Differential gene expression in cells from Fabry and Gaucher diseases: cell reprogramming and culture aging

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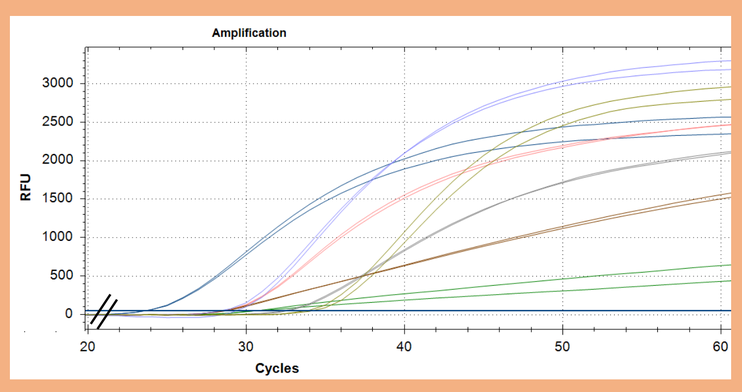
Abstract: Gene expression varies deeply, even in the same individual's cells, depending on stress factors, cell aging, gene variants or gene and cell manipulation. In this work, gene expression can be seen to shift in many genes, using fibroblasts from donors with Fabry and Gaucher type 3 genetic diseases. Tests were also made to compare the impact of aging cultures. Real-time qRT-PCR protocols for genes of interest were used for expression comparison after cell induction and reprogramming.

Introduction: Fibroblasts are easily obtained, these cells can be used in cell reprogramming to generate cells of difficult isolation, such as neuronal cells, while maintaining the patient's genetic background. For reprogramming, transcription factors (TFs) like OCT4, SOX2, KLF4, and MYC are vector-delivered to the cells. These TFs can induce fully differentiated cells in pluripotent stem cells (iPSCs), capable of self renewal and differentiation into almost all types of cells [**1**]. Neuronal progenitor cells can be achieved using TFs such as GSK, TGF and Notch inhibitors [**2**]. Cells can be studied by the gene expression, explaining the differences in morphology and functions.

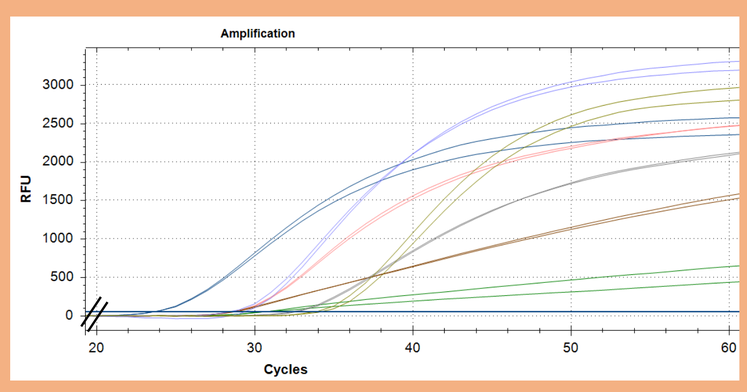
Methods: Human fibroblasts of FD and GD3 patients and HDFa fibroblasts (obtained from cell banks) were cultured and reprogramed into iPSCs. Subsequently GD3 iPSCs were differentiate into pre-neuronal cells. Human fibroblasts and resulting iPSCs were cultured and pelleted to extract RNA using Invitrogen Purelink RNA Mini kit or Trizol method. The cDNA was then synthetized and used in Real-Time qPCR for gene expression analysis.
FAM probes for the following genes were used:
- GAPDH, Actin A, TUBB3 (cytoskeleton)
- GLA, GBA1, HEXA (lysosomal storage related)
- OCT4, SOX2 (TFs)
- ZEB2 (aging)
- MAP2, NES, OTX2 (neurogenesis)

Results:

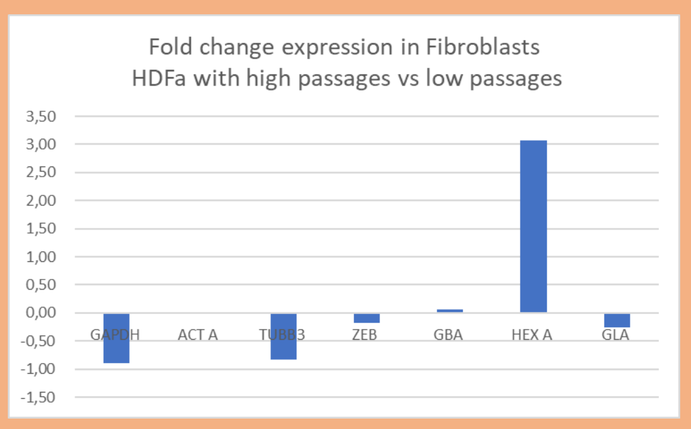
Actin A, TUBB3, ZEB2, GBA1, HEXA and GLA expression variations between HDFa cell lines with low and high passage numbers, using Actin A as a housekeeping:



GADPH, Actin A, TUBB3, ZEB2, GBA1, HEXA and GLA expression in Fibroblast HDFa low passage cell line with RT-PCR.

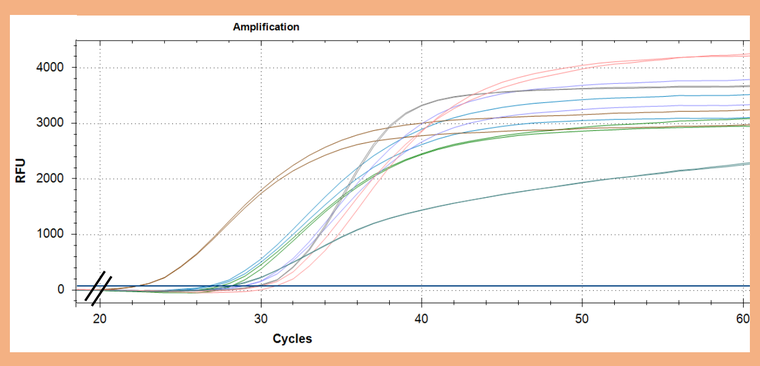


GADPH, Actin A, TUBB3, ZEB2, GBA1, HEXA and GLA expression in Fibroblast HDFa high passage cell line with RT-PCR.

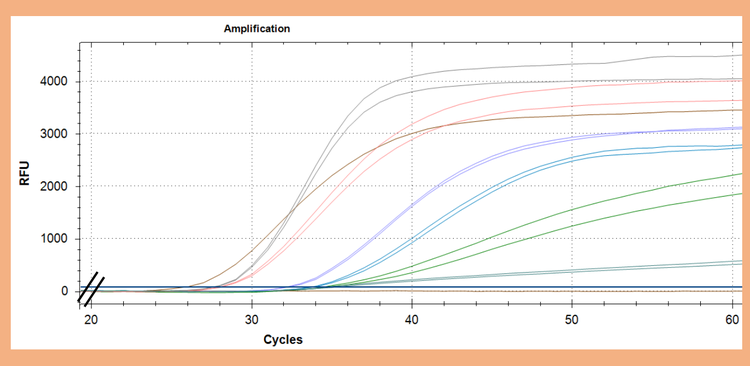


- HEXA increase: experimental artifact or not?

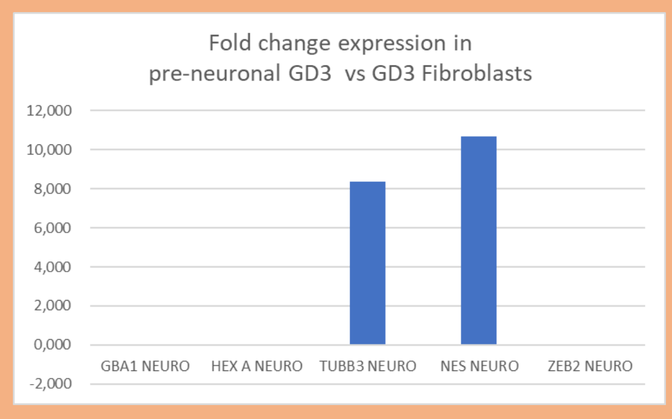
GBA1, TUBB3, NES, SOX2, OCT4 and ZEB2 expression variations between GD3 Fibroblasts GD3 and pre-neuronal cell lines, using HEXA as a housekeeping:



GBA1, HEXA, TUBB3, NES, SOX2, OCT4 and ZEB2 expression in GD3 Fibroblast cell line with RT-PCR.

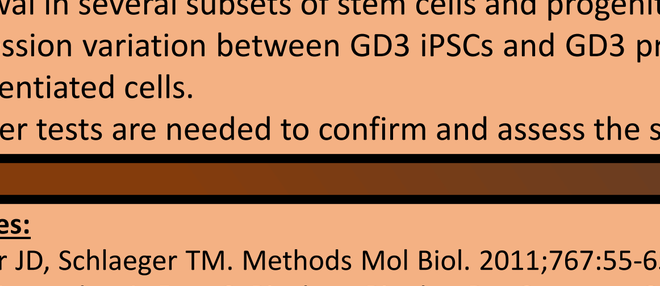


GBA1, HEXA, TUBB3, NES, SOX2, OCT4 and ZEB2 expression in GD3 pre-neuronal cell line with RT-PCR.

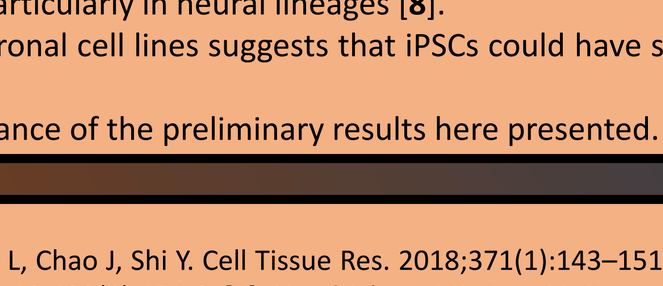


- Higher expression of TUBB3 and NES.

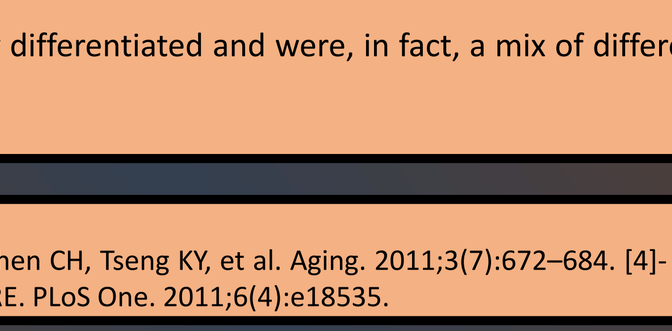
NES, MAP2, TUBB3 and OTX2 expression variation between GD3 iPSCs and GD3 pre-neuronal cell lines, using GADPH as a housekeeping:



GADPH, NES, MAP2, TUBB3 and OTX2 expression in GD3 pre-neuronal cell line with RT-PCR.



GADPH, NES, MAP2, TUBB3 and OTX2 expression in GD3 iPSCs cell line with RT-PCR.



- Lower expression of NES, MAP2, TUBB3, OTX2 in pre-neuronal cell line.

Discussion and Conclusion:
- **Increase in HEXA expression** could be an experimental **artifact or an upregulation** event with aging of cell cultures. Since glycosphingolipids are a major component of lipid rafts (important in signal transduction and cell proliferation), higher expression of HEXA might enhance the degradation of the glycosphingolipids, thus affecting signaling pathways [**3**].
- **Pre-neuronal cells showed higher expression, of TUBB3 and NES**, than Fibroblasts. TUBB3 is a marker for neurons and like NES it is a cytoskeleton protein [**4**]. The higher expression in neuronal progenitor cells (NPCs), and even in iPSCs, when compared to fibroblasts, is explained by NES being characteristic of NPCs and related to self-renewal in several subsets of stem cells and progenitors, particularly in neural lineages [**8**].
- Expression variation between GD3 iPSCs and GD3 pre-neuronal cell lines suggests that iPSCs could have spontaneously differentiated and were, in fact, a mix of differently differentiated cells.
- Further tests are needed to confirm and assess the significance of the preliminary results here presented.

References:
[1]- Miller JD, Schlaeger TM. Methods Mol Biol. 2011;767:55-65. [2]-Li L, Chao J, Shi Y. Cell Tissue Res. 2018;371(1):143–151. [3]-Jiang SS, Chen CH, Tseng KY, et al. Aging. 2011;3(7):672–684. [4]- Liu C, Zhong Y, Apostolou A, Fang S. Biochem Biophys Res Commun. 2013 Sep 13;439(1):154-9. [5]- Hendrickson ML, Rao AJ, Demerdash ON, Kalil RE. PLoS One. 2011;6(4):e18535.