

Identification of p66 Binding Partners

Amanda Collins, Dr. Zhenyi Ma PhD, and Dr. Lance Terada MD

Eastfield College, Mesquite, Texas, and Pulmonary Division, Department of Internal Medicine

The University of Texas Southwestern Medical Center, Dallas, Texas

Introduction

- The two main types of lung cancer are non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). SCLC makes up 10-15% of lung cancers. It often starts in the bronchi near the center of the chest, and spreads through the body, usually before symptoms even appear.

- Previous research has shown p66shc, a protein involved in anchorage dependent cell death, is absent in small cell lung cancer.

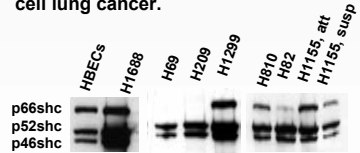


Fig1. p66shc is suppressed in SCLC cells (H69, H209 and H82).

- Shc refers to the Src Homology and Collagen) gene, which encodes three isoforms p52shc, p46shc and p66shc. As an adapter protein, Shc functions in integrin-dependent cell signal transduction which is necessary for cell growth and survival. However, metastasizing cancer cells do not require attachment for survival.

- The suppression of p66 in SCLC, poses the question "What's the function of p66shc in SCLC?"

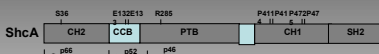


Fig2. Schematic of ShcA with transcription start sites of p66shc, p52shc and p46shc, Domains: Collagen homology region, CH2; cytochrome C binding, CCB; Phosphotyrosine-binding, PTB; Central region rich in proline and glycine residues, CH1 and Src homologue 2, SH2.

Methods & Results

1 Creation of Tagged p66shc

- Molecular-cloning was used to create the desired DNA:
 - pIPX-FF-ZZ (empty vector)
 - pIPX-p66FF-ZZ,
 - pIPX-p52FF-ZZ
 - pIPX-CH2-FF-ZZ.

- The constructs were sub-cloned into the 305 lentiviral vector. Fig3. shows the digestion pattern by BamH I and EcoR I.

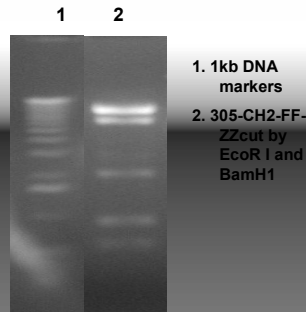


Fig3. Molecular cloning of CH2 domain into 305 vector

- The constructs were sequenced to verify the cloning.

2 Production of Tagged p66shc

- The lentivirus was created by transfecting ϕ Nx-293 packaging cells with the lentiviral constructs and helper plasmids:
 - Lentiviral Vectors: 305-p66 FF ZZ, 305-p52 FF ZZ or 305-FF ZZ
 - Helper vectors: REV, pMDL, and VSV-G

- HUVECs were then transduced with the lentivirus.
 - Their expression was then checked using the Anti-Flag Western Blot

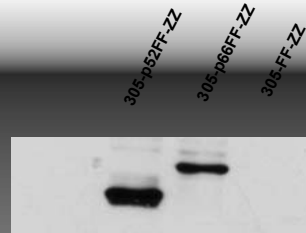


Fig4. Immunoblot for Flag showing p66shc and p52shc expression in HUVECs stably infected with lentiviral empty vector FF-ZZ, p66shc-FF-ZZ or p52-FF-ZZ.

3 Identification of Binding Partners

- The Tandem Affinity Purification, TAP Approach was used for Immuno Precipitation
 - Experimental steps:
 - IP: α -protein A IgG, TEV cleavage, IP : α -flag and Elute by 3XFLAG peptide

- Proteins were separated with SDS-PAGE and then silver stained to reveal the protein bands.

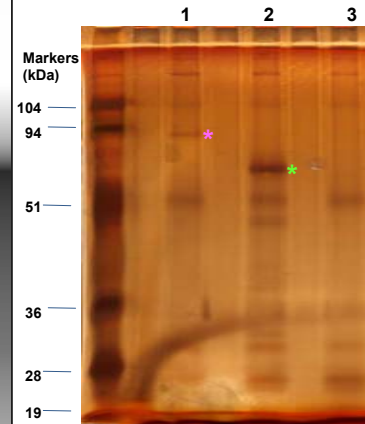


Fig5. Silver stain analysis of FLAG elute samples from HUVECs stably infected with lentivirus. 1. FLAG elute of p66, 2. FLAG elute of p52, and 3. FLAG elute of empty vector p66shc-2xFlag and p52shc-2xFlag

Conclusion

- No significant binding partners could be identified using the TAP Protocol.
- Future efforts to identify the binding partners may include:
 - Changing the DTBP Crosslinker: There was less soluble protein, so its possible DTBP is not suitable for the binding partner identification.
 - Using a cancer cell line: related research identified p66 binding partners in melanoma cancer cell line.
- Testing the intermolecular interactions: it's possible intermolecular interactions inhibit or active p66 binding partners.

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References

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