In vitro models for Parkinson’s research

The death of dopamine-producing neurons leads to Parkinson’s disease, but the cause of this selective cell degeneration is unknown. Dr. Stefan Schildknecht from the University of Konstanz is therefore developing human cell culture models to investigate the early molecular processes in the development of Parkinson’s disease. The models can also be used to develop new approaches for the treatment of Parkinson’s.

Parkinson’s patients with symptoms such as slowness of movement, muscular rigidity and tremor are already in an advanced stage of the disease. However, the selective degeneration of dopamine-producing neurons in a specific region of the midbrain (i.e. substantia nigra), which is the main pathological hallmark of the disease, usually starts many decades before the first symptoms appear. Only when the lack of dopamine becomes so significant that the substantia nigra is no longer able to fulfil its role of conveying signals to the periphery, e.g. signals that enable hand movement, will the primary movement disorders of Parkinson’s disease become obvious. The central question in Parkinson’s research relates to the causes of the selective degeneration of the dopaminergic neurons in the substantia nigra and it is a question that remains largely unanswered. In order to investigate the causes of the disease, Dr. Stefan Schildknecht, a biologist in Prof. Dr. Marcel Leist’s laboratory at the University of Konstanz, is developing in vitro models to investigate the molecular processes that occur in Parkinson’s patients.

“First of all, we need a model that can represent the situation in the human brain as accurately as possible,” Schildknecht explains. The model system that Schildknecht was involved in developing is based on so-called LUHMES (LUnd Human MESencephalic) cells, a cell line derived from foetal neuronal cells that have been immortalised and can be specifically differentiated into dopaminergic neurons. “It only takes us around a week to produce a homogenous culture of human dopaminergic neurons. We then use this culture to explore the molecular and biochemical mechanisms of Parkinson’s disease,” explains Schildknecht.
Dr. Stefan Schildknecht is developing in vitro models at the University of Konstanz in order to investigate the molecular and biochemical foundations of Parkinson’s disease. © Stefan Schildknecht
LUHMES cells shown as undifferentiated neuronal precursor cells (left) and as a network of dopaminergic neurons after differentiation. © Stefan Schildknecht

Alpha synuclein and oxidative stress – a dangerous mixture

Schildknecht uses the LUHMES cell model for investigating the protein alpha synuclein (ASYN) and its role in the development of Parkinson’s disease. ASYN is an essential component of aggregates known as Lewy bodies. They are a characteristic feature of the disease found in the brains of Parkinson’s patients. Although ASYN can be found throughout the brain, it plays a particular role in the degeneration of dopaminergic neurons. So the question to be answered is what causes the death of these particular nerve cells? “It is known from literature that oxidative modifications affect the aggregation and binding of ASYN to membranes,” explains Schildknecht. Oxidative modifications are caused, amongst other things, by free radicals such as those arising from dopamine as the result of so-called auto-oxidation processes.

Nitration, i.e. the attachment of an NO₂ group to one or several of the four tyrosines in ASYN, is one such oxidative modification. “In the brain, the nitration of ASYN can usually be observed in association with inflammatory responses regularly found in the affected brain regions in Parkinson’s patients,” says Schildknecht, describing the relevance of the modification. In order to investigate the effect of nitration on the aggregation of ASYN in vitro, Schildknecht produces recombinant ASYN and treats the protein with peroxynitrite, a highly reactive and toxic radical. “However, the disadvantage here is that the four tyrosines are differentially nitrated and peroxynitrite can produce a number of other oxidative modifications in ASYN, with the result that the specific effect of nitration is not clearly visible,” explains Schildknecht. He is therefore working on the production of ASYN with exactly a single defined nitration and no further oxidative modifications. “We are using an unnatural amino acid, 3-nitrotyrosine, for the production of recombinant ASYN in bacteria. This particular amino acid can then be inserted into a particular site in the modified bacterial DNA,” Schildknecht explains.

Nitration as potential starting point for new therapies

The specifically modified ASYN variants will subsequently be used to assess the relevance of nitration in the development of Parkinson’s disease in the cell model. “There is evidence that nitrated ASYN promotes the aggregation of unmodified ASYN molecules by serving as catalyst for the process,” says Schildknecht. In order to substantiate this, LUHMES cells that express endogenous ASYN will be treated with recombinant, nitrated ASYN. If the scientists are able to
confirm the catalytic effect of ASYN, the selective pharmacological inhibition of nitration in the brain would be able to slow the progression of ASYN pathology.

Schildknecht has therefore investigated minocycline as a potential drug with the aforementioned effect. This antibiotic has already been used for the treatment of patients with neurodegenerative diseases characterised by the presence of misfolded proteins and has shown a neuroprotective effect. “With recombinant ASYN, we were able to show that minocycline is a very selective and powerful inhibitor of tyrosine nitration. It interacts efficiently with peroxynitrite,” explains Schildknecht. In the search for new drugs for the treatment of Parkinson’s disease, the inhibition of nitration represents a promising approach.

Further information:

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Neurodegenerative diseases