Improved protocol and characterization of neuronal differentiation of skin derived precursor cells

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To study the cellular mechanisms underlying hereditary neurodegenerative disorders of individual patients we tried to improve the culture of skin derived precursor cells and the protocol for neuronal differentiation. Skin derived precursor cells were isolated from small skin biopsies of control persons and patients with proven SPG4 and maintained in a 3:1 mixture of DMEM and F12 medium. Skin derived precursor cells growed as non adherent cell clusters that proliferated and self renewed as floating spheres in the presence of epidermal growth factor, fibroblast growth factor 2 and additional components including B-27 and N-2 supplement and chicken embryo extract. The spheres were passaged every 7 days and cultured for more than 8-10 weeks in total. Then differentiation into cells with a neuronal morphology was induced by removal of fibroblast growth factor 2 and epidermal growth factor and addition of the neurotrophic factors BDNF, beta-NGF, neurotrophin-3 and retinoic acid and laminine. 3 days after initiation of differentiation conditions more than 50% of cells co-expressed neuronal markers including nestin, beta III tubuline and MAP2c. 2 weeks later cells also co-expressed neurofilament-L, NeuN and synaptophysin. Electrophysiological analysis confirmed the expression of Na^+ and K^+ channels and the appearance of action potentials. Functional assays in comparison between control and patient derived neuronal like cells are currently under way to address basic cell features in neurodegenerative disorders.