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Rethinking the role of oncogenes in papillary thyroid cancer initiation

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Thyroid cancer originating from follicular epithelial cells accounts for approximately 1% of all new case of cancer each year and its incidence has increased significantly over the last two decades (Hodgson et al., 2004; Davies and Welch, 2006). Papillary thyroid carcinoma (PTC) accounts for approximately 85% of all cases, and it is responsible for the overall increase in incidence of thyroid cancer. Mortality in PTC is low and the majority of patients can be considered cured after thyroidectomy followed by ablation of thyroid remnant by 131-iodine (Cooper et al., 2009).

Molecular studies performed in the last decades, have elucidated in part the molecular mechanisms underlying thyroid cancer initiation and progression. Specific genetic alterations are associated to this thyroid tumor histotype: *RET/PTC* and *TRK* rearrangements and *BRAF* and *RAS* mutations.

The first genetic alteration discovered in PTC and also the most specific was the *RET/PTC* rearrangement (Fusco et al., 1987). *RET/PTC* is a chimeric gene generated by the fusion of the tyrosine kinase domain of the rearranged during transfection gene (*RET*) to the 5′terminal region of genes that are constitutively expressed in thyroid follicular cells (Pierotti et al., 1992; Santoro et al., 1992, 2006; Nikiforov, 2002). The chimeric proteins generated dimerize in a ligand-independent manner and result in a cytoplasmatic constitutively active tyrosine kinase. The higher frequency of PTC observed in the population exposed to the Chernobyl accident supports a role for the external radiations in the chromosome rearrangements observed in this tumor (Nikiforov, 2006). It has been proposed that the spatial proximity of translocation-prone gene loci may favor gene rearrangements. Indeed, proximity between *RET* and *H4*, and *NTRK1* and *TPR* has been reported in interphase thyroid nuclei. Thus, in this simplified model, radiations induce chromosome rearrangements and generation of *RET/PTC* or *TRK* oncogenes that will be initiator of thyroid carcinogenesis. The role of *RET/PTC* in thyroid carcinogenesis is supported by experimental evidences generated in cells in culture and in animal models. PCCl3, a differentiated rat thyroid cell line, stably transfected with a *RET/PTC3* expressing plasmid undergoes morphological alterations and is no longer TSH dependent for growth (Santoro et al., 1993). Thyroid-specific expression of the *RET/PTC1* or *RET/PTC3* in transgenic mice induces thyroid tumors with features resembling those of human PTC. These tumors are characterized by nuclear grooves and ground glass cells, continuous slow growth rate, and loss of iodide uptake (Jhiang et al., 1996; Santoro et al., 1996). However, some evidence suggest that *RET/PTC* alone is not sufficient to develop thyroid carcinoma, and other molecular events are needed. Thyroid cancer occurs only after a long latency period and only in a fraction of *RET/PTC* transgenic animals. At beginning, the majority of studies excluded the occurrence of *RET/PTC* in benign thyroid nodules. In following studies, *RET* rearrangements have been demonstrated in nodules diagnosed as benign at histology. Ishizaka et al. (1991) have been the first to detected *RET/PTC* in 21% of follicular adenomas. The use of highly sensitive detection methods contributed to definitively demonstrate that *RET* rearrangements occurs in a significant fraction of both radiation-induced and sporadic benign nodules (Bounacer et al., 1997; Cinti et al., 2000; Guerra et al., 2011; Marotta et al., 2011a; Sapio et al., 2011). Its presence in benign nodules, raised some queries about the role of *RET/PTC* in thyroid carcinogenesis. Doubts on the primary role of *RET/PTC* in thyroid carcinogenesis are also supported by the evidence that some irradiated PTC are composed of a mixture of cells with and without *RET* rearrangements. In sporadic microcarcinomas and post-Chernobyl PTC interphase fluorescence *in situ* hybridization (FISH) analysis demonstrated that *RET/PTC* rearrangements can occur only in a fraction of the cells, indicating that PTC can be composed of a mixture of cells with and without *RET* rearrangements (Corvi et al., 2001; Unger et al., 2004). These evidences are in favor of a secondary role of *RET/PTC* which would not be the initiating event in thyroid carcinogenesis.

BRAF is a protein-serine/threonine kinases that participate in the mitogen-activated protein kinase (MAPK) cascade (Wellbrock et al., 2004). By modulating the MAPK cascade, BRAF plays a pivotal role in many aspects of cell biology in nearly every cell type. More than 65 different mis-sense *BRAF* mutations have been detected in human cancer so far (Davies et al., 2002). The $BRAF^{V600E}$ mutation, resulting from the $BRAF^{T1799A}$ transversion, is nearly the only mutation of this kinase found in thyroid cancer and the most common genetic mutation in PTC, being detected in about 50% of cases (Kimura et al., 2003; Xing, 2005; Marotta et al., 2011b). This mutation occurring within the activation segment, disrupts the hydrophobic interaction between the glycine-rich loop of the N-terminal region and the activation segment of the kinase domain, and transforms BRAF in a constitutively activated kinase (Davies, et al., 2002; Brummer et al., 2006; Moretti et al., 2009). In the thyroid, this oncogene is restricted to papillary-patterned cancer and it does not occur in Hashimoto's thyroiditis, benign colloid nodules, thyroid adenomas, or other types of thyroid tumor (Xing, 2007).

Its restricted occurrence makes *BRAF*^{V600E} of clinical diagnostic utility (Xing, 2007; Zatelli et al., 2009). Its carcinogenetic potential has been demonstrated in several different cell types and in animal models. Thyroid-specific expression of *BRAF*^{V600E} obtained in transgenic mice by the bovine thyroglobulin promoter provided us with important information on the tumorigenic potential of this oncogene. By 12–22 weeks age, transgenic mice revealed a large goiter, well differentiated thyroid cancer foci, and poorly differentiated foci in some animals, depending on the level of expression of the *BRAF*^{V600E} mRNA. These tumors displayed a phenotype similar to that one of spontaneous human PTC, supporting a key role for this oncogene in the tumor initiation of this type of cancer and in the progression to poorly differentiated carcinomas (Knauf et al., 2005). In a more recent animal model, expression of *BRAF*^{V600E} was obtained in adult mice in already developed thyroid glands. After 1 month of induced expression of the oncogene, mice developed an hypercellular thyroid, up to 10 times larger in size than controls, whilst nodules of tumor cells displaying a characteristic papillary structure were readily apparent 6 months after *BRAF*^{V600E} expression (Charles et al., 2011). These experimental animal models demonstrate that *BRAF*^{V600E} can promote the transformation process of the thyroid follicular cell, however they do not demonstrate that this oncogene is the initiating event in spontaneous human PTC. Very recently a more detailed analysis of *BRAF*^{V600E} expression by means of a quantitative assay, demonstrated the heterogeneous intratumoral nature of spontaneous PTC. The analysis of the percentage of mutant *BRAF* demonstrated that clonal *BRAF*^{V600E} is a rare occurrence in PTC, while more frequently this cancer consists of a mixture of tumor cells with wild-type and mutant *BRAF*. This result demonstrates that *BRAF* mutation in PTC is a secondary subclonal event (Guerra et al., 2012a).

Thus, the original idea that a normal thyroid cell, under the effect of ionizing radiations or other mutagenic factors, acquires the *RET/PTC* rearrangement or *BRAF*^{V600E} mutation and consequently is transformed by these oncogenes in what we call a PTC cell, should be revised. Although heterogeneity is the rule in cancer, the identification of the genetic initiating event is important, not only to understand the molecular mechanisms of tumorigenesis, but also for practical purposes. A high percentage of *BRAF*^{V600E} alleles is associated with a higher frequency of recurrence (Guerra et al., 2012b). This makes a quantitative assessment necessary to use *BRAF*^{V600E} in clinical practice as a predictor of recurrence in PTC. Also targeted therapeutic interventions must take into account of this heterogeneity. The recent development of novel small-molecule inhibitors targeting one or more of these oncogenes may provide selective advantages for the treatment of advanced thyroid cancer harboring these mutations (Salerno et al., 2010; Nucera et al., 2011). Many of these promising drugs are currently being evaluated in clinical trials and the presence of target-negative subpopulations should be considered.

In conclusion, we have to revise our simplistic vision of thyroid carcinogenesis. Oncogenes known so far may play important role in the fate of a PTC, conferring specific biological and clinical features, but the genetic event initiating thyroid cancer is still to be identified.

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