The Cytoprotective Role of DJ-1

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Abstract

DJ-1 is a protein that plays a multiple roles in transcriptional regulation, antioxidant response, chaperone, protease, and mitochondrial regulation. Its activity is regulated by its oxidative status as excess oxidation of DJ-1 renders the protein inactive. In patients with sporadic Parkinson's and Alzheimer's diseases, the inactivation of DJ-1 by excess oxidation and cell death has been observed. We also analyzed the role of fibroblast growth factor receptor 1 (FGFR1), which belongs to the tyrosine kinase receptor. This protein is an important factor in cell repair and proliferation and wound healing.

Hypothesis

Extracellular recombinant human DJ-1 stimulates cell repair and wound healing through FGFR-1 activation.

Material and Methods

Cell culture

- Human alveolar A549 epithelial cells were cultured in DMEM medium with 10% fetal bovine serum and 1% penicillin/streptomycin.
- Four conditions were used: Control, Nontargeting (NT) siRNA, FGFR-1 siRNA and FGFR-1 siRNA +DJ1

Transfection

- A549 cells were transfected with non-targeting siRNA or FGFR-1 siRNA for 48h.

Scratch Assay

- We used 200 µL pipet tip to vertically scratch the wells with cultured cells. Old media was discarded, A549 cells were washed with PBS, and new media was added to the wells. Recombinant human DJ-1 was diluted in new media and added to A549 cells transfected with FGFR-1 siRNA.
- At 0 hours, 4 hours, 24 hours, and 48 hours after scratch, pictures were taken using bright field microscope (Zeiss) of the space caused by the 200 µL pipet tip.

Western Blot analysis

- Western Blot analysis was performed to check FGFR-1 protein expression in A549 cells transfected with FGFR-1 siRNA.

Cloning

- PCR amplification of genes of interest including Traf6, Lig3, XRCC4, and TDP1 using cDNA as template
- Restriction enzyme digestion of PCR fragments with restriction enzyme (Ecor1 and Kpn1)
- Purification of PCR product by running through agarose gel and using gel purification kit (Zymogen)
- Cut Bifc vn and Bifc vc plasmids with EcoR1 and Kpn1.
- Ligate PCR fragments into cut plasmids using T4 Ligase.
- 2-5 µL of ligation products were used to transfect DH5 alpha E. coli.
- Ampicillin resistant colonies were selected from agar plate and grown in LB media.
- Plasmids were purified using Miniprep kit (Biolab)
- Products were sent for sequencing (Genewiz) to confirm insertion

Results

- To test the transfection efficiency of FGFR-1, we used siRNA strategy to knockdown FGFR-1 gene. FGFR-1 protein expression was compared to control and NT siRNA. We observed decreasedFGFR-1 expression after A549 cell transfection with 25 nM FGFR-1 siRNA.

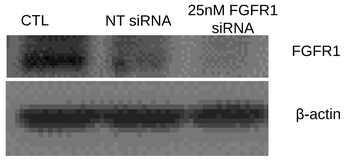
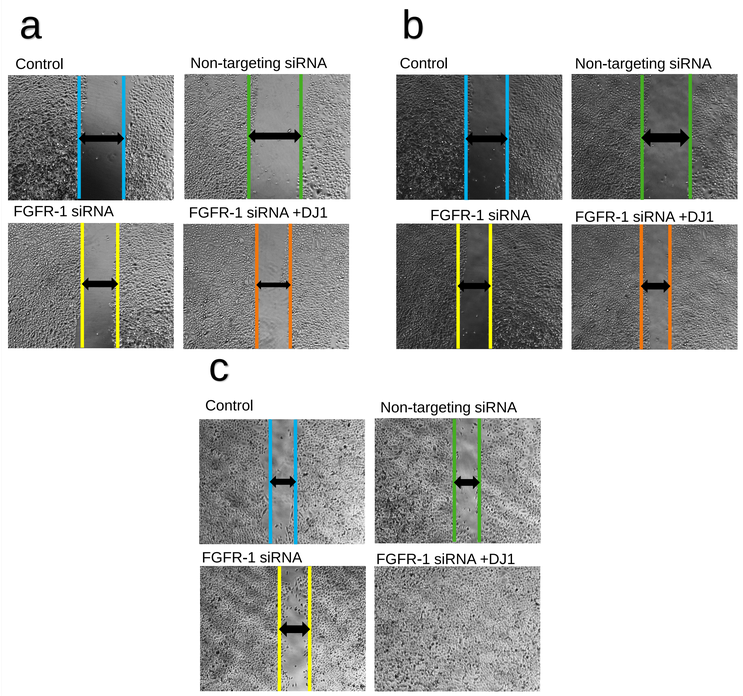


Figure 1: Analysis of FGFR-1 Knockdown. 5 x 10^5 A549 cells were plated onto a 6 well plate. 25 nM Non-targeting (NT) siRNA and FGFR-1 siRNA were used for cell transfection using Lipofectamine RNAiMAX Reagent according to manufacturer instructions. Western Blot was used to analyze FGFR-1 and β-actin protein expression (CTL – control).

- To determine if recombinant human DJ-1 stimulates cell proliferation in FGFR-1 silencing cells, we cultured A549 cells and transfected them with 25 nM FGFR-1 siRNA. Then we performed a scratch assay onto 100% confluent cells and added recombinant human DJ-1 to determine its effect on wound healing.



Results (cont.)

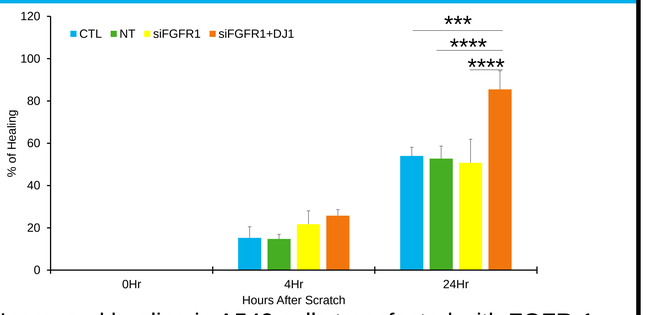


Figure 2. Increased healing in A549 cells transfected with FGFR-1 siRNA and treated with recombinant human DJ-1. Representative images of scratch assay at (a) 0 hrs, (b) 4 hrs and (c) 24 hrs using bright field microscope (magnification 20x). The distance of the opening was measured and analyzed with two way ANOVA. Data are mean ± s.e.m. *** P<0.0002; **** P<0.0001.

Conclusion

1. We knocked down FGFR-1 gene by using 25nM siRNA transfection into A549 cells.
2. Increased healing was observed in cells with FGFR-1 siRNA and treated with recombinant human DJ-1 for 24 hours.
3. To repeat experiments and have improved results, it is better to use 6 well plates rather than 24 well plates as it will be easier to have a consistent scratch.
4. Cloning was performed to further study the role of DJ-1 and its interaction with other proteins.

References

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Contact Information and Funding

This work is supported by NIH R01HL118171 (BK) and FAMRI CIA (BK).

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