Identifying Protein-Protein Interactions of DDX41 by BioID

Seyed Mohammad Mahdi Toliat Zavareh[1,2], Ananaya Charaya[1], Jim Xiang[2], and Yuliang Wu[1]

[1]Department of Biochemistry, Microbiology and Immunology, [2]Division of Oncology, University of Saskatchewan, Saskatoon, SK S7N 5E5, Canada

INTRODUCTION

Helicases are known as enzymes that separate double-stranded(ds) nucleic acids to single-strand(ss) nucleic acids by hydrolysis ATP; and some of them also can anneal ss nucleic acids to ds nucleic acids in an ATP-independent manner. DEAD-box helicases are characterized by containing an Asp-Glu-Ala-Asp (DEAD) sequence in their motif II that is required for ATP binding and hydrolysis. DEAD-box helicase 41 (DDX41) is a member of DEAD-box helicases with multiple functions, including acting as a sensor for intracellular DNA in myeloid dendritic cells[1] and for bacterial secondary messengers (c-di-GMP or c-di-AMP) to trigger type 1 interferon production[2]. Recently, the Dr. Wu's lab discovered that DDX41 modulates the balance of dsDNA and ssDNA, in which regulates the activation of the cyclic GMP–AMP synthase (cGAS)[3]. Mutations in DDX41 are linked with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML)[4], two blood caners. The most recurring mutation in DDX41 that lead to AML or MDS is c.1574G>A (p.R525H). Despite concrete evidence suggests that DDX41 acts as a DNA sensor in innate immunity[5,6]; no innate immunity-related protein has been identified as a DDX41-binding partner. Therefore, we established a BioID system to identify DDX41-biniding proteins under virus infections.

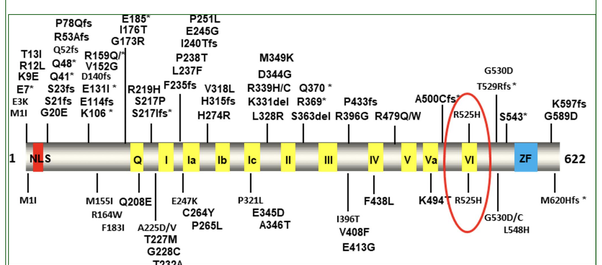


Figure 1. The somatic (bottom) and germline (above) mutations associated with DDX41 in AML and MDS patients. NLS (in red) stands for nuclear localizing sequence, the yellow boxes (Q to VI) are conserved helicase motifs, and ZF (blue) is zinc finger domain.

OBJECTIVES

- To construct plasmid vectors that constitute FLAG tag, DDX41 (WT or R525H), and BirA gene.
- To transfect the recombinant plasmid vector into HeLa DDX41 knockout cells, and Western blot confirm the expression of FLAG, DDX41, and BirA.
- To identify DDX41-binding proteins within its proximity by mass spectrometry.
- To verify proteins that interact with DDX41.

MATERIALS AND METHODS

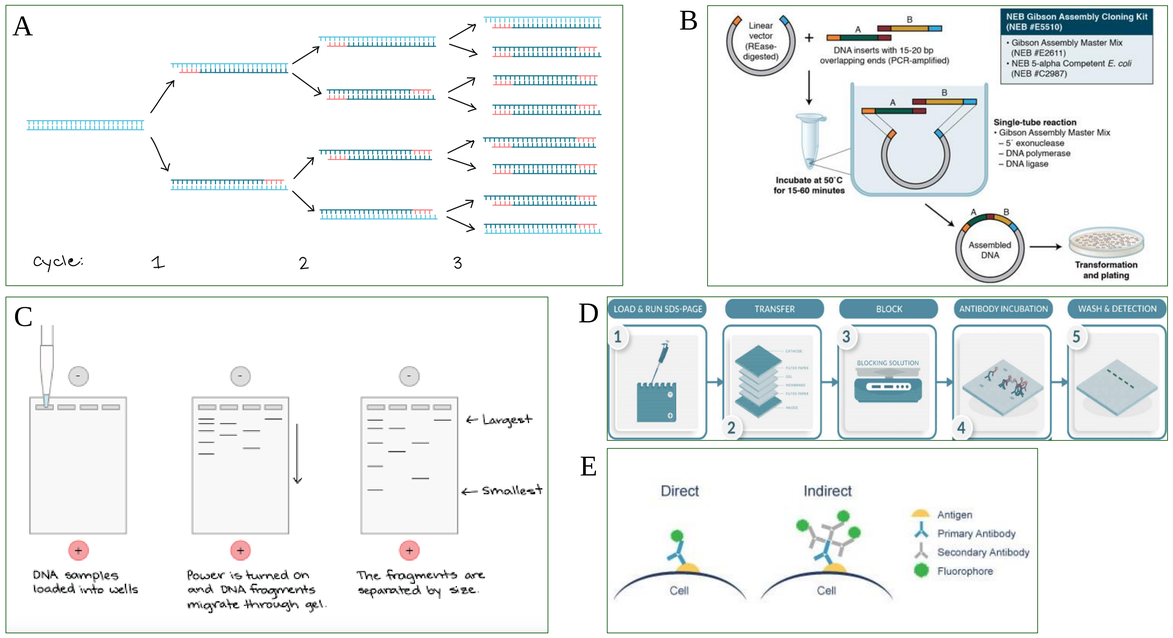
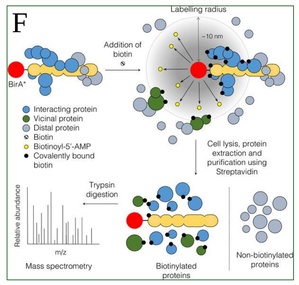


Figure 2. Schematic diagram of techniques used in this study. (A) Polymerase chain reaction (PCR)[7]. The light blue stand represents the original DNA samples, small red strands are the primers that were used for amplification and the dark blue strands are the synthetised DNA. (B) Gibson assembly[8]. The same coloured DNA insert ends were used to guide the proper annealing, then ligation of the inserts. (C) Agarose gel electrophoresis[9]. (D) Western blot[10]. (E) Immunofluorescence staining[11]. Represents the method used for immunofluorescence staining using primary and secondary antibody. (F) Overall process of BioID[12]. Depicts the steps of detection and analysing the interacting proteins with DDX41 by BioID.



RESULTS

Confirmation for vector construction:

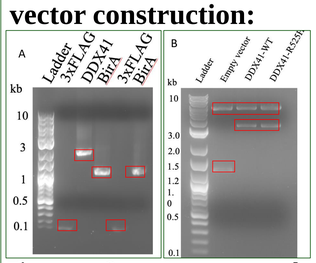


Figure 3. Agarose gel electrophoresis of PCR and cloned vector. (A) PCR amplified fragments of FLAG, DDX41, and BirA before cloning into vector. The size of fragments are: Flag: 111 bp; DDX41: 1906 bp; BirA: 1044 bp. (B) The EcoRI and HindIII digested vectors. The size of digested DNA fragments are: vector pcDNA3: 5400 bp; insertion DDX41-WT : 3094 bp; insertion DDX41-R525H: 3094 bp; Empty vector (FLAG+BirA): 1098 bp. Ladder: 1 kb Plus DNA Ladder from NEB (N3200).

Confirmation of protein expression:

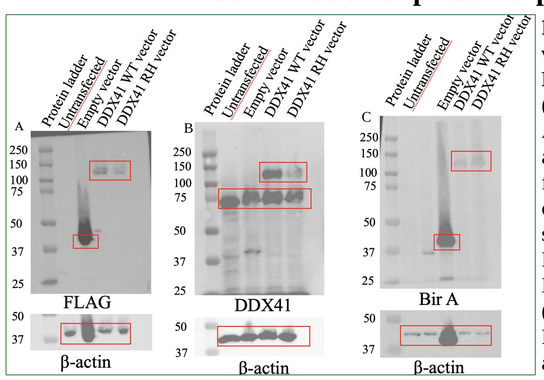


Figure 4. Western blot analysis of vectors transfected into DDX41 KO HeLa cells with indicated antibody (A) FLAG, (B) DDX41, and C(BirA). After probing with the primary antibody and secondary antibody, the fluorescence of secondary antibody was captured using a ChemiDoc imaging system (Bio-Rad). The protein size of FLAG is 2.7 KDa, BirA is 35.3 KDa, DDX41 is 70 KDa, empty vector (FLAG+BirA) is 38 kDa, FLAG+DDX41+BirA is 108 kDa. β-actin is a loading control and sized at 42 kDa.

Confirmation for protein localization:

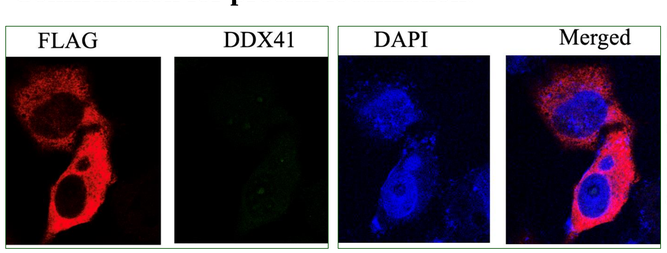


Figure 5. Immunostaining of HeLa DDX41 KO cells transfected with FLAG tagged DDX41-WT vector. DAPI stains DNA (nucleus), FLAG fluorescence emitting antibody (only noise), The green dye is DDX41 fluorescence emitting antibody.

CONCLUSIONS

- Construction of vectors consisting of FLAG, DDX41, and BirA.
- Confirmation the expression of fusion proteins.
- Subcellular localization of DDX41 is unclear, which needs to be repeated for validation.

Future directions

BioID/Mass spectrometry: After performing a large-scale transfection, the cells should be treated with stress conditions such as Infrared Radiation or sodium arsenite. This will then be followed by the incubation of HeLa cells with biotin after which the samples are purified with streptavidin. The samples are then sent for Mass Spectrometry analysis.

Bioinformatic analysis: The general analysis of the collected primary data will be conducted. As an example, pathways analysis will be performed using Gene Ontology term enrichment tools.

Characterization of DDX41-binding proteins: The first one or two top hits obtained from the mass spectrometer and data analysis will be selected for further studies. Proteins specifically involved in innate immune response or P-bodies formation will be especially considered.

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