The role of a cysteine residue within an ERK1/2 substrate docking site on signaling and proliferation of melanoma cells containing BRAF mutations

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Abstract:

The role of extracellular signal-regulated kinase 1/2 (ERK1/2) in signaling pathways in cells is crucial for cell proliferation. Within specific types of cancers, a member of this pathway, BRAF, is mutated at the valine (V)600 position so that this pathway is continuously activated, leading to uncontrolled proliferation. A docking site in ERK1/2 is of interest for inhibitors to control activation of downstream proteins responsible for transcription.[1] A compound has been developed to target a substrate docking site and was found to target a specific cysteine[2]. This residue has been mutated via CRISPR CAS9 in both ERK1 and ERK2 and the proposed studies investigate the effects the ERK1/2 cysteine mutations have on A375 cell melanoma cells regarding cell signaling and proliferation.

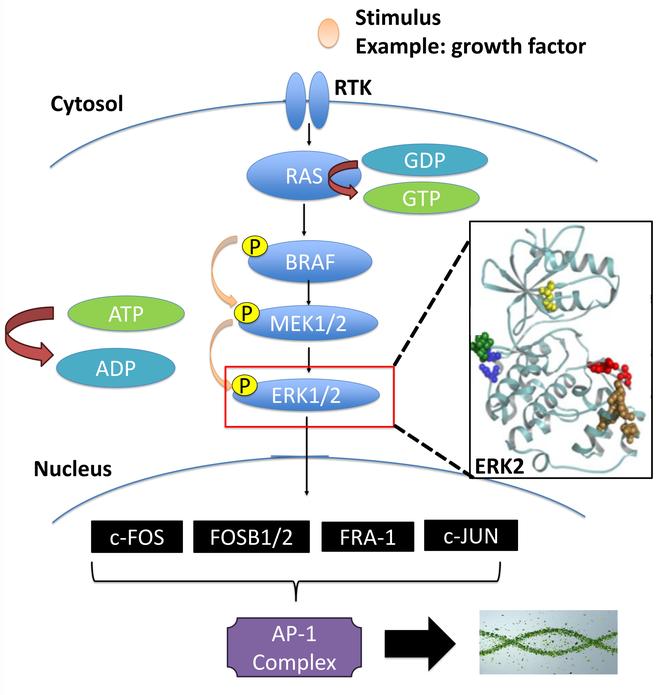

***Figure 1**: ERK1/2 pathway and Structure of ERK2. Ribbon structure of ERK2 shows substrate docking sites (green, blue, and brown) as well as activation site (red) and ATP-competitive site (yellow).*

***Figure 2:** Image adapted from Martinez III, et al to show the mechanism by which SF3-030 interacts with C252 in ERK2.[2] On the right, figure shows the other locations of cysteine residues (green), substrate docking site (red), activation site (magenta), and cysteine of interest in red font.*

Methodology:

- A375 melanoma cell line containing a BRAF mutation that leads to constitutive activation of ERK1/2 signaling was used as a model for this study
- A375 parent cells were mutated via CRISPR/Cas9 system to generate A375 cells containing mutations at C271A/C252A of ERK1/2
- Mutants were characterized and compared to parents via viability and immunoassay

Results:

- Slower growth of ERK1 $^{C271A-/-}$ cell line
- There may be different ERK content in each mutation
- Increase in FOSB expression in the ERK1 $^{C271A-/-}$ mutation

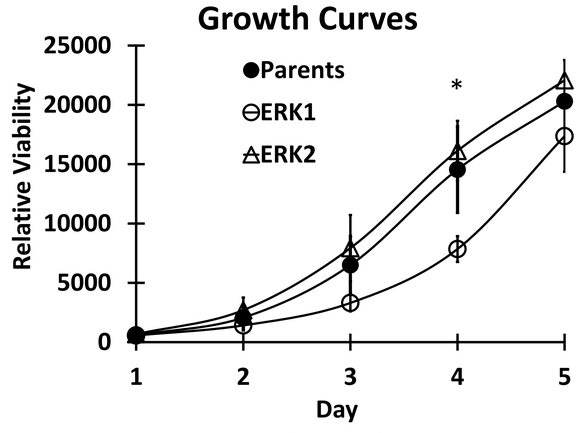

***Figure 3:** Viability data over time for the ERK1/2 cysteine mutants. Unpublished data. Significance is for a p-value<0.05 for ERK1 $^{C271A-/-}$ compared to WT parent A375 cells.*

Results Continued

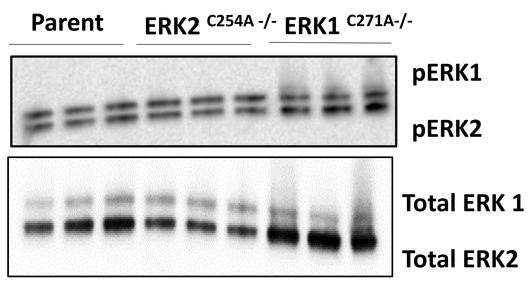

***Figure 4:** Immunoblot of total ERK protein in technical triplicate of the ERK1/2 cysteine mutants. Unpublished data*

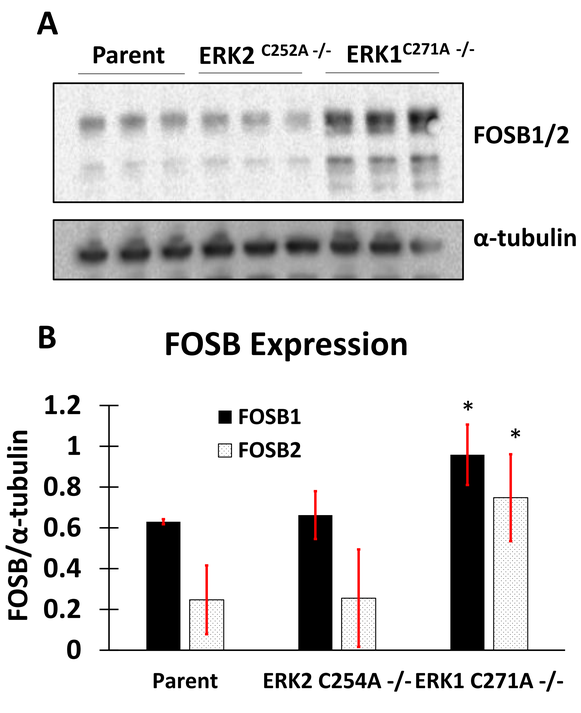

***Figure 5:** Quantification of FOSB proteins in the homozygous ERK1/2 cysteine mutants. Unpublished data. Significance is for a p-value<0.05 for ERK1 $^{C271A-/-}$ compared to WT parent A375 cells.*

Conclusions:

- There is a slower growth rate and an increase in expression of the transcription factor FOSB/B2, suggesting a signaling role of this residue in the ERK1 $^{C271A-/-}$ mutant.
- Visual levels of total ERK expression vary among the ERK1/2 cysteine mutants.
- Future studies needed to investigate signaling and proliferation.

Future Directions:

- Repeat experiments with new mutation when it is created (ERK1$^{C271A-/-}$ and ERK2$^{C254A-/-}$) .
- AP-1 expression and activity through immunoblotting and luciferase reporter assay needs to be investigated.
- Global proteomic studies using mass spectrometry on all ERK1/2 cysteine mutants to identify adducts and AP-1 protein regulation.

References:

(1) Defnet, A. E; et al, *FASEB J* **2019**, *33* (10), 10833-10843. DOI: 10.1096/fj.201900680R

(2) Martinez, R., et al. *J Pharmacol Exp Ther* **2021**, *376* (1), 84-97. DOI: 10.1124/jpet.120.000266

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