Targeting MSH2 using CRISPR/Cas9

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Introduction

- Clustered regularly interspaced short palindromic repeats (CRISPR) and their associated protein9 (Cas9) is an adaptive mechanism found in some bacteria1.
- In genome editing CRISPR/Cas complexes have been modified to allow for the identification targeted genomic loci using a 20 nucleotide RNA guide (gRNA), complementary to its target DNA sequence1.
- Synthesized gRNA combines to the DNA and recruits Cas protein, which cleaves the DNA1.

Motivation

- Colorectal cancer is the third most common cancer in the U.S., accounts for 8% of all cancer1.
- The 5 year survival rate is 64.9%1.
- MSH2 gene provides instructions for making the MSH2 protein which repairs DNA.
- Mutated MSH2 increases the risk of Lynch syndrome, which increases the risk of colorectal cancer4.
- Target and cleave MSH2 at specific exons using CRISPR/Cas system.
- Determine the cleaving efficiency at these sites may be use to insert therapeutic donor.

Methodology

Transformation of gRNA/SpCas9 dual expressing plasmid into bacterial cells

Transfection of GFP and gRNA/SpCas9 plasmids to HEK293Ts. DNA(MSH2 area) gets cleaved by Cas protein.

Harvest of genomic DNA from the cells

Amplify the MSH2 area and perform T7 and detect cleavage on MSH2.

Results

A

Fig2. (A) Bright field images of HEK293Ts. Plasmids expressing gRNA, spCas9 and GFP were transfected. (B) GFP images of HEK293Ts. GFP is expressed in the cells. Scale bar = 100μm.

B

Untransfected

Exon3.1

Exon3.2

Exon3.3

Exon4.1

Fig3. Flow cytometer analysis of transfected HEK293Ts cells. The selected region P1 shows the percentage of viable cells, while M1 shows GFP positive cells for each treatment.

Future Work

- Correcting the mutation in MSH2 site using Exon3.2
- Applying the methods to other diseases such as endometrial cancer, where MSH6 gene is mutated5.

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Conclusions

Exon 3.3 cleaved the MSH2 most efficiently, which its NHEJ percentage was 19.1%. The results of Exon 4 were inconclusive.

References

[1] Patrick D. Hsu, Cell (2014)