Endocrine (2019) 64:406–413
https://doi.org/10.1007/s12020-019-01890-4

ORIGINAL ARTICLE

Check for updates

BRAF^{V600E}-mutant cancers display a variety of networks by SWIM analysis: prediction of vemurafenib clinical response

Rosa Falcone[1] · Federica Conte[2,3] · Giulia Fiscon[2,3] · Valeria Pecce[1] · Marialuisa Sponziello[1] · Cosimo Durante[1] · Lorenzo Farina[4] · Sebastiano Filetti[1] · Paola Paci[2] · Antonella Verrienti[1]

Received: 30 January 2019 / Accepted: 1 March 2019 / Published online: 8 March 2019

Abstract

Purpose Several studies have shown that different tumour types sharing a driver gene mutation do not respond uniformly to the same targeted agent. Our aim was to use an unbiased network-based approach to investigate this fundamental issue using *BRAF*^{V600E} mutant tumours and the BRAF inhibitor vemurafenib.

Methods We applied SWIM, a software able to identify putative regulatory (switch) genes involved in drastic changes to the cell phenotype, to gene expression profiles of different *BRAF*^{V600E} mutant cancers and their normal counterparts in order to identify the switch genes that could potentially explain the heterogeneity of these tumours' responses to vemurafenib.

Results We identified lung adenocarcinoma as the tumour with the highest number of switch genes (298) compared to its normal counterpart. By looking for switch genes encoding for kinases with homology sequences similar to known vemurafenib targets, we found that thyroid cancer and lung adenocarcinoma have a similar number of putative targetable switch gene kinases (5 and 6, respectively) whereas colorectal cancer has just one.

Conclusions We are persuaded that our network analysis may aid in the comprehension of molecular mechanisms underlying the different responses to vemurafenib in *BRAF*^{V600E} mutant tumours.

Keywords *BRAF*^{V600E} · Vemurafenib · Network medicine · Prediction of response

Introduction

Cancer is a complex, heterogeneous, and dynamic disease which is driven by genomic and epigenomic abnormalities. The number of mutations found in any cancer can vary from a handful to hundreds of thousands. While the majority of mutations are 'passengers' (i.e. without any significant consequences for the cell), a small number are functionally relevant 'drivers' [1]. Drivers are responsible for carcinogenesis and clonal expansion, and provide cancer cells with a selective survival advantage.

One of the best known driver genes is the *BRAF* oncogene, which encodes for a member of the RAF kinase family. The RAF kinase family, in turn, constitutes a core component of the ERK signalling pathway which is involved in cell growth, differentiation, and survival. The most frequent mutation in the *BRAF* gene is the point mutation c.1799T>A, which is a substitution that results in an aminoacid change from valine (V) to glutamic acid (E) in the activation segment of the BRAF kinase (V600E), promoting its hyperactivation and resulting in constitutive activation of downstream ERK signalling.

BRAF mutations are present in ~8% of human tumours [2]. *BRAF*^{V600} mutations occur in almost 100% of hairy cell leukaemias (HCL), in ~50% of cutaneous melanomas (SKcm), and with different frequencies in various non-melanoma cancers: in 37–50% of papillary thyroid cancers

These authors contributed equally: Rosa Falcone, Federica Conte, Giulia Fiscon

Supplementary information The online version of this article (https://doi.org/10.1007/s12020-019-01890-4) contains supplementary material, which is available to authorised users.

✉ Paola Paci
paola.paci@iasi.cnr.it

[1] Department of Translational and Precision Medicine, Sapienza University of Rome, Rome, Italy

[2] Institute for Systems Analysis and Computer Science "Antonio Ruberti", National Research Council, Rome, Italy

[3] ACT Operations Research, Research & Development, Roma, Italy

[4] Department of Computer, Control and Management Engineering, Sapienza University of Rome, Rome, Italy

(THca), in 5–8% of metastatic colorectal cancers (CRC), and in 1–2% of lung adenocancarcinomas (LUad) [3, 4]. In some of these cancers, the $BRAF^{V600}$ mutations are associated with poor prognosis due to an aggressive disease phenotype, shortened overall survival, and minor response rates [5–8]. For instance, in papillary thyroid cancer the role of the $BRAF^{V600E}$ mutation confers an increased risk of cancer recurrence and extrathyroidal disease in tumours of >10 mm [9] but not in tumours of ≤10 mm [10]. In metastatic thyroid cancer, the presence of the $BRAF^{V600E}$ mutation can impair iodine metabolism, rendering the tumour more likely to be refractory to treatment with radioactive iodine [11]. Moreover, $BRAF^{V600E}$ mutation may be present alone or in combination with other genetic or epigenetic modifications. In particular, in thyroid cancer, the coexistence of $BRAF^{V600E}$ with *TERT* mutations or amplification has been more strongly associated with high-risk clinicopathologic features than either mutation alone was, higher risk of recurrence and cancer-related mortality [12–14].

Since the driver gene is considered responsible for cancer initiation and progression, the idea that blocking this gene with an inhibitor would interfere with tumour growth has caused a shift in focus from tumour histology to molecular profile, with pharmaceutical companies rushing to develop targeted therapy. The targeted agent vemurafenib was approved by the FDA in 2011 for the treatment of patients with $BRAF^{V600E}$ mutant metastatic melanoma [15]. In these patients, it showed a response rate of ~50% and improved survival. However, several studies have shown that $BRAF^{V600E}$-mutated tumour types do not respond uniformly to *BRAF*-targeted therapy (Table 1). Impressive responses (ORR 96–100%) have been observed in $BRAF^{V600E}$ hairy cell leukaemia [16], and intermediate activity has been observed in advanced *BRAF*-mutated non-small cell lung cancer [17] and metastatic thyroid cancer [18]. However, in marked contrast to these results, single-agent vemurafenib did not show meaningful clinical activity in patients with $BRAF^{V600E}$ mutant colorectal cancer [5].

To investigate why different tumours with the same driver gene do not respond uniformly to the same inhibitor, we computationally explored gene expression profiles of a variety of $BRAF^{V600E}$ mutant cancers available on The Cancer Genome Atlas (TCGA) (https://cancergenome.nih.gov/) using the software package SWItchMiner (SWIM) [19] (Information about SWIM are available in Supplementary S1). Our aim was to identify the switch genes that could potentially explain the heterogeneity of tumour response to targeted agents. In order to do this, we identified: (1) switch genes by comparing $BRAF^{V600E}$ mutant cancers; (2) switch genes putatively involved in $BRAF^{V600E}$ carcinogenesis; (3) the function of proteins coding switch genes; and (4) additional kinase targets of vemurafenib.

Table 1 Response to BRAF inhibitor monotherapy in $BRAF^{V600E}$ mutant tumours

Cancer	ORR (%)	PFS (months)
SKcm	51	7.3
CRC	4.8	2.1
HCL	96–100	9
NSCLC	33	5.5
THca	38.5	8.9–18.2

SKcm melanoma, *CRC* colorectal cancer, *HCL* hairy cell leukaemia, *NSCLC* non-small cell lung cancer, *THca* papillary thyroid cancer, *ORR* overall response rate, *PFS* progression free survival

Methods

Identification of switch genes from the comparison of $BRAF^{V600E}$ mutant cancers

RNA-sequencing data, available on TCGA, obtained from 294 thyroid cancers (papillary thyroid cancers), 205 melanomas, 36 colorectal cancers, and 9 lung adenocarcinomas were analysed using SWIM software. The Hamming distance was used to group cancer types based on their degree of switch gene similarity (Fig. 1).

Analysis of switch gene function

Function of switch genes has been analysed by Gene ontology (GO, http://www.geneontology.org/) and Kyoto Encyclopaedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg/) databases. Pathway enrichment analyses were conducted by using Search Tool for the Retrieval of Interacting Genes (STRING, https://string-db.org/). The GO terms refer to the following items: biological process (BP), cellular component (CC), and molecular function (MF). GO analysis uses hypergeometric tests to perform enrichment analysis on gene sets. KEGG pathway analysis aims to identify and visualise significantly enriched pathways of molecular interactions, reactions, and relations. STRING is a freely accessible biological database to evaluate protein–protein interaction information. A $P < 0.05$ was considered statistically significant and indicative of significant enrichment.

Identification of additional kinase targets of vemurafenib

The sequence of the kinases previously identified as targets of vemurafenib, reported in the Selleckchem database (http://www.selleckchem.com), and the sequence of the kinases codified by the switch genes were downloaded from the UniProt database (http://www.uniprot.org). In particular, the sequences of the canonical isoform for each protein have

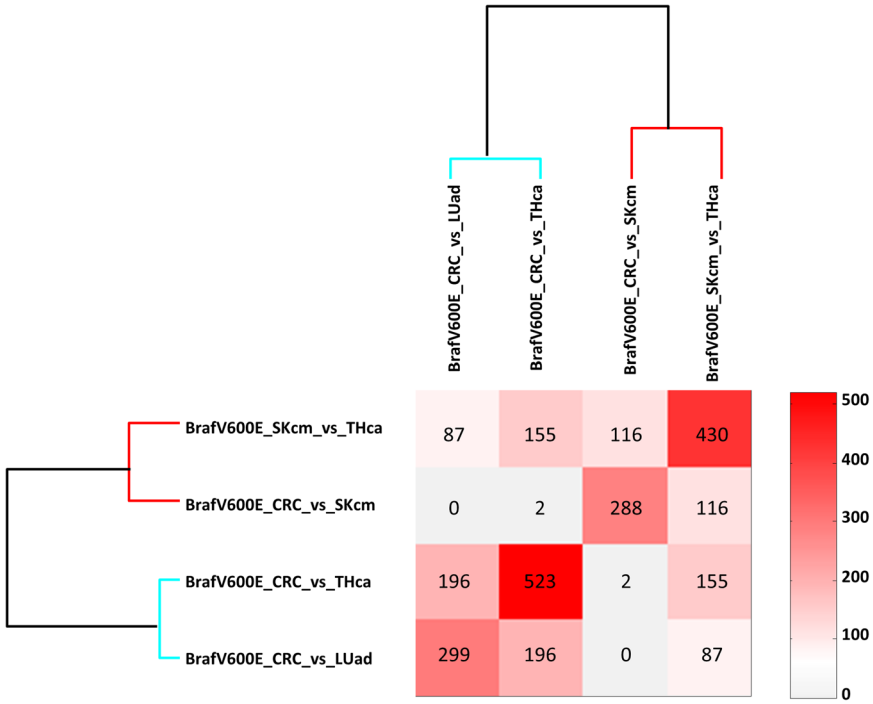

Fig. 1 Hamming distance for comparisons among different tumours harbouring *BRAF*^{V600E} mutation. In the diagonal are shown the switch genes emerged from the comparison between two tumours, i.e. the switch genes equivalent to the differences between the tumours. The greater the reported number, the greater the difference between the two tumours compared. Instead, in other boxes of the figure, there are common genes, emerged from two comparisons. CRC colorectal adenocarcinoma, THca thyroid cancer, LUad lung adenocarcinoma, SKcm melanoma

Table 2a Characteristics of switch genes in comparisons between *BRAF*^{V600E}-mutant tumours

Cancer	Switch genes	Up-regulated (%)	Down-regulated (%)	Protein-coding vs. non-coding RNAs
CRC vs. SKcm	288	15 (5)	273 (95)	254 protein-coding 3 non-coding 3 pseudogenes
CRC vs. THca	523	522 (99.8)	1 (0.2)	474 protein-coding 2 pseudogenes
CRC vs. LUad	299	296 (99)	3 (1)	270 protein-coding 1 non-coding RNA 1 pseudogene
SKcm vs. THca	430	427 (99)	3 (1)	379 protein-coding 4 non-coding RNAs 4 pseudogenes

CRC colorectal cancer, *SKcm* melanoma, *THca* papillary thyroid cancer, *LUad* lung adenocarcinoma

been selected and uploaded in the Geneious R11 desktop platform and compared using the MUltiple Sequence Comparison by Log-Expectation (MUSCLE) alignment method [20]. The Nextprot database (https://www.nextprot.org) was used to analyse the domains in each kinase.

Results

Identification of switch genes from the comparison of BRAF^{V600E}-mutant cancers

We analysed RNA-sequencing data, available on TCGA, of 294 thyroid cancers, 205 melanomas, 36 colorectal cancers, and 9 lung adenocarcinomas with the *BRAF V600E* mutation. Clinical data concerning these tumours are available in Supplementary Data S2. Almost all the examined tissues come from patients who have not received any treatment before surgery. Furthermore, in all four cancers, the percentage of metastatic tumours is low compared to non-metastatic disease. We did not compare statistically the stages because they refer to tissue-specific staging systems. Our algorithm identified 288 switch genes out of 2000 (14%) differentially expressed genes (DEGs) in the comparison of colorectal cancer versus melanoma, 523 out of 2314 DEGs (23%) between colorectal and thyroid cancer, 299 out of 2018 DEGs (15%) between colorectal cancer and lung adenocarcinoma, and 430 out of 2224 DEGs (19%) between melanoma and thyroid cancer. Switch genes included protein coding genes, as well as long non-coding

Table 2b Characteristics of switch genes in comparisons between *BRAF* ^{V600E} tumours and their normal counterpart

Cancer vs. normal	Switch genes	Up-regulated (%)	Down-regulated (%)	Protein-coding vs. non-coding RNAs
CRC	183	183 (100)	0	166 protein-coding 1 pseudogene 1 non-coding RNA
LUad	298	293 (98)	5 (2)	258 protein-coding 1 pseudogene 6 non-coding RNAs
THca	227	184 (81)	43 (19)	211 protein-coding 1 pseudogene 3 non-coding RNAs

CRC colorectal cancer, *THca* papillary thyroid cancer, *LUad* lung adenocarcinoma

RNAs and pseudogenes. The number, the percentage, and the type of up-regulated and down-regulated switch genes in these comparisons are reported in Table 2a. We used the Hamming distance to represent cancers similarity based on the number of switch genes (Fig. 1). We assumed that the greater the number of switch genes in each comparison, the greater the difference between tumours. Therefore, colorectal and thyroid cancers, which have the largest number of switch genes (523), appear as the most different (*distant*) from each other. On the contrary, colorectal cancer and melanoma are the most similar (*closest*) to each other (288 switch genes).

Switch gene function

We investigated the function of proteins coding switch genes by performing an enrichment analysis in KEGG pathway (KEGG, http://www.genome.jp/kegg/) and Gene Ontology (GO, http://www.geneontology.org/) biological processes (see Supplementary Data S3 online). Significant enrichment pathways in GO emerged in all four cancer comparisons, while KEGG pathway enrichment was found to be statistically significant in all comparisons except colorectal cancer versus melanoma. Both enrichment analyses showed that switch genes were mainly involved in cell cycle, system/organ/tissue development, signal transduction, response to stimuli, and CC organisation. Some enriched pathways were only present in one comparison, such as pigmentation and neurogenesis in the colorectal cancer versus melanoma comparison, or digestion in the colorectal and thyroid cancer comparison.

Identification of switch genes putatively involved in BRAF ^{V600E} carcinogenesis

Using SWIM, we compared data of *BRAF*^{V600E} mutant thyroid, colorectal, and lung cancers with their corresponding normal samples (59 for thyroid, 50 for colorectal, and 59 for lung). We were not able to analyse RNA-sequencing data from hairy cell leukaemia and normal skin

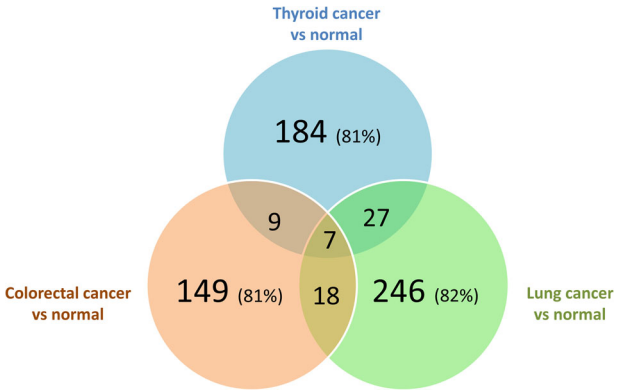

Fig. 2 Switch genes in *BRAF*^{V600E}-mutant cancers. Venn diagram showing shared and non-shared switch genes for each *BRAF*^{V600E} cancer compared to its normal tissue

because not available. 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. The number, the percentage, and the type of up-regulated and down-regulated genes in these comparisons are reported in Table 2b. The number and percentage of shared and non-shared switch genes for each tumour compared with its normal tissue are shown in Fig. 2. Seven genes (*CDH3, PLEKHN1, LEMD1, SPTBN2, ETV4, C1orf170, C8orf73*), all of them up-regulated in comparison to normal tissue, are shared among thyroid, lung, and colorectal cancer.

Switch gene function

We investigated switch gene function by performing an enrichment analysis in KEGG pathways and GO BP (see Supplementary Data S4 online). Significant enriched KEGG pathways emerged only for lung adenocarcinoma versus normal tissue and involved metabolic pathways and

glycosphingolipid biosynthesis. In GO, DNA conformation change was the most significant enriched pathway in lung adenocarcinoma. On the contrary, system/organ/tissue development and tissue morphogenesis are common enriched pathways when comparing colorectal and thyroid cancers with their normal tissue.

Kinase-encoding switch genes

To investigate why different tumours with a mutation in the same driver gene *(BRAF)* do not respond uniformly to the same inhibitor (vemurafenib), we hypothesised that other kinases, in addition to the known ones, may be targeted by vemurafenib. Indeed, in vitro data showed that vemurafenib, although widely recognised as a selective tyrosine kinase inhibitor, also inhibits other kinases with different IC50. Therefore, we identified the switch genes that encode for kinases: 14 in lung adenocarcinoma, 7 in thyroid cancer, and 3 in colorectal cancer (Table 3). Moreover, all kinase-encoding switch genes are up-regulated, with the only exception being *PFKFB2* in thyroid cancer. 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 we identified three common motifs belonging to the kinase domain, HRD, KXXDFGX, and WXAPE (Fig. 3). Among the 24 kinase-encoding switch genes, 12 had at least one homology motif (Fig. 4). Enrichment analysis for these kinases in KEGG and GO showed involvement in the following pathways: axon guidance, Ras signalling pathway, Rap1 signalling pathway, PI3K-Akt signalling pathway, phosphorylation, transmembrane receptor protein, tyrosine kinase signalling pathway, ephrin receptor signalling pathway, and regulation of programmed cell death (see Supplementary Data S5 online). Just one kinase, (*EPHB3*), had all three homology sequences (HRD, KXXDFGX, WXAPE) and it has been associated with thyroid cancer. In lung adenocarcinoma, four out of six kinases had two homology sequences (HRD, KXXDFGX or HRD, WXAPE). On the contrary, in colorectal cancer there were no kinases with two or three homology sequences, just one kinase (*TYRO3*) sharing one homology sequence (HRD). HRD was the most frequent shared homology sequence among kinases encoded by switch genes (10 out of 12 kinases). In 6 out of the 10 kinases sharing the HRD sequence, the aspartate (D) in the HRD sequence may have an important role as a proton acceptor in the kinase domain.

Table 3 Kinases encoded by switch genes in *BRAF* ^{V600E} mutant tumours

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		

CRC colorectal cancer, *THca* papillary thyroid cancer, *LUad* lung adenocarcinoma

Discussion

Driver mutations, responsible for both tumour initiation and progression, are considered attractive targets for the development of selective inhibitors in cancer treatment. For instance, in $BRAF^{V600E}$ mutant thyroid cancers, several successful targeted agents have been developed [21, 22]. However, these inhibitors are not equally effective in other cancers harbouring the same driver mutation. The advent of large-scale genome sequencing has revealed that cancer is a complex disease in which genetic and epigenetic alterations act together. The analysis of the cancer molecular landscape, using bioinformatics tools, may help to reveal hidden, tissue-specific pathways. This approach may be helpful to personalise treatment, selecting more active and less toxic pharmacologic agents, to use alone or in a combination strategy.

We applied SWIM algorithm to analyse gene expression data from several cancers sharing $BRAF^{V600E}$ mutation. Our aim was to identify the most important differences, in terms of switch genes, that may contribute to cancer-specific pharmacological response to vemurafenib.

The highest number of switch genes (523) was found by comparing colorectal and thyroid cancers, while the lowest number of switch genes (288) was found between colorectal cancer and melanoma. Assuming that the greater the number of switch genes in the comparison, the greater the difference between tumours, these data suggest that colorectal cancer is more different from thyroid cancer than from lung adenocarcinoma and/or melanoma and that thyroid cancer is quite different from melanoma.

When we looked at differences between tumours and their normal tissue, we found that lung adenocarcinoma is the tumour with the highest number of switch genes (298), followed by thyroid cancer (227) and colorectal cancer

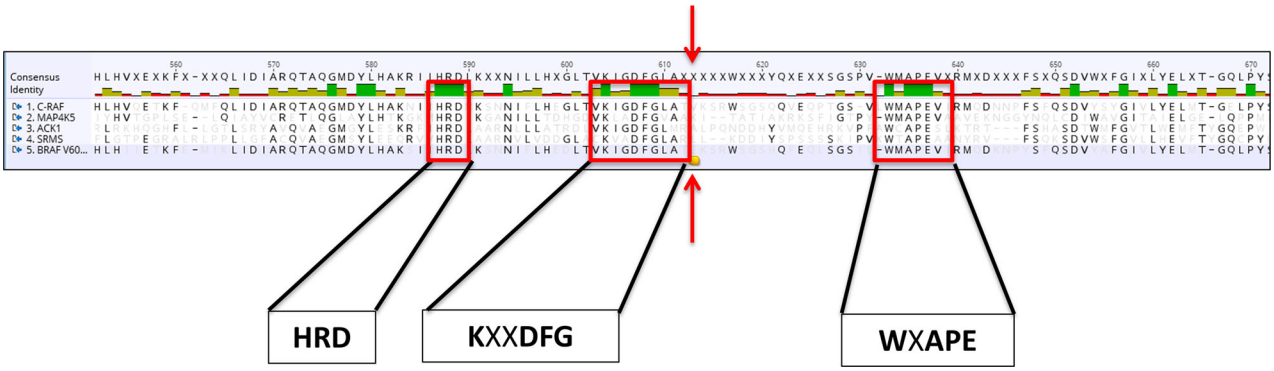

Fig. 3 Sequence homology among known vemurafenib targets. The figure shows three motifs belonging to the kinase domain of BRAF (HRD, KXXDFGX, WXAPE) shared among known vemurafenib targets

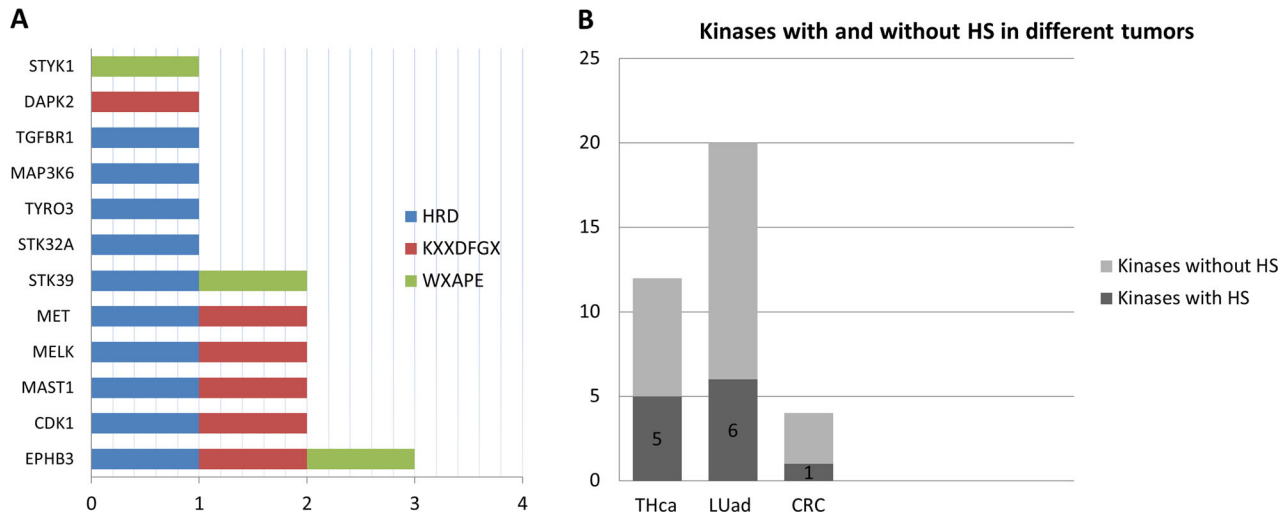

Fig. 4 Kinase-encoding switch genes. **a** List of kinases with one or more homology sequences. **b** Kinases encoded by switch genes with and without homology sequences in different tumours

(183). A higher number of switch genes may suggest a more complex tumourigenesis process. On the whole, the vast majority of switch genes are unshared between tumours, which further highlights tumour heterogeneity. Switch genes are usually protein-coding genes but they may also be long non-coding RNAs (lncRNAs) or pseudogenes. This is not surprising since both lncRNAs and pseudogenes have recently been demonstrated to play an important role in human diseases, especially in cancer [23, 24]. In particular, pseudogenes, that represent a significant proportion of the transcriptome [24], are able to alter the sequence and/or the transcriptional activity of their parental genes and, once they are transcribed in RNA, they can act as antisense RNA, lncRNA, endogenous short interfering RNAs, competing endogenous RNAs, chimeric RNA and can compete with their parental messenger RNAs for RNA-binding proteins and/or translational machinery binding [25, 26]. Moreover, it has been demonstrated that some pseudogenes can be translated in proteins that can interfere with their parental protein functions [25].

Most of these switch genes are up-regulated in tumour versus normal tissue. Indeed, all switch genes are up-regulated in colorectal cancer, 98% are up-regulated in lung adenocarcinoma, and 81% are up-regulated in thyroid cancer, suggesting that the gene's activation plays a large role in the BRAF-guided carcinogenesis process.

Lastly, we looked at sequence homology across known vemurafenib targets and we identified three common motifs (HRD, KXXDFGX and WXAPE) repeated in all sequences analysed. When we searched for these motifs in the kinases encoded by switch genes in all cancers, we found one or more of these sequences in 12 kinases: 6 in lung adenocarcinoma, 5 in thyroid cancer, and one in colorectal cancer. It is likely that kinases with the highest homology sequence number (three) are preferential targets of vemurafenib, as compared with kinases with two or fewer homologous sequences.

Taking into account the number of kinases encoded by switch genes and the presence of vemurafenib putative target motifs, we could hypothesise that papillary thyroid cancer and lung adenocarcinoma have a comparable response to vemurafenib while colorectal cancer has a worse response. Indeed, this hypothesis is in accordance with clinical trial data that report a better response rate to vemurafenib in papillary thyroid cancer patients than in colorectal cancer patients. The importance of the putative targetable kinases has been previously reported [27, 28]. Notably, EPHB3 seems to have a key role in cancer. EPHB3 is the kinase encoded by the switch gene with the highest homology sequence. Preclinical studies showed that the expression of *EPHB3* was significantly elevated in papillary thyroid cancer. Li et al. suggested that EPHB3 acts as a tumour promoter in THca by increasing the in vitro migration, as well as the in vivo metastasis of THca cells by regulating the activities of Vav2 and Rho GTPases in a kinase-dependent manner [29]. Interestingly, *EPHB3* was investigated even in other cancers: in gastric cancer, upregulation of *EPHB3* was associated with acquired resistance to a selective inhibitor of FGFR 1–3 [30], whereas in CRC its downregulation has been proposed as a prognostic indicator [31].

Cancer is a complex and dynamic disease, and its evolutionary nature makes it hard to achieve success with targeted agents. Indeed, cancer clone evolution involves contemporaneous sub-clones with distinctive mutational profiles and this concept has practical implications for targeted therapy. In the rush to produce targeted therapy, focus has shifted from tumour histology to molecular profile, but clinical trials results show that this shift is not entirely justified. Histology is considered a surrogate for potentially important differences in biology, meaning that tumour histology is an important determinant of sensitivity to targeted agents, which may be due to factors related to tissue lineage, epigenetic features, and mechanisms of resistance. Our results highlight a substantial difference between BRAF-mutant tumours supporting the issue that a single mutational event, although a major one, is not enough to predict the clinical benefit of target therapy.

Some limitations of this study must be acknowledged. First of all, we have not included any data on skin and hairy cell leukaemia. The latter is the tumour with the highest response rate to vemurafenib, followed by melanoma. Unfortunately, since hairy cell leukaemia and skin RNA-sequencing data were not available, we were not able to analyse data regarding two of the most common tumours with $BRAF^{V600E}$ mutations. Nevertheless, we preferred to analyse homogeneous data at the expense of losing some information. Secondly, the analyses conducted using computational and bioinformatics tools need to be validated by in vitro studies.

In summary, in this report we used a computational approach to explain a clinical observation. It is for the first time that someone analyzes through a network approach the use of targeted therapy in a histology-agnostic way. In particular, this preliminary analysis may provide additional insights to understanding the molecular mechanisms underlying the different responses to vemurafenib in several tumours harbouring $BRAF^{V600E}$ mutation. In vitro data are needed to validate these results.

Data availability

Data generated or analysed during this study are included in this published article (and in the Supplementary Information files).

Author contributions S.F., A.V., and L.F. conceived and designed the project. P.P. developed the software. F.C. and G.F. performed computational data analysis. A.V., R.F., V.P., and M.S. performed biological analysis. R.F. wrote the manuscript. C.D. prepared figures and tables. All authors have read and approved the final article.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the author.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. M. Greaves, C. Maley, Clonal evolution in cancer. Nature **481** (7381), 306–313 (2012)
2. H. Davies, G.R. Bignell, C. Cox, P. Stephens, S. Edkins, S. Clegg, J. Teague, H. Woffendin, M.J. Garnett, W. Bottomley, N. Davis, E. Dicks, R. Ewing, Y. Floyd, K. Gray, S. Hall, R. Hawes, J. Hughes, V. Kosmidou, A. Menzies, S. Hooper, R. Wilson, H. Jayatilake, B.A. Gusterson, M.R. Stratton, P.A. Futreal, Mutations of the BRAF gene in human cancer. Nature **417**(6892), 949–954 (2002)
3. B. Falini, M.P. Martelli, E. Tiacci, Review article BRAF V600E mutation in hairy cell leukemia: from bench to bedside. Blood **128** (15), 1918–1928 (2019)
4. P.A. Ascierto, J.M. Kirkwood, J. Grob, E. Simeone, A.M. Grimaldi, M. Maio, G. Palmieri, A. Testori, F.M. Marincola, N. Mozzillo, The role of BRAF V600 mutation in melanoma. J Transl Med. **10**, 85 (2012)
5. S. Kopetz, J. Desai, E. Chan, J.R. Hecht, P.J. O'Dwyer, D. Maru, V. Morris, F. Janku, A. Dasari, W. Chung, J.P.J. Issa, P. Gibbs, B. James, G. Powis, K.B. Nolop, S. Bhattacharya, L. Saltz, Phase II pilot study of vemurafenib in patients with metastatic BRAF-mutated colorectal cancer. J. Clin. Oncol. **33**(34), 4032–4038 (2015)
6. G. Safaee Ardekani, S.M. Jafarnejad, L. Tan, A. Saeedi, G. Li, The prognostic value of BRAF mutation in colorectal cancer and melanoma: a systematic review and meta-analysis. PLoS ONE **7** (10), e47054 (2012)

7. G. Tallini, D. De Biase, C. Durante, G. Acquaviva, M. Bisceglia, R. Bruno, M.L. Bacchi Reggiani, G.P. Casadei, G. Costante, N. Cremonini, L. Lamartina, D. Meringolo, F. Nardi, A. Pession, K.J. Rhoden, G. Ronga, M. Torlontano, A. Verrienti, M. Visani, S. Filetti, BRAF V600E and risk stratification of thyroid microcarcinoma: a multicenter pathological and clinical study. Mod. Pathol. **28**(10), 1343–1359 (2015)
8. E. Puxeddu, S. Filetti, BRAF mutation assessment in papillary thyroid cancer: are we ready to use it in clinical practice? Endocrine **45**(3), 341–343 (2014)
9. M. Xing, A.S. Alzahrani, K.A. Carson, Y.K. Shong, T.Y. Kim, D. Viola, R. Elisei, B. Bendlová, L. Yip, C. Mian, F. Vianello, R.M. Tuttle, E. Robenshtok, J.A. Fagin, E. Puxeddu, L. Fugazzola, A. Czarniecka, B. Jarzab, C.J. O'Neill, M.S. Sywak, A.K. Lam, G. Riesco-Eizaguirre, P. Santisteban, H. Nakayama, R. Clifton-Bligh, G. Tallini, E.H. Holt, V. Sýkorová, Association between BRAF V600E mutation and recurrence of papillary thyroid cancer. J. Clin. Oncol. **33**(1), 42–50 (2015)
10. E. Puxeddu, C. Durante, N. Avenia, S. Filetti, D. Russo, Clinical implications of BRAF mutation in thyroid carcinoma. Trends Endocrinol. Metab. **19**(4), 138–145 (2008)
11. C. Durante, E. Puxeddu, E. Ferretti, R. Morisi, S. Moretti, R. Bruno, F. Barbi, N. Avenia, A. Scipioni, A. Verrienti, E. Tosi, A. Cavaliere, A. Gulino, S. Filetti, D. Russo, Brief report: BRAF mutations in papillary thyroid carcinomas inhibit genes involved in iodine metabolism. J. Clin. Endocrinol. Metab. **92**(7), 2840–2843 (2007)
12. T. Zhang, G. Zhu, M. Xing, Regulation of mutant TERT by BRAF V600E/MAP kinase pathway through FOS/GABP in human cancer. Nat. Commun. **9**, 579 (2018)
13. S.W. Cho, J.H. Moon, Effects of coexistent BRAF V600E and TERT promoter mutations on poor clinical outcomes in papillary thyroid cancer. Thyroid **27**(5), 651–660 (2017)
14. V. Maggisano, M. Celano, G.E. Lombardo, S.M. Lepore, M. Sponziello, F. Rosignolo, A. Verrienti, F. Baldan, E. Puxeddu, C. Durante, S. Filetti, G. Damante, D. Russo, S. Bulotta, Silencing of hTERT blocks growth and migration of anaplastic thyroid cancer cells. Mol. Cell. Endocrinol. **448**, 34–40 (2017)
15. P.B. Chapman, A. Hauschild, C. Robert, J.B. Haanen, P. Ascierto, J. Larkin, R. Dummer, C. Garbe, A. Testori, M. Maio, D. Hogg, P. Lorigan, C. Lebbe, T. Jouary, D. Schadendorf, A. Ribas, S.J. O'Day, J.A. Sosman, J.M. Kirkwood, A.M.M. Eggermont, B. Dreno, K. Nolop, J. Li, B. Nelson, J. Hou, R.J. Lee, K.T. Flaherty, G.A. McArthur, Improved Survival with Vemurafenib in Melanoma with BRAF V600E Mutation. N. Engl. J. Med. **364**(26), 2507–2516 (2011)
16. E. Tiacci, J.H. Park, L. De Carolis, S.S. Chung, A. Broccoli, S. Scott, F. Zaja, S. Devlin, A. Pulsoni, Y.R. Chung, M. Cimminiello, E. Kim, D. Rossi, R.M. Stone, G. Motta, A. Saven, M. Varettoni, J.K. Altman, A. Anastasia, M.R. Grever, A. Ambrosetti, K.R. Rai, V. Fraticelli, M.E. Lacouture, A.M. Carella, R.L. Levine, P. Leoni, A. Rambaldi, F. Falzetti, S. Ascani, M. Capponi, M.P. Martelli, C.Y. Park, S.A. Pileri, N. Rosen, R. Foà, M.F. Berger, P.L. Zinzani, O. Abdel-Wahab, B. Falini, M.S. Tallman, Targeting mutant BRAF in relapsed or refractory hairy-cell leukemia. N. Engl. J. Med. **373**(18), 1733–1747 (2015)
17. D. Planchard, T.M. Kim, J. Mazieres, E. Quoix, G. Riely, F. Barlesi, P.-J. Souquet, E.F. Smit, H.J.M. Groen, R.J. Kelly, B.C. Cho, M.A. Socinski, L. Pandite, C. Nase, B. Ma, A. D'Amelio, B. Mookerjee, C.M. Curtis, B.E. Johnson, Dabrafenib in patients with BRAFV600E-positive advanced non-small-cell lung cancer: a single-arm, multicentre, open-label, phase 2 trial. Lancet Oncol. **17**(5), 642–650 (2016)
18. M.S. Brose, M.E. Cabanillas, E.E.W. Cohen, L.J. Wirth, T. Riehl, H. Yue, S.I. Sherman, E.J. Sherman, Vemurafenib in patients with BRAFV600E-positive metastatic or unresectable papillary thyroid cancer refractory to radioactive iodine: a non-randomised, multicentre, open-label, phase 2 trial. Lancet Oncol. **17**(9), 1272–1282 (2016)
19. P. Paci, T. Colombo, G. Fiscon, A. Gurtner, G. Pavesi, L. Farina, SWIM: a computational tool to unveiling crucial nodes in complex biological networks. Sci. Rep. **7**, 44797 (2017)
20. R.C. Edgar, MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. **32**(5), 1792–1797 (2004)
21. S. Bulotta, M. Celano, G. Costante, D. Russo, Emerging strategies for managing differentiated thyroid cancers refractory to radioiodine. Endocrine **52**(2), 214–221 (2015)
22. S.P. Weitzman, S.I. Sherman, Novel drug treatments of progressive radioiodine-refractory differentiated thyroid cancer. Endocrinol Metab Clin North Am. **48**(1), 253–268 (2018)
23. J.C.R. Fernandes, S.M. Acuña, J.I. Aoki, L.M. Floeter-winter, S. M. Muxel, Long non-coding RNAs in the regulation of gene expression: physiology and disease. Noncoding RNA. **5**(1) (2019). https://doi.org/10.3390/ncrna5010017
24. L. Poliseno, L. Salmena, J. Zhang, B. Carver, J. William, P.P. Pandolfi, A coding-independent function of gene and pseudogene mRNAs regulates tumour biology. Nature **465**(7301), 1033–1038 (2011)
25. L. Xiao-jie, G. Ai-mei, J. Li-juan, X. Jiang, Pseudogene in cancer: real functions and promising signature. J Med Genet. **52**(1), 17–24 (2015)
26. J.R. Kho, HHS public access. Pac Symp Biocomput. **23**, 536–547 (2018)
27. C.W. Chien, P.C. Hou, H.C. Wu, Y.L. Chang, S.C. Lin, S.C. Lin, B.W. Lin, J.C. Lee, Y.J. Chang, H.S. Sun, S.J. Tsai, Targeting TYRO3 inhibits epithelial-mesenchymal transition and increases drug sensitivity in colon cancer. Oncogene **35**(45), 5872–5881 (2016)
28. S. Hu, D. Liu, R.P. Tufano, K.A. Carson, E. Rosenbaum, Y. Cohen, E.H. Holt, K. Kiseljak-Vassiliades, K.J. Rhoden, S. Tolaney, S. Condouris, G. Tallini, W.H. Westra, C.B. Umbricht, M.A. Zeiger, J.A. Califano, V. Vasko, M. Xing, Association of aberrant methylation of tumor suppressor genes with tumor aggressiveness and BRAF mutation in papillary thyroid cancer. Int. J. Cancer **119**(10), 2322–2329 (2006)
29. J.J. Li, Z.J. Sun, Y.M. Yuan, F.F. Yin, Y.G. Bian, L.Y. Long, X. L. Zhang, D. Xie, EphB3 stimulates cell migration and metastasis in a kinase-dependent manner through Vav2-Rho GTPase axis in papillary thyroid cancer. J. Biol. Chem. **292**(3), 1112–1121 (2017)
30. S. Lee, Y. Jin, Y.A. Jeong, J. Lim, S. Cheul, Upregulation of EphB3 in gastric cancer with acquired resistance to a FGFR inhibitor. Int. J. Biochem. Cell. Biol. **102**, 128–137 (2018)
31. Z. Xuan, J. Huang, L. Gao, Y. Wang, J. Wang, Receptor tyrosine kinase EphB3: a prognostic indicator in colorectal carcinoma. Pathol Oncol Res. (2018). https://doi.org/10.1007/s12253-018-0562-x
32. M.C. Palumbo, S. Zenoni, M. Fasoli, M. Massonnet, L. Farina, F. Castiglione, M. Pezzotti, P. Paci, Integrated network analysis identifies fight-club nodes as a class of hubs encompassing key putative switch genes that induce major transcriptome reprogramming during grapevine development. Plant Cell Online **26** (12), 4617–4635 (2014)
33. G. Fiscon, F. Conte, V. Licursi, S. Nasi, P. Paci, Computational identification of specific genes for glioblastomastem-like cells identity. Sci. Rep. **8**(1), 41598 (2018)