Identification of the components of long noncoding mediated aggregation

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Introduction

Nucleolar detention of proteins, such as the tumor suppressor von-Hippel Lindau (VHL), occurs in response to acidification of the extracellular milieu, a general consequence of lactic acid production during anaerobic metabolism. Detained molecules are targeted to the nucleolus by long noncoding RNA (IGS_{28}RNA and IGS_{22}RNA), sequestering them in the nucleolus away from their downstream effectors rendering them functionally inert. A return to normal physiological conditions triggers the release of these proteins, allowing them to rejoin their functional networks. Currently, the proteome of the detained proteins is unknown. Thus, we will attempt to identify these molecules using the promiscuous biotin ligase (BioID) and exogenously-expressed long noncoding RNA molecules. Proteins in close proximity to VHL-BioID fusion protein will be biotinylated for further identification.

Methods and Materials

1) Transformation Experiment:
→ pcDNA MCS-BioID-HA, pGEM-IGS_{28}, pGEM-IGS_{22}(p), pcDNA-IGS_{22}, pcDNA-IGS_{28}, pPOL1-IGS_{28}-term and pPOL1-IGS_{22}-term
2) IGS Experiment:
→Transfect plasmids with pF-VHL-GFP, RNA extraction, RT-PCR for IGS-RNA
3) BioID Experiment:
→PCR amply VHL, subclone into BioID containing plasmid, screen for positive clones
→Co-transfect pF-VHL-BioID and pPOL1-IGS28-term, add biotin, precipitate biotin labeled proteins and mass spectrometry

Results

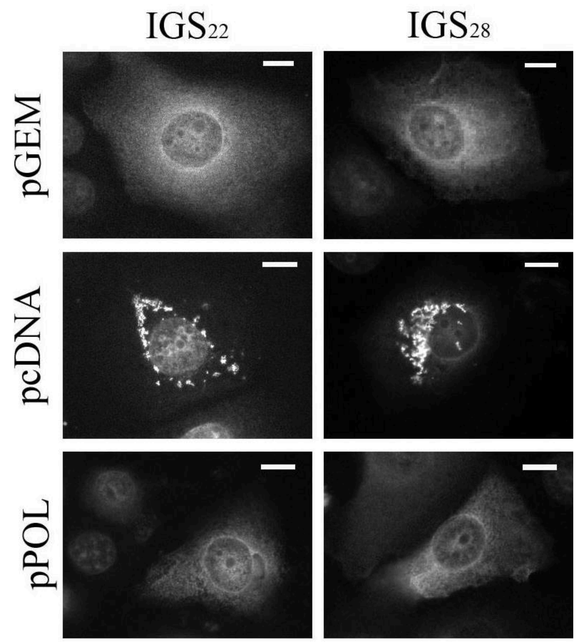


Figure 1. Only pcDNA-IGS_{22} and pcDNA-IGS_{28} plasmids triggered protein aggregation. Breast adenocarcinoma cancer cells expressing pF-VHL-GFP were transfected with pGEM-IGS_{22}, pGEM-IGS_{28}, pcDNA-IGS_{22}, pcDNA-IGS_{28} or pPOL1-IGS_{22}-term, pPOL1-IGS_{28}-term.

Results

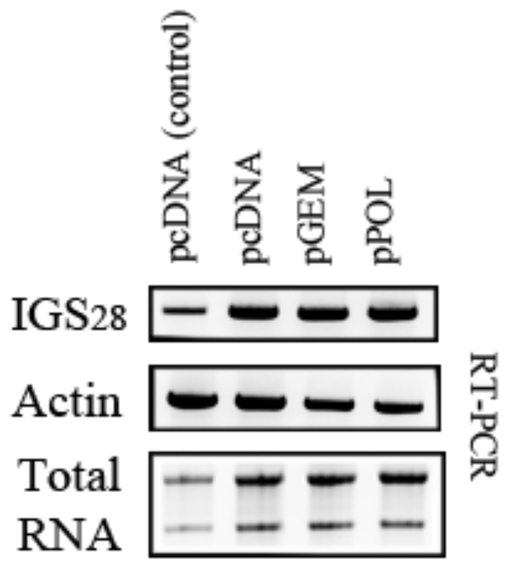


Figure 2. Exogenously-expressed IGS_{28}RNA (pcDNA-IGS_{28}) is present throughout all the cells. Vectors used for the RT-PCR for IGS_{28}RNA were pcDNA3.1 (control), pcDNA-IGS_{28} (RNA), pGEM-IGS_{28} (no RNA) and pPOL1-IGS_{28}-term(RNA).

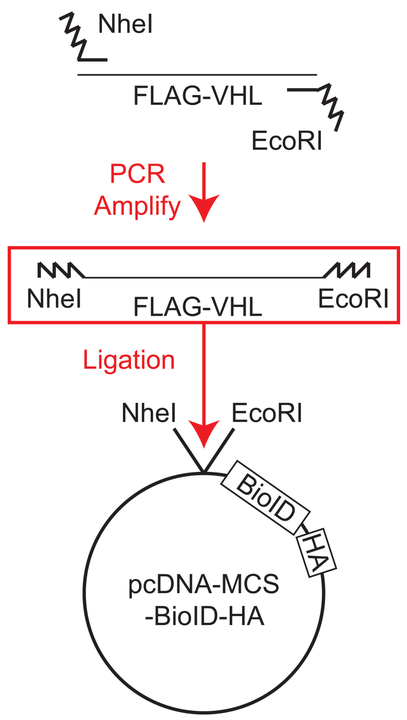


Figure 3. Cloning strategy to produce a VHL-BioID fusion protein expressing plasmid. Plasmid will be co-transfected with an IGSRNA expressing plasmid.

Results

- The results for pGEM were as expected; did not show aggregation. pGEM is negative control, which does not have a promoter in front of the IGS RNA coding sequence, thus transcription did not occur.
- The results for pcDNA were as expected; pcDNA shows cytoplasmic aggregation of proteins. pcDNA contains a CMV promoter (strong RNA PolII promoter) and a polyA tail (termination sequence). The addition of the PolyA tail causes RNA to move into the cytoplasm, which is demonstrated in Figure 1.
- The results for pPOLI were not as expected: it did not show aggregation (negative results). We were ideally hoping for nucleolar aggregates with this plasmid (RNA PolI transcribed in nucleolus) as the transcript did not contain a polyA tail (should have remained within the nucleolus)
- pFLAG-VHL-GFP are aggregates formed by RNA being expressed and allows for VHL to bind around the RNA, however aggregates were not present

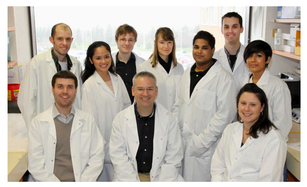
Discussion

The absence of nucleolar aggregation with pPOLI may have occurred due to two reasons, either the RNA was expressed and VHL does not form aggregates or RNA was not expressed due to numerous reasons. Semi-quantitaive RT-PCR results showed bands in all lanes for the IGS_{28}RNAtranscript. This suggests there was considerable DNA contamination as the pcDNA (control) and pGEM would not be expressing the RNA. This experiment would need to be repeated with a longer DNase treatment to show meaningful data.
If no RNA is expressed from the pPOLI plasmids the reasons could be the promoter failed, the terminator failed, the plasmid did not transfect or the plasmid did not go to the nucleolus.
Once these issues have been resolved we can proceed with the co-transfection experiment with the VHL-BIOID construct that was successfully constructed.

Conclusion

The immobilization of proteins observed in response to IGSRNA is reminiscent of the physiological aggregates seen in; mad cow disease, Alzheimers and other amyloid plaque conditions.
To date, the only one of these conditions known to be reversible is nucleolar detention, suggesting a greater understanding of the proteome and underlying mechanism of detention would improve our chances of treating these other debilitating conditions.

Acknowledgements



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References

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