hiPSC-Derived Sensory Neurons and their Role in Skin Pigmentation: A 'Disease-in-a-Dish' Model for Hyperpigmentation Research

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INTRODUCTION

- Senile lentigines are hyperpigmented skin patches with stable, localized pigmentation and well-defined edges, commonly triggered by UV exposure. However, the mechanisms behind their development and persistence remain unclear.
- Sensory neurons and melanocytes, both derived from the neural crest, interact closely in the skin, with recent studies (1) highlighting the modulation of melanocytes by sensory neurons.
- This neuron-melanocyte interaction offers new insights into pigmentation disorders and potential targeted therapies.
- Yashraj Biotechnology aims to develop a hyperpigmented "disease-in-a-dish" model, providing a valuable tool for understanding disease etiology and enabling the development of targeted therapeutics.
- Concentrations of SN-CM exhibit increased extracellular melanin content with no change in their viability. RGMB at the reported concentrations of 125 ng/mL also show ~20% increase in melanin content.
- The study exploits the role of sensory neuron-secreted factors in developing an *in vitro* disease platform for research and high-throughput screening.

MATERIALS AND METHODS

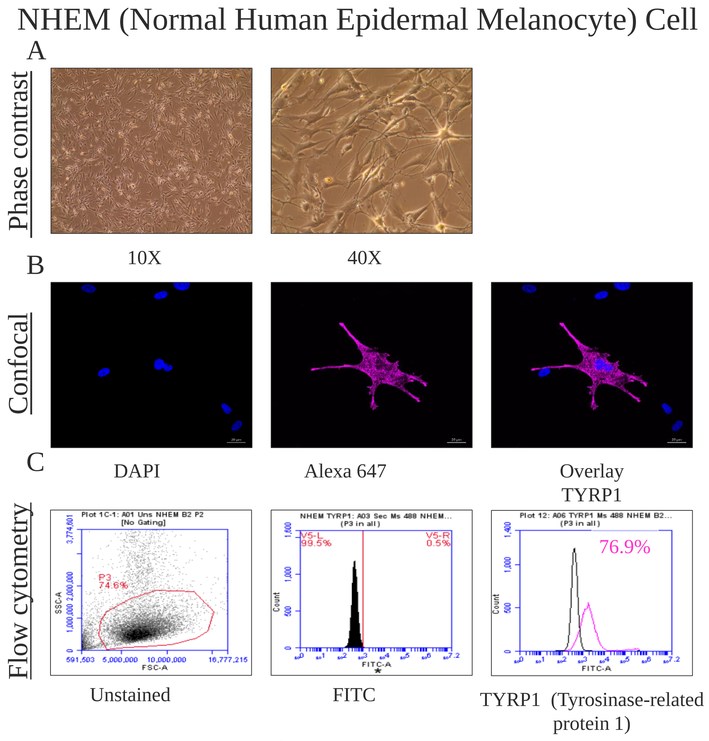
iPSC-derived sensory neurons were generated using the STEMCELL Technologies protocol. Melanocytes were isolated from human skin tissue by using lab protocol. Co-culture of Sensory Neurons (SN) and melanocytes was done using different combinations of SN media (10%, 25%, 50%) and MGM for 48 hrs. Calcein-AM, intracellular and extracellular melanin content was quantified in Melanocytes treated with SN-CM (Sensory Neuron – Condition Media) (10% SN-CM+90% MGM, 25% SN-CM+75%MGM, 50%SN-CM+50%MGM and 100%SN-CM) using lab standardized protocols.

References

1. Chow, Siu Yu A., et al. "Human sensory neurons modulate melanocytes through secretion of RGMB." *Cell Reports* 40.12 (2022).
2. Chung, Soobin, et al. "Quantitative analysis of melanin content in a three-dimensional melanoma cell culture." *Scientific Reports* 9.1 (2019): 780.

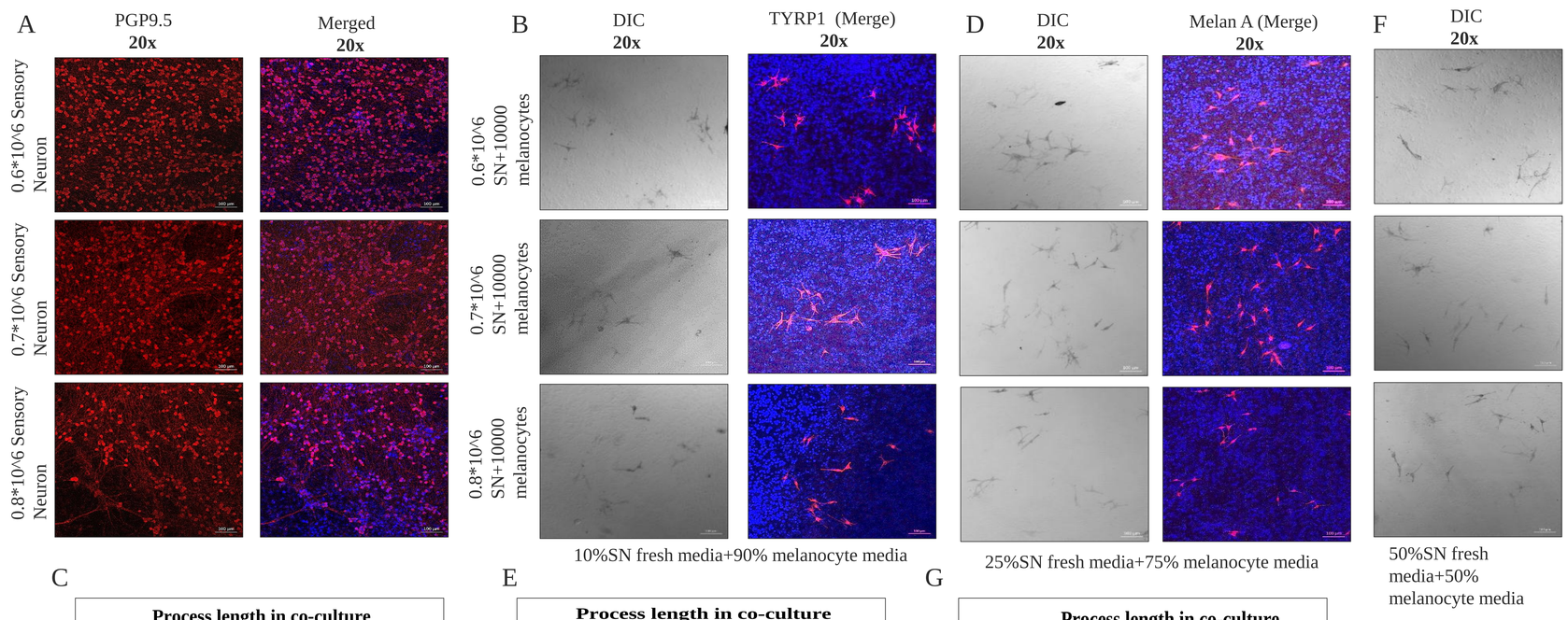
RESULTS

Figure -1



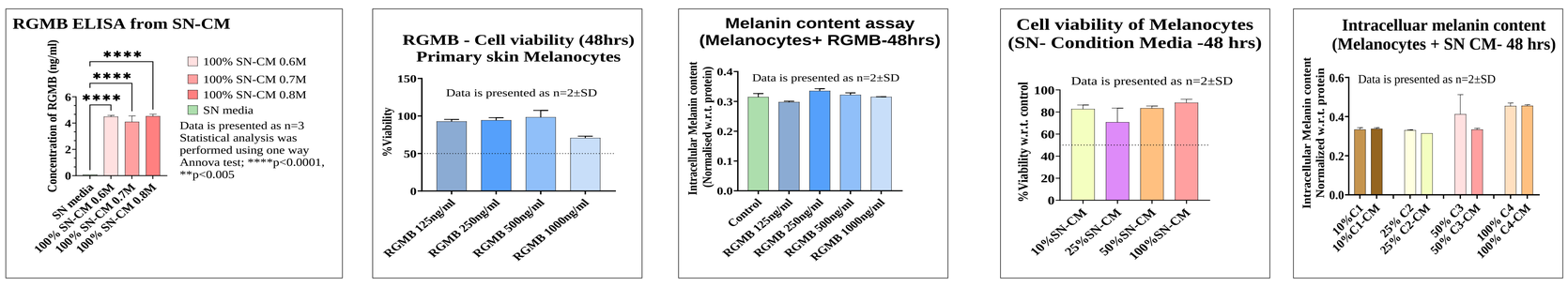
A. Phase contrast images of human skin-derived melanocytes (NHEM) were captured at 10X and 40X.
B. Confocal image of NHEM immunostained with melanocyte-specific marker, TYRP1, staining the cytoplasm (magenta) and counterstained nucleus with DAPI(blue). The data is representative of N=2, n=5(each N).
C. Flow cytometry analysis of NHEM cells specific marker, TYRP1 demonstrates 76.9% purity. The data is representative of N=2.

Figure -2



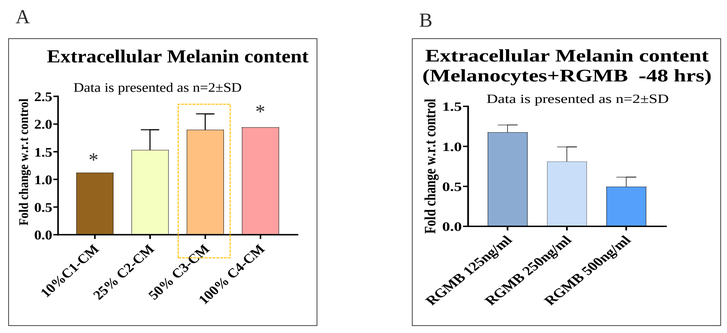
A. Confocal images showing iPSC-derived sensory neurons grown for a period of 20 days and immunostained for Pgp9.5, a sensory neuron marker staining the neuron nucleoplasm and neurite lengths (red) and counterstained with DAPI(blue). The data is representative of N=1, n=5.
B. Confocal images showing a co-culture of SN with NHEM in different media combinations, immunostained with TYRP1 (cytoplasm-red) and counterstained nucleus with DAPI(blue). The data is representative of N=1, n=6.
C. Quantification of process length from the DIC images of co-culture of SN and NHEM. The data is representative of N=1, n=20±s.d. Statistical analysis was performed using one-way ANOVA test, ***p<0.0004, **p<0.005.
D. Confocal images showing a co-culture of SN with NHEM in different media combinations, immunostained with MelanA (cytoplasm-red) and counterstained nucleus with DAPI(blue). The data is representative of N=1, n=6.
E. Quantification of process length from the DIC images of co-culture of SN and NHEM. The data is representative of N=1, n=50±s.d. Statistical analysis was performed using one-way ANOVA test, ****p<0.0001.
F. Confocal images (DIC) showing a co-culture of SN with NHEM in different media combinations. The data is representative of N=1, n=6.
G. Quantification of process length from the DIC images of co-culture of SN and NHEM. The data is representative of N=1, n=35±s.d. Statistical analysis was performed using one-way ANOVA test, ns-non significant.

Figure -3



A. Graphical representation of RGMB ELISA performed using SN-condition media (SN-CM) from iPSC-derived sensory neurons grown for 20 days. The data is representative of N=1, n=3±s.d. Statistical analysis was performed using one way ANOVA test; ****p<0.0001
B. Graphs representing cell viability of NHEM treated with RGMB using Calcein-AM.
C. Graphs representing intracellular melanin content of NHEM treated with different concentrations of RGMB using MCA reagent and normalized against total protein content (BCA method)
D. Graphs representing cell viability of NHEM treated with different concentrations of SN-CM using Calcein -AM
E. Graphs representing intracellular melanin content of NHEM treated with different concentrations of SN-CM and normalized against total protein content (BCA method)

Figure -4



A. Graphs representing extracellular melanin content of NHEM treated with different concentrations of SN-CM (* outlier)
B. Graphs representing extracellular melanin content of NHEM treated with different concentrations of RGMB

CONCLUSION

- Our initial investigation reveals the presence of extracellular melanin in the SN condition media.
- This clearly indicates vesicular shedding of melanosome possibly due to overexpression of vesicle transporters in SN-CM culture.
- Further investigation is required to determine the mechanism behind vesicular shedding.

ACKNOWLEDGMENTS

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