

## Vitamin D binding protein-derived macrophage activating factor stimulates proliferation and signalling in a human neuronal cell line

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Vitamin D (vitD), vitD binding protein-derived macrophage activating factor (DBP-MAF), and vitD receptor (VDR) are essential for adult neurogenesis [1], and this effect could be responsible for the recently reported effects of DBP-MAF on autism spectrum disorders (ASD) [2]. In order to test this hypothesis, we challenged a human neuronal cell line (SH-SY5Y, IZSLER) with DBP-MAF (Immuno Biotech), and we studied cAMP formation (cAMP EIA kit, Abnova), cell proliferation (MTT assay, Sigma Aldrich), apoptosis (human caspase 3 act, Invitrogen) and cell morphology. SH-SY5Y cells represent a validated *in vitro* model of human neurons in neurodegenerative diseases [3]. DBP-MAF induced rapid (15 min) formation of cAMP in a dose-dependent manner (0.4-40 ng/ml) as well as increase in cell proliferation at 24-48 and 72 h. Cell morphology was consistent with neurogenesis and an increase in the number of cells with high synthetic activity was observed. No apoptosis following DBP-MAF treatment was observed. Our results open the way to exploit these newly described effects to treat neurodegenerative disorders from Parkinson's and Alzheimer's diseases to Myalgic Encephalomyelitis and ASD.

### References

- [1] Zhu et al. (2012) Abnormal neurogenesis in the dentate gyrus of adult mice lacking 1,25-dihydroxy vitamin D3 (1,25-(OH)<sub>2</sub> D<sub>3</sub>). *Hippocampus* 22: 421-33.
- [2] Bradstreet et al. (2012) Initial Observations of elevated Alpha-n-Acetylgalactosaminidase Activity Associated with Autism and Observed Reductions from GC Protein-Macrophage Activating Factor Injections. *Autism Insights* 4: 31-38.
- [3] Xie et al. (2010) SH-SY5Y human neuroblastoma cell line: in vitro cell model of dopaminergic neurons in Parkinson's disease. *Chin Med J* 123: 1086-92.

### Key words

Vitamin D, macrophages, vitamin D receptor, vitamin D binding protein-derived macrophage activating factor, human neurons, autism spectrum disorders.