Editorial

Oncogenic changes in a new transgenic model of BRAF^{V600E} in thyroid

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Thyroid cancer is the most common endocrine cancer and its incidence has increased worldwide in the last two decades (1). Papillary thyroid carcinoma (PTC) is the most common type of thyroid cancer, representing up to 80% of all malignant thyroid tumors. The genetic background of PTC comprises the mutually exclusive activating mutations in effectors genes of MAPK signal transduction pathway such as RET, RAS, BRAF (2,3). Among them, the most frequent is the *BRAF*^{V600E} mutation (mutBRAF), resulting from a T→A transversion at nucleotide position 1799 in *BRAF* gene, which accounts for 45% of mutations in PTC and elicits a highly sustained activation of MAPK pathway (4).

Since the high incidence of mutBRAF in PTC was first described in 2003, much progress has been made in uncovering its influence in MAPK pathway signaling and transcriptional networks (5,6). Several genetically engineered models of thyroid cancer were generated, including the mutBRAF as PTC driver mutation (7). Up to now, the transgenic animal models to recapitulate thyroid cancer were based on mice background closely related to humans. However, depending of the *BRAF*^{T1799A} constructs, the transgenic strains present a variable efficiency in transforming thyrocytes to malignancy (8-11) (*Table 1*).

In a recent report in *Elife*, Anelli *et al.* (12) presented a model of human mutBRAF drived transgenic zebrafish (*Danio rerio*) that recapitulates thyroid tumorigenesis. Zebrafish shares 70% of orthologues genes with human genome, and this model has been widely utilized to study human genetic diseases, including carcinogenesis (13). This new model in zebrafish shows the involvement of the highly conserved MAPK signaling pathway, selectively stimulated by human mutBRAF, in the zebrafish thyroid follicular cells tumorigenesis. Furthermore, in the present model the thyrocytes were fluorescently labeled allowing live imaging of tumors formation and growth. The study was carried out following the probands originated from muBRAF mother in the larvae stage 5 days post-fertilization and in the adult zebrafishs (5 and 12 months post-fertilization) (12).

Several interesting questions are raised by this work. In the larvae, as soon as 5 days post-fertilization (dpf), the thyroid follicle disorganization and loss of thyroid hormones production were observed, while the expression profile of proliferation markers was not changed. Moreover, this zebrafish model requires a long-time period for thyroid cancer progression. The invasion features were observed at 5 months, even though, the characteristics of PTC cells such as nuclear grooves were detected just after 1 year (12). At this late stage, the proliferation and metabolism signaling pathways were enriched in cancer cells.

These observations could be attributed to the morphological characteristic a zebrafish thyroid gland, which lacks a precise delimiting capsule, favoring the expansion out of the gland (14). Nevertheless, it is tempting to suggest an association with clinical feature such as invasiveness prior to the enlargement of primary tumor growth, observed in patient with mutBRAF metastatic microcarcinoma (15,16)

Taking advantage of this slow growing PTC model, a comparative study of gene expression profile between adult mutBRAF-zebrafish (12 months) and the TCGA human PTC database was performed and identified high expression of Twist family. Upon deleting twist 2, ortholog of human

Table 1 Transgenic model leading to thyroid $BRAF^{V600E}$ expression

Thyroid $BRAF^{V600E}$ expression	Animal	*$BRAF^{T1799A}$* construct	PTC features	Reference
Constitutive model	Mice	*Tg* promoter-*$BRAF^{T1799A}$*	Papillary structure	(8)
			Tall cell	
			Frequent invasion	
	Zebrafish	*tg* promoter-*$BRAF^{T1799A}$*-*pA*	Nuclear groove	(12)
			Overcrowding	
			Frequent invasion	
Conditional and/or inducible *knock in* model	Mice	*TgrtTA/tetO-$BRAF^{T1799A}$* + doxycycline	Nuclear groove	(9)
			Overcrowding	
			Solid growth pattern	
			Poor differentiated with invasion	
	Mice	*LoxP-flanked $BRAF^{T1799A}$ /Tg:CreER* + tamoxifen	Papillary structure	(10)
			Nuclear groove	
			Pseudonuclear inclusion	
			Invasion	
	Mice	*LSL-$BRAF^{T1799A}$/TPO-Cre*	Papillary structure	(11)
			Tall cell	
			Pseudonuclear inclusion	
			Frequent invasion	

TWIST3, using *CRISPR/Cas9* gene editing, the adult muBRAF zebrafish, interestingly, recovered the follicle morphology and restored thyroid hormone synthesis. Furthermore, the authors tested whether the inhibition of MAPK signaling pathway could block the mutBRAF-induced alteration in the follicle structure at the larvae stage. Indeed, the combined treatment with dabrafenib (a $BRAF^{V600E}$ inhibitor) and selumetinib (a MEK inhibitor) restored the normal thyroid morphology (12). The ability to respond to the specific targeting compounds makes this less expensive model extremely useful to address the efficacy of emerging agents.

In contrast with the mice model in which the EMT genes were expressed at the late stage of thyroid cancer development (17,18) and as aggressive thyroid cancer biomarkers in human thyroid cancer (19,20), the muBRAF-thyroid zebrafishs elicit the upregulation of genes associated with EMT and TGF-β signaling at early larvae stage that persist through the adult stages (12). The authors suggest their participation in the thyroid follicle morphogenesis observed at earliest time. However, it is intriguingly that even though the precocious enrichment of gene related with EMT as soon as in larvae stage, the adult transgenic zebrafish still preserve the histological epithelial follicular structures in the gland and maintain thyroglobulin expression for at least 1 year, period covered by this study.

Overall, the work by Anelli *et al.* presents a challenging approach using zebrafish to recapitulate the mutBRAF-induced oncogenesis. This study shed light in the promising model that could be useful to manipulate genes and molecules in the understanding of thyroid cancer progression behavior.

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Footnote

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