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REVIEWS

EXPERIMENTAL WORKS

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HIPSC-DIFFERENTIATED DOPAMINERGIC NEURONS ARE A USEFUL TOOL FOR STUDYING THEIR NEUROPHYSIOLOGY AND MATURATION

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Dopaminergic (DA) neurons play a crucial role in motor control, motivation, and cognition, with their degeneration in Parkinson's disease leading to severe motor deficits. While rodent models are widely used, species-specific differences necessitate human-relevant models.

Aim. This study investigates the functional maturation of DA neurons derived from human induced pluripotent stem cells (IPS).

Methods. DA differentiation was performed using a mCherry-based TH reporter iPS line. Immunocytochemistry confirmed neuronal identity, while patch-clamp recordings assessed electrophysiological properties, including firing rate, action potential duration, Ih current, and dopamine sensitivity.

Results. TH expression was detected from day 10, reaching 64% by day 30. Electrophysiological maturation followed a distinct timeline, with spontaneous activity emerging around day 20 and stable pacemaking developing by day 40, along with D2 receptor-mediated autoinhibition.

Conclusions. Our findings demonstrate that hIPSC-derived DA neurons attain an adult-like profile by day 40, making them a viable model for studying Parkinson's disease mechanisms and testing potential therapies. Further research will focus on slow pacemaking mechanisms in these neurons.

Keywords: Dopaminergic neurones, human induced pluripotent stem cells, electrophysiology, pacemaking, Parkinson's disease.

Dopaminergic (DA) neurons are essential for modulating motor control, motivation, and various cognitive functions. Predominantly located in the substantia nigra pars compacta and the ventral tegmental area, these neurons (at least part of them) undergo progressive degeneration in Parkinson's disease, resulting in severe motor deficits1. While rodent models are widely used to study DA neurobiology, species-specific differences limit the direct translation of findings to humans2. Consequently, investigating the electrophysiological and functional properties of DA

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neurons derived from human induced pluripotent stem cells (IPS) represents a crucial strategy for advancing our understanding of their physiology and potential therapeutic interventions to prevent their degeneration.

Aim. This work aims to investigate the changes in the functional activity of human DA neurons in the process of its differentiation from hIPSC.

Methods. Differentiation of DA from mCherry-based human TH reporter iPS line was performed according to a published protocol 3 with minor changes. The DA profile of neurons in the process of maturation was confirmed by immunocytochemistry as described earlier 4 with the use of TH (Merck, AB152), βIII tubulin/ TUJ1 (BioLegend, MMS-435P) antibodies. For functional aspects, patch clamp recordings were performed, as described in Borgs et al.4 and Seutin, 20215. SNc DA neurons were identified and assessed based on their electrophysiological properties, including low frequency of firing (0.5–5 Hz), AP duration (width at half-amplitude > 1.35 ms), the presence of a strong hyperpolarization-activated inward current (Ih) and sensitivity to dopamine (100 µM) (hyperpolarization > 5 mV from -60 mV).

Results and Discussion. We found that cells that expressed βIII tubulin/ TUJ1 start expressing TH from day 10 of terminal differentiation (TD), and the percentage progressively increases to 64%

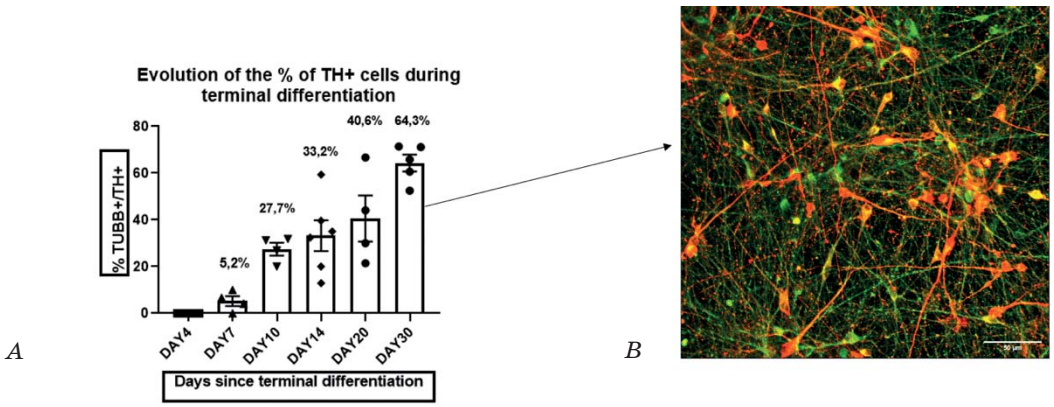

Fig. 1. **(*A*) Percentage of TUBB/TH ratio on day 4, 7, 10, 14, 20, 30 of terminal differentiation of dopaminergic neurons (*n* = 4). (*B*) — immunocytochemistry of neurons on day 30 of terminal differentiation TUBB(green)/TH(red)**

Bar = 50µm

by TD 30 (Fig. 1).

Regarding electrophysiological maturation, the ability to evoke action potentials appeared on days 10–14 of TD, as did the Ih current. After TD 20, cells demonstrated irregular spontaneous activity. At the same time, after TD 40, they had stable pacemaking activity both in the "on-cell" mode (Fig. 2) and in the "whole-cell" mode, which was reversibly blocked by 100 µM dopamine (Fig. 3), which indicates the presence of somatodendritic D2-type receptors. Activation of these receptors by dendritically released dopamine leads to a hyperpolarization mediated by GIRK-type K+ channels and is a hallmark of DA neurons. The coefficient of variation for firing was CV = 0.18; all together, this corresponds to a mature DA profile.

Conclusions. In this study, we characterized the maturation of dopaminergic neurons during their differentiation from human-induced stem cells. We obtained a relatively high percentage of

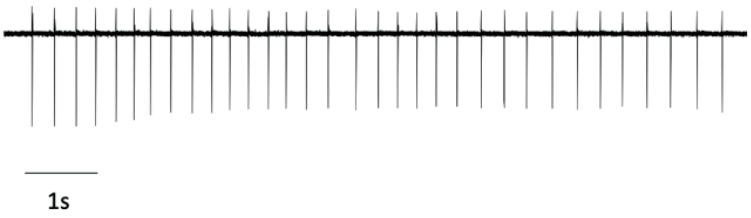

Fig. 2. **Representative cell-attached recording of a spontaneous firing dopaminergic neuron on day 49 of terminal differentiation**

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THE MULTIFACETED ROLE OF ENDOPLASMIC RETICULUM STRESS IN THE REGULATION OF GENE EXPRESSION IN NORMAL ASTROCYTES AND GLIOBLASTOMA CELLS

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Aim. To determine the role of the endoplasmic reticulum (ER) in regulating gene expression in normal astrocytes and glioblastoma cells under the application of carbon nanoparticles as anti-tumor agents and inhibition of ERN1 (endoplasmic reticulum to nucleus signaling 1).

Methods. Aqueous dispersions of graphene oxide (GO) and aqueous suspensions of single-walled carbon nanotubes (SWCNTs) were used to study the impact of carbon nanoparticles. Experiments were conducted on regular human astrocytes line NHA/TS and U87MG glioblastoma cells. Two sublines of ERN1 knockdown cells were applied to determine the role of ERN1 in the control of gene expression. Quantitative RT-PCR detected the expression level of mRNA and microRNA.

Results. It was shown that GO and SWCNTs affect the expression of numerous regulatory genes in both normal human astrocytes and glioblastoma cells, but the effect was less pronounced in glioblastoma cells than in normal astrocytes and preferentially mediated by ERN1. The expression of serine biosynthesis genes is controlled by ERN1 protein kinase or endoribonuclease activity. Furthermore, the inhibition of functional activity of ERN1 modifies the effects of hypoxia on gene expression.

Conclusions. Thus, these results demonstrated that ER stress plays an essential role in controlling the expression of various regulatory genes in glioblastoma cells and that normal astrocytes are more sensitive to the effect of carbon nanoparticles than glioblastoma cells.

Keywords: ER stress, carbon nanoparticles, mRNA and microRNA expression, DNAJB9, serine synthesis genes, glioblastoma cells, normal astrocytes.

Endoplasmic reticulum (ER) stress is an essential factor in the development of many pathological conditions, including the growth of malignant tumors, as well as various complications due to the action of nanoparticles. Previous studies have shown that inhibition of ERN1 (endoplasmic reticulum to nucleus signaling 1), a key sensor in the ER stress response, suppressed glioma cell proliferation and decreased the effect of nanoparticles. The exact mechanisms of this influence remain poorly understood, but a possible pathway for their action may involve the recruitment of both protein kinase and endoribonuclease activity of ERN1 signaling protein. Moreover, ER stress is responsible for nanoparticle toxicity as well as for regulation of the expression of pro-oncogenic gene expression.

Aim. To investigate the comparative effects of carbon nanoparticles on the expression of key regulatory genes in normal astrocytes and glioblastoma cells and find out the molecular mechanism of regulation of serine synthesis gene expression in glioblastoma cells with different ERN1 knockdowns.

Methods. Experiments were conducted on U87MG glioblastoma cells and their sublines with different ERN1 knockdown and regular human astrocytes line NHA/TS. Aqueous dispersions of

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graphene oxide (GO) and aqueous suspensions of single-walled carbon nanotubes (SWCNTs) were used to study the impact of carbon nanoparticles. The measurement of mRNA and microRNA expression levels was carried out using quantitative real-time polymerase chain reaction.

Results and Discussion. Our studies indicate that even small doses of graphene oxide (1 and 4 ng/mL) and SWCNTs (2 and 8 ng/mL) disrupt the expression of numerous regulatory genes in regular human astrocytes line NHA/TS [1–4]. Thus, the treatment of normal human astrocytes with GO nanoparticles in a dose of 1 ng/ml of medium) introduced a substantial increase in *DNAJB9* (heat shock protein family member B9 DnaJ) gene expression (by 126%, $P < 0.001$) as compared to the control [1]. A much stronger effect on the expression of *the DNAJB9* gene (203%, $P < 0.001$) was observed when using a bigger dose (1 ng/ml of medium) of these nanoparticles [1].

At the same time, the effect of GO nanoparticles on the expression of this gene was significantly lower (+41%, $P < 0.01$, and +82%, $P < 0.001$, respectively, for 1 and 4 ng/ml of nanoparticles) in glioblastoma cells with native ERN1 (Figure). It is interesting to note that the knocking down of ERN1 signaling protein in U87MG glioblastoma cells reduced the effect of these nanoparticles on *DNAJB9* gene expression close to a control level [1]. Similar results were shown for genes encoded transcription factors ATF3 and ATF4 [1]. These results demonstrated that the expression of different genes in normal astrocytes is more sensitive to GO nanoparticles than glioblastoma cells and that the effect of these nanoparticles is mainly mediated by ERN1 signaling protein, a major signaling pathway of ER stress. Thus, the impact of GO nanoparticles on different gene expressions is dose-dependent and gene-specific in both normal astrocytes and glioblastoma cells. Moreover, the gene specificity of the action of these nanoparticles is determined mainly by ER stress [1]. These carbon nanoparticles also affect microRNA expression levels, which have binding sites with target mRNAs, indicating the presence of post-transcriptional regulatory mechanisms for these genes [2].

It was also found that carbon nanotubes affect the expression of numerous genes in normal human astrocytes and glioblastoma cells. It was shown a more pronounced increase in the expression level of *BRCA1* (breast cancer type 1 susceptibility protein) and *DNAJB9* in normal cells [4]. Suppression of ERN1 signaling protein in glioblastoma cells decreased the effect of SWCNTs, thereby confirming the role of ER stress in affecting these gene expressions by carbon nanoparticles [4]. Furthermore, the treatment of normal human astrocytes with carbon nanotubes significantly affects the expression level of numerous microRNAs, which have binding sites with target mRNAs, indicating the possibility of a post-transcriptional mechanism for the regulation of studied gene expressions [3].

It has been shown that the expression level of genes responsible for serine synthesis was generally decreased in glioblastoma cells with inhibited both enzymatic activities of the ERN1, except *SHMT1* (serine hydroxymethyltransferase 1) gene, whose expression was sharply increased [5]. At the same time, the suppression of ERN1 endoribonuclease activity affects the expression of the *ATF4* (activating transcription factor 4) gene but does not significantly change the expression of *PHGDH* (phosphoglycerate dehydrogenase), *SHMT1*, and *SHMT2* genes. These results demonstrate that the expression of the *ATF4* gene is controlled by ERN1 endoribonuclease activity and that the expression of *PHGDH, SHMT1*, and *SHMT2* genes is regulated by ERN1 protein kinase activity [5]. It was also shown that silencing of XBP1 (X-box binding protein 1) by specific siRNA also suppresses only *ATF4* gene expression in glioblastoma cells. Still, silencing of ERN1 by specific siRNA decreases the expression of *PHGDH, SHMT2,* and *ATF4* genes. silencing of ERN1 by specific siRNA decreases the expression of *PHGDH, SHMT2,* and *ATF4* genes. At the same time, silencing of ERN1 strongly increases the expression of *SHMT1* [5].

The inhibition of ERN1 signaling protein also affects the expression of several microRNA expressions, which is responsible for post-transcriptional control of serine synthesis mRNA expressions in glioblastoma cells and possibly contributes to the suppression of these cell proliferation [5].

The significant results of analyzed data concerning the multifaceted role of ER stress in the regulation of gene expression in normal astrocytes and glioblastoma cells are summarized in Fig.

Conclusions. Changes in gene expression induced by graphene oxide nanoparticles and single-walled carbon nanotubes are mainly associated with the induction of ER stress and alterations in microRNA expression, which are key regulators of ER homeostasis and critical players in UPR (unfolded protein response) signaling. These changes are gene-specific and depend on ER stress because the inhibition of ERN1 significantly modifies the effects of carbon nanoparticles on the expression of studied genes. Meanwhile, normal human astrocytes are more sensitive to both graphene oxide nanoparticles and SWCNTs exposure compared to glioblastoma cells in a dose-

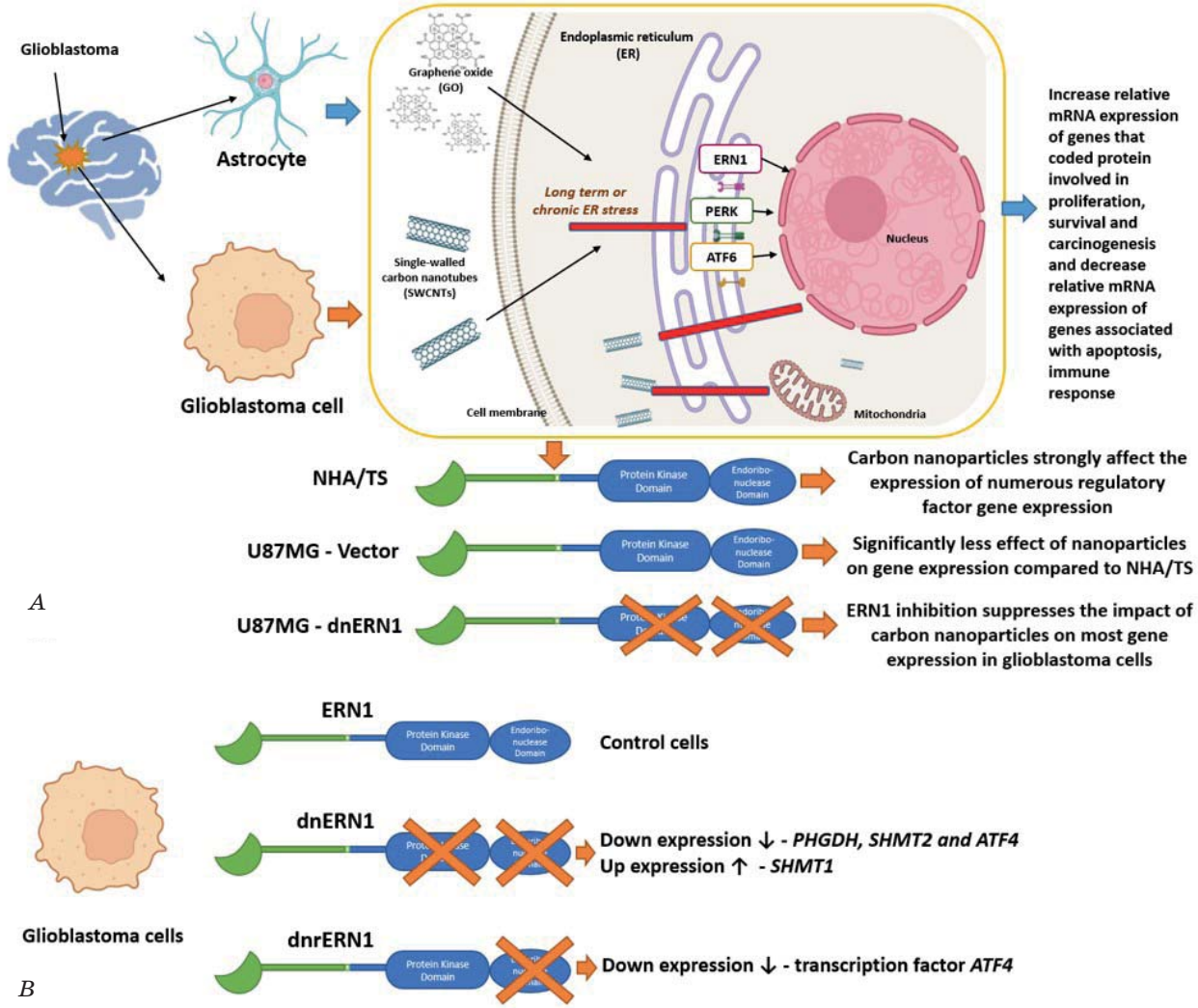

Figure. A — **Effect of ERN1 enzymatic activities on the expression level of genes in U87MG glioblastoma cells with suppressed both enzymatic activities (endoribonuclease and protein kinase) of ERN1 (dnERN1) and in cells without only endoribonuclease activity of ERN1 (dnrERN1) compared to control cells transfected with "empty" vector; *B* — Schematic representation of the effect of carbon nanoparticles on the expression level of genes associated with proliferation and survival processes in normal human astrocytes line NHA/TS, U87 glioblastoma cells transfected with a vector (U87-Vector) and cells with a dominant-negative ERN1 construct (U87-dnERN1)**

dependent manner, which is likely due to the tumor cell resistance to various external factors. This serves as a cautionary point regarding the biomedical applications of carbon nanoparticles. It has been established that the protein kinase activity of ERN1 is an essential regulator of serine biosynthesis gene expression, such as *PHGDH*, *SHMT1*, and *SHMT2* genes. At the same time, the ERN1 endoribonuclease activity controls the level of ATF4 gene expression.

Authors' Contribution
The authors contributed to the experimental work equally.

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