Hgf/Met activation mediates resistance to BRAF inhibition in murine anaplastic thyroid cancers

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In Brief
Anaplastic thyroid cancer (ATC) is a devastating disease. Outcomes are dire with a median survival of approximately 6 months. Treatment options are limited due to the likelihood of inadequate surgical resection and cytotoxicity of chemotherapeutic agents, therefore, the quest for innovative therapies is necessary. Previous work has identified BRAFV600E as a common mutation in papillary thyroid cancer (PTC) and ATC (1). BRAFV600E has been heralded as a primary driver of thyroid oncogenesis mediated through activation of the mitogenic MAP kinase pathway and previous studies have, therefore, evaluated BRAF inhibition for new treatment regimens. Overall, these studies have had disappointing results. Response rates lack durability likely due to development of tumor adaptation that overcomes MAP kinase pathway dependence on BRAFV600E activity (1, 2).

The primary purpose of the study published by Knauf and colleagues was to explore the mechanisms of acquired resistance to inhibition of BRAFV600E in ATC. In order to investigate this important phenomenon, ATC was induced in transgenic mice using doxycycline-mediated thyroid specific expression of a BRAFV600E transgene in animals carrying a p53 tumor suppressor gene knockout. Following oral doxycycline administration over a 9-week period, transgenic mice developed ATC in 50% of cases. BRAFV600E transgene expression was then deinduced in these mice by doxycycline-withdrawal. These animals' tumors were assessed by magnetic resonance imaging (MRI) 3-4 weeks later to evaluate disease response. Lack of BRAFV600E transgene expression resulted in disease regression in all surviving mice. BRAFV600E deinduction was associated with increased progression-free survival and overall survival relative to control mice, for which maintenance doxycycline had been continued (all of which succumbed to their disease).

However, after an 18-month period, 87% of deinduced/dox-free diet mice developed tumor recurrence, predominately in the primary tumor bed. Most recurrences were categorized as ATC, although other tumor types were also identified in a subset of these mice, including mucinous adenocarcinoma and poorly differentiated thyroid cancer. The majority of ATC recurrences (17 of 20 mice) were BRAFV600E transgene independent. Analysis of BRAFV600E independent recurrent ATC tumors revealed an increase in MAP kinase pathway activity (MAP kinase output score) compared to normal thyroid tissue, indicating bypass of MAP kinase pathway dependence on BRAFV600E expression in these cancers.
Cancer cell dependence on MAP kinase pathway activation was maintained in some recurrent tumors, indicated by the disruption of MAP kinase pathway signaling via use of MEK protein inhibitors, which resulted in regression in 3 out of 4 of recurrent tumors. Five out of 11 tumors had focal chromosome 6 amplification, which correlated with 7 different genes, one in particular being Met. Because of the known pre-existing relationship between Met and MAPK signaling, the role of Met overexpression in BRAFV600E independent ATC recurrence was explored. The investigators found that recurrent tumor cells did overexpress Met, as well as its ligand Hgf, and were subsequently exquisitely sensitive to Met inhibition. The non-Met amplified recurrences were found to be resistant to this inhibition.

This study concluded that tumors that develop resistance to BRAFV600E gene product inhibitors may benefit from additional upstream inhibition of Met/Hgf or downstream inhibition of MEK that ultimately result in decreased MAPK expression and/or activity and, therefore, decreased cell proliferation.

**Critique and Future Directions**

MET gene overexpression-mediated bypass of BRAFV600E activation of the MAP kinase pathway in ATC, as highlighted in this article, represents a potentially clinically significant target for future research and therapies. Indeed, this mechanism has been previously described by other authors using in vitro/cell line-based thyroid cancer models (3, 4). This work is particularly important given the very poor clinical outcomes among patients diagnosed with ATC. The study design employed by the authors (an inducable transgenic mouse model) represents accepted and well-established methodology. The experiments described were carefully executed and well controlled.

Nonetheless, although MET gene overexpression has been implicated in the development of BRAFV600E independence in other tumor models (i.e. melanoma), its in vivo clinical relevance in ATC remains unclear. In particular, validating studies assessing MET gene expression in human ATC samples remain necessary. In addition, previous work by other investigators (5) suggests that MET gene overexpression is a common feature of PTC, calling into question its relative importance as a promoter of ATC development. It is also imperative to note that although focal amplification of chromosome 6 was the most common genetic alteration associated with BRAFV600E independent ATC recurrence in this study (5 of 11 recurrent tumors), this finding was not present in the majority of recurrences. This finding indicates multimodal mechanisms for development of BRAFV600E independence in ATC, thus suggesting that MET targeting may only be clinically useful in a subset of ATC patients. Similarly, 6 genes in addition to MET were identified in the focal chromosome 6 amplification described. The significance of these genes in the development of BRAFV600E independence may be crucial and remains an uncharted area of research. Finally, this murine model simplifies ATC compared to the human disease process by re-creating tumors via BRAF and p53 mutations only, eliminating the implications of other known mutations that may impact the tumor biology, aggressiveness, and, thus, responsiveness to MET-targeting therapeutics.

Overall, this vital research portends future contributions to the realm of BRAF independent ATC tumor development and offers exciting new targets for the advancement of ensuing therapeutic agents.

**References:**
5. (C) Luigi Ruco, Stefania Scarpino. The pathogenetic role of the Hgf/c-Met system in papillary carcinoma of the thyroid. Biomedicines. 2014; 2(4), 263-274.