Generation of Mutant Galectin-3 Binding Protein (LG3BP): a Crucial Resource for Characterizing LG3BP/Sulf-2 Interaction

Amy Lum[1,2*], Julius Benicky[1,2], and Radoslav Goldman[1,2,3]

[1]Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University, Washington, District of Columbia, USA; [2]Clinical and Translational Glycoscience Research Center, Georgetown University, Washington, District of Columbia, USA; [3]Department of Biochemistry and Molecular & Cellular Biology, Georgetown University, Washington, District of Columbia, USA

Introduction

The galectin-3 binding protein (LG3BP) was identified as an interacting partner for extracellular 6-O-endosulfatase Sulf-2. Interaction with LG3BP inhibits Sulf2 activity, leading to changes of heparan sulfation that is involved in numerous biological processes, including cancer cell proliferation, migration and/or invasion. Generation of LG3BP F357W mutant is based on structure models generated by Dr. C. Barinka. We generated the F357W mutant to test the hypothesis that the mutant LG3BP disrupts Sulf-2-LG3BP interactions and eliminates the LG3BP-mediated inhibition of Sulf-2 activity.

Methods and Materials

Cell Culture: Wild-type human embryonic kidney HEK293F cells (Invitrogen) were grown in suspension culture in FreeStyle293 medium (Invitrogen) at 37ºC / 5% CO2 atmosphere on a rotating platform at 140 rpm.

Sulf-2 and Mutant LG3BP/Sulf-2 AlphaFold Models: Predicted structures of LG3BP (Uniprot, Q08380) and Sulf-2 (Uniprot, Q8IWU5) were generated in PyMOL.

Generation of LG3BP F357W Mutant: pCMV6 expression vector containing wild-type LG3BP ORF (Origene # RC204918) was subjected to inverse PCR to introduce F357W mutation.

Assembly of Lentiviral Vector: restriction digested pHR-CMV-TetO2_3C-TwinStrep_IRES-EmGFP lentiviral vector and LG3BP mutant DNA was isolated with 1% agarose gel electrophoresis and visualization by SafeGreen DNA stain (IntellixBio), then assembled through in-fusion cloning.

Generation of LG3BP Mutant Viral Particles was done through in-fusion cloning with 23.3 µg assembled transfer plasmid DNA, 23.3 µg envelope vector pMD2.G (Addgene # 12259) DNA, 23.3 µg packaging vector psPAX2 (Addgene #12260)) DNA, 175 µl polyethylenimine (PEI), and 325 µl DMEM/F12/SFM, and confirmed with green fluorescent protein (GFP) fluorescence.

Concentration of viral particles was measured using a sandwich enzyme-linked immunosorbent assay (ELISA).

Infection of HEK293F Cells were infected by viral particles as described (Elegheert, 2018).

Confirmation of Mutant LG3BP in HEK293F Cells was done with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western Blot analysis.

Results

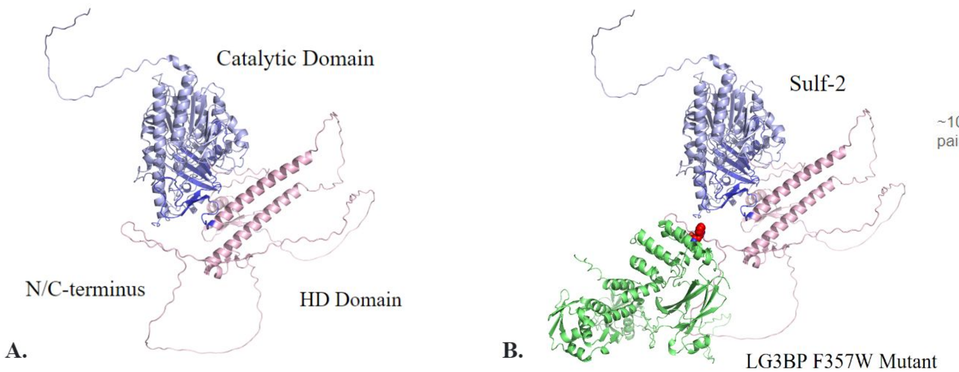

Fig 1. AlphaFold model of LG3BP-F357W/Sulf-2 complex. A, Structure of Sulf-2, the catalytic core, the hydrophilic domain, and the N/C-termini of Sulf-2 are colored light blue, pink, and blue, respectively. B, The predicted LG3BP-F357W/Sulf-2 complex; LG3BP-F357W is colored green and Sulf-2 is colored as in Panel A. The F357W (phenylalanine to tryptophan) mutant is colored red.

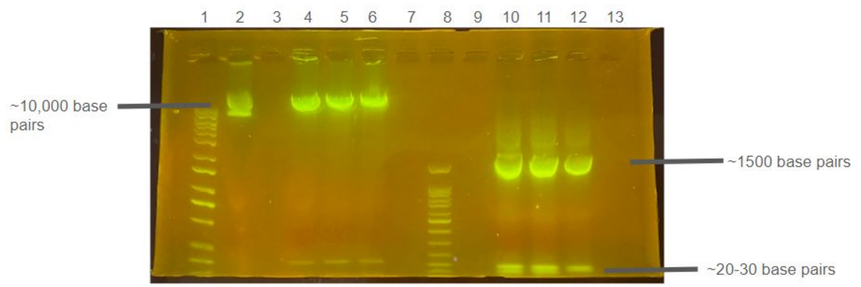

Fig 2. Lentiviral Vector Components Used for In-Fusion Cloning. CMV-pHR control (lane 2) Digested lentiviral plasmid (lanes 4-6) and LG3BP-F357W mutant (lanes 10-12). 20 µl of DNA sample and 4 µl loading dye was loaded lanes 2, 4-6, and 10-12. Lanes 1 and 8 contain 5 µl 1 to 10 kilobase (kb) protein ladders and 5 µl 100 base pairs to 1 kb ladders, respectively. 10 kb, 1500 base pairs, and 20 to 30 base pair bands are shown in lanes 4-6, 10-12, and 4-6 & 10-12, respectively.

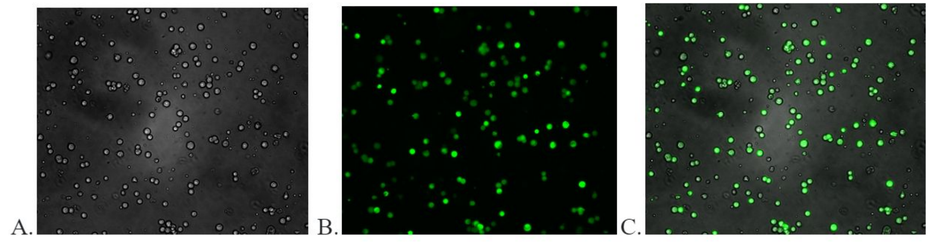

Fig 3. Infected HEK293F cells with LG3BP mutant lentiviral particles. A, Phase contrast image of infected HEK293F cells. B, Fluorescent image of infected HEK293F cells. C, Overlay of phase contrast image and fluorescent image of infected HEK293F cells.

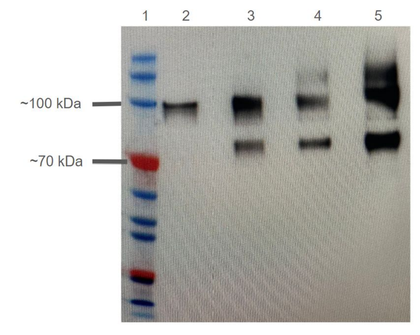

Fig 4. Confirmation of our mutant LG3BP gene in HEK293F cells. SDS-PAGE and Western Blot were done on conditioned media harvested from infected HEK293F cells. 30 µl HEK293F media (negative control), 2 µl LG3BP (positive control), 30 µl HEK293F media containing wildtype LG3BP protein, and 30 µl HEK293F media containing mutant protein were loaded together with protein loading dye to Lanes 2-5, respectively. A 10 kDa to 250 kDa ladder was loaded in Lane 1.

Conclusions & References

- LG3BP is a highly N-glycosylated secreted protein associated with tumor invasion and metastasis (Grassadonia et. al, 2004). However, there is no information on the Sulf2-mediated impact of LG3BP on any living system.
- In this study, we generated a mutant cell line of LG3BP at the predicted LG3BP F357 binding site of the LG3BP/Sulf-2 complex, which provides a resource crucial for further characterization of the LG3BP/Sulf-2 interaction site.
- Future research should involve the purification of mutant and wild-type LG3BP protein, followed by in vitro assays with the F357W mutant LG3BP/Sulf-2 and wild-type LG3BP/Sulf-2 for characterization of the LG3BP/Sulf-2 interaction site.