

Alix co-localizes with galectin-3 and traffics Tyrp-1 in melanocytes

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Background

Melanocytes play a role in melanin synthesis, as well as many other physiologic processes. The main role of melanin is to protect the skin from UV damage. Errors in melanogenesis have been seen in a variety of congenital hypopigmentary disorders. Many proteins play a role in trafficking cargo needed for melanin synthesis from the Golgi to the melanosome, with an endosome intermediate. Galectin 3 has been shown to interact with Tyrp1 and help traffic it to the melanosome.

Aims

The goals of this project are to determine if Galectin 3 and Alix co-localize within melanocytes, as well as Alix and Tyrp1 in order to better understand the proteins involved in melanogenesis, leading to treatments for affected patient populations.

Methods

Indirect immunofluorescence was performed on melanocytes and melanoma cells to determine levels of co-localization for proteins of interest. Images were captured via confocal microscope and analyzed using Image J software to measure Mander's coefficients, which quantitate co-localization. Melanocytes were also silenced for Galectin and Alix respectively and the immunofluorescence was measured via the above method, as well as confirmed via Western Blot Analysis.

Results

Galectin and Alix showed the strongest Mander's coefficient of 0.905 and .916 for melanocytes and melanoma cells respectively. Also, Alix showed slightly higher Mander's coefficients when compared with Tyrp1 (0.908 and 0.899) than tyrosinase (0.889 and 0.863). Also, the galectin silenced cells showed a statistically significant difference in Alix levels ($p=0.000169513$) while the Alix silenced cells did not show a significant difference in respective Galectin 3 levels ($p=0.131037503$).

Conclusion

Galectin 3 and Alix show strong co-localization, and Alix preferentially co-localizes with Tyrp1 versus tyrosinase, which is consistent with Galectin 3's preferential co-localization. In addition, because the levels of Alix in Galectin 3 silenced cells seemed to be affected more than the levels of Galectin 3 in Alix silenced cells it is predicted that Galectin 3 cargo transport is upstream of Alix cargo transport in melanogenesis.

Acknowledgements

MSSRP Program and Cincinnati Children's Hospital Medical Center

Funding from The National Institute of Diabetes and Digestive and Kidney Disease (Grant # 2T35DK060444-11A1)

Janay Woolridge and other members of the research staff in the Department of Dermatology