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Studies on improving the embryonic stem cell test for an assessment of neuroembryotoxic effects

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Introduction:

The Embryonic Stem Cell Test (EST) represents a validated in vitro system for the classification of chemical compounds according to their embryotoxic potential. It is based on the evaluation of beating cardiomyocytes in embryoid body (EB) outgrowths in comparison to cytotoxic effects on D3 murine embryonic stem cells and 3T3 fibroblasts. To improve the predictive potential of the EST and to avoid false-negative classifications, further cell type-specific endpoints of differentiation have to be established. Recent attempts employ RT-PCR methods or FACS analyses based on antibody staining of neuronal markers, but these techniques are cost-intensive and need a lot of hands-on time. Both aspects are disadvantageous in future routine applications. Hence we tested the feasibility of detecting neuronal differentiation by using a simple fluorescent staining of Nissl substance, which is specific for neuronal cells and can therefore be used to distinguish them from non-neuronal cells.

Materials and Methods:

EBs were produced from D3 suspension cultures within 4 days. Neuronal differentiation was induced by incubating EBs for 4 days with retinoic acid (RA). Then EBs were transferred to TC-dishes and further cultured for at least 9 days. To detect neuronal cells in EB outgrowths, NeuroTrace? fluorescent Nissl stain was used together with DAPI. To confirm the specificity of staining, a coculture of embryonic neuronal cells from chicken retina with a human Nissl-negative keratinocyte cell line was used. Additionally, RT-PCR was performed to monitor the expression of other neuronal markers such as tyrosine hydroxylase.

Results:

RA treatment of EBs led to a pronounced spot-like staining of the Nissl substance similar to that usually found in fully differentiated neuronal cells. A few neuronal cells spontaneously developed also in RA-untreated EBs and could clearly be detected by Nissl staining. In monolayer cocultures, a weak homogeneous background staining of nuclei and cytosol could also be observed in HaCaT keratinocytes, but chicken embryo retina cells displayed a significantly stronger but still homogeneous staining of cells.
Discussion and Conclusion:

A reliable identification of neuronal cells by fluorescent Nissl staining is possible in combination with morphological criteria. For future applications in routine testing, image analysis employing threshold-based algorithms or FACS analysis should be used to standardize the evaluation procedure.