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Live imaging of cell motility and mitochondrial dynamics provides a new energy-consuming mechanism required for NGF-induced differentiation

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Keywords: NGF, differentiation, NAD^+, energy, metabolism, mitochondrial dynamics, mitophagy, motility

Abstract:
Background: Neuronal differentiation is a complex cellular process requiring extensive changes in biochemical and cytoskeletal morphology. Nerve Growth Factor (NGF) is a neurotrophic factor crucial for differentiation and maintenance of specific neuronal populations through a complex TrkA and MAPK/PI3K-Akt/PLCy signalling.
We published that NGF-induced differentiation involves active mitochondrial remodelling, linked to their distribution along the new neurites, as well to the control of energy and redox homeostasis in response to the higher energy demand. Thus, implying a functional link between boosted energy metabolism and molecular events modulating mitophagy, mitochondrial biogenesis and dynamics. NAD^+ is also known to boost energy metabolism and mitophagy thus playing a critical role in several biological processes, such as in neuroprotection against several toxic stimuli. NAD^+ seems also to be involved in control of mitochondrial dynamics through the activation of NAD^+-dependent enzymes.
Aim and methods: Based on this knowledge, we aimed to compare the effect of NGF and NAD^+ supplementation on neuronal differentiation and mitochondrial dynamics in PC12 cell. To this end, we used bright-field and fluorescence live imaging of cells and of mitochondrial morphology and mitophagy.
Results: Interestingly, we found that NAD^+ alone is able to induce PC12 differentiation to an extent comparable to NGF, and potentiates NGF-induced neurites sprouting by increasing mitochondrial number, volume and mitophagy, as determined by increased colocalization of mitochondria with lysosomes. Nonetheless, NAD^+ effect in neuronal differentiation is not persistent and does not produce a post-mitotic state.
Moreover, we show that NGF-induced differentiation is associated with an increased cellular motility, as compared to both CTR and NAD^+-dependent differentiation. The velocity and distance covered by cells peaked at 24h. These data indicate that, in addition to neurite processes extension, phyllopodia protrusion is an important component of differentiation by increasing motility.