The role of an invasive phenotype in promoting resistance to MAPK-directed therapies in thyroid cancer

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Abstract

Purpose: Mutations in the MAP kinase (MAPK) pathway are common in advanced papillary thyroid cancer (PTC) and anaplastic thyroid cancer (ATC) , especially in *BRAF* (40-60%). Despite the approved combination therapy of dabrafenib (BRAFi) and trametinib (MEKi) for $BRAF^{V600E}$-mutated ATC, PTC patients fail to benefit from the BRAFi/MEKi combination over single agent therapy and many ATC patients develop resistance over time. An emerging mechanism of resistance to targeted therapies involves a more invasive phenotype in which cells transition from a proliferative, therapy sensitive population to an invasive, therapy resistant population.

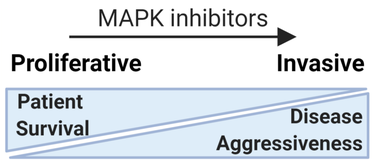
Experimental Design: Here, we examined the effects of BRAF and ERK inhibition on signaling, cell growth, and invasion and investigated the role of fibronectin (FN) in a more invasive phenotype in response to inhibition of the MAPK pathway.

Results: We found that in response to BRAFi, cells with intrinsic resistance to BRAFi exhibit an increase in invasion while sensitive cells do not. We identified an increase in the extracellular matrix protein, FN, at 72hrs in response to BRAFi. Using ELISA assays, we found that secreted FN increases in response to BRAFi in resistant cell lines, but decreases in response to BRAFi in sensitive cell lines. Further, addition of FN-supplemented media phenocopies treatment with BRAFi by increasing invasion in BRAFi-resistant cell lines. MAPK pathway reactivation occurs by 72hrs following BRAFi treatment, as indicated by rebound of pERK levels. However, combination treatment with BRAFi and ERKi prevents pERK rebound. Further, combined BRAFi/ERKi mitigates the increase in invasion observed in response to single-agent BRAFi or FN treatment in BRAFi-resistant cell lines. Importantly, treatment of sensitive cell lines with conditioned media from BRAFi-treated resistant cells increases invasion in the sensitive cells, suggesting the secretome of resistant cells promotes invasion in response to BRAFi.

Conclusions: These data indicate that a more invasive phenotype characterized by MAPK pathway reactivation and alterations in the secretome, including an increase in FN, play a role in resistance to BRAF inhibition in thyroid cancer.

Introduction

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



- An emerging mechanism of resistance in response to single agent BRAF inhibition is a more invasive phenotype during which they transition from a proliferative, drug sensitive phenotype to an invasive, drug resistant phenotype in order to survive therapy

BRAF inhibition increase invasion in BRAFi-resistant thyroid cancer cell lines

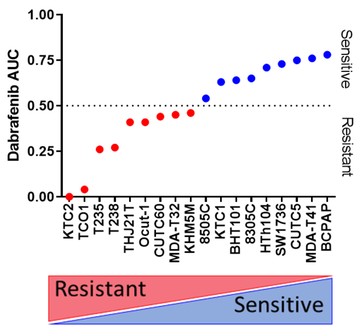


Figure. 1. BRAF mutant thyroid cancer cell lines exhibit varying sensitivity to BRAF inhibition. A panel of 18 thyroid cancer cell lines with BRAF-V600E mutations were treated with increasing concentrations of dabrafenib (BRAF inhibitor) for 72 hours. Cell viability was measured using CellTiter-Glo 2.0 assay. Area under the dose response curve (AUC) values were calculated with greater AUC signifying greater sensitivity.

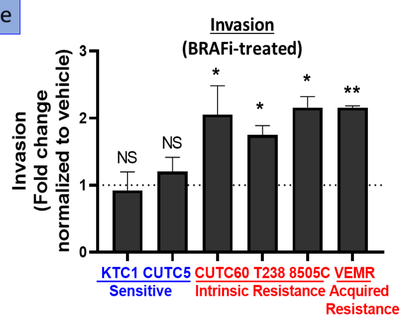
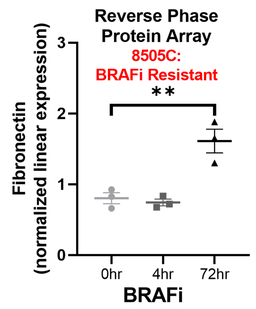


Figure 2. BRAF inhibition increases invasion in cell lines with intrinsic or acquired resistance. BRAFV600E cell lines that are sensitive (KTC1, CUTC5), have acquired resistance (KTC1-VEMR), or intrinsic resistance (T238, 8505C, CUTC60) to BRAF inhibition were treated with BRAFi for 24 hrs then plated in Matrigel-coated Boyden chambers for 24 hrs. Invading cells were stained with DAPI and counted using ImageJ. Results displayed as averages normalized to DMSO treated control, indicated with a dotted line, +/- SEM. *, p<0.05; **, p<0.01.

Results

BRAF inhibition increases fibronectin levels and secretion in BRAFi-resistant thyroid cancer cells



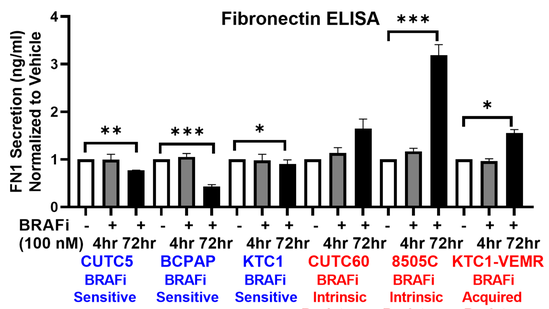


Figure 3. BRAF inhibition increases fibronectin expression. 8505C cells were treated with 1 μM vemurafenib for 4 hrs or 72 hrs and protein expression was quantified using RPPA (MD Anderson Functional Proteomics Reverse Phase Protein Array), *, p<0.05; **, p<0.01.

Figure 4. BRAF inhibition decreases FN secretion in sensitive cell lines but increases FN secretion in resistant cell lines. Cells were treated with vehicle or BRAFi for 4 hrs or 72 hrs and secreted FN was quantified using an ELISA assay (ThermoFisher). *p<0.05; **p<0.01; ***, p<0.001.

BRAF inhibition and fibronectin promote invasion in BRAFi-resistant cell lines

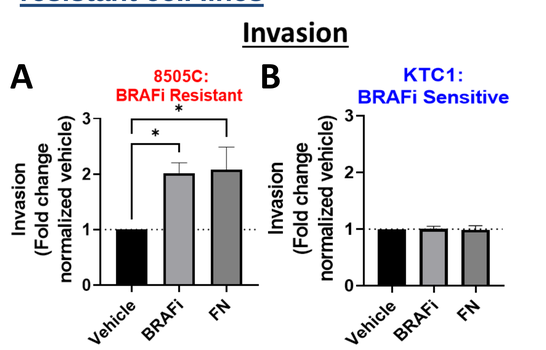


Figure 5. In resistant cells, FN treatment phenocopies BRAFi by increasing invasion. A) 8505C or B) KTC1 cells were treated with the indicated drugs, serum starved for 24 hrs, and plated in Matrigel-coated Boyden chambers for 24 hrs. Invaded cells were stained with DAPI and counted using ImageJ. Results displayed as averages normalized to DMSO-treated control, indicated with a dotted line, +/- SEM.

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

MAPK pathway reactivation occurs in response to BRAF inhibition.

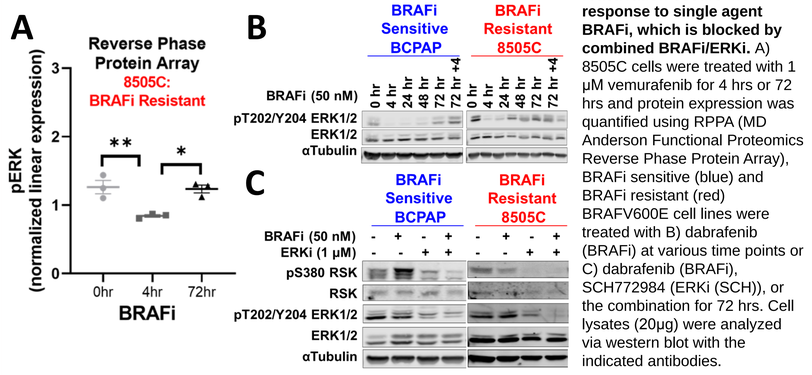
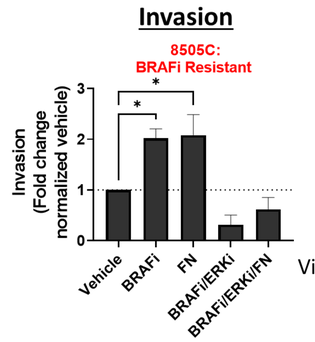


Figure 6. MAPK pathway reactivation occurs in response to single agent BRAFi, which is blocked by combined BRAFi/ERKi. A) 8505C cells were treated with 1 μM vemurafenib for 4 hrs or 72 hrs and protein expression was quantified using RPPA (MD Anderson Functional Proteomics Reverse Phase Protein Array), BRAFi sensitive (blue) and BRAFi resistant (red) BRAFV600E cell lines were treated with B) dabrafenib (BRAFi) at various time points or C) dabrafenib (BRAFi), SCH772984 (ERKi (SCH)), or the combination for 72 hrs. Cell lysates (20μg) were analyzed via western blot with the indicated antibodies.

Results

Fibronectin is required for BRAF inhibitor-induced invasion.



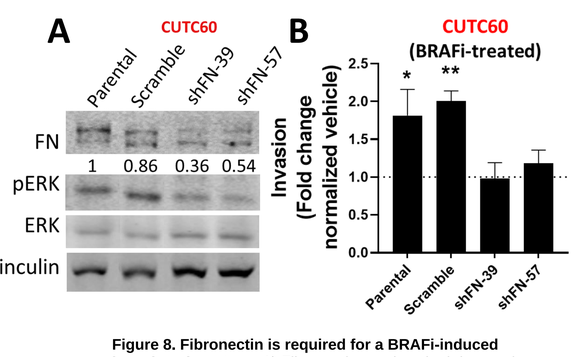


Figure 7. In resistant cells, FN treatment phenocopies BRAFi by increasing invasion, which can be blunted by ERKi. 8505C cells were treated with the indicated drugs, serum starved for 24 hrs, and plated in Matrigel-coated Boyden chambers for 24 hrs. Invaded cells were stained with DAPI and counted using ImageJ. Results displayed as averages normalized to DMSO-treated control +/- SEM.

Figure 8. Fibronectin is required for a BRAFi-induced invasive phenotype. A) Fibronectin was knocked down using shRNA in BRAFi-resistant CUTC60 cells. Cell lysates were analyzed via Western Blot. B) Indicated cell lines 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 averages normalized to DMSO treated control +/- SEM. *, p<0.05; **, p<0.01.

BRAF inhibition promotes a pro-invasive secretome in BRAFi-resistant cell lines

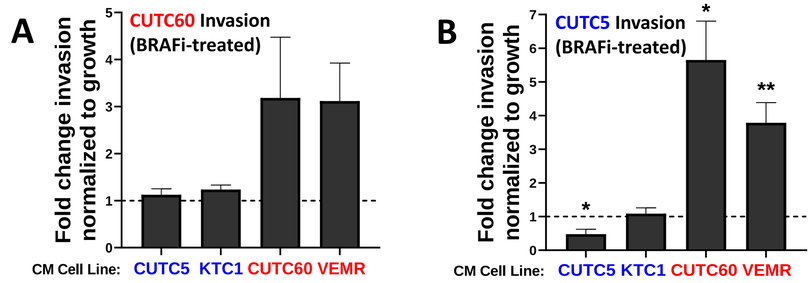
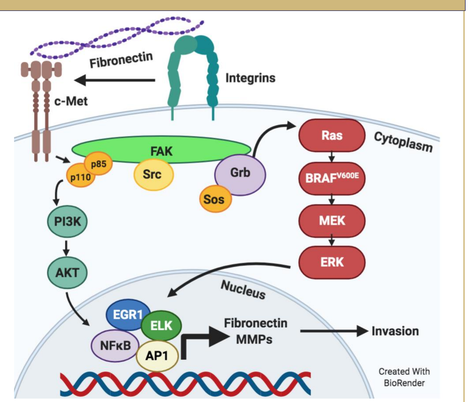


Figure 9. Conditioned media from resistant, but not sensitive, cells is pro-invasive. BRAFV600E cell lines that are A) BRAFi-resistant or B) BRAFi-sensitive were treated with conditioned media from indicated cell lines for 24 hrs then plated in Matrigel-coated Boyden chambers for 24 hrs. Invading cells were stained with DAPI and counted using ImageJ. Results displayed as averages normalized to DMSO treated control (i.e. invasiveness of cells treated with CM from a vehicle-treated cell line), indicated with a dotted line, +/- SEM. *, p<0.05; **, p<0.01.

Conclusions & Future Directions

- BRAF inhibition increases invasion in BRAFi-resistant cell lines.
- Fibronectin levels are increased in response to BRAFi
- Inhibition of the MAPK pathway using single-agent therapies results in pERK rebound.
- Combined BRAF and ERK1/2 inhibition prevents pathway reactivation and synergistically inhibits cell growth.
- BRAF inhibition and fibronectin treatment increases invasion in resistant cell lines, which can be overcome by inhibiting ERK.
- Resistant cell lines can exhibit a pro-invasive phenotype in response to BRAFi.



Future Directions:

- Fully characterize components of a pro-invasive secretome driven by BRAFi *in vitro*,
- 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: N Engl J Med. 2016 Sep 15;375(11):1054-67. N Engl J Med. 2011;364:2507–16. Clin Cancer Res. 2012;18(7):2056-65. Expert Opin Emerg Drugs. 2014:1–17. Nature. 2010;468:968-972. J Clin Oncol. 2018;36(1):7-13. J Clin Oncol 35, 2017 (suppl; abstr 6022). Cancer Discov. 2013;3(7):742-50.