LRRK2-induced Neuronal Toxicity

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Introduction:

Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene have been identified as a unambiguous cause of rare autosomal dominant forms of PD. However, the pathogenic role and associated biochemical pathways responsible for LRRK2-linked disease still remains unclear. To investigate the neurotoxicity induced by LRRK2, we transfected LRRK2 WT and G2019S mutant into mouse cortical neuronal culture by METAFECTENE EASY reagents.

Materials and methods:

Transfect reagent: Metafectene EASY and Metafectene SI (Biontex, Munich, Germany);
Plasmids: pcDNA3.1/MYC-HIS-LRRK2 –WT/G2019S, pcDNA3.1-eGFP;
Cells: mouse cortical neuronal culture

Experimental procedures / transfection protocol:

1). The mouse cortical neuronal culture grew on 24-well plates for one week.
2). Dilute 10XEASY buffer to 1X
3). Add 5ul METAFECTENE EASY in 100ul 1XEASY buffer and mix gently
4). Add 2.2ug DNA (2ug LRRK2, 0.2ug eGFP) into above mix, incubate for 15 mins at RT
5). Change the culture media to OPTI-MEM media (400ul per 24-well)
6). Add the mix from step 4 to the culture
7). Chang the media to neuronal growth media next morning
8). The viability of eGFP positive neurons using TUNEL staining or nuclear condensation/fragmentation by fluorescence microscopy were quantified to assess the neuronal toxicity.

Results and discussion:

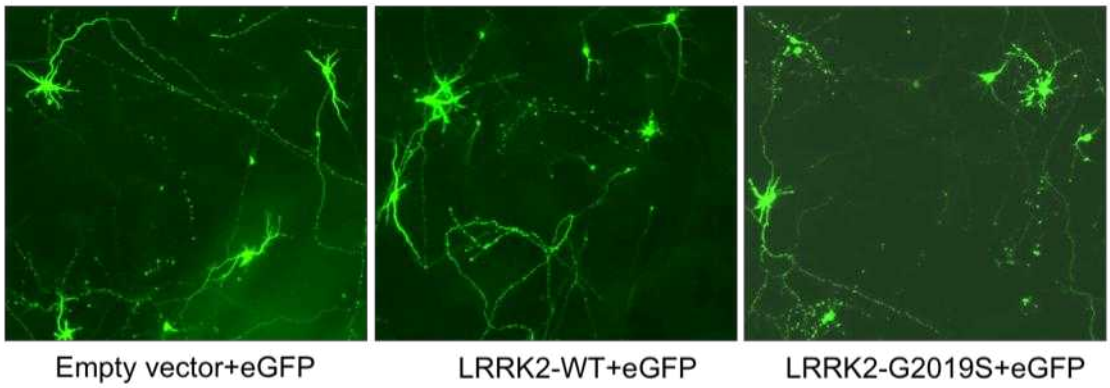
Transfection of 7-days old cortical neurons using Metafectene EASY resulted in a transfection efficiency around 1-2%, which is similar to the efficiency of Lipofectamine 2000 (Invitrogen, data not shown)

Conclusion / summary:

Transfection efficiency was as high as compared to results achieved using transfection reagents of other manufacturers. We didn’t see obvious decreased cytotoxicity compared to other transfection reagents.

Appendix: Tables and/or figures:

Cell code	Primary	Class	Species	Organ	Reagent	Growth Properties	Genetic Material	Efficiency	Toxicity
Neurons	yes	Mam-malia	mouse	cortex	METAFECTENE EASY	adherent	Plasmid	1-2%	low



Empty vector+eGFP LRRK2-WT+eGFP LRRK2-G2019S+eGFP

Figure 1. LRRK2 induced neuronal toxicity. 7-days old mouse cortical neurons were co-transfected with LRRK2 and eGFP (10:1 ratio) by Metafectene Easy reagent. Viability was analyzed at 48 hrs post-transfection (DIV 9) with non-viable neurons exhibiting obvious neurite process and/or nuclear fragmentation. Representative images are showing eGFP postive neurons.