Analysis of Isoliquiritigenin effect on mitoNEET expression

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Overview of Melanoma

Melanoma represents the most aggressive and deadliest form of skin cancer. It is the fifth most common cancer among men and women with an estimated 196,000 new cases in the United States in 2021.[1] It occurs from genetic mutations in melanocytes, cells that produce pigments.[2] Melanocytes are located in the basal layer of the epidermis. Their growth is regulated by the surrounding keratinocytes in a variety of pathways.[3] Treatments for this cancer are limited. Most common practice includes surgery, chemotherapy, immunotherapies, and other targeted therapies none of which provide a cure.[4] Alternative and effective therapies are greatly needed to potentially cure this aggressive cancer.

MitoNEET

MitoNEET is a newly discovered mitochondrial protein. It is an integral protein localized in the outer mitochondrial membrane (OMM). Its name is based on its subcellular localization and the presence of the amino acid sequence Asn-Glu-Glu-Thr (NEET). MitoNEET is also identified as part of the unique 39 amino acid sequence, CDGSH, a domain in residues 55-93 that act similarly, to a zinc finger and is likely involved with iron binding. The protein also contains a N-terminal α-helix with a redox active iron-sulfur domain, [2Fe-2S]. These components have shown that mitoNEET is able to play a role in the regulation of energy metabolism in the mitochondria.[6]

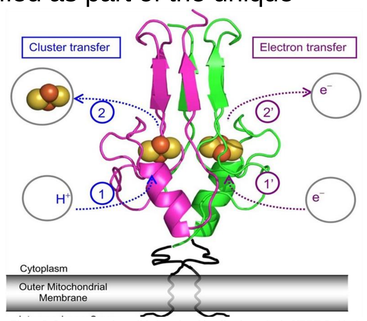

Figure 3. Structure and possible functional implications of mitoNEET. Paddock, Mark L.; Wiley, Sandra E.; Axelrod, Herbert L.; Cohen, Aina E.; Roy, Melinda; Abresch, Edward C.; Capraro, Dominique; Murphy, Anne N.; Nechushtai, Rachel; Dixon, Jack E.; Jennings, Patricia A. MitoNEET is a uniquely folded 2Fe-2S outer mitochondrial membrane protein stabilized by pioglitazone. *PNAS*. 2007. 104(36). 14342-14347.

Isoliquiritigenin

Isoliquiritigenin (ISL), is a bioreactive compound that is derived from licorice root. It is commonly found in foods and Chinese herbal medicines.[7] This root is a member of the flavonoids, which promote health and disease prevention. Many studies have revealed that they are affective against cardiovascular disease and cancer.[8] More specifically they are known to have anti-tumor effects in vitro and in vivo.[9] ISL has a promising outlook when considering cancer treatment options.

ACKNOWLEDGMENTS

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Preliminary Studies

Initial plating of the A375 cells were done using 10,000, 25,000, 50,000, 75,000, and 100,000 cells per 35 mm dish. After examination, it was concluded approximately 50,000 cells per dish was optimal. The amount of lipofectamine 2000 for transfection was tested with 3, 6, 9, 12, 15, and 18 µL per dish. Only the 3 µL and 6 µL survived the transfection. The results after imaging showed using 3 µL was most ideal for the experiment. Figure 2 shows representative images from the transfection optimization experiment.

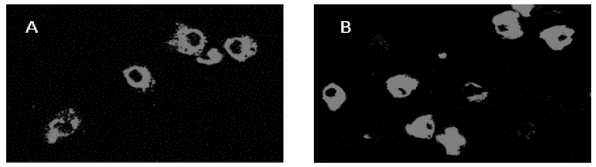

Figure 2: A375 cells expressing mitoNEET-GFP after transfection with 3 µL lipofectamine 2000 (A) or 6 µL lipofectamine 2000 (B).

The concentration of mitoNEET-GFP was varied using four concentrations including 100, 200, 500, and 1000 ng/µL. It was determined that the ideal concentration was 150 ng/µL for each dish of cells. Figure 3 shows each concentration of mitoNEET-GFP after imaging.

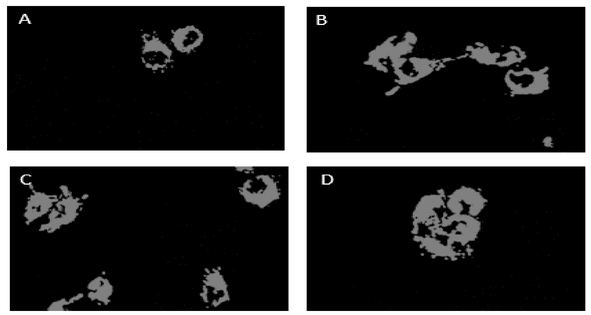

Figure 3: (A) A375 cells with 100 ng/µL mitoNEET-GFP. (B) A375 cells with 200 ng/µL mitoNEET-GFP. (C) A375 cells with 500 ng/µL mitoNEET-GFP. (D) A375 cells with 1000 ng/µL mitoNEET-GFP.

MitoNEET in the presence of ISL

Trials of 0.1, 1, 10, and 100 µM and 0.5, 5, 50, and 500 µM were added to the A375 cells tagged with the mitoNEET-GFP. Results are shown in Figure 4 and Figure 5.

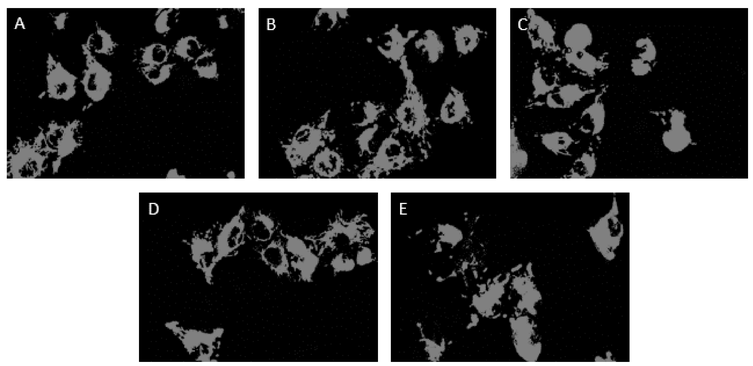

Figure 4: (A) Control, A375 cells expressing mitoNEET-GFP. (B) A375 cells expressing mitoNEET-GFP after exposure to 0.1 µM ISL. (C) A375 cells expressing mitoNEET-GFP after exposure to 1 µM ISL. (D) A375 cells expressing mitoNEET-GFP after exposure to 10 µM ISL. (E) A375 cells expressing mitoNEET-GFP after exposure to 100 µM ISL.

MitoNEET in the presence of ISL

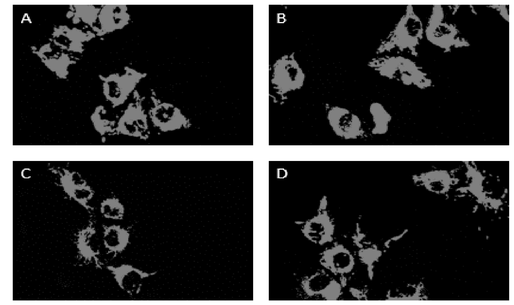

Figure 5: (A) Control, A375 cells expressing mitoNEET-GFP. (B) A375 cells expressing mitoNEET-GFP after exposure to 0.5 µM ISL. (C) A375 cells expressing mitoNEET-GFP after exposure to 5 µM ISL. (D) A375 cells expressing mitoNEET-GFP after exposure to 50 µM ISL.

The mean fluorescence intensity (a.u) was examined for each trial which is shown in Figure 6. In the experiment using 0.1, 1, 10, and 100 µM the results were 318 ±14, 286 ±15, 274 ±17, and 236 ±15, respectively with the control being 337 ±10. For the trial using 0.5, 5, 50 µM the fluorescence intensities were 167 ±7, 165 ±14, and 142 ±9, respectively with the control being 235 ±10. The raw integrated densities were measured and are shown in Figure 7.

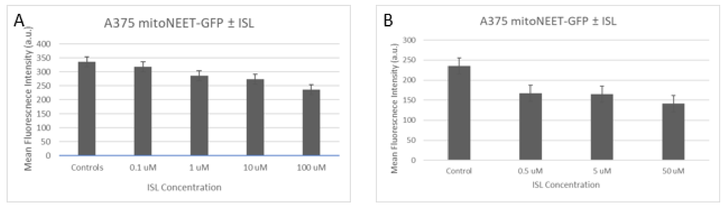

Figure 6: (A) Mean fluorescence intensity of A375 cells expressing mitoNEET-GFP for control, 0.1 µM ISL, 1 µM ISL, 10 µM ISL, and 100 µM ISL. (B) Mean fluorescence intensity of A375 cells expressing mitoNEET-GFP for control, 0.5 µM ISL, 5 µM ISL, and 50 µM ISL. (n= 50,000), (Data are mean values ± S.D.)

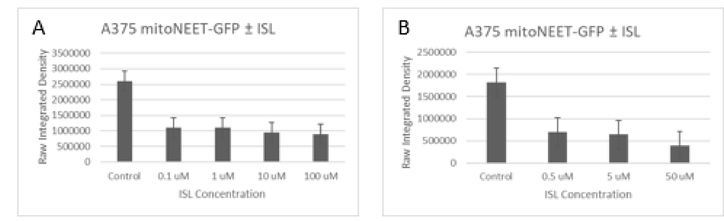

Figure 7: (A) Mean raw integrated density of A375 cells expressing mitoNEET-GFP for control, 0.1 µM ISL, 1 µM ISL, 10 µM ISL, and 100 µM ISL. (B) Mean raw integrated density of A375 cells expressing mitoNEET-GFP for control, 0.5 µM ISL, 5 µM ISL, and 50 µM ISL. (n= 50,000), (Data are mean values ± S.D.)

Conclusion

Overall, treatment of A375 cells with ISL downregulated the expression of mitoNEET making it a promising target for future cancer treatments. Additional studies will measure the effect of mitoNEET on cell viability and production of ROS in the presence of additional treatments in combination with ISL.

References

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