Characterization of GRB2 Binding Site Mutation in Oncogenic Fusion Protein BCR-FGFR1

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Introduction

Receptor Tyrosine Kinases (RTKs) are surface cell receptors which control signaling pathways and regulate various cell processes such as cell proliferation. The breakpoint cluster region-fibroblast growth factor receptor (BCR-FGFR1) fusion results from a chromosomal translocation, leading to unregulated and uncontrolled cell proliferation. This causes the development of cancer, such as leukemia. The adapter protein growth factor receptor bound protein 2 (Grb2) creates a multimeric signaling complex by joining a variety of signaling molecules. The goal of this study was to determine if mutating tyrosine to phenylalanine (Y177F) would disable or significantly reduce signaling or foci formation in cells with this oncogenic fusion protein. This study found that the mutation, Y177F, decreases the signaling in the oncogenic cells. However, the mutation did not completely stop signaling because foci were observed in the focus assay plate with BCR(Y177F)-FGFR1.

Methods

Designed Plasmid
pLXSN

Polymerase Chain Reaction (PCR)

Figure 1: Plasmid Map and Sequence

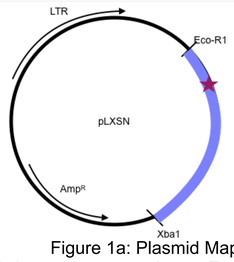


Figure 1a: Plasmid Map
Restriction enzymes used were Eco-R1 and Xba1. The plasmid contained an ampicillin resistant gene. An LTR promoter was used. The purple highlighted region is the BCR-FGFR1 gene

Y177F Mutation

Y
GAG AAG CCC TTC TAC GTG AAC GTC GAG TTT C
GAG AAG CCC TTC TTC GTG AAC GTC GAG TTT C
F

Figure 1b: Sequence
Highlighted codon shows the intended mutation. The top strand is the original sequence, the bottom strand is the mutated sequence. The A was mutated to a T through site-directed mutagenesis. This mutation allows for Y177F.

Bacterial Colonies

Plate PCR Product

Mini Prep

Digest
EcoR1-HF
Xba1

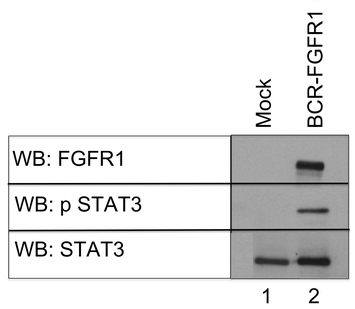
DNA Gel

SDS Page/Protein Gel

Western Blot
FGFR1
P-STAT3
STAT3

Results

Figure 2: Western Blotting



Western blot used HEK293T cells.

The FGFR1 blot confirmed BCR-FGFR1 in cells. The p STAT 3 blot checked for phosphorylation of STAT3. The STAT 3 blot confirmed total STAT3 in mock and BCR-FGFR1 cell lysates.

Figure 3: Focus Assay
The focus assay used NIH3T3 cells. Foci only appeared when the cells were transfected with an oncogenic construct.

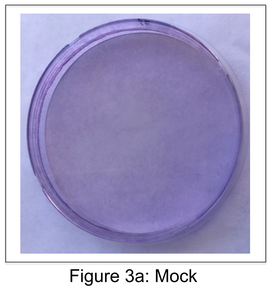


Figure 3a: Mock

The mock plate (Figure 3a) did not have foci growth.

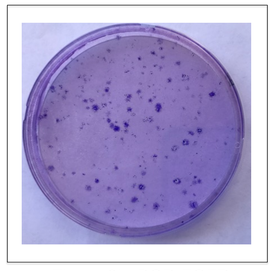


Figure 3b: BCR-FGFR1

The BCR-FGFR1 plate (Figure 3b) had the most number of foci.

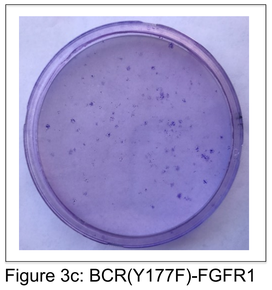
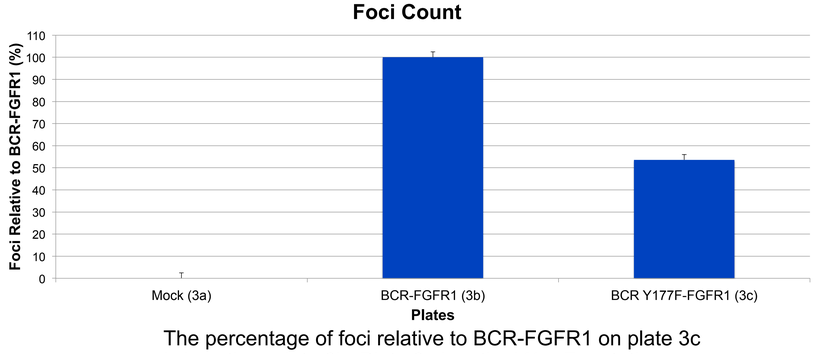


Figure 3c: BCR(Y177F)-FGFR1

The BCR(Y177F)-FGFR1 plate (Figure 3c) had fewer foci than the BCR-FGFR1 plate.

Figure 4: Focus Assay Graph



The percentage of foci relative to BCR-FGFR1 on plate 3c is less than plate 3b. Plate 3c has the Y177F mutation.

Discussion/Conclusion

According to the National Cancer Institute, cancer affects about 38.4% of men and women. This research will enable more people who are diagnosed with cancer to receive better treatment. This study, focused on the BCR-FGFR1 fusion which is found in some leukemias. Specifically, the Y177F mutation was investigated in order to abolish the Grb2 binding site in BCR.

This study used NIH3T3 (mouse) cells and HEK293T (human) cells.

Western Blotting was done on the BCR-FGFR1 fusion protein in 293T cells. FGFR1 was present in the cells meaning we were able to successfully place the plasmid in cells. First, BCR-FGFR1 expression was confirmed through western blot. Then p STAT3 activation was confirmed through western blot. The third test confirmed total STAT3 in the mock and BCR-FGFR1 cell lysates.

The amino acid tyrosine can be phosphorylated however, phenylalanine is not able to be phosphorylated. The BCR(Y177F)-FGFR1 mutant while able to signal for cell proliferation, reduced the number of foci on the petri dish (Figure 3c) compared with the BCR-FGFR1 plate (Figure 3b).

It is crucial to identify and treat cancer in patients quickly and effectively. This research will someday help patients receive lifesaving treatment for leukemia. This study shows that mutating the BCR fusion protein does lower its signaling ability; the BCR(Y177F)-FGFR1 mutated fusion protein showed a decrease in signaling ability. Patients positive for BCR-FGFR1 fusion protein may benefit from drugs that target Grb2 signaling pathways.

Future

While there some mutations that are well studied, there are also many that are not well studied. In the future I would like to look at additional mutations in the BCR-FGFR1 gene.

References

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