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**COMPUTATIONAL ANALYSIS OF EXPRESSION PROFILING DATA IN PAPILLARY  
THYROID CANCER**

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## Abstract

Papillary thyroid carcinoma (PTC) is the most prevalent form of thyroid cancer, making up about 85% of the cases. Although in recent years several genes (e.g. BRAF, RAS, RET/PTC, TERT) have been demonstrated to play crucial roles in PTC, the molecular basis of carcinogenesis is not well understood. Using a bioinformatics tool, we investigated, through a network unbiased approach, the genes and pathways involved in this complex landscape. We exploited SWItchMiner (SWIM) software to analyze gene expression profiles available on The Cancer Genome Atlas. SWIM is able to identify a small pool of regulatory genes (switch genes), which are likely to be critically associated with drastic changes in cell phenotypes. We built networks based on switch genes (nodes) and their Pearson correlation value  $> |0.7|$  (connection). Finally, we selected those genes that were present only in the tumor or in the normal network. Comparing RNA-sequencing data between thyroid cancers and thyroid normal tissue samples, we identified 131 switch genes out of 1718 differentially expressed genes. We selected forty of them: four were present only in normal network while the others 36 in cancer network. Most of the selected genes are involved in cAMP-dependent pathway and phospholipase C signaling, and suggest a potential inhibition of the former and activation of the latter, with a release of  $\text{Ca}^{2+}$  from the endoplasmic reticulum into the cytoplasm. Moreover, there was an over-expression of genes promoting cellular cycle, NFkB and Wnt/beta catenin pathways and a down-regulation of genes involved in apoptosis and in the non-homologous end joining. Genes involved in metabolism suggest an increase in glycine and lipid synthesis, with an increase in the storage of the latter in lipid drops. Network analysis may provide an additional approach to explore the molecular basis of cancer and to select the main mechanisms involved in carcinogenesis. Although the data obtained need to be validated by vitro experiments, they may result in significant progress in diagnosis, prognosis and therapy of PTC.

*Key words:* papillary thyroid cancer, network medicine, SWIM.

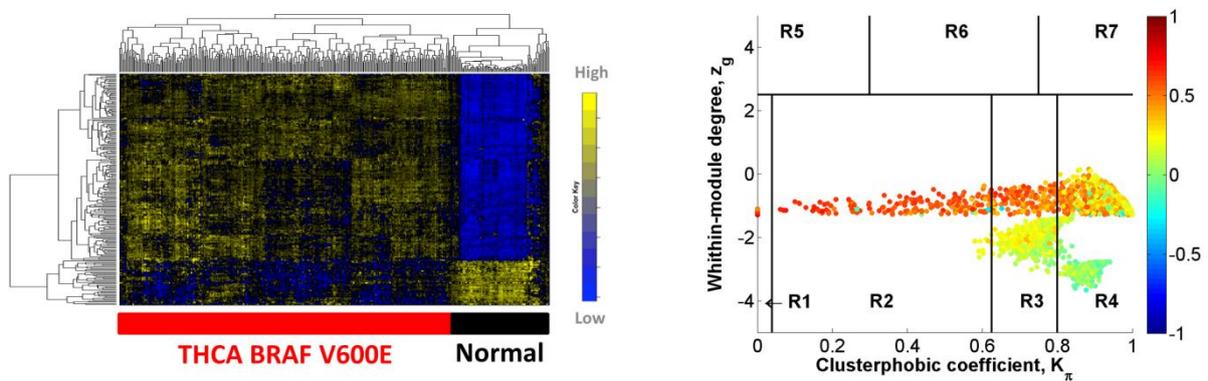
## 1. Introduction

Papillary thyroid carcinoma (PTC) accounts for approximately 85% of all thyroid malignancies [1]. In recent years, several gene alterations (e.g., BRAF, RAS, TERT mutations, RET/PTC rearrangements) have been identified as crucial players in PTC, but the molecular basis of PTC carcinogenesis is not well understood [2-4]. We used a novel bioinformatics tool to explore the gene network that characterizes PTCs harboring the BRAFV600E mutation and compared its features with those of the gene network characterizing BRAFV600E melanomas.

## 2. Methods

We downloaded RNA-sequencing data for BRAFV600E PTCs (n=302), normal thyroid tissues (n=59) and of BRAFV600E melanomas published in The Cancer Genome Atlas (TCGA) [5].

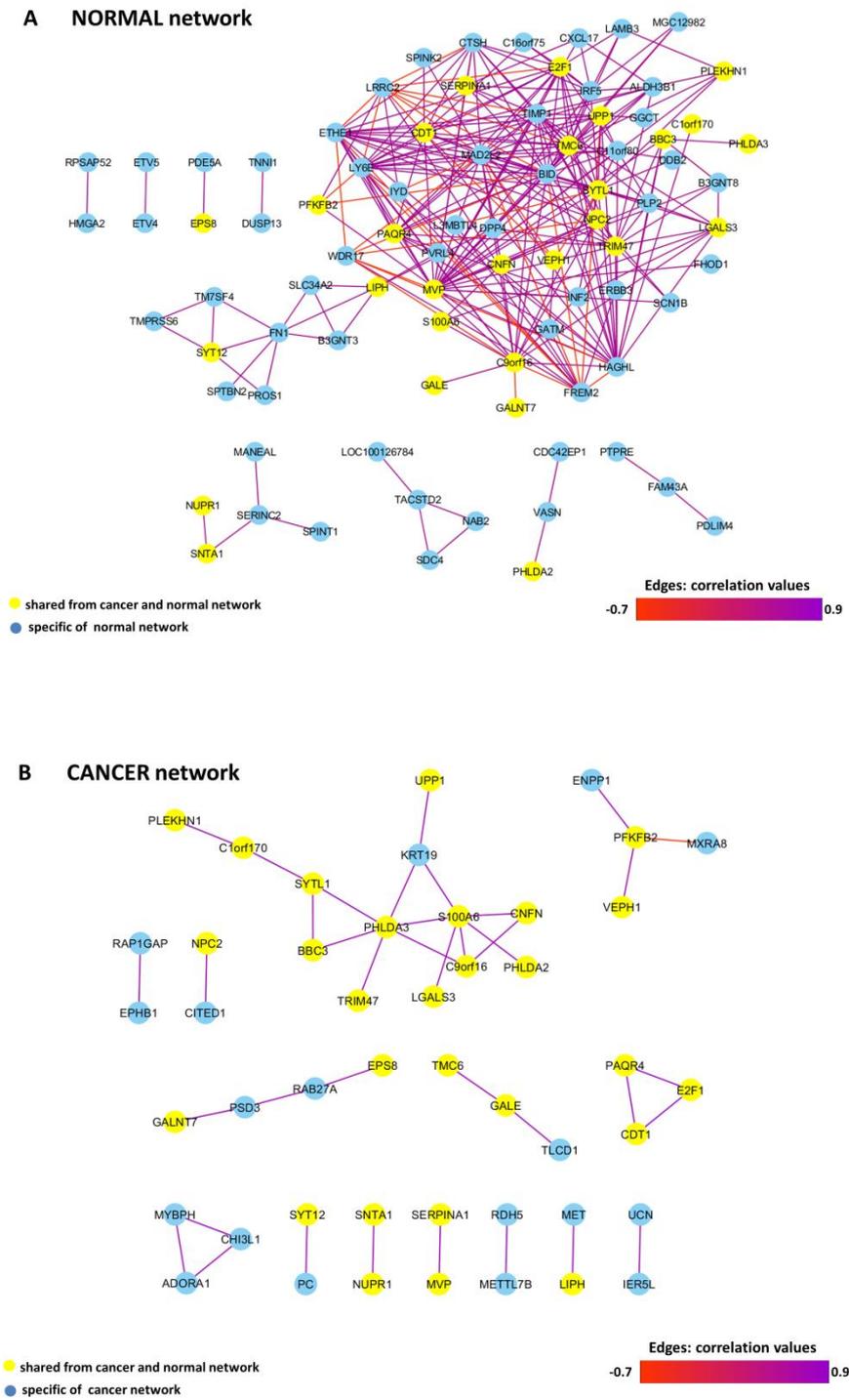
We used SWitchMiner (SWIM) software [6] to analyze the gene expression profiles of the BRAFV600E PTCs samples in comparison with the normal ones (Fig.1). SWIM is capable of identifying a small pool of regulatory genes known as switch genes, which are critically associated with drastic changes in cell phenotypes. Networks were then constructed based on switch genes (nodes) and their correlation values (those  $> |0.7|$ ) (connection), and KEGG pathway enrichment analysis was used to explore the switch genes' functions ([www.genome.jp/kegg/](http://www.genome.jp/kegg/)). For comparison purposes, we also used SWIM to analyze TCGA gene expression data on BRAFV600E melanomas.



**Fig.1. Identification of switch genes between BRAFV600E mutant PTC and normal thyroid**

### 3. Results

Comparison of TCGA RNA-sequencing data on BRAFV600E PTC and normal thyroid tissues revealed 2122 genes that were differentially expressed in the cancers, 227 of which were switch genes. We constructed switch gene correlation networks for the normal (Fig.2a) and cancerous thyroid tissues (Fig.2b).



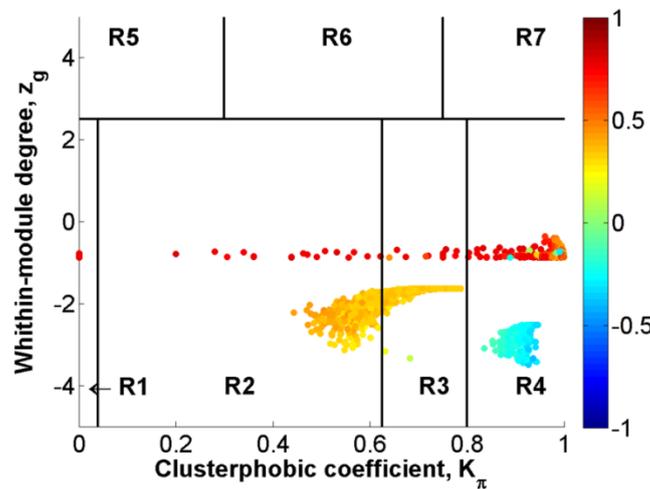
**Fig.2 Switch-gene networks in (a) normal thyroid and (b) BRAFV600E PTC (Person correlation  $\rho \geq |0.7|$  p-value > 0.05).**

The apparently simpler network found in the cancers suggests a wide carcinogenic process involving switch genes separated from one another by substantial distances. This conclusion is supported by KEGG pathway enrichment analysis of the switch-gene set, which revealed over-representation of only a few general pathways involved in cancer (Table 1).

KEGG Pathways			
Pathway ID	Pathway description	Count in gene set	False discovery rate
05200	Pathways in cancer	11	0.0412
05202	Transcriptional misregulation in cancer	8	0.0412

**Table.1 KEGG pathways enriched in switch genes**

Analyzing gene expression data on from BRAFV600E forms of PTC and melanomas, SWIM identified 428 differentially expressed switch genes, which highlight the marked differences between these cancers, despite their common driver mutation (Fig.4 and Table 2).



**Fig.4 Differences between BRAFV600E PTCs and BRAFV600E Melanomas.**

SUMMARY	
fold-change (FC) threshold	5
FDR threshold	0.05
Number RNAs DE	2224
Pearson Correlation threshold	0.74 (85 <sup>th</sup> prc)
Number of clusters	3
Number of switch genes	428

**Table 2. Summary of SWIM analysis on BRAFV600 PTC versus Melanoma datasets**

## 4. Conclusion

Network analysis may provide an additional approach to explore the molecular basis of cancer. PTCs driven by BRAFV600E seem to be characterized by an extensive molecular network in which the switch genes are widely separated from one other. The switch genes found in this network differ markedly from those identified in the network operating in BRAFV600E melanomas, suggesting that these two forms of cancer are profoundly different, de-spite the fact that they share the same driver mutation. This finding might explain why their reported rates of response to the BRAF inhibitor vemurafenib are different (Table 3).

BRAF V600E mutant Tu- mor	Overall response rate (%)
Melanoma	51
Thyroid cancer	38.5

**Table.3 Responses of BRAFV600E thyroid cancers and melano-mas to vemurafenib.**

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