Exploring prevention of cancer metastasis through the DGKι – RhoC interaction: deletion of DGKι exon 25

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Introduction

Metastasis, the migration of cancer cells from the primary tumour to other body parts, is the leading cause of cancer-related death. Cells need actin protein for migration, which is regulated in part by the RhoC enzyme. In earlier studies, RhoC overexpression was shown to drive metastasis, while its genetic deletion resulted in almost complete metastasis prevention. Inhibiting RhoC can therefore lead to treatments reducing metastasis. Recent findings from Dr. Gee's lab suggest that an enzyme called diacylglycerol kinase iota (DGKι) regulates RhoC's abundance and enzymatic activity. The ACC-1 domain of DGKι was shown to be necessary for DGKι-RhoC binding. Part of this domain's amino acid sequence is encoded by an alternatively-spliced exon, named exon 25. The inclusion of this exon is hypothesized to promote metastasis by allowing DGKι to bind to and regulate RhoC, which in turn would regulate actin organization and allow the molecular switch to a metastatic state.

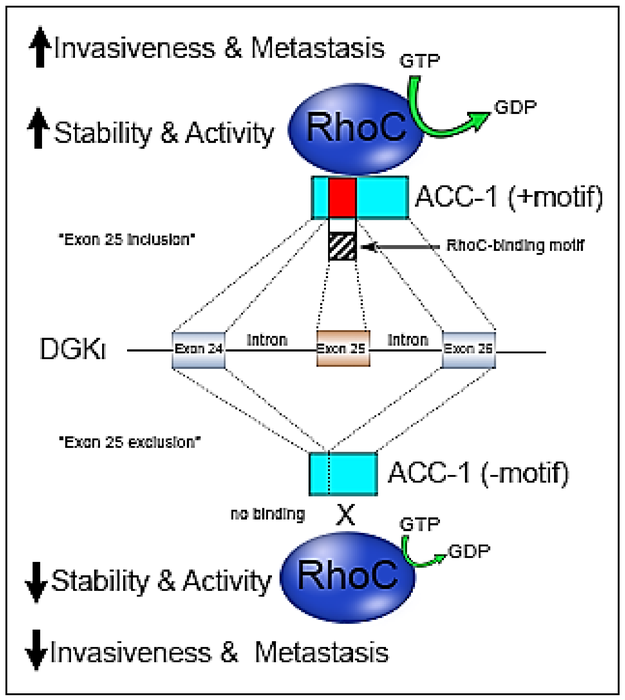

Figure 1: Hypothesized results of exon 25 alternative splicing

Objective

Create a plasmid containing DGKι lacking exon 25 so that mutant DGKι protein can be expressed and its interaction with RhoC can be studied. This would help determine if exon 25 is required for DGKι binding to RhoC and whether its deletion could decrease the molecular changes in the cell leading to metastasis.

Methodology

Assembling DGKι construct lacking exon 25:

1. Using PCR, get fragments of the C-term and N-term ends of DGKι, leaving out exon 25 that is in the middle.
2. Obtain plasmid (pCMV-HA) backbone without DGKι by digesting plasmid-DGKι with *Xho*I and *Eco*RI restriction enzymes. Separate the backbone fragment from the DGKι fragment by agarose gel electrophoresis, cut it out of the gel and purify.
3. Perform Gibson assembly of the two DGKι ends and the plasmid backbone.
4. Transform assembled recombinant plasmid into competent cells and plate onto agar plates containing ampicillin.
5. Obtain colonies from the plate, isolate their plasmid DNA and screen the recombinants by restriction analysis to make sure the correct construct was produced.

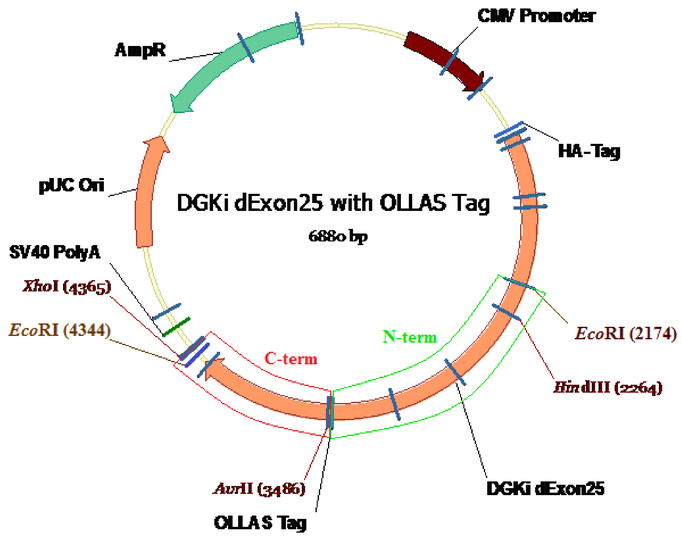

Figure 2: Desired DGKι construct lacking exon 25

Results

- Colonies grew on the agar + ampicillin plate, confirming that the transformation was successful.
- So far, the recombinants have not yet been screened to determine that they contain the correct construct.

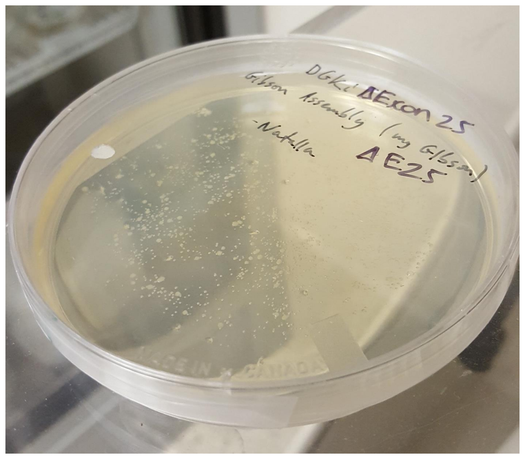

Figure 3: My transformation result of the recombinant plasmid

Discussion

Growth of colonies on the agar + ampicillin plate indicates that the transformation was successful and the competent cells took up the plasmid. Without the AmpR gene from the plasmid, which confers ampicillin resistance, the cells would not have been able to grow on the ampicillin-containing plate. It is not yet clear whether the correct construct was obtained. It is possible that during the Gibson assembly, the plasmid backbone had closed in on itself, or that the C-term or N-term end of DGKι had been omitted during ligation. To test this, the next step will be to isolate and perform a restriction digest on the plasmid DNA from selected colonies and determine if the sizes of the fragments obtained match the hypothesized sizes.

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