The Role of a More Invasive Phenotype in Response to MAPK-Directed Therapies in Thyroid Cancer

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Abstract

Purpose: Advanced papillary thyroid cancer (PTC) and anaplastic thyroid cancer (ATC) are the leading causes of endocrine cancer death. Mutations in the MAP kinase (MAPK) pathway are common in PTC and ATC, especially in *BRAF*. However, therapies targeting the MAPK pathway are not approved for PTC patients, and despite the approved combination of BRAF and MEK inhibition to treat *BRAF*-mutant ATC, these patients often progress. An emerging mechanism of resistance to targeted therapies is an invasive phenotype switch in which cells transition from a proliferative, therapy sensitive population to an invasive, therapy resistant population.

Results: Using Matrigel Chamber Invasion assays, we showed that *BRAF*-mutant PTC and ATC cells resistant to BRAFi exhibit an increase in invasion when treated with BRAFi while sensitive cells do not. We further identified an increase in the levels and secretion of fibronectin (FN1) in response to BRAFi treatment in resistant cells. Treatment with either FN1 or conditioned media from BRAFi-treated resistant cells phenocopies BRAFi-treatment by also increasing invasion. However, depletion of FN1 blocks this response. Interestingly, ERK inhibition also mitigates the invasiveness observed in response to BRAFi or FN1 in resistant cells. We further observed that dual BRAF and ERK inhibition slows tumor growth *in vivo* in a BRAFi-resistant patient-derived xenograft model.

Conclusions: These data indicate that thyroid cancer cells resistant to BRAF inhibition exhibit a more invasive phenotype characterized by increased FN1 and a pro-invasive secretome. Further, dual inhibition of BRAF and ERK ablates BRAFi-induced invasion and slows tumor growth *in vivo,* providing a potential therapeutic strategy for *BRAF*-mutant thyroid cancer patients.

Introduction

An invasive phenotype is an emerging mechanism of resistance to targeted therapies

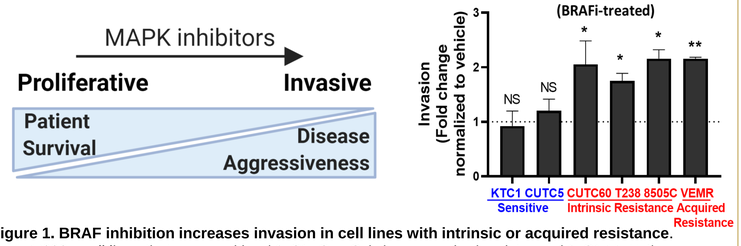


Figure 1. BRAF inhibition increases invasion in cell lines with intrinsic or acquired resistance. BRAFV600E cell lines that are sensitive (KTC1, CUTC5), have acquired resistance (KTC1-VEMR), or intrinsic resistance (T238, 8505C, CUTC60) to BRAF inhibition were treated with dabrafenib (BRAFi) for 24 hrs then plated in Matrigel-coated Boyden chambers for 24 hrs. Invading cells were stained with DAPI and counted using ImageJ. Results displayed as mean normalized to DMSO treated control, +/- SEM. *, p<0.05; **, p<0.01.

BRAF inhibition increases fibronectin, which promotes invasion in BRAFi-resistant thyroid cancer cells

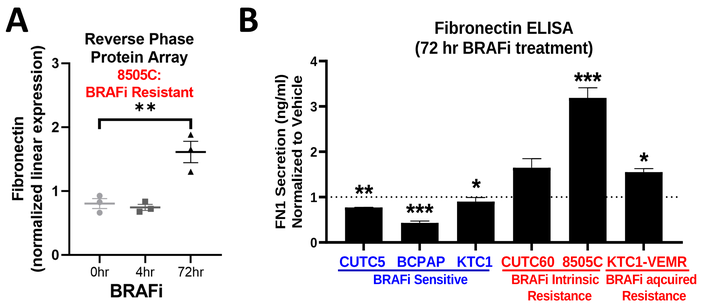


Figure 2. BRAF inhibition increases fibronectin (FN1) expression and secretion. A) 8505C cells were treated with 1 μM vemurafenib for 4 hrs or 72 hrs and protein expression was quantified using RPPA (MD Anderson Functional Proteomics Reverse Phase Protein Array) B) Cells were treated with vehicle or BRAFi for 4 hrs or 72 hrs and secreted FN1 was quantified using an ELISA assay (ThermoFisher). *p<0.05; **p<0.01; ***, p<0.001.

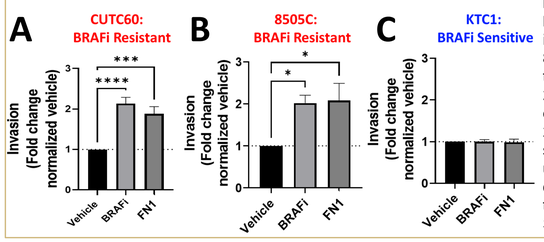


Figure 3. In resistant cells, FN1 treatment phenocopies BRAFi by increasing invasion. A) CUTC60 B) 8505C or B) KTC1 cells were treated with BRAFi or FN1 for 24 hrs and plated in Matrigel-coated Boyden chambers for 24 hrs. Invaded cells were stained with DAPI and counted using ImageJ. Results displayed as mean normalized to DMSO-treated control +/- SEM. *, p<0.05

Results

Hypothesis: BRAF inhibition increases the production and secretion of fibronectin to promote a pro-invasive secretome.

Fibronectin is required for BRAF inhibitor-induced invasion.

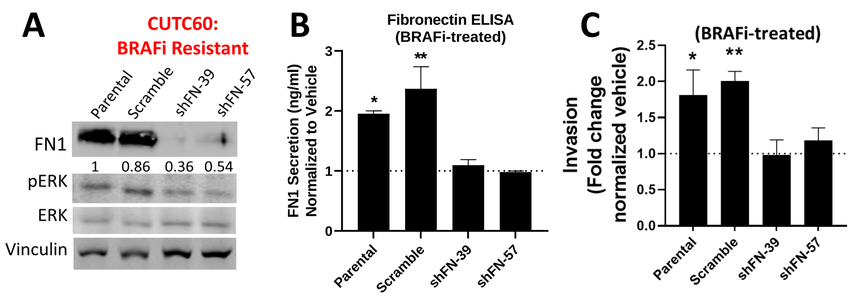


Figure 4. FN1 is required for a BRAFi-induced invasive phenotype. A) FN1 was knocked down using shRNA in CUTC60 cells. Cell lysates were analyzed via Western Blot for indicated antibodies. B) Cells were treated with vehicle or BRAFi for 72 hrs and secreted FN1 was quantified using an ELISA assay (ThermoFisher). C) Indicated cells were treated with BRAFi for 24 hrs then plated in Matrigel-coated Boyden chambers for 24 hrs. Invading cells were stained with DAPI and counted using ImageJ. Results displayed as mean normalized to DMSO treated control +/- SEM. *, p<0.05; **, p<0.01.

MAPK pathway reactivation occurs in response to BRAF inhibition.

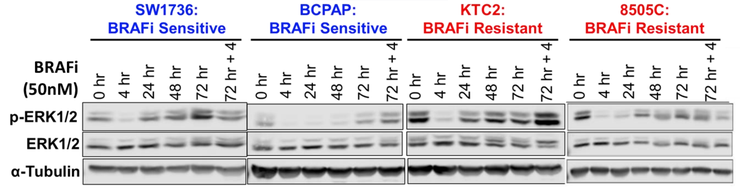


Figure 5. MAPK pathway reactivation occurs in response to single agent BRAFi. BRAFi sensitive and BRAFi resistant BRAFV600E cell lines were treated with BRAFi at various time points. Cell lysates were analyzed via western blot with the indicated antibodies. *, p<0.05; **, p<0.01.

Dual Inhibition of BRAF and ERK prevents MAPK reactivation, blocks invasion, and slows tumor growth.

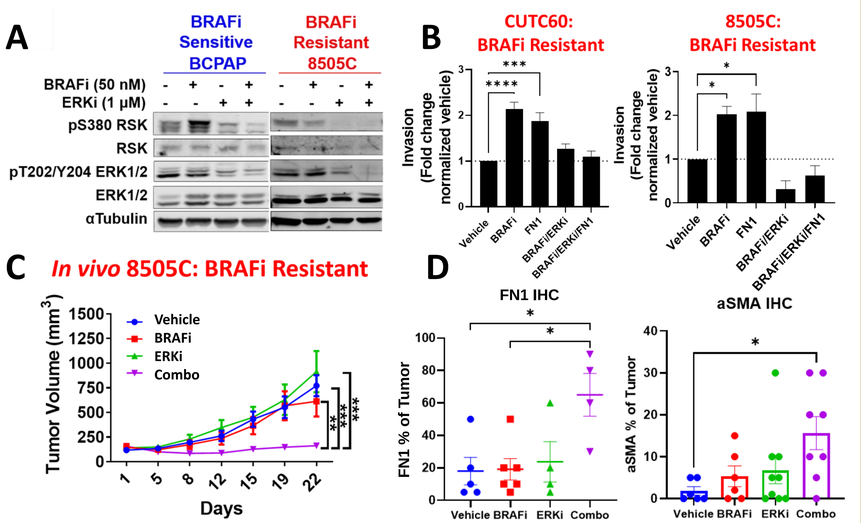


Figure 6. Combined BRAFi/ERKi prevents MAPK pathway reactivation and slows tumor growth. A) BRAFi sensitive and resistant cells were treated with BRAFi, ERKi, or the combination at various time points. Cell lysates were analyzed via western blot with the indicated antibodies. B) BRAFi resistant cells were treated with indicated compounds for 24 hrs then plated in Matrigel-coated Boyden chambers for 24 hrs. Invading cells were stained with DAPI and counted using ImageJ. C) BRAFi resistant 8505C cells were injected into the flanks of nude mice. Upon tumor establishment, mice were randomized and treated daily with vehicle, BRAFi, ERKi, or the combination via oral gavage. D) At study end, tumors were resected and IHC was performed for the indicated antibodies. *, p<0.05; **, p<0.01; ***, p<0.001.

Results

BRAF inhibition promotes a pro-invasive secretome in BRAFi-resistant cells

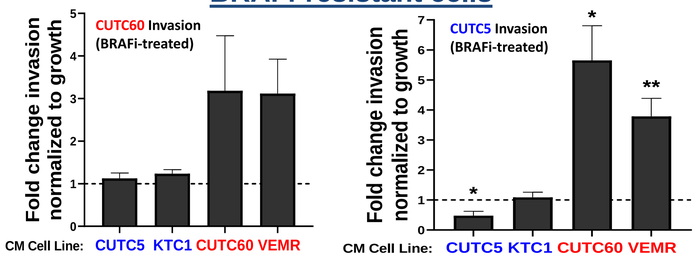


Figure 7. Conditioned media from resistant, but not sensitive, cells is pro-invasive. A) BRAFi-resistant or -sensitive cell lines were treated with conditioned media from indicated cell lines for 24 hrs then plated in Matrigel-coated Boyden chambers for 24 hrs, stained with DAPI and counted using ImageJ. Results displayed as mean normalized to DMSO treated control (i.e. invasiveness of cells treated with CM from a vehicle-treated cell line) +/- SEM. *, p<0.05; **, p<0.01.

Collagen is increased in response to BRAFi in resistant cells and promotes formation of invasive protrusions

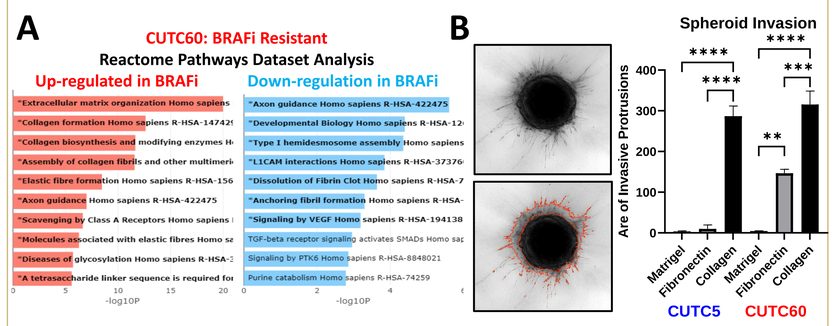
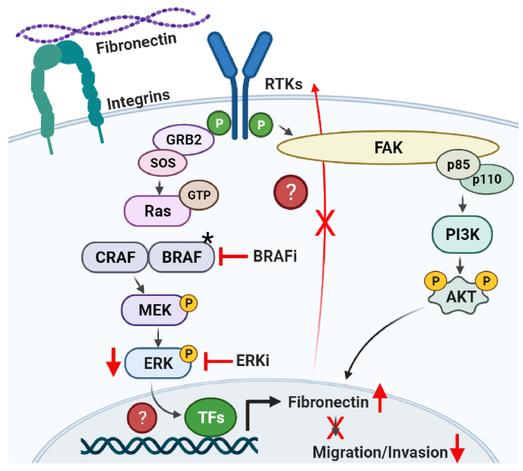


Figure 8. Collagen promotes formation of invasive protrusions. A) RNA-sequencing was performed on CUTC60 cells treated with BRAFi for 48 hrs (Anschutz Medical Campus Functional Genomics Core). Pathway analysis was then performed using BioJupies. B) Indicated cell lines were plated in ultra-low attachment, round-bottom, 96 well plates (Sbio). Spheroids formed for 24 hrs. 50% Matrigel, 20 ug/ml FN1, or 30% Rat Tail Collagen I was added to each spheroid. After 5 days, Z-stacked images were projected, and spheroids/protrusions were quantified using the Cytation 5 Imaging Multi-Mode Reader. *, p<0.05; **, p<0.01; ***.p<0.001; ***,P<0.0001.

Conclusions & Future Directions

Conclusions:

- BRAF inhibition and FN1 treatment increases invasion in BRAFi-resistant cells.
- FN1 is necessary for BRAFi-induced invasion, but not reactivation of the MAPK pathway.
- Combined BRAF and ERK1/2 inhibition prevents MAPK pathway reactivation, blocks invasion, and slows tumor growth.
- Resistant cell lines can exhibit a pro-invasive secretome in response to BRAFi.
- Collagen promotes spheroidal invasion in sensitive and resistant cells



Future Directions:

- Characterize the role of EGR1 in an invasive phenotype in response to BRAFi
- Determine the role of a BRAFi-driven invasive phenotype in promoting invasion and metastasis *in vivo,* and whether ERK inhibition can block this phenotype.

References: Subbiah, V. *et al. J Clin Oncol* **36**, 7-13(2017); Shah, M. H. *et al. Journal of Clinical Oncology* **35**(2017); Welsh, S. J.,*et al. V. Eur J Cancer* **62**, 76-85 (2016); Emmons, M. F., Faiao-Flores, F. & Smalley, K. S. M. *Biochem Pharmacol* **122**, 1-9(2016); Kemper, K.,*et al. Cancer Res* **74**, 5937 5941(2014); Lovly, C. M. & Shaw, A. T. *Clin Cancer Res* **20**, 2249-2256(2014); Liu, F., Yang, X., Geng, M. & Huang, M. *Acta Pharm Sin B* **8**, 552-562 (2018); Ahronian, L. G. *et al. Cancer Discov* **5**, 358-367(2015); Hicks HM, McKenna LR, et al. Molecular Carcinogenesis (2021).