Studying the Interaction of GluT-1 and HRas

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Introduction

GluT-1 is a ubiquitously expressed glucose transporter that functions to maintain basal levels of glucose in cells. The collaborative project on GluT-1 has looked into modes of expression and activation of the transporter, one such way being association with lipid rafts, a more dense and protein-packed portion of the cell membrane. It was found that GluT-1 appeared to be interacting with the lipid raft associated human Ras isoform hRas not only better than other raft proteins, but also better than a second human isoform kRas, which does not associate with lipid rafts. Keeping in mind the role of Ras proteins as cell signaling molecules and commonly mutated oncogenes in cancer, we want to look further into this relationship by comparing these interactions with the final human Ras isoform, nRas, and hypothesize further how this interaction works and what it causes.

Objectives

- Clone nRas vectors to be used in BRET analysis
- Compare the interactions of hRas, kRas, and nRas with GluT-1 through BRET analysis
- Identify what may be causing differences in interaction between the three Ras isoforms

Methods

Subcloning nRas from a library vector:
- nRas gene was inserted into a TOPO vector and then cloned into destination vectors including bioluminescent nanoluciferase and fluorescent mCherry
- This DNA was transfected in live cells and analyzed by Western Blot to confirm creation of the protein vector (see Figure 3).

BRET Analysis

- Protein A is attached to a luminescent “donor” (nanoluciferase)
- Protein B is attached to a fluorescent

Comparison of Ras tails

<table>
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<tr>
<th>Ras</th>
<th>Linear domain targeting domain</th>
<th>hRas</th>
<th>kRas</th>
<th>nRas</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>HKLKLNPQESGPGCGCCKCYS</td>
<td>EALKK1GKKEEKGPGCVPK1KCKT</td>
<td>HKONKLSNADQTGCGLPVCVN</td>
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hRas: interacts with acidic lipids
kRas: palmitoylated in all Ras isoforms
nRas: palmitoylated in hRas to direct it to lipid rafts
nRas: farnesylated in all Ras isoforms

Figure 2: The differences between the human Ras isoforms are largely in their tails called hyper variable regions (HVR). The HVR determines the isoform’s placement in the cell such as the membrane of lipid rafts in the membrane. This is determined by the attachment of fatty acids such as palmitate or a farnesyl group.

Results

Figure 3: Western Blot confirmation of nRas fusion proteins. Labeled are the proteins nRas is fused to. All proteins showed up at their respective size confirming successful cloning nRas-mCherry was used for BRET analysis. PTAPS is a dual affinity tag that can be used for immunoprecipitation experiments. Size markers are indicated on the left side.

Figure 4: BRET analysis of GluT-1 and human Ras isoforms. The signals given off by the donor and acceptor are measured for samples with increasing amounts of acceptor DNA added. The ratio of the donor signal and the acceptor signal was plotted against the ratio of their respective concentrations. If an interaction is occurring, an increased amount of acceptor, attached to Ras, will create a higher signal ratio because of more excitation of the acceptor mCherry. hRas shows a clear increase in acceptor signal as the amount of acceptor-hRas complex is increased, indicating an interaction between GluT-1 and hRas. The donor and acceptor were developed from the same parent plasmid.

Conclusions

Western Blot analysis of nRas fusion proteins showed bands and the correct size indicating successful cloning of the proteins.

BRET analysis showed strong interaction between GluT-1 and hRas and little or no interaction at all between the other two human Ras isoforms. This interaction between a commonly mutated oncogene and a protein controlling metabolic fuel is very interesting as it pertains not only to diabetes but cancer as well.

Future Work

We would like to optimize the BRET analysis by quantifying protein expression in the live cells to normalize for protein amount.

We are also in the process of performing mutagenesis to swap tails on Ras isoforms and mutate the Cys residue on the hRas tail responsible for trafficking to lipid rafts. These mutated proteins will be analyzed by BRET to determine how both the body and tail of Ras proteins may affect interaction with GluT-1.

References

- Present and past student researchers of the Arnoys, Louters, and Looyenga Labs
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