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Novel Interaction Between ECD and EIF4A1 Indicates ECD Regulates Eukaryotic Translation

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Summer Undergraduate Research Program

Novel Interaction Between ECD and EIF4A1 Indicates ECD Regulates Eukaryotic Translation

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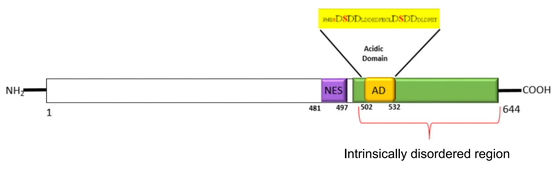
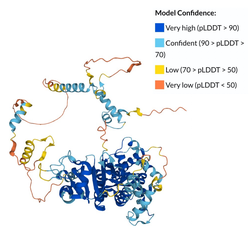
Background/Introduction

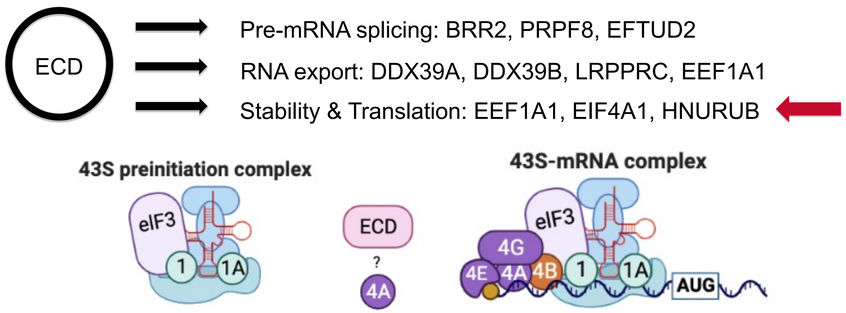
- ECD, Ecdysoneless protein, is evolutionarily conserved. It was identified as a human homologue of Drosophila ecdysoneless, interacting with HPV16 E6 in a yeast two-hybrid assay.[1]
- ECD has been proven to be essential for cell cycle progression from G1 to S phase, mitigating endoplasmic reticulum (ER) stress, and embryogenesis.[2,6,8]
- ECD KO mice are embryonic lethal as it halts the cell cycle at G1.[2,6] ECD interacts with p53[1] and Rb[2,4]. ECD also associates with pre-mRNA splicing factor PRPF8.[3,6]
- ECD is overexpressed in breast[4], pancreatic[5], gastric, and Human Papilloma-driven[7] cancers and is correlated with shorter patient survival.
- ECD interacts with co-chaperone complex R2TP involved in protein assembly and folding.[6]
- ECD overexpression has been proven to increase oncogenesis regulated by c-MYC in a recent mice model.[8]
- Biochemical analyses showed ECD to have a role in mRNA splicing and nucleus to cytoplasm export.[3,7,8,9]

ECD Domain Structure





Mass Spectrometry identified ECD binds to RNA binding proteins[3]



Methods

- Cell culture: HEK293T and SUM159
- Transfection
- Western Blot
- S-35 Labeling
- Immunoprecipitation (IP)

Objective/Purpose

- Purpose: to determine if there is an interaction between ECD and EIF4A1 and determine ECD's effect on global translation
- HEK293T (human embryonic kidney) and SUM159 (breast cancer) cell lines were used

Results

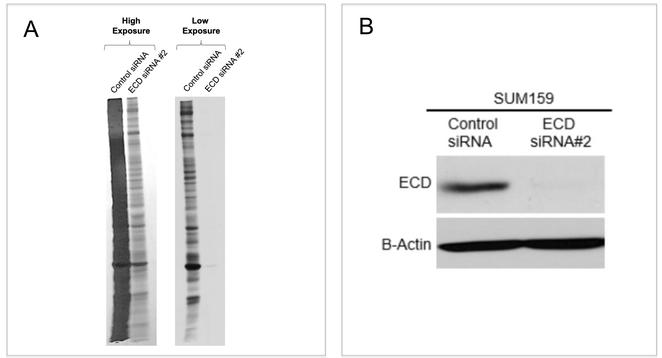


Fig. 1: ECD Regulates Global Translation. (A) S 35 Metabolic labeling of SUM159 control and ECD knockdown cells were performed to examine the effect of ECD on global translation. The cells were grown in 10 cm dishes and treated with control and ECD siRNA (siRNA#2) for 72 hours. The medium was removed and washed three times with PBS and incubated with methionine-and cysteine-free DMEM media for 60 min at 37 ° C. A 35S-labeled methionine/cysteine mixture (cat. NEG772, Perkin Elmer, Waltham, MA, USA) was added to a final concentration of 0.25 mCi/mL. After 30 min, cells were washed three times with cold PBS. The lysates (50 ug) were resolved by SDS-PAGE. The gel was fixed, incubated with Auto-Flour (LS-315; National Diagnostics), and dried. Signals were visualized by autoradiography. (B) Demonstration of ECD KD by WB and B-actin was used as loading control.

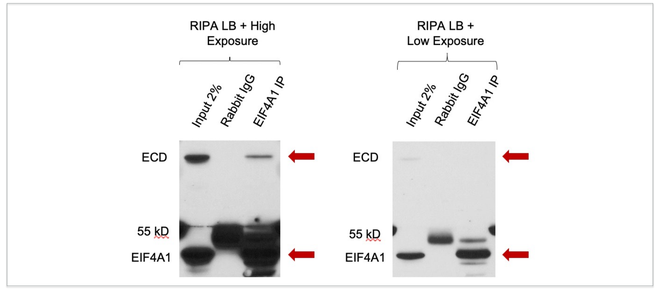


Fig. 2: ECD Interacts with EIF4A1 in HEK293T Cell Line. 1 mg of HEK-293T cell lysates were immunoprecipitated (IP) with the antibodies indicated at the top using Radioimmunoprecipitation Assay Buffer (RIPA LB) followed by Western blotting (WB) with the antibodies shown on the left. Mouse IgG, and rabbit IgG were used as negative controls; 25 µg aliquots of lysate protein were used in the input lane.

Results Continued

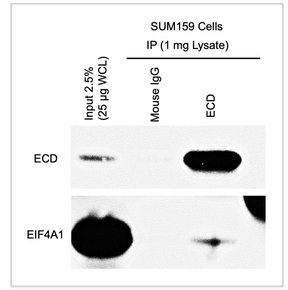


Fig. 3: ECD Interacts with EIF4A1 in SUM159 Cell Line. 1 mg of SUM159 cell lysates were immunoprecipitated with the antibodies indicated at the top using RIPA LB followed by WB with the antibodies shown on the left. Mouse IgG, and rabbit IgG were used as negative controls; 25 µg aliquots of lysate protein were used in the input lane.

Discussion/Conclusion/Future Directions

- IPs (Fig. 2 & 3) show a novel interaction between ECD and EIF4A1 in RIPA lysis buffer (LB), but did not show in Triton-X or CHAPS LB. Further testing in these and other buffers should be performed in future. This interaction was seen in both HEK293T and SUM159 cells.
- Level of translation was decreased when ECD was knocked down (Fig. 1A) by siRNA transfection in SUM159 cells, indicating that ECD regulates mRNA translation. Successful method of transfection was indicated by lower protein levels compared to the control HSC-70 in Fig. 1B.
- Future experiments include determining if ECD controls the cap-dependent translation initiation of MYC in dox-inducible MCF10A and 76NTERT cell lines. This will further the understanding of the mechanism by which ECD increases oncogenesis.
- The interaction between ECD and EIF4A1 should be further confirmed using GST-Pull Down Assay.
- The interaction between ECD and other translation initiation and elongation proteins such as EIF4E and EEF1A1 should be explored.
- Overall, the interaction between ECD and EIF4A1 supports a novel mechanism by which ECD protein regulates eukaryotic mRNA translation. This mechanism may contribute to the resistance of cancer cells over-expressing ECD to the translation-inhibitory effect of endoplasmic reticulum stress prevalent in tumors.

References

- Ying et al. (2006). The Human Orthologue of Drosophila Ecdysoneless Protein Interacts with p53 and Regulates its Function. (1)
- Jun kim et al. (2009). Role of Mammalian Ecdysoneless in Cell Cycle Regulation. *JBC* (2)
- Claudis et al. (2014). Unexpected Role of the Steroid-Deficiency Protein Ecdysoneless in Pre-mRNA Splicing. *Plos Genetics* (3)
- Zhao et al. (2012). Overexpression of a novel cell cycle regulator ecdysoneless in breast cancer: A marker of poor prognosis in HER2/neu-overexpressing breast cancer patients. *BCRT* (4)
- Dey et al. (2012). Overexpression of Ecdysoneless in Pancreatic Cancer and its Role in Oncogenesis by Regulating Glycolysis. *CCR* (5)
- Mirza et al. (2016). A Novel Interaction of Ecdysoneless (ECD) Protein with R2TP Complex Component RUVBL1 is Required for the Functional Role of ECD in Cell Cycle Progression. *MCB* (6)
- Mirza et al. (2021). Ecdysoneless Protein Regulates Viral and Cellular mRNA Splicing to Promote Cervical Oncogenesis. *MCR*. (7)
- Mohapatra et al. (2022). Ecdysoneless overexpression drives mammary tumorigenesis through upregulation of c-MYC and glucose metabolism. *MCR* (8)
- Saleem et al. (2021). The Mammalian Ecdysoneless Protein Interacts with RNA Helicase DDX39A To Regulate Nuclear mRNA Export. *MCB* (9)
- Yartseva, V. (n.d.). Biorender. Retrieved August 3, 2022, from https://app.biorender.com/illustrations/62eaa211f95d0d86b90c45ce (10)
- UniProt. (n.d.). O95905 · ECD_HUMAN. Retrieved August 5, 2022, from https://www.uniprot.org/uniprotkb/O95905/feature-viewer (11)