Putative tumor suppressor ELL2 is required for proliferation and survival of AR-negative prostate cancer cells

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Background: Elongation Factor for RNA Polymerase II, 2 (ELL2) was reported as a putative tumor suppressor in the prostate. ELL2 is frequently down-regulated in prostatic adenocarcinoma specimens, and loss of ELL2 induced murine prostatic intraepithelial neoplasia and enhanced AR-positive prostate cancer cell proliferation. However, ELL2 gene appears to be amplified in AR-negative neuroendocrine prostate tumors, suggesting a potential oncogenic role for ELL2 in AR-negative prostate cancer cells. In this study, we explored potential function of ELL2 in PC-3 and DU145, two AR-negative prostate cancer cell lines.

Methods: The role of ELL2 in PC-3 and DU145 cells was studied using siRNA-mediated ELL2 knockdown. Genes regulated by ELL2 knockdown in PC-3 cells were identified and analyzed using RNA-Seq and bioinformatics. Expression of representative genes was confirmed by Western blot and/or quantitative PCR. Cell growth was determined by BrdU, MTT and colony formation assays. Cell death was analyzed by 7-AAD/Annexin V staining and trypan blue exclusion staining. Cell cycle was determined by PI staining and flow cytometry.

Results: ELL2 knockdown inhibited proliferation of PC-3 and DU145 cells. RNA-Seq analysis showed an enrichment in genes associated with cell death and survival following ELL2 knockdown. The interferon-γ pathway was identified as the top canonical pathway comprising of 55.6% of the genes regulated by ELL2. ELL2 knockdown induced an increase in STAT1 and IRF1 mRNA and an induction of total STAT1 and phosphorylated STAT1 protein. Inhibition of cell proliferation by ELL2 knockdown was partly abrogated by STAT1 knockdown. ELL2 knockdown inhibited colony formation and induced apoptosis in both PC-3 and DU145 cells. Furthermore, knockdown of ELL2 caused S-phase cell cycle arrest, inhibition of CDK2 phosphorylation and cyclin D1 expression, and increased expression of cyclin E.

Conclusions: ELL2 knockdown in PC-3 and DU145 cells induced S-phase cell cycle arrest and profound apoptosis, which was accompanied by the induction of genes associated with cell death and survival pathways. These observations suggest that ELL2 is a potential oncogenic protein required for survival and proliferation in AR-negative prostate cancer cells.

1. Cell Proliferation In ELL2-knockdown AR-negative Cells

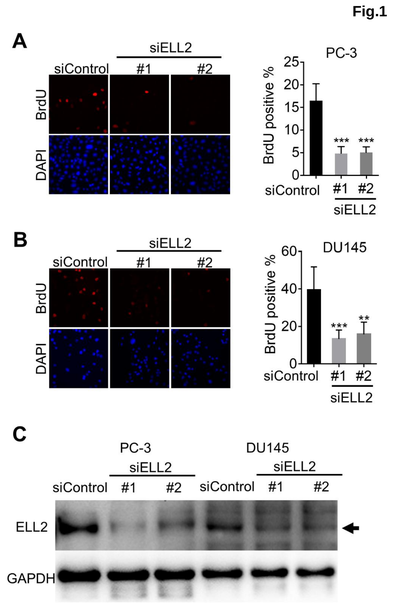

Fig. 1 Impact of ELL2 knockdown on BrdU incorporation in AR-negative prostate cancer cells. Images shown are BrdU-positive nuclei in PC-3 cells (A) or DU145 (B) transfected with 25 nM nontarget control siRNA (siControl) or two different siRNAs targeting ELL2 (#1 or #2). DAPI staining shows all the nuclei. BrdU incorporation was quantified by determining the mean percentage ± SD of BrdU-positive cells relative to the total number of cells. Cells were counted from two different fields for each well from triplicate wells and 30-130 cells per field. C.Efficiency of siELL2 knockdown in PC-3 cells was verified by western blotting. ELL2 band denoted by black arrow. GAPDH was used as a loading control. Results are representative of three individual experiments. ** p < 0.01, *** p < 0.001.

2. ELL2 Is Associated With A Set Of Genes And Pathways

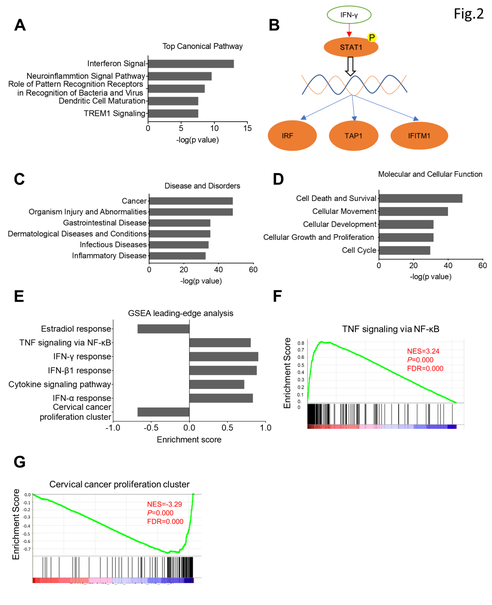

Fig. 2. A. Pathway identification by Ingenuity Pathway Analysis (IPA®) software based on differentially expressed genes (DEGs) upor ELL2 knockdown in PC-3 cells This analysis shows DEGs mainly were enriched in the IFN-γ pathway, neuroinflammation signal pathway, and other indicated pathways. B. IFN-γ pathway involving STAT1 phosphorylation and transcriptional activation of IRF1, TAP1, and IFITM1. C. Ontological annotation of DEGs in disease and disorders. D. Molecular function of ELL2-modulated genes. E. GSEA of DEGs between ELL2-depleted and siControl treated PC-3 cells. Genes in the TNF-α and IFN-γ pathways were upregulated and genes in cervical cancer proliferation cluster were downregulated. F. & G. GSEA analysis of the DEGs enriched in the TNF-α pathway and cervical cancer proliferation cluster, respectively.

3. STAT1 Activation In Response To Loss Of ELL2 And Regulates ELL2-induced Cell Short-term Viability

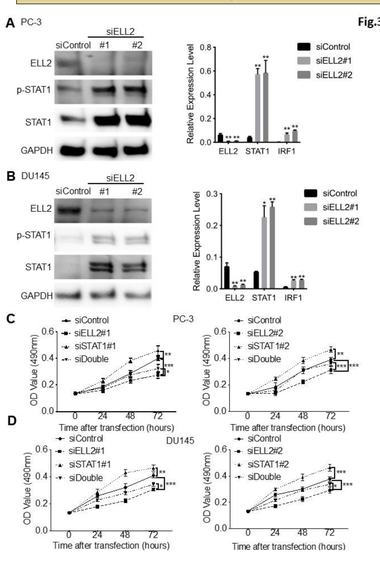

Fig.3 Western blot analysis of ELL2, STAT1 and phospho-STAT1 (Tyr701) , Quantitive real-time PCR analysis of ELL2, STAT1, and IRF1 mRNA after ELL2 knockdown in PC-3 cells (A) and DU145 cells (B).
Effect of ELL2 and STAT1 knockdown individually or in combination (siDouble) on cell viability in (C) PC-3 and (D) DU145 cells. MTT assay was performed at indicated hours after transfection with indicated siRNAs. Data were shown as mean ± S.D. * p < 0.05; ** p < 0.01; *** p < 0.001.

4. Deletion Of ELL2 Inhibits PC-3 And DU145 Long-term Viability Dramatically, Overwhelming Induced Proliferation By STAT1 Knockdown

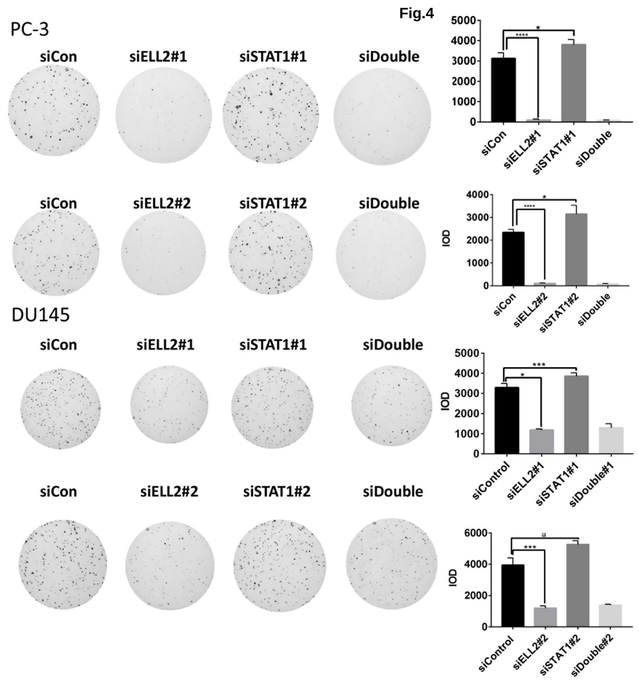

Fig.4 Effect of ELL2 and STAT1 knockdown individually or in combination (siDouble) on colony formation. PC-3 (A) and DU145 (B) cells were treated with indicated siRNAs as descdribed in Fig. 5, prior to colony formation assay. The colonies were visualized by crystal violet staining. The colonies in each dish were imaged by ChemiDoc™ Touch Imaging System and counted by Image Pro Plus software for each group of cells. *, p < 0.05, *** p < 0.001, **** p < 0.0001.

5. ELL2 Interacts With STAT1 for Co-regulating PC-3 and DU145 Cell Proliferation

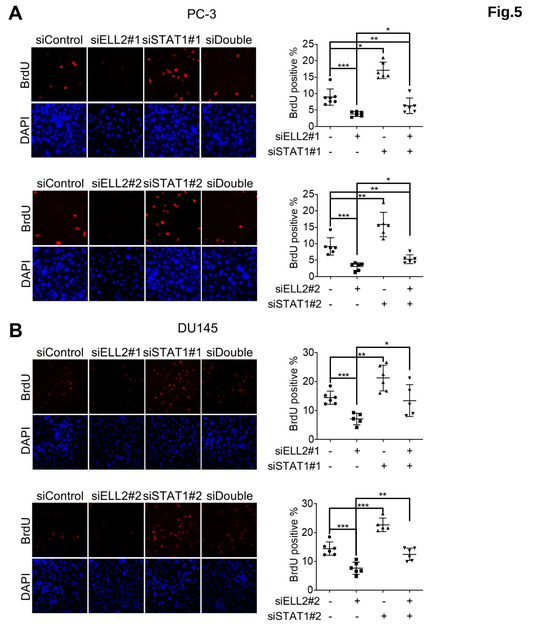

Fig.5 BrdU incorporation in PC-3 (A) and DU145 (B) cells transfected with nontargeted control (50 nM siControl) siRNA, targeted to ELL2 (25 nM siELL2+25 nM siControl), target to STAT1 (25 nM siSTAT1+25 nM siControl), and both (25 nM siELL2+25 nM siSTAT1) (siDouble). Upper panel shows BrdU-positive nuclei (red), and lower panel shows nuclear staining with DAPI (Blue). Images were acquired using an 40x objective. Quantification of BrdU incorporation is shown as mean percentage ± SD of BrdU-positive cells relative to the total number of cells. Cells were counted from two different fields for each well from triplicate wells and 50-170 cells per field. * p < 0.05; ** p < 0.01, *** p < 0.001.

6. ELL2 Deletion Causes S Phase Arrest In PC-3 And DU145 Independent Of STAT1

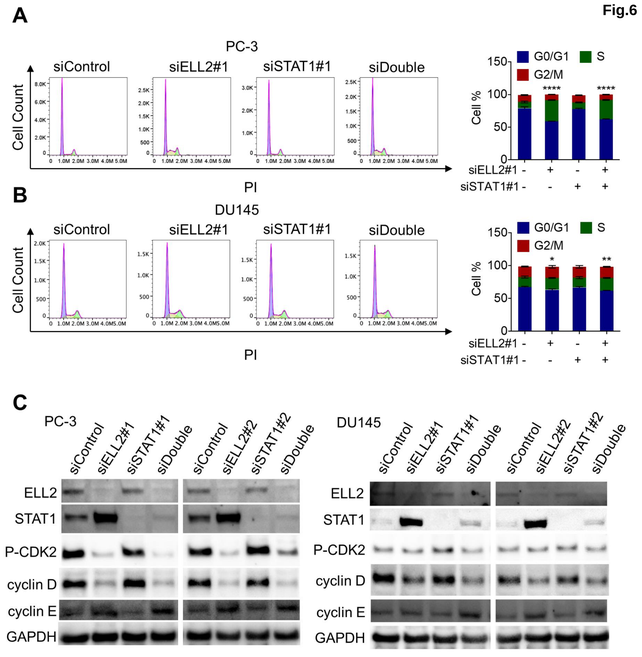

Fig 6. PC-3 cells (A) were treated with 25nM siELL2 or combination of siELL2 and siSTAT1, while DU145 cells (B) were transfected with 25nM siELL2, with 1.3 to 3-fold increase of cell populations of ELL2-knockdown group in S phase compared to control group, as assessed by flow cytometry. *p<0.05, ** p< 0.01, *** p < 0.001, ****p<0.0001). **C.** PC-3 and DU145 cells were untreated or were transfected siELL2, siSTAT1or both for 72 h and analyzed for the expressions of cyclin D,p-CDK2, and cyclin E by Western blotting. GAPDH was used as a loading control. ELL2 knockdown induces cyclin Eexpression and repressed cell cycle related p-CDK2 and Cyclin D

7. ELL2 Deletion Induces Apoptosis In PC-3 And DU145 Independent Of STAT1

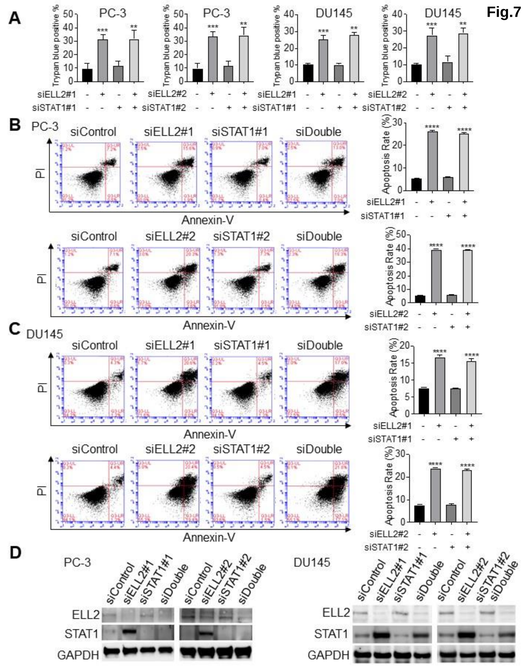

Fig. 7. A. Trypan blue exclusion assay of cell death of PC-3 and DU145 cells transfected with indicated siRNA as described in Fig. 5. Data represent the means ± SD. Cell apoptosis was analyzed with Flow cytometry apoptosis analysis using Annexin V-FITC/7-AAD in PC-3 (B) and DU145 (C) cells transfected with indicated siRNAs. D. PC-3 and DU145 cells were untreated or were transfected siELL2, siSTAT1or both for 72 h and analyzed for knockdown efficiency. ELL2 band denoted by black arrow. GAPDH was used as a loading control. ** p< 0.01, *** p < 0.001, **** p < 0.0001.