Accelerated and efficient neuronal differentiation of *Sox1*GFP mouse embryonic stem cells *in vitro* using nicotinamide

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A major challenge for advancement of clinical neuronal replacement therapies is the production of high yields of purified neuronal populations of appropriate phenotype with control over proliferation to prevent tumorigenesis. We previously reported that treatment of mouse embryonic stem cell (mESC; 46C *Sox1*GFP reporter cell line) monolayer cultures with the vitamin B_3 metabolite nicotinamide at the early onset of development not only increased the efficiency of neuronal generation by two-fold but also enriched the ratio of purified neurons to non-neuronal cells in culture. This study aimed to investigate if nicotinamide enhances neural induction in this model and whether it also promotes the production/differentiation of specific neuronal subtypes. To address these aims, monolayer mESC cultures were treated with nicotinamide (10 mM) for different durations and immunocytochemistry/fluorescence microscopy was performed to assess the expression of stem cell, neural progenitor (NP) and neuronal subtype markers. Morphometric analyses were also performed to assess the extent of neuronal differentiation. Nicotinamide treatment significantly decreased $Oct4^+$ pluripotent cells and concomitantly increased GFP^+cells at day 4, suggesting enhanced neural lineage commitment. By day 14, nicotinamide treatment (from day 0-7) reduced both $Oct4^+$ and GFP expression concomitant with enhanced expression of neuron-specific β-tubulin. Nicotinamide selectively enhanced the production of catecholaminergic, serotonergic and GABAergic neurons and, moreover, enhanced various aspects of neuronal morphology and maturation. Collectively, these data demonstrate a direct effect of nicotinamide at the initial stages of embryonic stem cell differentiation which could be critical for rapidly and efficiently promoting neural commitment to highly enriched neuronal lineages. The strong clinical potential of nicotinamide could successfully be applied to future neural cell-based therapies, including Parkinson's and Huntington's disease, both to eradicate proliferating cells and for a more enhanced and specific differentiation.

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