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THE BRAF V600E MUTATION DOES NOT MAKE PAPILLARY THYROID CANCERS MORE VASCULAR OR EXPRESS MORE VEGF-A OR VEGF RECEPTORS

Durante C, Tallini G, Puxeddu E, Sponziello M, Moretti S, Ligorio C, Cavaliere A, Rhoden KJ, Verrienti A, Maranghi M, Giacomelli L, Russo D, Filetti S. **BRAFV600E mutation and expression of proangiogenic molecular markers in papillary thyroid carcinomas.** Eur J Endocrinol 2011;165;455-63. Epub June 8, 2011; doi: 10.1530/EJE-11-0283.

SUMMARY

BACKGROUND

If a papillary thyroid cancer (PTC) has invaded the lymphatic nodes or blood vessels, the patient's risk of having a recurrence, of distant metastases, and of death is increased. Therefore, it would be important to learn what factors are involved in lymphovascular growth in PTCs. Vascular endothelial growth factors (VEGFs) and platelet-derived growth factors (PDGFs) act via tyrosine kinase receptors to promote growth of lymph and blood vessels. Certain tyrosine kinase inhibitors (TKIs) are known to inhibit tumor vascularity and growth, but the mechanisms involved remain unclear. Some TKIs that block VEGF and PDGF receptors (VEGFRs, PDGFRs) also inhibit the serine/threonine kinase BRAF. The most common point mutation in thyroid cancers is BRAF (V600E), which is present in about 40% of all PTCs, and can be twice as common in some geographic regions. This mutation constitutively activates the downstream MEK/ERK pathway and can cause cell transformation. The authors of this paper studied a collection of PTCs previously obtained from several Italian centers to address whether the V600E mutation is associated with tumors that are more vascular; that express higher levels of VEGF-A, VEGFRs 1–3, or PDGFR-β mRNAs; or have histologic or clinical features associated with increased risk of tumor spread or recurrence.

METHODS

The levels of mRNAs encoding VEGF-A, VEGFRs 1–3, the co-receptor neuropilin-1, and PDGFR-β were measured by reverse transcriptase–polymerase chain reaction (RT-PCR) in 55 selected PTCs bearing the V600E mutation and in 35 PTCs without this mutation. Smaller numbers of histologic samples were assayed for VEGF-A and VEGFR, and for microvessel and lymphatic vessel density from the two groups, using a biotin-free horseradish peroxidase detection method for immunostaining. In addition, the V600E mutant was induced/silenced in rat/human cell lines in vitro to study the effects on pro-angiogenic gene expression.

RESULTS

The mRNA levels of the pro-angiogenic factors were actually lower in the PTCs that expressed the V600E mutation. Vessel densities and VEGFR staining intensities were not significantly different between PTCs expressing V600E and those expressing wild-type (wt) BRAF. In both wt and mutant BRAF tumors, lymphatic density was higher outside the tumors, whereas microvascular density was higher within the tumors. In the rat cell line, inducing V600E BRAF caused the level of VEGF-A mRNA to fall, while silencing V600E increased VEGF-A mRNA levels in the human cell line.

CONCLUSIONS

The V600E-containing tumors actually expressed lower levels of VEGF-A, VEGFRs, and PDGFR-β mRNA. Although the vessel densities and VEGFR staining were similar in both V600E-positive and V600E-negative tumors, one probably should not draw any firm conclusions, because of the small number of cases examined in the present study. In contrast, PDGF-β (not the receptor) was reported to be increased in V600E-positive tumors in a Chinese study, while unspecified VEGF isoform(s) were reported to be increased in a Korean study.

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COMMENTARY

A recent meta-analysis of the literature found that PTCs bearing the V600E mutation were more likely to be associated with advanced tumor–node–metastasis (TNM) staging, regardless of the geographic region being studied (1). The association with extrathyroidal invasion, however, was greater in geographic regions where the mutation occurred in less than half the PTC population, and the association with lymph-node metastases was significant only in geographic regions where the mutation occurred in less than half the PTC population. Thus, it might be informative to look for markers that are associated with lymph-node metastases in geographic regions where the V600E mutation occurs in more than half of PTCs.

The decreased tissue expression of VEGF-A, VEGFRs 1–3, and PDGFR-β mRNA levels in V600E expressing PTCs suggests that these tumors have made compensatory responses to the constitutive activation of BRAF, prior to any treatment. Phase 2 trials with several TKIs have shown promising partial responses in PTC, but the low frequency of sustained complete remissions suggests that simply blocking the oncogenic effect of the BRAF mutation—while concurrently inhibiting VEGFR and PDGFR tyrosine kinases—still permits other oncogenic changes in the complex feedback loops that regulate the Ras/BRAF/MAPK/ERK and other pathways (2).

It remains to be established whether the V600E mutation is associated with the circulating levels of other pro-angiogenic cytokines, platelets, and soluble VEGFRs and with the tissue levels of other VEGF family members, other angiogenic factors, other V600 BRAF mutations, BRAF fusion proteins, heterodimer partners or alternatively spliced isoforms, as well as to miRNAs and the BRAF pseudogene.

Clinically, the detection of the V600E mutation appears to improve identification of a PTC preoperatively, and can help in surgical decision-making if a fine-needle aspiration (FNA) biopsy is equivocal, by analyzing fluid obtained by rinsing the FNA needle (3). Postoperatively, if V600E is present in a PTC that is <1 cm in diameter, the risk of lymph-node metastases or tumor recurrence is increased if certain histopathologic features are also present (4).

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