Forschungsprojekt mit humanen embryonalen Stammzellen /
Projet de recherche utilisant des cellules souches embryonnaires humaines
R-FP-S-2-0002-0002

Referenznummer / numéro de référence	R-FP-S-2-0002-0002
Projekttitel / titre du projet	*Neuronale Differenzierung von humanen embryonalen Stammzellen*
Projektstand / état du projet	beendet
Projektleiter_in / direction du projet	Prof. Dr. Yves Alain Barde
Institut, Firma / institut, société	Biozentrum, Universität Basel, Abt. Pharmakologie/Neurobiologie Klingelbergstr. 50-70 CH-4056 Basel
Projektbeginn / début du projet	Oktober 2005
Voraussichtliche Dauer / durée probable	84 Monate
Ziele des Projekts / but du projet	Ziel des Projektes ist die neuronale Differenzierung von humanen embryonalen Stammzellen. Unsere Arbeit mit Mausstammzellen hat gezeigt, dass reine und definierte Populationen von Neuronen entstehen können. ähnliches soll nun mit humanen Zellen versucht werden. Dies ist wichtig, da es bis heute keine geeigneten Modelle von humanen Neuronen gibt und nur Tumorzellen wie z.B. Neuroblastomen zur Verfügung stehen. Aus diesem Grund bleibt es schwierig, z.B. die zelluläre Basis von neurodegenerativen Prozessen zu untersuchen. Nicht alle molekularen Vorgänge verlaufen in Maus und Mensch identisch, wie z.B. die Expression von manchen Genen, die für einige neurodegenerative Krankheiten von grosser Bedeutung sind. Die Charakterisierung von humanen embryonalen Stammzellen ist in diesem Zusammenhang sehr wichtig.

Verwendete hES Zelllinien / Lignées de cellules utilisées	
H1 (WA01)	BAG-hES-IMP-0001
HS181	BAG-hES-IMP-0009
Mel-1	BAG-hES-IMP-0003
Edi-2	BAG-hES-IMP-0032

Projektergebnis / résultat du projet	The goal of the project was to explore whether human embryonic stem (ES) cells can be used to reproducibly generate homogenous populations of neurons, just as we have previously shown with mouse ES cells (see Bibel et al. 2004 Nat Neurosci.7,1003-1009). There were a number of reasons to tackle this project which, if successful, would represent a significant step forward towards screening and testing drugs

and their mode of action on human neurons. Indeed, there is currently no alternative to this approach which is now even more attractive and important given the possibility to reprogram accessible human cells carrying mutations of interest. Screening for drugs correcting gene defects in human neurons is now a real option, but for this objective to be reached, reasonably pure populations of human neurons will need lo be reproducibly generated. We appreciated from the start the difficulties of the project having worked for several years with mouse ES cells. Our goal could be reached with mouse ES cells in large part because we of course benefited from more than 20 years of research and solid results obtained by others defining ever better the conditions to successfully grow mouse ES cells. This is still not the case with human ES cells. lndeed the very existence of human ES cells, i.e. of truly pluripotent human cells is still debated. lnstead, a consensus begins to appear that such cells may correspond to "primed" cells as opposed to genuine pluripotent cells. lnstead of using fibroblast growth factor as an agent stimulating cell division and survival because this factor also commits ES cells to differentiate, we focused much of our attention on (human) leukemia inhibitory factor (LIF) to grow human cells and keep them undifferentiated. lnitially, the project seemed to work and we did obtain neurons with up to about 70% purity. The procedure we developed with human cells closely followed the procedure we developed for mouse cells, including in particular deprivation of feeder cells, formation of aggregates and treatment with retinoic acid. However, human cells turned out to grow and differentiate very slowly indeed - neurons could only be generated well over after a month of aggregating the cells. More worryingly, the results were also quite variable. We used a totalof 4 human ES cell lines (BAG-hES-lMP-0001, 0003, 0009 and 0032) and the problems we encountered were similar with all lines. After initial successes, problems begun to appear which could not be finally satisfactory resolved.

ln conclusion, much more work is still needed to reach satisfactory, i.e. stable and reproducible results in terms of speed and quality of neuronal differentiation with human ES cells. However, the goal remains as important as it was at the start of the project and the work will be continued in the U.K. at Cardiff University.