Neuronal Cells as an Ideal Model for Neurodegenerative Diseases

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ABSTRACT

Neurodegenerative diseases are pathological conditions that have an insidious onset and chronic progression. Different models have been established to study these diseases in order to understand their underlying mechanisms and to investigate new therapeutic strategies. Although various in vivo models are currently in use, in vitro models might provide important insights about the pathogenesis of these disorders and represent an interesting approach for the screening of potential pharmacological agents.

In vitro models of these pathological conditions offer advantages over in vivo models in several aspects. First, it is possible to study the role of isolated cells of one particular type in an environment that simulates the disease and to investigate mechanisms of a possible deleterious or protective role of specific molecules and compounds. Second, screening for potential actions of drugs is also facilitated. Primary midbrain dopaminergic neurons are suitable to study dopaminergic cell survival and neurite retraction as well as regeneration. Usually, embryonic midbrain neurons from embryonic day 14 to 18 (E14-18) are ideal to culture initiation. A high yield of dopaminergic neurons can be obtained, which can be exposed to various neurodegenerative stimuli. In vitro models of neurodegenerative processes can provide important clues about mechanisms of the diseases and potential pharmacological targets.

Keywords: *Neurodegenerative disease, Neuronal cells, Dopaminergic, Pharmaceutical targets*.

Introduction

The nervous system is a complex, sophisticated system that regulates and coordinates the body's basic functions and activities. Our whole body is controlled by nervous system. Starting from the digestion up to endocrine function, everything is depending upon the complex neurophysiology. It is made up of two major divisions, including the central nervous system (consisting of the brain and spinal cord) and the peripheral nervous system (consisting of all other neural elements) [1].One of the major challenges in neuroscience is to translate basic findings in neurobiology to real clinical solutions.

Dissociated primary neuron cultures have been a popular research tool for decades [2] because they allow easy access to individual neurons for electrophysiological recording and stimulation, pharmacological manipulations, and high-resolution microscopic analysis.

It would be informative to follow individual cultured neurons for months, electro physiologically and morphologically, to help understand how neural activity and morphology interact with each other. While neuronal cell lines have been very useful in the study of neuronal cell cultures and continue to be used today, the use of primary cultures is desirable because they are not tumor-derived and hence are more likely to recapitulate the properties of neuronal cells *in vivo*. However, unlike cell lines that provide unlimited supplies of homogeneous cells, the preparation and culture of primary cells is much more challenging and this is especially true for neuronal cells [3],[4].

Primary cell cultures are not immortal and hence the number of cells available for experiments is much more limited. Furthermore, it is necessary to separate the cell type of interest from other cell types and to determine the purity of the resulting cultures, e.g., by immunocytochemistry with cell lineage-specific markers. In the case of primary neuronal cell cultures, it is necessary to separate them, as much as possible, from astrocytes and oligodendrocytes. Isolation and propagation of primary human and rodent embryonic neural progenitor cells and cortical neurons was describe by , Alexandra Nothnagel (2009) the preparation and culture of primary neuronal cell cultures and also include culturing of neural and oligodendrocyte progenitor cells ,(figure1)[5] . various markers were developed for classic lineage-specific markers for CNS cells include βIII-tubulin for neuronal cells, GFAP for astrocytic cells, O_4 for oligodendrocytic cells and OX-42/CD-11b for microglial cells. In the case of

cultures of stem or progenitor cells, lineage-specific markers also exist for the degree of differentiation, e.g., Nestin and SOX2 for neuroprogenitor cells, NG2 for oligodendrocyte precursor cells. Primary cell cultures

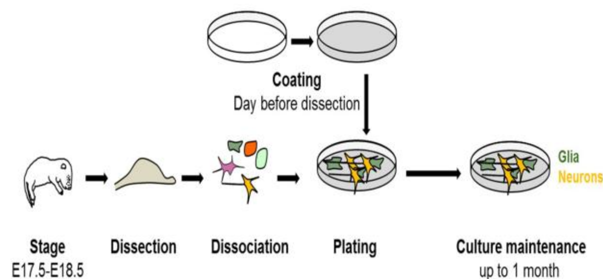

Figure 1: Schematic representation for primary neuronal cell culture. (Source, Alexandra Nothnagel e, 2009)

Continue to be important in neurotoxicology as they are considered to closely mimic the *in vivo* state and generate more physiologically relevant data [6]. However, where appropriate continuous cell lines are available they offer major advantages including reproducibility, ease of propagation, the availability of quality controlled cell banks and the ability to routinely use cells of human origin. Numerous types of immortalized cells now provide key tools for *in vitro* neurotoxicology studies [7],[8]. Figure 2 represents the generation of neural progenitors and neurons from non neuronal cells .

The induction of proliferate neuroblasts from astrocytes was achieved by lentiviral expression of SOX2 in the adult mouse striatum and spinal cord [9],[10]. These neuroblasts differentiated to a mature neuronal fate on treatment with VPA. The direct trans differentiation of NG2 glia and astrocytes to neurons by NEUROD1 in the mouse cortex, as well as, NG2 glia converted to neurons by SOX2 _ ASCL1 demonstrate a dominant role for transcription factors in cell fate determination [11], [12].The induction of neurons in the mouse striatum from endogenous nonneuronal cells and transplanted human astrocytes further demonstrates the plasticity of cells within the neural microenvironment [13] [14].

In the immature and mature brain, neocortical neurons degenerate after hypoxia-ischemia, trauma, and seizures, and subsets of neocortical neurons degenerate in Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, and Huntington's disease. Despite many advances in the understanding of mechanisms that cause abnormalities in neuronal development and loss of neuronal viability, effective restorative and therapeutic treatments for most developmental and for all of the neurodegenerative disorders of cortical neurons do not exist

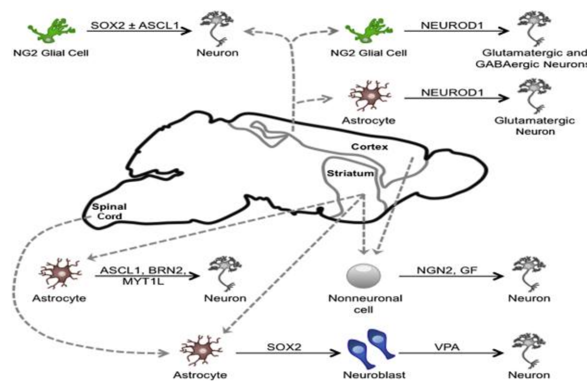

Figure 2 A selections of *in vivo* reprogramming methods demonstrate the generation of neural progenitors and neurons from non neuronal cells. GF, growth factor; VPA, valproic acid (Source, Smith and Zhang 2015).

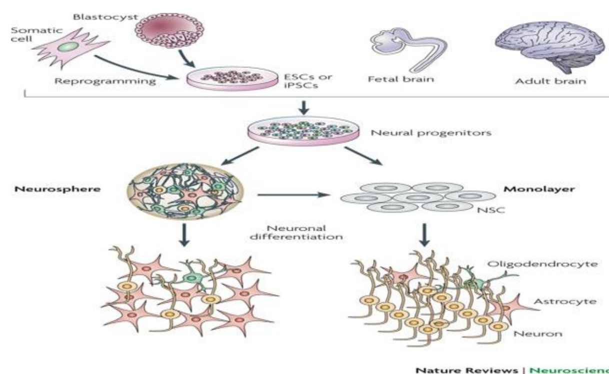

Figure 3 Sources and in vitro growth protocols for neural stem cell generation and expansion.(source , Luciano Conti & Elena Cattaneo 2010)

Animal and cell culture model systems are used to further understand mechanisms of neuronal dysfunction and degeneration. These *in vivo* or *in vitro* systems have particular advantages and limitations Figure 3 represent the neural stem cell generation and expansion *in vitro* conditions. Furthermore, cell death in populations of neurons in the CNS is not always uniform. For example, an apoptosis-necrosis cell death continuum has been identified in animal models of excitotoxicity, cerebral ischemia, and axotomy. The neuronal death continuum is influenced by the subtype of glutamate receptor that is activated and brain maturity [15], but the mechanisms are difficult to pinpoint *in vivo*. With *in vitro* systems, although devoid of an intact tissue environment, homogeneous populations of cells that are undergoing synchronous and uniform changes

can be studied, providing the appropriate system is used.

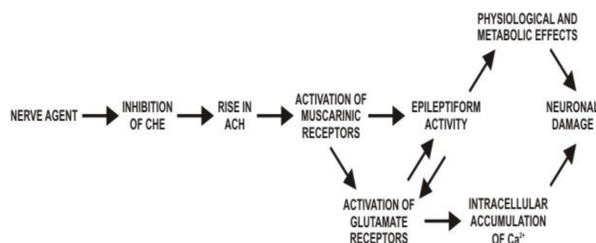

Figure 4: Mechanisms of nerve agent-induced neuronal damage. The proposed sequence of biochemical, neurochemical and electrophysiological events that occur after exposure to nerve agent that initiate and maintain seizure activity and subsequently lead to neuronal damage. CHE, cholinesterase; ACH, acetylcholine, (source, McDonough and Shih, 1997) .

Neuronal Degenerative Disease:
Parkinson's disease

PD is a slowly progressive neurodegenerative disease clinically characterized by motor impairment, namely bradykinesia, rigidity, resting tremor, and postural instability[16]. Synaptic and axonal degeneration within the striatum followed by loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) leads to reduced levels of dopamine in the nigrostriatal circuitry[17]. Besides dopaminergic cell loss, intracellular formation of Lewy bodies and Lewy neurites, consisting predominantly of aggregated alpha-synuclein (aSyn), has been suggested to be crucial in the pathogenesis of this disease [18].

Moreover, genetic factors contribute to the pathogenesis of PD [19]. To date, more than 16 loci and 11 associated genes have been identified. Among these, mutations in the gene for aSyn were the first ones to be mapped [20]. On the cellular level, research in PD focuses on protein aggregation, neurotoxicity, increased oxidative stress, excitotoxicity, mitochondrial dysfunction, and defects in the protein degradation machinery (including the ubiquitin- proteasomal system and autophagy pathways)[21].Several cell culture systems have been employed to study these possible disease processes. A homogeneous cell culture system that is easy to handle would be preferable. Cells should be easy to expand in order to generate large numbers of neuronal precursor cells. Next, these cells should be able to be transferred from a proliferate into a post-mitotic state. Finally, these cells should be easily directed towards a post-mitotic state in a synchronized manner with a mature neuronal (dopaminergic) phenotype [22].

Usually, embryonic midbrain neurons from embryonic day 14 (E14) are dissected [23] a high yield of dopaminergic neurons can be obtained, which can be exposed to various neurodegenerative stimuli. Several neurotoxins are employed to study neurodegeneration. In particular, 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenylpyridinium (MPP+) are widely accepted to induce neurotoxicity. Both neurotoxins are thought to induce dopaminergic toxicity by intra- and extra cellular oxidation, hydrogen peroxide formation, and direct inhibition of the mitochondrial respiratory chain [24]. On the one hand, this cell model is very suitable to study methods of neurodegeneration and neurite retraction; on the other hand, possible neurorestorative capacities by pharmacological compounds and the underlying mechanisms can be nicely illustrated in figure 4.

Alzheimer's disease

AD is a slowly progressive neurodegenerative disorder and the most common cause of dementia in the elderly. Neurodegenerative process is characterized by early damage to synapses with retrograde degeneration of axons and eventual atrophy of the dendritic tree In fact, loss of synapses is the best correlate of the cognitive impairment in patients with AD[25],[26].

The brain regions involved early in the course of the disease are the entorhinal cortex and the CA1 region of the hippocampus, followed by limbic structures and, at later stages, all isocortical areas [27]. Neuropath logical changes include abundant extra cellular amyloid plaques and neurofibrillary tangles, comprised of hyperphoshorylated tau [28]. Deciphering mechanisms leading to neuronal dysfunction and cell loss are the main advantages of *in vitro* model systems.

Therefore, different cell lines were generated. Different neuronal cell lines are commonly used for neuronal in vitro culture system, such as PC12, HEK293, and SH-SY5Y cell lines. These cells can be transfected with wild-type amyloid-precursor proteins, tau, or mutant forms of these molecules. In addition to cell lines, primary cortical and hippocampal cultures play a valuable tool in AD research. The addition of amyloid beta to the medium of primary neuronal cells induces apoptosis[29],[30]. It is widely accepted that glial cells also contribute to the pathogenesis of AD. It has been shown that, besides neuronal loss, reactive astrocytes and

activated microglial cells can be associated with amyloid plaques and neurofibrillary tangles[31]-[33]. Although amyloid-beta itself can be toxic to neurons, it also activates microglia, leading to neuronal damage[34].These cell lines were briefly discussed in present review.

HEK293 (human embryonic kidney 293)

A commonly human cell line used in PD research is the HEK293 (human embryonic kidney 293) cell line. These cells can be easily transfected (e.g., via calcium phosphate, liposome based, electroporation). In one of the study, the kinetics of aSyn aggregation was analyzed with respect to aggregation formation. Increased expression of wild-type aSyn was shown to result in the formation of cytoplasmic aggregates[35]. Time-lapse imaging illustrated how cells form and accumulate aggregates of aSyn in HEK293 cells [36]. HEK293 cell line proves to be suitable model system for the effect of aSyn mutations and other PD associated genes. The expression of a mutant A53T form of aSyn caused an increased susceptibility to dopamine [35]. Recently, it was demonstrated that over expression of leucine-rich repeat kinase 2 (LRRK2) does not result in altered gene expression in HEK293 cells [36]. Mutations in LRRK2 are strongly associated with late-onset autosomal dominant PD, and HEK293 may be suitable to go for candidate pharmacological screening for LRRK2 inhibitors [37]. Moreover, mechanisms of possible in vitro transfer of aSyn and its modified species may be studied in this cell line. Despite the common usage of HEK293 cells in PD research, there are some drawbacks, including the fact that these cells lack a neuronal phenotype.

SH-SY5Y cell line

Another cell line that is widely used in the field of PD research is the SH-SY5Y cell line, which is derived from human neuroblastoma cells. These cells are widely used to study mechanisms of neurodegeneration. For example, over expression of wild-type human aSyn was shown to promote inclusion formation in SH-SY5Y cells [38]. Moreover, extra cellular addition of aSyn oligomers caused transmembrane seeding of aSyn aggregation in a dose- and time-dependent manner [39]. However, SH-SY5Y cells are hard to differentiate into a post-mitotic mature dopaminergic state [40]. Several other human cell lines mainly derived from embryonic carcinomas (NT2, hNT) are currently used, and they can be directed towards a post-mitotic neuronal phenotype[41],[42] .

Human H4 neuroglioma

The human H4 neuroglioma cell line has been used to study the oligomerization of intracellular aSyn by fluorescence lifetime imaging (FLIM) for the first time [43]. Moreover, the role of aSyn in the autophagy pathway has been illustrated with the help of human H4 neuroglioma cell line [44]. It documented that dysfunction of the autophagy pathway may lead to exosome-mediated release of aSyn oligomers in order to clear these toxin aSyn species [45]. However, all these cell lines are derived from tumorous cells and only moderately show a distinct neuronal phenotype.

LUHMES cells

LUHMES cells are widely catching the acceptance for neuronal degeneration and regeneration studies. They are derived from 8- week-old human fetal ventral mesencephalic cells. To induce immortalization these mesencephalic cells were transformed with the help of LINXvmyc vector with tetracycline-regulated v-myc expression [46]. This vector also contains a tetracycline transactivator that enhances the expression of v-myc from a minimal promoter from human cytomegalovirus (CMV) fused to the tetracycline operator sequence. Addition of tetracycline inactivates the transactivator and thereby abolishes v-myc expression. Supplementation with GDNF and cAMP induces a dopaminergic phenotype after 5 days of differentiation [47]. Differentiated LUHME cells showed a high degree of dopaminergic phenotype, including release of dopamine and neuronal electric properties [48],[49]. The LUHMES cell line has been widely used to study dopamine-related cell death mechanisms [48],[50].A drawback of this cell line is that classical transfection methods showed very low transfection efficiency. Thus, a lentiviral approach to efficiently transfect these cells are necessary.

Recent reports support the hypothesis that extra cellular aSyn plays an important role in PD-associated neurodegenerative processes [51],[52].There is accumulating evidence for inflammatory processes in the progression of PD derived from.

1) serum and cerebrospinal fluid (CSF) analyses,
2) genetic analyses, and
3) Epidemiological studies (Hirsch *et al.,* 2009).

Microglia and astrocytes in AD and PD

Primary microglial cells from rat or mice are commonly used to study inflammatory processes. For instance, primary microglial cells can be isolated from cerebral cortices of 1-day-old Wistar rat [53]-[57]. It is important to take extreme care to avoid lipopolysaccharide contamination, thus to keep microglia in a resting or ''surveying'' state instead of an activated state. Floating microglia can be harvested from 10- to 14-day-old mixed astroglial and microglial primary cultures. Finally, the purity of the microglial culture should be determined. Several microglial markers can be obtained to perform immunocytochemistry or FACS analysis, e.g., Iba1, CD68 (ED1), CD11b (OX-42), tomato lectin, or isolectin-B4. Primary microglial cultures have been used to study whether and by what means extra cellular aSyn can activate microglial cells. Indeed, consistent and permanent microglial activation and subsequent production of pro-inflammatory cytokines have been shown in primary microglial cells [58]-[62].

The production of inflammatory mediators might contribute to the formation of amyloid-beta plaques [63] Also, primary microglial cells may be used to study inflammatory processes and anti-inflammatory approaches. For example, the role of prostaglandins and underlying cell signaling after activation of lipopolysaccharide led to novel insights[64],[65]. Since AD and PD are age-related disorders and microglia may change their functional properties in the aging brain [66] protocols are in demand for the isolation of microglia from adult rodents.

Besides the primary microglia cell system, one microglial cell line is widely used, i.e., the BV-2 cell line. Microglial cells from C57Bl/6 were immortalized with vmyc. The BV-2 cell was recently characterized, and transcriptome and proteome analysis revealed a high similarity to primary microglial cells [67]. Since BV-2 are easy to culture, they are a valuable tool to study not only inflammatory processes, but also phagocytosis [68]. In addition, astrocytes may contribute to the activation of microglial cells and vice versa [69].

Astrocytes are the most abundant cells in the CNS, and show a wide variety of functions including regulation of blood flow and synaptic function, but may also play an important role in mediating neuroinflammation in neurodegenerative diseases indeed, astrocytes play an important role in initiating and regulating CNS immune response through the release of pro-inflammatory cytokines and chemokine [70]. Recently, it could be shown that aSyn is directly transferred from neurons to primary astrocytes in vitro. Interestingly, aSyn was up taken by astrocytes via endocytosis and showed an increase in TNFa gene expression [71].

Primary astrocytes cultures are relatively easy to prepare. Astrocytes can be obtained from every region of the CNS and at any age, although the optimal time point would be in rodents from 2-3 days postnatal when astrogenesis is at its peak. Several astrocytes isolation protocol exist[72]. However, a caution needs to be taken when dealing with astrocytic cultures because these cells may be ''contaminated'' with a high amount of microglia, oligodendrocytes, neurons, and endothelial cells [73]. Thus, it is important to use specific markers for the cell types. Commonly employed astrocyte markers are GFAP, GLAST, vimentin, glutamine synthetase, glutamate transporter 1, aldehyde dehydrogenase 1 L1, and S100bet [74],[75].

To determine the percentage of microglial cells, immunocytochemical or FACS analysis for common microglial markers should be performed. Several methods can be used to reduce the number of microglial cells. First, frequent medium changes, shaking, and sub culturing all reduce the number of microglial cells. Secondly, laminin enhances astroglial growth and inhibits microglial growth[76].Also, application of cytosine arabinoside (Ara-C) or L-leucine methyl ester may effectively deprive the astrocytes cultures from microglial cells. In addition to primary cell cultures, a few astroglial cell lines exist, such as the human U373 astrocytoma cell Line [77].

Conclusion

The present review had discussed the possibilities of using cells and tissues in the investigation of neurodegenerative disorders. Importantly, these models might offer advantages in various aspects Moreover, they complement the animal to understand and investigate the mechanisms involved in the pathogenesis of neurodegeneration. While culture models are not a replacement for animal or human research, they have long been engrained in the beginnings of basic neuroscience research and yet are archaic in many respects. While much is to be learned using established fetal cultures (and cell lines), there is a tremendous need to increase complexity in a rational, systematic way, without compromising feasibility and reproducibility. The challenges are to choose the most appropriate building blocks to create complex 3D neural cultures, and to define and measure physiological homeostasis and functional fluctuations. These challenges stem from specific needs: current in vitro systems are limited and many processes and mechanisms cannot be isolated *in vivo*, given the complexity.

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