

set



**Stiftung zur Förderung
der Erforschung von
Ersatz- und
Ergänzungsmethoden
zur Einschränkung von
Tierversuchen**

Stiftung zur Förderung der Erforschung von Ersatz- und Ergänzungsmethoden zur Einschränkung von Tierversuchen

Mainzer Landstraße 55, 60329 Frankfurt am Main
www.stiftung-set.de

Project

Establishment of in vitro models for neuroprotection with molecular connotations to human neurodegenerative diseases

Prof. Dr. André Schrattenholz, ProteoSys AG, Mainz

March 2006 - February 2007

The logo for 'set' consists of the lowercase letters 'set' in a bold, sans-serif font. Below the letters are two vertical columns of horizontal bars. The left column has 10 blue bars, and the right column has 10 pink bars. The bars in each column are of varying lengths, creating a stepped effect.

Stiftung zur Förderung
der Erforschung von
Ersatz- und
Ergänzungsmethoden
zur Einschränkung von
Tierversuchen

Establishment of in vitro models for neuroprotection with molecular connotations to human neurodegenerative diseases

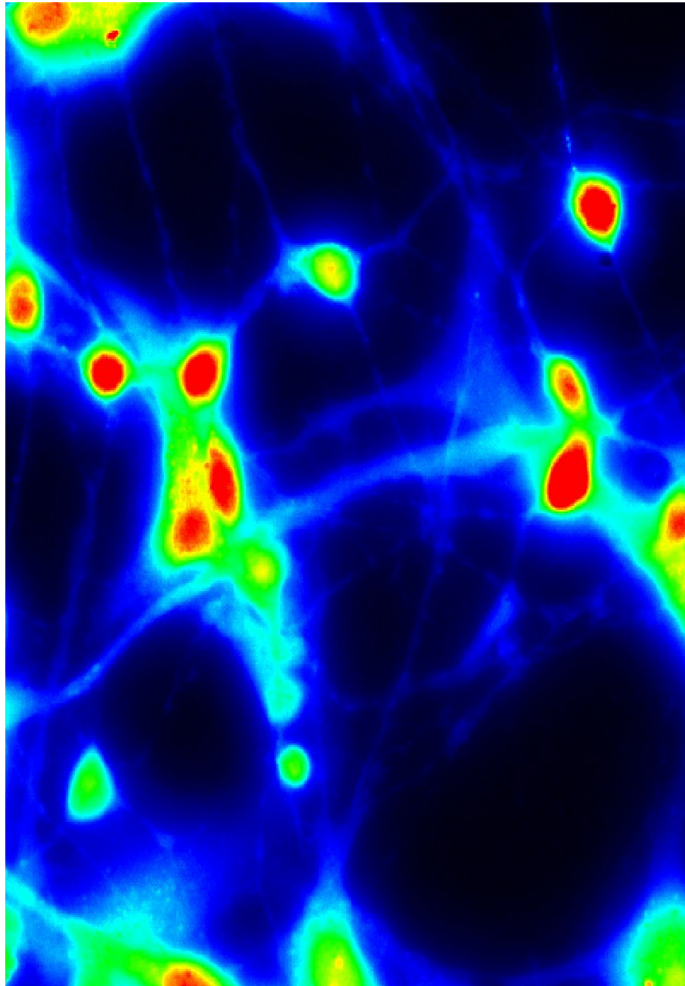
General background: Neural derivatives of human and murine embryonic stem cells (hESC and mESC) as potential replacement of animal models for stroke, Alzheimer, multiple sclerosis and ALS.

The aim of this project is the characterization of endpoints from murine and human stem cell models for the quantification of neuroprotective effects of therapeutic compounds. Previous work with these models has so far aimed at a precise molecular and functional description of neurotoxic conditions (protein biomarker signatures for neuronal stress). Based on established protocols for neural differentiation in both models, it is planned to further examine various stages of neuronal maturation with regard to neurotransmitters, known marker proteins and pharmacologic properties, and subsequently to define appropriately correlated endpoints for both species. Functional endpoints of this type would then be differently screened for surrogate markers by advanced Proteomics technologies. Previous work of the team conducting this study has shown that the approach adequately represents molecular events and processes underlying certain human diseases of the central nervous system and can be successfully used for the quantification of compound effects [1, 2, 3]. Briefly, neuronal stress is induced by three different conditions, representing human pathomechanisms, namely ischemia, excitotoxicity and toxicity induced by amyloidogenic peptides. Functional and molecular events observed so far in the murine ESC and other models [4, 5, 6] encourage us to extend these studies to human ESC models, with the ultimate aim to develop a human in vitro system representing crucial aspects of human neurodegenerative diseases, which thus could at least partially replace corresponding animal models like e.g. MCAO-models for stroke, transgenic mouse models for Alzheimer's disease and ALS, or the extremely irksome models for multiple sclerosis (MOG-EAE) [7]. First results of the project have been accepted for publication [8].

set



Stiftung zur Förderung
der Erforschung von
Ersatz- und
Ergänzungsmethoden
zur Einschränkung von
Tierversuchen



Fluorescence measurements on neurons derived from human stem cells: The uptake of calcium ions (shown in yellow and red) into the cells is induced by neuronal stress. This calcium influx mechanistically relates to stroke, therefore this method also allows for substance screening.

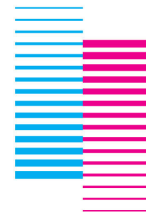
Literature

[1] Sommer S, Hunzinger C, Schillo S, Klemm M, Biefang-Arndt K, Schwall G, Pütter S, Hoelzer K, Schroer K, Stegmann W, Schrattenholz A (2004) Molecular analysis of homocysteic acid-induced neuronal stress. *Journal of Proteome Research* 3(3), 572-581

[2] Schrattenholz A, Wozny W, Klemm M, Schroer K, Stegmann W, Cahill MA (2005) Differential and Quantitative Molecular Analysis of Ischemia: Complexity reduction by isotopic labeling of proteins using a neural embryonic stem cell model; *J. Neurological Sciences*, 229-230 (1), 261-267

[3] Schrattenholz A and Šoškić V (2006) NMDA receptors are not alone: Dynamic regulation of NMDA receptor structure and function by neuregulins and transient cholesterol-rich membrane domains leads to disease-specific nuances of glutamate-signalling *Current Topics in Medicinal Chemistry*, 6(7), 663-686

set



**Stiftung zur Förderung
der Erforschung von
Ersatz- und
Ergänzungsmethoden
zur Einschränkung von
Tierversuchen**

- [4] Falsig J, Pörzgen P, Lund S, Schrattenholz A, Leist M (2006) The inflammatory transcriptome of reactive murine astrocytes and implications for their immune functions. *J. Neurochem.* 96(3), 893-907
- [5] Lund S, Christensen KV, Hedtjörn M, Mortensen AL, Hagberg H, Falsig J, Hasseldam H, Schrattenholz A, Pörzgen P and Leist M (2006) The dynamics of the LPS triggered inflammatory response of murine microglia under different culture and *in vivo* conditions. *J Neuroimmunol.* 2006 Sep 19; [Epub ahead of print]
- [6] Schillo S, Pejović V, Hunzinger C, Hansen T, Poznanović S, Kriegsmann J, Schmidt WJ and Schrattenholz A (2005) Integrative Proteomics: functional and molecular characterization of a particular glutamate-related neuregulin isoform. *Journal of Proteome Research* 4 (3), 900-908
- [7] Schrattenholz A, Klemm M (2006) How Human Embryonic Stem Cell Research Can Impact In Vitro Drug Screening Technologies of the Future; in: *Drug testing in vitro: Achievements and trends in cell culture techniques* (Marx U, Sandig V, eds.) Wiley-VCH, Weinheim, 205-22
- [8] Schrattenholz A, Klemm M (2007) Neuronal Cell Culture from Human Embryonic Stem Cells as *in vitro* Model for Neuroprotection. *ALTEX* in print.

December 2006