets of the drug, we looked for the presence of sequence homologies between switch gene kinases and known vemurafenib targets and identified three commons motifs belonging to the kinase domain, HRD, KXXDFGX and WXAPE (Figure 2).

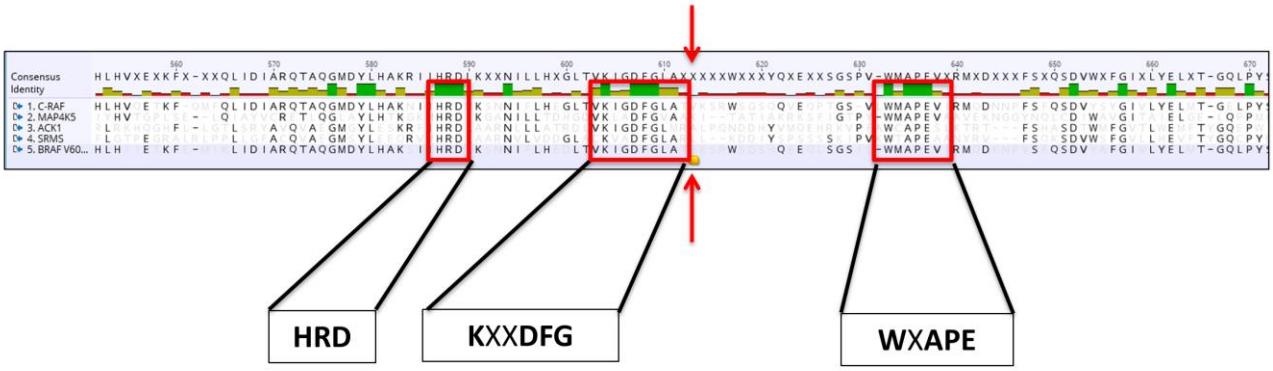

Figure.2 Three homologous sequences identified across vemurafenib targets.

Among the 24 kinase-encoding switch genes, 12 had at least one of these homologous sequences (Figure 3).

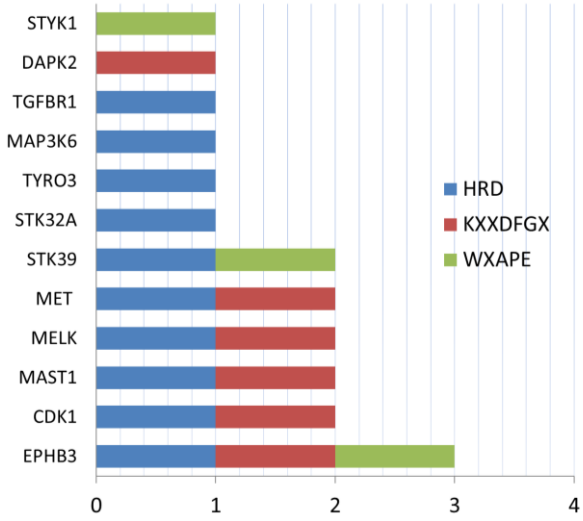

Figure 3. Switch gene kinases with one or more homology sequences.

In particular, we found that THca and LUad have a similar number of putative targetable switch genes kinases (5 and 6), whereas CRC has just one, with a minor homology sequence.

Altogether, these results prompt us to hypothesize that the presence of these homologous sequences in switch genes coding for kinases could potentially explain the heterogeneity of tumours' responses to vemurafenib and help to predict their drugability.

4. Conclusions

Our network analysis may provide additional approaches to exploring the molecular mechanisms underlying the different response to vemurafenib in BRAF V600E mutant tumors and highlight the importance of tumor histology in precision medicine. It is likely that although different cancers share the same major driver event, the response to therapy varies based on the number of kinases that share sequence homology with the druggable kinase tar-gets. Of course, experimental data are needed to validate this prediction.

Reference

[1] Davies, H., Bignell, G. R., Cox, C., Stephens, P., Edkins, S., Clegg, S., et al. (2002). Mutations of the BRAF gene in human cancer. Nature, 417(6892), 949.

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