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Embryonic stem cell-derived neurons as a novel cellular model system to study neurodegenerative and neuroregenerative processes *in vitro*

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Introduction:

The isolation and culture of embryonic stem (ES) cells has opened the possibility of generating unlimited numbers of any cell type. This is of particular importance in neurobiology as homogeneous cell populations are not available in sufficient quantities. In addition, ES cells can be genetically manipulated or isolated carrying relevant mutations. Thus, wild-type and mutant neurons may be compared and mechanisms causing the loss of specific cell types in neurodegenerative diseases identified.

Materials and Methods:

We have devised a neuronal differentiation procedure of mouse ES cells *in vitro*. Our procedure is novel in that we can differentiate ES cells into a defined neuronal lineage. More precisely, we obtain an essentially pure population of neural progenitors, defined as radial glial cells. These progenitors differentiate subsequently into a uniform population of neurons with characteristics of glutamatergic pyramidal neurons, thus

recapitulating a recently described *in vivo* lineage (Bibel et al., Nat Prot 2:1034, 2007; Bibel et al., Nat Neurosci 7:1003, 2004).

Results:

We use this system to analyze signaling pathways in neurodegeneration. In contrast to neuronal cell lines we can address synaptic function and neurite de- and regeneration. For example, using ES cells that we isolated from *app^{-/-} aplp2^{-/-}* mice we identified a novel function of APP in glutamate uptake/release that we could confirm *in vivo* studying EPSP curves in organotypic slice cultures of wild type versus knockout mice. In other examples we introduce disease-relevant mutations in ES cells (huntingtin, synuclein, tau, A β) and compare wild-type and mutant differentiated neurons to analyze mechanisms of neuronal cell death applying gene and protein profiling technologies, including epigenetic as well as miRNA profiling.

Discussion and Conclusions:

Our studies show the highly predictive value of the differentiation system for the *in vivo* situation and the unique possibility to identify new targets and gene functions. In this regard, we also have successfully established bi-allelic targeting of genes using Blmtet/tet Es cells. In addition, lentiviral infection of candidate genes in the neural progenitors allows us to study signaling networks in a given mutant ES cell background. Importantly, we have optimized conditions for long-term cultures for neurons to mature with high synaptic activity, form spines and express all isoforms of tau as neurons do in the adult brain, thus we can analyze degeneration processes of mature neurons.

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