Ultrastructural Heterogeneity in Melanocytes Cultured from Patients with Chediak-Higashi Syndrome

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ABSTRACT AND INTRODUCTION

Chediak Higashi syndrome (CHS) is due to mutations in the *CHS/LYST* gene that affect organelles such as lysosomes, platelet dense granules, cytolytic granules and melanosomes. The oculocutaneous albinism in CHS may result from enlarged melanosomes that can contain minimal amount of melanin. Ultrastructural observations of the skin of two CHS patients demonstrated dramatically reduced transfer of melanosomes to epidermal keratinocytes. In addition, we developed cultured melanocytes from three different CHS patient, CHS 4 carried two truncating *LYST* mutations; a nonsense (R514X) and a frameshift (F3298delT), CHS 6 carried a truncating frameshift (E805delG) and a relatively milder missense mutation (N3376S), CHS 2 that was not genetically defined. These cultured melanocytes were characterized at the ultrastructural level to assess melanosome size, amount of endogenous and inducible melanin production, presence of DOPA-positive 50 nm vesicles and tubular elements, and the location of melanosomes in the cells. All three cell lines contained melanosomes ranging between 2-4X normal size. Two lines (CHS 2 & 6) contained minimal amount of endogenous melanin and the other (CHS 4) contained moderate amount of melanin with distinct focal deposition within melanosomes. Melanin production could be induced in all three lines after DOPA incubation. Two lines (CHS 2 & 6) contained 50 nm vesicles present throughout the cell and all three lines contained tubular elements. Melanosomes in one line (CHS 4) were restricted to the perinuclear region and in the other two lines (CHS 2 & 6) were distributed throughout the cell. In conclusion, these CHS cell lines demonstrate heterogeneity in ultrastructural morphology.

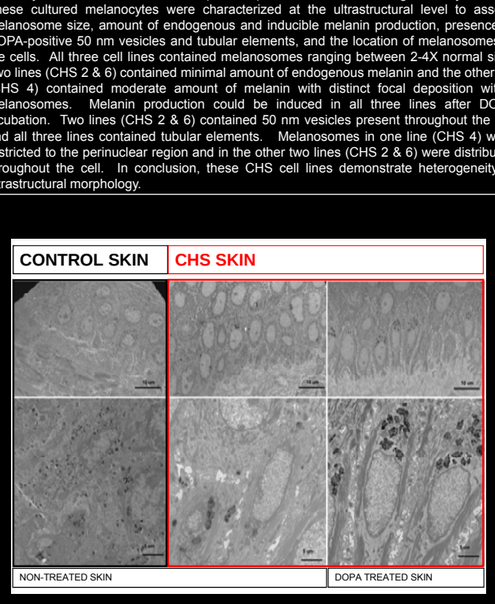

Fig 1. **EM images of Control and CHS skin.**

SPURR embedded samples were sectioned into 90um thick sections and viewed using JEM-1230 transmission electron microscope. Images were captured at a low mag (2Kx for upper panels) and at a higher magnification (8Kx for lower panels). Melanosomes were detected in CHS skin basal and super basal keratinocytes, but at a lower frequency than in control skin. The far right upper and lower panels show DOPA incubated skin. The melanosomes appeared to contain more reaction product. Shown in this figure is patient CHS#5

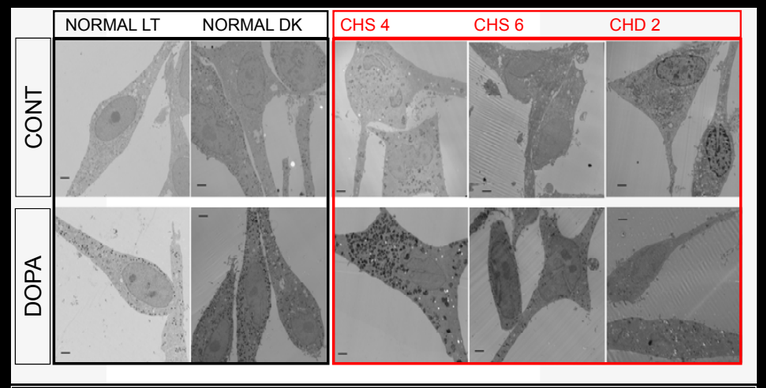

Fig 2. **Low Magnification of Normal Melanocytes (light & dark skin derived) compared to CHS Melanocytes demonstrating variation in pigmentation of the CHS cultured cells.**

SUMMARY TABLE

Cell Line/ Skin EM ID #	Normal Human Melanocytes	CHS Cell Line -CHS 4 HM 733	CHS Cell Line -CHS 6 HM 828	CHS Cell Line –CHS 2 HM 2	HM 1539 CHS 3 SKIN	HM 1562 CHS 5 SKIN
Melanosome Size	Defined as Normal	++Larger Than NMH	+Larger Than NHM	++Larger than NHM	MC-N/D KC- not present	MC-N/D KC-normal
Endogenous Melanin	Yes, amount dependent on donor type	At close to NHM levels	↓	↓	Minimal by visual assessment	Moderate by visual assessment
50 nM vesicles	Not detectable away from Golgi area	Not detectable away from Golgi area	Throughout cell body	Throughout cell body	N/D	N/D
Tubular elements	Not detectable under normal conditions	Present	Present	Present	N/D	N/D
Location of melanosomes Within melanocytes	From perinuclear to extremities of dendrites	Restricted to perinuclear region	From perinuclear to extremities of dendrites	From perinuclear to extremities of dendrites	N/D	In perinuclear region and dendrites
Melanosome transfer to keratinocytes	In situ and In Vivo	N/D	N/D	N/D	Not present	Present in a subset of super basal KC clustered

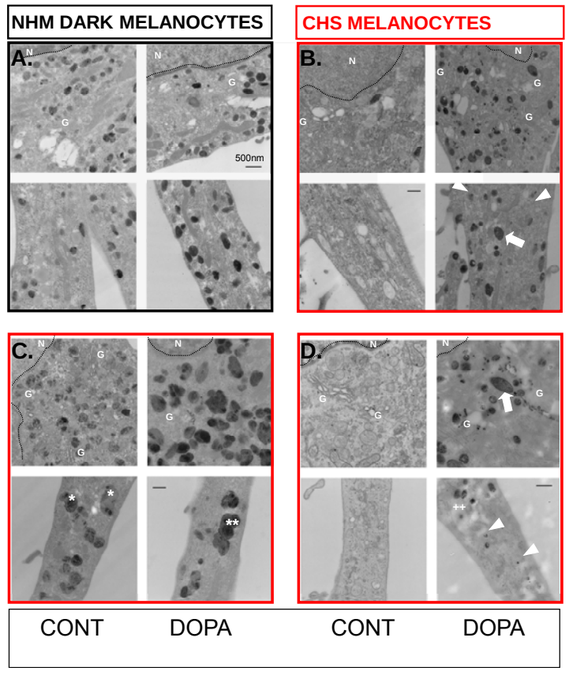

Fig 3. Ultrastructural Comparison of the Three CHS cell lines.
Cells were fixed, dehydrated and embedded in Epon resin as described. During the embedding process, cells were incubated in either cacodylate buffer or in buffer including L-3,4-dihydroxyphenylalanine (DOPA). Panel A represents control melanocytes derived from dark neonatal foreskin (NHM). Panel B, C and D are CHS 6, CHS 4 and CHS 2, respectively. CHS 6 and CHS 2 have reduced endogenous melanin as compared to NHM and to CHS 4. In addition, CHS 6 and CHS 2 contain 50 nm vesicles (arrowheads) outside of the Golgi area (G). All three lines had a subset of melanosomes that were larger (arrows) than those in NHM, especially within CHD 2 and CHS 4 . (++) indicates an amelanatic enlarged melanosome in CHS 6. Melanosomes in cell line CHS 4 were also found as clusters (*),

METHODOLOGY

4mm punch biopsies were obtained from the skin of CHS patients or from the skin of control patients, transported to tissue culture facilities at either the National Institute of Health or the University of Cincinnati and processed for cell cultures or for electron microscope analysis. To obtain cell cultures, the epidermis was split from the dermis using either dispase or trypsin. The epidermis was incubated in melanocyte growth media containing 8% fetal bovine serum, BPE, insulin, TPA, bFGF, and Vitamin E for 48 hours to allow cell attachments. The derived cultures were propagated in this media until adequate cells numbers were achieved. For each cell type, two wells were plated at $1x10^5$ cells per well. One well was treated with L-3,4-dihydroxyphenylalanine (DOPA). The cells were then further processed for TEM analysis by fixation in Karnovsky's ½ strength fixative, dehydration, embedding in EPON resin, sectioning and examination at the ultrastructural level using a JEM-1230 transmission electron microscope fitted with an AMT Advantage Plus 2K x 2K digital camera. For skin analysis, the punch biopsies were cut into six equal pieces, then fixed in Karnovsky's ½ strength fixative. Half of the punch was incubated in DOPA, then processed as described for the isolated cells and embedded in SPURR medium.

Conclusions and Future Direction

The goal of this study was to characterize at the ultra-structural level the phenotype of cell lines derived from patients with Chediak-Higashi Syndrome. Genetic analysis of two of the lines, CHS 4(C) and CHS 6(B) has been done. CHS 4(C) carries two truncating *LYST* mutations; a nonsense (R514X) and a frameshift (F3298delT) and CHS 6(B) carries a truncating frameshift (E805delG) and a relatively milder missense mutation (N3376S). To date, CHS 2(D) has not been genetically defined. The difference in the causal mutations may explain the difference in the severity of the phenotypes seen in the two cell lines. In CHS 4(C) , melansomes are not found in the dendrites implying that transfer is not occurring. Also the melanosomes form large clusters further preventing proper transfer. In CHS 6(B), the melanosomes are found in the dendrites and are not in large clusters. So transfer may be occurring, even at a reduced rate. Co-culture experiments can be done to determine if this is the case. CHS 2(D) appears to have a phenotype more similar to CHS 6(B), and perhaps the genetic analysis may show that the mutations are the same in these two lines. In conclusions, the three cell lines examined in this study demonstrated distinct phenotypes.

References

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