Many forms of hearing loss is the result of the inner ear’s inability to replace lost sensory hair cells. Here, we apply a differentiation strategy to differentiate human adipose stem cells into cells of the otic lineage using chemically-defined conditions. Generation of human otic progenitor cells was dependent on FGF signaling and extended culture led to the upregulation of markers inner ear cells, at protein and gene level. Even though defined conditions must be optimized, this work demonstrate that hASCs have the ability to differentiate into the otic lineage.

We isolated and cultured the stromal vascular fraction (SVF) of 3 samples of human adipose tissue. We differentiated human adipose stem cells (hASCs) to otic lineage, with a two step protocol using a differentiation medium containing DMEM HG, F12, N2, B27 and different growth factors like FGF3, FGF10, EGF and IGF-1 (Figure 1). The differentiated cells were fixed and RNA extracted at day 10 and 38 to analyze the expression of otic progenitor, neuronal or inner ear hair cell markers (Figure 2).

After 10 days of differentiation we detected otic placode markers expression: Pax2 (90.9 ± 0.16%), Pax8 (44.2 ±0.03%) and BMP7 (86.4 ± 0.24%), Consistent with the presence of progenitors of sensorineural and inner ear hair cells, Brn3.c (24.2 ± 0.05%), MyoVIIa (10.8 ± 0.05%) and Bill tubulin (28.8 ± 0.37%) were also detected. At day 38, an increase of inner ear hair cell markers (33.2 ± 0.04% MyoVIIa, 33.9 ± 0.3% Brn3.c and 14.3% Math1), a decreased of otic progenitor gene markers (46.2% Pax2 and 15.9% Pax8) and absence of neural markers were seen (Figure 3). Another otic progenitor gene markers (p27kip1 and Eya1) were upregulated after the first step of differentiation at mRNA level (Figure 4).

Even though these results are preliminary and a protocol improvement is necessary, this work demonstrates the ability of hASCs to differentiate into otic lineage, opening a door to a future therapeutic option for sensorineural hearing loss.

References