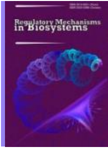

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.

Tumorigenicity assay of adrenal medulla derived cell culture

G. A. Bozhok*, O. S. Sidorenko*, N. A. Komaromi*, O. V. Pakhomov*, O. S. Protsenko**, N. O. Remnyova**, L. I. Chumak**, V. D. Ustichenko*, Y. O. Bozhkova*, Y. I. Legach*

**Institute for Problems of Cryobiology and Cryomedicine of the NAS of Ukraine, Kharkiv, Ukraine*
***V. N. Karazin Kharkiv National University, Kharkiv, Ukraine*

Article info

Received 07.09.2025
Received in revised form 21.10.2025
Accepted 10.11.2025

Institute for Problems of Cryobiology and Cryomedicine of the NAS of Ukraine, Pereyaslavska st., 23, Kharkiv, 61016, Ukraine.
Tel.: +38-095-149-55-36.
E-mail: nkomaromig@gmail.com

V. N. Karazin Kharkiv National University, Svobody sq., 4, Kharkiv, 61022, Ukraine.
Tel.: +38-067-258-16-13.
E-mail: chumak@karazin.ua

Bozhok, G. A., Sidorenko, O. S., Komaromi, N. A., Pakhomov, O. V., Protsenko, O. S., Remnyova, N. O., Chumak, L. I., Ustichenko, V. D., Bozhkova, Y. O., & Legach, Y. I. (2025). Tumorigenicity assay of adrenal medulla derived cell culture. Regulatory Mechanisms in Biosystems, 16(4), e25198. doi:10.15421/0225198

Neurodegenerative disorders involve a continual and permanent deterioration of specific neuronal populations, leading to severe and debilitating neurological impairments. Despite the development of various therapeutic strategies, current treatments largely provide only symptomatic relief and are unable to halt or reverse disease progression. In this context, cell-based therapies using stem or progenitor cells offer a promising alternative. Neural crest-derived cells (NCDCs) represent a multipotent cell population that originates during embryogenesis and can give rise to diverse cell types. Importantly, these cells or their derivatives persist in certain adult tissues such as skin, dental pulp, and the adrenal medulla, making them accessible for biomedical applications. Their high plasticity and availability from multiple sources make NCDCs a valuable candidate for regenerative medicine, including treatment of neurodegenerative disorders. However, one of the key safety requirements for cellular therapeutics is the evaluation of tumorigenic potential. In this study, we assessed the tumorigenicity of a cell culture derived from the adrenal medulla of neonatal pigs after subcutaneous transplantation into Balb/c mice. Immunodeficiency was induced by intraperitoneal administration of cyclosporine A. The Neuro2a neuroblastoma cell line was used as a tumorigenic positive control. Tumors developed in the groups that received Neuro2a cells (in 100% of the animals under immunosuppression and in 40% of the animals without it). No neoplasms were detected in the groups that received AGCC. Histological analysis confirmed the presence of malignant tumors only in the groups injected with Neuro2a cells. The obtained results indicate the absence of tumorigenic potential in AGCC and justify the feasibility of their further preclinical studies.

Keywords: neurodegenerative diseases; neural crest derived cells; tumorigenicity; cell therapy; adrenal gland cell culture; Neuro2a cell line.

Introduction

Neurodegenerative diseases are characterized by the progressive and irreversible loss of certain neuronal populations. Current treatments for severe neurodegenerative diseases provide only partial symptomatic relief. Given the limited availability of therapies capable of halting disease progression, transplantation of stem or specialized neural cells has emerged as a promising alternative.

For example, fetal striatal allotransplantation has been reported to improve motor and cognitive function in patients with Huntington's disease, although no sustained functional effect was observed (Barker et al., 2013). Neural grafts derived from human embryonic stem cells (hESCs) or induced pluripotent stem cells (iPSCs) have shown encouraging results in replacing dopaminergic neurons in Parkinson's disease (Hayashi et al., 2013; Jang et al., 2020). A number of clinical trials are currently underway to evaluate the efficacy of stem or progenitor cell transplantation for neurodegenerative disorders (Barker et al., 2015; Athauda et al., 2017; Studer et al., 2017).

Stem cell-based therapies, particularly those utilizing mesenchymal stem cells, have demonstrated potential benefits in various neurological conditions, including CNS injuries, stroke, multiple sclerosis, and brain tumors, although most studies remain in the early stages of development (Namiot et al., 2022). Transplantation of human neural progenitor cells engineered to secrete glial cell line-derived neurotrophic factor in patients with amyotrophic lateral sclerosis was shown to be safe, with long-term graft survival and sustained neurotrophic support for up to 42 months (Baloh et al., 2022). Similarly, in progressive multiple sclerosis, intrathecal transplantation of human fetal neural precursor cells proved feasible and safe, correlating with reduced brain atrophy and increased neuroprotective factors (Genchi et al., 2023). Autologous MSC-NTF transplantation in amyotrophic lateral sclerosis patients slowed motor and respiratory decline (Petrou et al., 2016), while Schwann cell transplantation after spinal cord injury promoted axonal regeneration, remyelination, and partial functional recovery (Lu et al., 2025).

Recent advances have also reinforced the translational potential of neuronal replacement strategies. A clinical study employing iPSC-derived dopaminergic neurons in Parkinson's disease reported preliminary improvements in motor function over 18–24 months (Chang & Lee, 2025), underscoring the promise of pluripotent stem cell-derived grafts for restoring neuronal function in degenerative disorders. Together, these findings highlight the growing clinical relevance of stem cell-based therapies and their potential to transform the treatment landscape of neurodegenerative diseases.

The neural crest is a transient embryonic population of multipotent cells that migrate and differentiate into peripheral nervous system components, melanocytes, adrenal medullary cells, craniofacial cartilage, bone and several other cell types (Leonard et al., 2020). It has been demonstrated that stem and progenitor cells originating from the neural crest are preserved in the adult organism (Soto et al., 2021). Neural crest-derived stem cells (NCDSCs) therefore represent a promising source for cell-based therapies targeting severe neurodegenerative diseases (Achilleos et al., 2012; Lim et al., 2021).

Their advantage in regenerative medicine lies in their remarkable plasticity – the ability to generate multiple cell lineages – and the wide range of accessible sources, including skin, dental pulp, and nasal septum cartilage. Recent clinical studies have begun exploring NCDSCs derived from dental pulp. A phase II randomized, double-blind, placebo-controlled trial of allogeneic dental pulp stem cells in Huntington's disease demonstrated favorable safety and preliminary clinical benefits (Fernandes et al., 2025). Mapping studies identifying neural-crest-derived populations in adult dental pulp and dermal papilla further support the feasibility of isolating these cells for therapeutic applications (Alzer et al., 2024).

Encouraging results have also been reported for other neural-crest-derived cell types. A phase I/IIa trial using autologous chondro-

progenitor cells from nasal septum cartilage demonstrated safety, functional stabilization, and joint preservation over 12 months (Tesch et al., 2024). Moreover, stem/progenitor cells isolated from nasal turbinate mucosa promoted dopaminergic neuron protection in a Parkinson's disease model, underscoring their potential for neuroregenerative therapy (Choi et al., 2024).

Collectively, these findings highlight the translational potential of neural crest-derived stem cells as a versatile and clinically accessible source for regenerative strategies in neurodegenerative and other chronic disorders.

The first step in implementing cell therapy is thorough preclinical and clinical trials (Marei et al., 2025). Modern preclinical testing protocols for cell-based therapies primarily include analysis of basic characteristics: the phenotype of the obtained cells, their viability and biological activity, homogeneity of the cell population, and the absence of pathogenic contamination. A tumorigenicity assay is also a crucial specific test for stem/progenitor cell-based products, as there is always a risk of de-/transdifferentiation of transplanted cells and the formation of a malignant tumor. (Du et al., 2017; Yogi et al., 2022).

In our previous studies, we obtained and characterized a cell culture from the adrenal medulla of neonatal piglets that expressed molecular markers of progenitor neural crest-derived cells and had the ability to form spheroids (Sidorenko et al., 2011; Bozhok et al., 2016). It was found that this culture is heterogeneous in its cellular composition. After being placed under cultivation conditions, the cells form a monolayer, upon which attached spheroids subsequently appear. Immunohistochemical analysis revealed the presence of cells expressing the marker of adrenal chromaffin cells (chromogranin A) and the neuroblast marker (β-III-tubulin), while lacking the expression of the glial marker S100 and the mature neuronal marker NeuN (Bozhok et al., 2016).

The ability of cells to undergo anchor-independent growth and form spheroids is known to be a hallmark of their tumorigenic potential. Moreover, the properties of the culture surface and the composition of the culture medium influence the rate and morphological characteristics of spheroid formation from neuronal progenitors (Mimura et al., 2010; Chojnacki & Weiss, 2013; Shamsnajafabadi et al., 2022). For example, in our previous studies, it was shown that in adrenal cell cultures under normal surface adhesion conditions, attached spheroids are formed, and the presence of fetal serum in the culture medium is essential. Under low-adhesion conditions, floating spheroids are formed regardless of the presence of serum in the culture medium (Plaksina et al., 2015). Such plasticity indicates the possible presence of stem / progenitor cells – neural crest derivatives – in the adrenal cell culture of neonatal piglets.

In this study, we assessed the risk of tumor formation when these cells were injected *in vivo* into laboratory mice.

The aim of the work is to study the tumorigenic potential of adrenal medulla derived cell culture at subcutaneous transplantation into mice with an immunodeficiency model.

Materials and methods

Experimental animals. All experiments on animals were performed in the compliance with the Law of Ukraine "On the Protection of Animals from Cruel Treatment" (№ 3447-IV dated 02/21/2006 with amendments) in accordance with the requirements of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986). Balb/c mice were used in experiments. The animals were kept in the animal house of the Institute for Problems of Cryobiology and Cryomedicine (IPC&C) of the NAS of Ukraine. The animals were given the standard laboratory chow and water ad libitum. The experimental protocol followed the Guide for Care and Use of Laboratory Animals and was approved by the Committee for Ethics in Animal Experimentation of the (IPC&C) of the NAS of Ukraine.

Cell cultures. The primary adrenal gland cell culture (AGCC) was obtained from the adrenal medulla of neonatal piglets using the method of Bozhok et al. (2016) and Plaksina et al.(2017). Briefly, adrenal fragments were placed in a solution with 1 mg/mL collagenase type IA (Sigma, USA) and 0.1 mg/mL DNase (Sigma). After three incubations, detached cells were collected, washed twice and placed under culture conditions. On average, cell viability when obtained by the enzymatic method was 85 ± 5% (trypan blue exclusion assay). Cultivation was performed in 25 cm² flasks (SPL Life sciences, Korea) using DMEM/F12 (Biowest, France) culture medium with the addition of 200 U/mL benzylpenicillin (Arterium, Ukraine), 200 μg/mL streptomycin (Arterium, Ukraine), 5 μg/ml amphotericin B and 10% fetal bovine serum (FBS, Biowest) at 37 °C in an atmosphere with 5% CO_2. The seeding cell concentration was 2.5–5 x 10^5 cells/mL. The medium was completely replaced every 3–4 days. The culture was subcultured by treating with a 1:1 mixture of 0.5% trypsin (Sigma, USA) and Versene solution (PAA, USA). The culture was incubated for 5 minutes, and detached cells were washed with DMEM/F12 medium. Cells from the second passage were used in further experiments.

Neuroblastoma cell line Neuro2a was used as a positive control to confirm the sensitivity of the selected model (Wu et al., 2021). The cell line obtained after the second passage from a cryopreserved culture stored at –196 °C was cultured in DMEM/F12 medium supplemented with antibiotics and FBS at 37 °C in an atmosphere with 5% CO_2.

Cell inoculation. Neuro2a cells or AGCC at a concentration of 0.5×10^6 cells were injected subcutaneously into the right thigh of the Balb/c mice. Three days before cell inoculation, three groups of animals were injected intraperitoneally with cyclosporine A (CsA) (Sandimmun Neoral, Novartis, Switzerland) at a dose of 35 mg/kg of body weight in 200 μL of physiological saline (PS) to induce immunodeficiency. The remaining groups were injected with PS. After cell inoculation, the mice were monitored daily, which included visual inspection, palpation of the injection site, and blood sampling. Blood smears were prepared and stained using the Romanowsky-Giemsa method. The cell count was performed across the entire smear. 100 leukocytes were counted per smear. The groups of animals studied in the experiment are presented in Table 1.

Table 1
Groups of experimental animals

Groups	Experimental conditions		Number of animals
	cell's type	CsA	
1	–	–	7
2	–	+	7
3	Neuro2a	+	7
4	Neuro2a	–	7
5	AGCC	+	7
6	AGCC	–	7

Histological analysis. On day 20, the animals were sacrificed, and tissue was collected from the injection site. All major organs were also examined. Any tumors found were removed and fixed in 10% formalin. Paraffin sections (7 μm thick) were prepared using standard techniques and stained with hematoxylin and eosin. Histological specimens were visually assessed using an AmScope XYL-403 light microscope and analyzed using AxioVision Rel. 4.8 software.

Statistical analysis. The proportions of white blood cells were represented as mean ± standard deviation; differences were considered significant at $P < 0.05$. ANOVA and the Tukey test for multiple comparisons were used on arcsine square root-transformed data. Survival of animals were calculated using the Kaplan–Meier method, differences between groups were compared with a log-rank test.

Results

The primary AGCC culture was heterogeneous in its cellular composition. On day 3, adherent large polygonal cells and rounded cells with vacuolated cytoplasm were observed in the culture. On day 7, a confluent monolayer of polygonal cells formed, tightly adjacent to each other, acquiring a more elongated shape and forming "streams" (Fig. 1a). With further cultivation, attached spherical formations appeared on the monolayer. Subsequently, tiny spindle-shaped cells with small nuclei- poor cytoplasm and neurite-like structures appeared around the spheroids. These formed a layer on the surface of the polygonal cells.

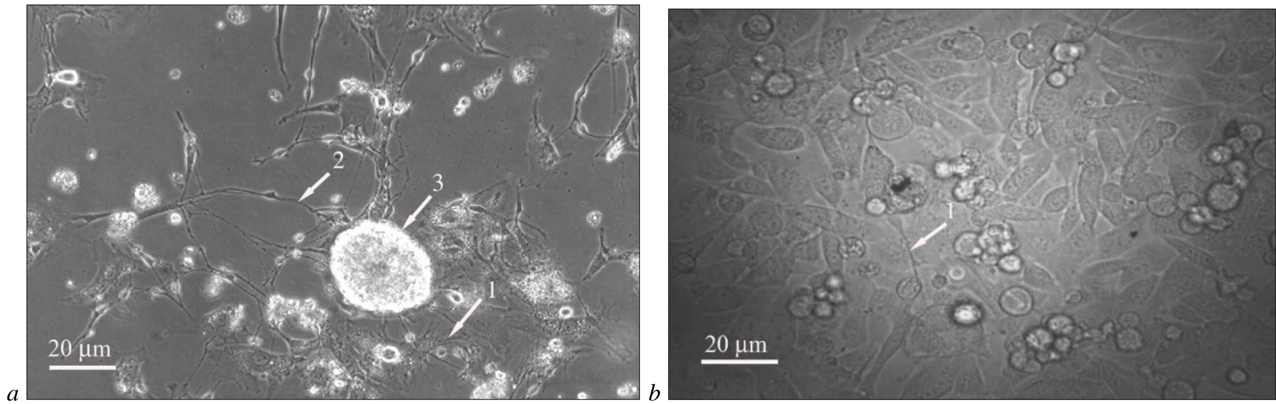

Fig. 1. Representative micrographs of cell cultures used in the experiment: adrenal gland cell culture (*a*), 7th day of cultivation: monolayer of polygonal cells (*1*); spindle-shaped cells with neurite-like structures (*2*); spheroid (*3*); Neuro2a cells (*b*), 6th day of cultivation: cells with neurite-like structures (*1*); phase contrast microscopy

Neuro2a cells, like adrenal medulla cells, are neural crest-derived. They are capable of differentiating into either cholinergic or dopaminergic neurons depending on culture conditions (Tremblay et al., 2010; Namsi et al., 2018). In serum-containing media, they are primarily composed of adherent cells with an "amoeboid" morphology (Fig. 1b). Cells with neurite-like structures are also found. Under certain conditions, they are also capable of forming spheroids (Choi et al., 2013; Liu et al., 2021).

The immunodeficiency in mice treated with CsA was confirmed by examining blood smears. A statistically significant decrease in lymphocyte counts was observed in the CsA-treated compared to the untreated groups (Table 2). Furthermore, chronic CsA administration significantly increased neutrophil counts in these groups, but had no effect on other leukocyte subsets.

The combination of reduced lymphocytes and increased neutrophils during chronic cyclosporine A (CsA) treatment is due to its dual effects on the immune system. CsA suppresses T lymphocyte proliferation by inhibiting the calcineurin/NFAT pathway, reducing IL-2 production (López-Flores et al., 2011; Xu et al., 2023). At the same time, it affects neutrophil metabolism and survival through the SIRT6-PDK4 pathway, altering their apoptosis and migration (Lu et al., 2021). Similar results have been observed in other mouse studies, showing lymphopenia alongside increased neutrophil activity (López-Flores et al., 2011; Lu et al., 2021; Xu et al., 2023).

Table 2
Peripheral blood leukocyte composition (%)
in animals from the experimental groups (n = 7)

No. groups	Lymphocytes	Neutrophils	Monocytes	Eosinophils	Basophils
1	74.6 ± 5.5	25.0 ± 5.2	0.25 ± 0.09	0.09 ± 0.01	0.06 ± 0.01
2	44.4 ± 4.1[a]	55.1 ± 4.2[a]	0.34 ± 0.05	0.09 ± 0.01	0.07 ± 0.01
3	31.5 ± 4.4[a]	68.0 ± 5.1[a]	0.32 ± 0.08	0.12 ± 0.01	0.06 ± 0.01
4	65.8 ± 6.6[b]	33.7 ± 6.8[b]	0.31 ± 0.09	0.14 ± 0.08	0.05 ± 0.01
5	45.0 ± 6.5[ab]	54.5 ± 7.1[a]	0.32 ± 0.08	0.12 ± 0.09	0.06 ± 0.01
6	69.3 ± 5.9[c]	30.3 ± 9.3[c]	0.25 ± 0.07	0.10 ± 0.05	0.05 ± 0.01

Note: [a], the values are statistically different from the corresponding values of group 1; [b], the values are statistically different from the corresponding values of group 3; [c], the values are statistically different from the corresponding values of group 5; values between groups were not statistically different for monocytes, basophils, and eosinophils.

Palpation during daily examination revealed the formation of masses in the femur area in some mice as early as days 13–15. After sacrifice (on day 20), tumors were detected in 100% of animals in Group 3 (Neuro2a+CsA) and in 40% of animals in Group 4 (Neuro2a). The tumors ranged in diameter from 5 to 15 mm. Figure 2 shows photographs of tumors removed from the cell injection site. No similar tumors were detected in the other groups.

Animal survival is shown in Figure 3. It is noticeable that in the group receiving Neuro2a against the background of immunodeficiency (Group 3), a 28% decrease in survival was observed during the experiment. In the groups receiving CsA, a 14% decrease in survival was observed, which is likely related to complications arising from immunosuppression.

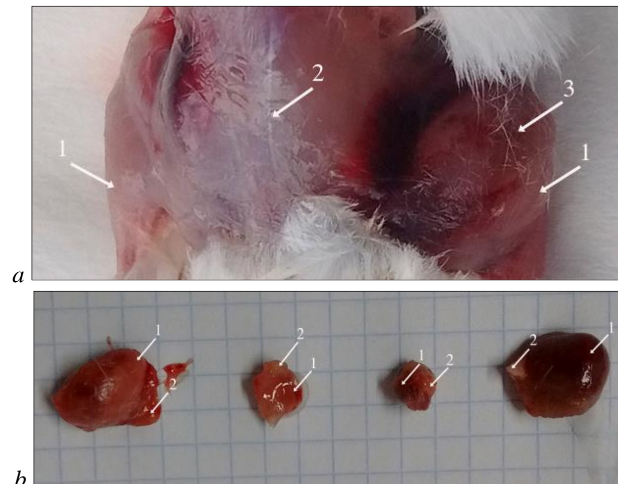

Fig. 2. Representative photographs of neoplasms removed at the site of inoculation of Neuro2a cells in animals of group 3 (with the introduction of CsA): the site of the neoplasm during visual examination (*a*): *1* – femur, *2* – abdominal surface, *3* – tumor; removed tumors (*b*): *1* – tumor, *2* – connective tissue; the distance between the grid lines in the photo = 5 mm

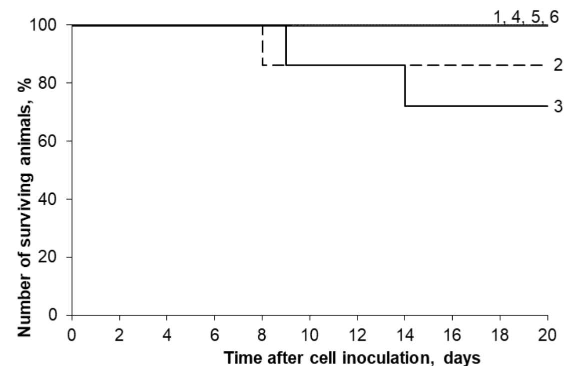

Fig. 3. Mouse survival after cell inoculation: numbers indicate animal group number

Histological examination of tissue removed from the cell inoculation site revealed that all samples from Group 3 (Neuro2a+CsA) and 40% of animals from Group 4 (Neuro2a) harbored a tumor. At the site of inoculation, the tumor consisted of small, round, spindle or polygonal cells with a thin rim of basophilic cytoplasm with moderate nuclear polymorphism and increased mitotic activity (Fig. 4a, 4b).

Large and small vessels vascularized the tumors. Lymphocytic infiltration was observed around the tumor.

No pathologically altered tissue was detected at the cell injection site in the Groups 5 (AGCC + CsA) and 6 (AGCC). The histological samples contained intact muscle tissue, displaying long, cylindrical, multinucleated fibers with nuclei located peripherally (Fig. 4c, 4d). Characteristic striated patterns were observed in the muscle fibers. The muscle fibers were grouped into bundles surrounded by connective tissue interlayers.

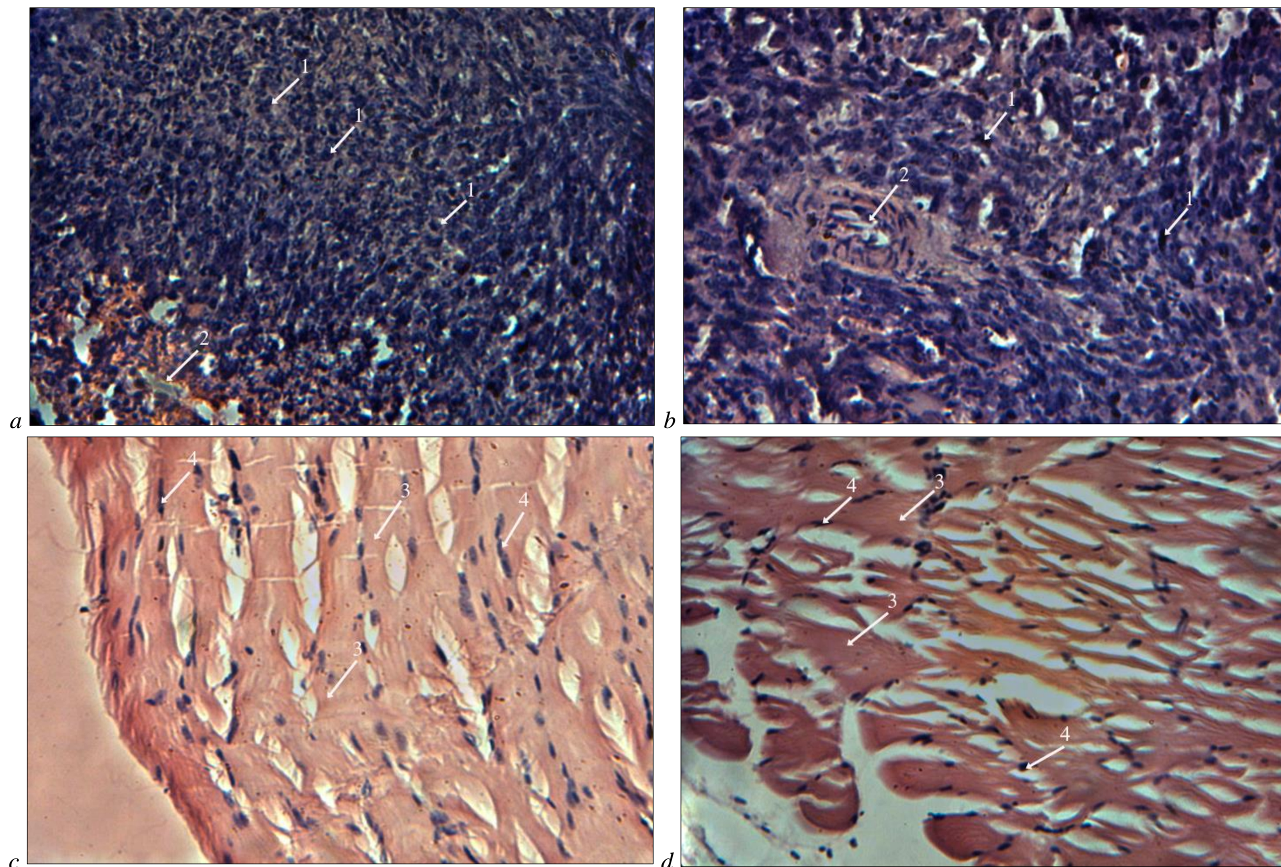

Fig. 4. Histological sections of tissue removed from the thigh area of mice from different experimental groups on the 20th day after cell inoculation: group 3 (Neuro2a+CsA) (*a*); group (Neuro2a) (*b*); group 5 (AGCC +CsA) (*c*); group 6 (AGCC) (*d*); arrows show: *1* – nuclear polymorphism in tumors; *2* – tumor vascularization; *3* – muscle fibers; *4* – muscle fibers nuclei

Discussion

Neural crest-derived cell transplantation is being studied for potential use in the treatment of severe neurodegenerative diseases (Müller et al., 2015; Schizas et al., 2018; Rahman et al., 2024). However, the risk of tumor formation from transplanted cells remains a key safety concern when using cell-based therapies. Several studies have demonstrated the tumorigenic activity of neural crest-derived cells. For example, in the work of Vega et al. (2019) it has been shown that the subpopulation of CD44-high neural crest stem-like cells demonstrates an increased tendency to form spheroids in serum-free conditions and correlates with aggressive overgrowth of tumor. Xu et al. (2021) confirmed that neural stem cells provide the potential for transformation and initiation of tumors in xenografts, while other types of stem cells do not have the same property.

The ability to form spheroids *in vitro* is called the "sphere-forming assay". It is a functional characteristic of neural crest-derived stem/progenitor cells that allows them to be identified in various tissues of the body in the absence of specific phenotypic markers (Pastrana et al., 2011). Chung K.F. and colleagues described the formation of spheroids from progenitor cells of the bovine adrenal medulla in cattle (chromospheres). Under culture conditions with 10% FBS, the chromospheres showed reduced expression of markers of differentiated chromaffin cells and increased expression of neural crest progenitor cell markers (Sox1, Sox9, nestin, vimentin) (Chung et al., 2009). There are reports on the generation of floating chromospheres from human adrenal chromaffin cells in the presence of FGF-2 and EGF. The chromospheres were characterized by the expression of several progenitor markers, including nestin, CD133, Notch1, NGFR, Snai2, Sox9, Sox10, Phox2b, and Ascl1 at the molecular level, and Sox9 at the immunohistochemical level. In contrast, the level of phenylethanolamine N-methyltransferase (PNMT), a marker of differentiated chromaffin cells, was markedly decreased after 12 days of cultivation (Santana et al., 2012). Neurospheres derived from oral mucosa cells expressed neural crest markers (nestin, CD44, Slug, Slim, and MSX1). These cells showed increased expression of neural crest–related genes (EDNRA, Hes1, and Sox9) and demonstrated the ability to generate ectopic bone tissue in the subcutaneous area (Abe et al., 2016). Spheroids containing neural crest progenitor cells have been successfully isolated from a variety of postnatal tissues.

These include adipose tissue, which provides an easily accessible and rich source of multipotent neural crest–derived stem cells (Nagoshi et al., 2008; Zavan et al., 2010). Similar spheroid-forming progenitor cells have been obtained from the respiratory epithelium of the inferior nasal turbinate and from the gingiva, demonstrating the widespread presence of neural crest remnants in adult craniofacial tissues (Hauser et al., 2012; Fournier et al., 2016). In addition, dental pulp and skin harbor neural crest-derived populations capable of forming neurosphere-like aggregates and differentiating toward neural and glial phenotypes (Zhang et al., 2020; Bosch et al., 2021). Further evidence of such progenitor reservoirs has been reported in bone marrow, dorsal root ganglia, vibrissae follicles, and carotid bodies, emphasizing the broad anatomical distribution of neural crest-derived cells in the adult organism (Nagoshi et al., 2008; López-Barneo et al., 2009). However, there are almost no studies that have investigated the tumorigenic potential of spheroid-forming cell cultures derived from

the adrenal glands. We previously obtained a cell culture from the adrenal gland medulla of neonatal pigs, in which an active spheroid formation process was observed (Sidorenko et al., 2011; Bozhok et al., 2016). This indicated the presence of post-migratory subpopulations of neural crest-derived cells with possible tumorigenic potential.

The gold standard for testing tumorigenicity is experiments on immunodeficient laboratory rodents. One important aspect is comparative studies of cell cultures with known tumorigenic potential and cultures with a theoretically possible risk of tumor formation. The Neuro-2a cell line, derived from mouse neuroblastoma, is a cell line with high tumorigenic potential and the ability to rapidly grow tumors *in vivo* (Feuerecker et al., 2015; Hergenhahn et al., 2024). Based on these assumptions, we conducted a comparative analysis of two cell cultures. This analysis revealed that, during the study period, Neuro-2a cells developed tumors at the site of inoculation in mice with immunodeficiency, while AGCC did not exhibit similar tumorigenic potential. This allows us to continue preclinical trials of AGCC as a potential source of neural crest-derived stem/progenitor cells for further practical application in the treatment of neurodegenerative diseases.

Conclusion

This study demonstrates that adrenal medulla–derived cell cultures (AGCC) obtained from neonatal pigs are non-tumorigenic *in vivo*. Unlike the Neuro2a neuroblastoma line, AGCC transplantation did not induce tumor formation or abnormal proliferation in immunodeficient mice. Histological evaluation confirmed the absence of neoplastic structures, indicating the biosafety and phenotypic stability of these neural crest–derived progenitors.

The adrenal medulla represents a distinctive postmigratory neural crest niche capable of generating both neuronal and endocrine derivatives. The absence of tumorigenic potential highlights the translational value of AGCC as a safe and versatile cell source for modeling neuroendocrine differentiation, stress physiology, and regenerative processes. Their ability to maintain stable progenitor populations *in vitro* without malignant transformation also opens prospects for developing 3D organoid and spheroid systems that replicate adrenal and neural crest tissue dynamics. Beyond their biological significance, AGCC offer a promising platform for integration with biomaterials and trophic factors to enhance survival, differentiation, and functional recovery in neural and endocrine tissues. Their adaptability to genetic modification further expands their potential for creating cell- and gene-based therapeutic constructs targeting neurodegenerative and adrenal disorders.

Future research should address long-term *in vivo* behavior, differentiation pathways, and molecular mechanisms underlying their stability. Collectively, these results position adrenal medulla-derived progenitors as a valuable and safe neural crest–origin cell source bridging developmental biology and regenerative medicine.

This research was carried out within the framework of regular state budget projects of the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine and did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abe, S., Yamaguchi, S., Sato, Y., & Harada, K. (2016). Sphere-derived multipotent progenitor cells obtained from human oral mucosa are enriched in neural crest cells. Stem Cells Translational Medicine, 5(1), 117–128.

Achilleos, A., & Trainor, P. A. (2012). Neural crest stem cells: Discovery, properties and potential for therapy. Cell Research, 22(2), 288–304.

Alzer, H., Shahin, N. A., & Alsoleihat, F. (2024). Neural and neural crest-derived stem cell distribution in the adult human dental pulp. International Journal of Morphology, 42(2), 510–515.

Athauda, D., Maclagan, K., Skene, S. S., Bajwa-Joseph, M., Letchford, D., Chowdhury, K., Hibbert, S., Budnik, N., Zampedri, L., Dickson, J., Li, Y., Aviles-Olmos, I., Warner, T. T., Limousin, P., Lees, A. J., Greig, N. H., Tebbs, S., & Foltynie, T. (2017). Exenatide once weekly versus placebo in Parkinson's disease: A randomised, double-blind, placebo-controlled trial. Lancet, 390(10103), 1664–1675.

Baloh, R. H., Johnson, J. P., Avalos, P., Allred, P., Svendsen, S., Gowing, G., Roxas, K., Wu, A., Donahue, B., Osborne, S., Lawless, G., Shelley, B., Wheeler, K., Prina, C., Fine, D., Kendra-Romito, T., Stokes, H., Manoukian, V., Muthukumaran, A., Garcia, L., Bañuelos, M. G., Godoy, M., Bresee, C., Yu, H., Drazin, D., Ross, L., Naruse, R., Babu, H., Macklin, E. A., Vo, A., Elsayegh, A., Tourtellotte, W., Maya, M., Burford, M., Diaz, F., Patil, C. G., Lewis, R. A., & Svendsen, C. N. (2022). Transplantation of human neural progenitor cells secreting GDNF into the spinal cord of patients with ALS: a phase 1/2a trial. Nature Medicine, 28(9), 1813–1822.

Barker, R. A., Mason, S. L., Harrower, T. P., Swain, R. A., Ho, A. K., Sahakian, B. J., Mathur, R., Elneil, S., Thornton, S., Hurrelbrink, C., Armstrong, R. J., Tyers, P., Smith, E., Carpenter, A., Piccini, P., Tai, Y. F., Brooks, D. J., Pavese, N., Watts, C., Pickard, J. D., Rosser, A. E., Dunnett, S. B., NEST-UK Collaboration (2013). The long-term safety and efficacy of bilateral transplantation of human fetal striatal tissue in patients with mild to moderate Huntington's disease. Journal of Neurology, Neurosurgery, and Psychiatry, 84(6), 657–665.

Barker, R. A., Studer, L., Cattaneo, E., Takahashi, J., & G-Force PD Consortium (2015). G-Force PD: A global initiative in coordinating stem cell-based dopamine treatments for Parkinson's disease. NPJ Parkinson's Disease, 1, 15017.

Bosch, B. M., Salero, E., Núñez-Toldrà, R., Sabater, A. L., Gil, F. J., & Perez, R. A. (2021). Discovering the potential of dental pulp stem cells for corneal endothelial cell production: A proof of concept. Frontiers in Bioengineering and Biotechnology, 9, 617724.

Bozhok, G. A., Sidorenko, O. S., Plaksina, E. M., Gurina, T. M., Sukach, A. N., Kholodnyy, V. S., Ustichenko, V. D., Bilyavskaya, S. B., Bondarenko, T. P., & Legach, E. I. (2016). Neural differentiation potential of sympathoadrenal progenitors derived from fresh and cryopreserved neonatal porcine adrenal glands. Cryobiology, 73(2), 152–161.

Chang, M. Y., & Lee, S. H. (2025). Human pluripotent stem cell-based therapies for Parkinson's disease: Challenges and potential solutions. Yonsei Medical Journal, 66(7), 395–404.

Choi, S. K., Kim, J. H., Park, J. K., Lee, K. M., Kim, E., & Jeon, W. B. (2013). Cytotoxicity and inhibition of intercellular interaction in N2a neurospheroids by perfluorooctanoic acid and perfluorooctanesulfonic acid. Food and Chemical Toxicology, 60, 520–529.

Chojnacki, A., & Weiss, S. (2013). Culturing fetal precursor cells using free floating serum-free conditions. Methods in Molecular Biology, 1059, 3–12.

Chung, K. F., Sicard, F., Vukicevic, V., Hermann, A., Storch, A., Huttner, W. B., Bornstein, S. R., & Ehrhart-Bornstein, M. (2009). Isolation of neural crest derived chromaffin progenitors from adult adrenal medulla. Stem Cells, 27(10), 2602–2613.

Du, F., Zhao, X., & Fan, D. (2017). Tumorigenicity assay in nude mice. Bio-protocol, 7(13), e2364.

Fernandes, J. M. S., Pagani, E., Wenceslau, C. V., Ynoue, L. H., Ferrara, L., & Kerkis, I. (2025). Phase II trial of intravenous human dental pulp stem cell therapy for Huntington's disease: A randomized, double-blind, placebo-controlled study. Stem Cell Research and Therapy, 16(1), 432.

Feuerecker, B., Pirsig, S., Seidl, C., Aichler, M., Feuchtinger, A., Bruchelt, G., Senekowitsch-Schmidtke, R. (2015). DCA promotes progression of neuroblastoma tumors in mice bearing Neuro-2a xenografts. American Journal of Cancer Research, 5(2), 639–650.

Fournier, B. P., Loison-Robert, L. S., Ferré, F. C., Owen, G. R., Larjava, H., & Häkkinen, L. (2016). Characterisation of human gingival neural crest-derived stem cells in monolayer and neurosphere cultures. European Cells and Materials, 31, 40–58.

Genchi, A., Brambilla, E., Sangalli, F., Radaelli, M., Bacigaluppi, M., Furlan, R., Andolfo, A., Drago, D., Magagnotti, C., Scotti, G. M., Greco, R., Vezzulli, P., Ottoboni, L., Bonopane, M., Capilupo, D., Ruffini, F., Belotti, D., Cabiati, B., Cesana, S., Matera, G., Leocani, L., Martinelli, V., Moiola, L., Vago, L., Panina-Bordignon, P., Falini, A., Ciceri, F., Uglietti, A., Sormani, M. P., Comi, G., Battaglia, M. A., Rocca, M. A., Storelli, L., Pagani, E., Gaipa, G., & Martino, G. (2023). Neural stem cell transplantation in patients with progressive multiple sclerosis: An open-label, phase 1 study. Nature Medicine, 29(1), 75–85.

Hauser, S., Widera, D., Qunneis, F., Müller, J., Zander, C., Greiner, J., Strauss, C., Lüningschrör, P., Heimann, P., Schwarze, H., Ebmeyer, J., Sudhoff, H., Araúzo-Bravo, M. J., Greber, B., Zaehres, H., Schöler, H., Kaltschmidt, C., & Kaltschmidt, B. (2012). Isolation of novel multipotent neural crest-derived stem cells from adult human inferior turbinate. Stem Cells and Development, 21(5), 742–756.

Hayashi, T., Wakao, S., Kitada, M., Ose, T., Watabe, H., Kuroda, Y., Mitsunaga, K., Matsuse, D., Shigemoto, T., Ito, A., Ikeda, H., Fukuyama, H., Onoe, H., Tabata, Y., & Dezawa, M. (2013). Autologous mesenchymal

stem cell-derived dopaminergic neurons function in parkinsonian macaques. The Journal of Clinical Investigation, 123(1), 272–284.
Hergenhahn, L., Padutsch, N., Azawi, S., Weiskirchen, R., Liehr, T., & Rinčic, M. (2024). Cytogenomic characterization of murine neuroblastoma cell line Neuro-2a and its two derivatives Neuro-2a TR-alpha and Neuro-2a TR-beta. Cells, 13(22), 1889.
Jang, S. E., Qiu, L., Chan, L. L., Tan, E. K., & Zeng, L. (2020). Current status of stem cell-derived therapies for Parkinson's disease: From cell assessment and imaging modalities to clinical trials. Frontiers in Neuroscience, 14, 558532.
Leonard, C. E., & Taneyhill, L. A. (2020). The road best traveled: Neural crest migration upon the extracellular matrix. Seminars in Cell and Developmental Biology, 100, 177–185.
Lim, J. Y., In Park, S., Park, S. A., Jeon, J. H., Jung, H. Y., Yon, J. M., Jeun, S. S., Lim, H. K., & Kim, S. W. (2021). Potential application of human neural crest-derived nasal turbinate stem cells for the treatment of neuropathology and impaired cognition in models of Alzheimer's disease. Stem Cell Research and Therapy, 12(1), 402.
Liu, W. W., Chen, S. H., & Li, P. C. (2021). Functional photoacoustic calcium imaging using chlorophosphonazo III in a 3D tumor cell culture. Biomedical Optics Express, 12(2), 1154–1166.
López-Barneo, J., Pardal, R., Ortega-Sáenz, P., Durán, R., Villadiego, J., & Toledo-Aral, J. J. (2009). The neurogenic niche in the carotid body and its applicability to antiparkinsonian cell therapy. Journal of Neural Transmission, 116(8), 975–982.
López-Flores, R., Bojalil, R., Benítez, J. C., Ledesma-Soto, Y., Terrazas, C. A., Rodríguez-Sosa, M., & Terrazas, L. I. (2011). Consecutive low doses of cyclosporine A induce pro-inflammatory cytokines and accelerate allograft skin rejection. Molecules, 16(5), 3969–3984.
Lu, H., Lin, J., Xu, C., Sun, M., Zuo, K., Zhang, X., Li, M., Huang, H., Li, Z., Wu, W., Feng, B., & Liu, Z. (2021). Cyclosporine modulates neutrophil functions via the SIRT6-HIF-1α-glycolysis axis to alleviate severe ulcerative colitis. Clinical and Translational Medicine, 11(2), e334.
Lu, M. F., Liu, J. P., Xu, Y. S., Zuo, C., Liu, S. C., & Zhang, W. J. (2025). Application and challenge of Schwann cells transplantation in spinal cord injury and clinical trial. International Journal of Surgery, in press.
Marei, H. E. (2025). Stem cell therapy: A revolutionary cure or a Pandora's box. Stem Cell Research and Therapy, 16(1), 255.
Mimura, T., Yamagami, S., Uchida, S., Yokoo, S., Ono, K., Usui, T., & Amano, S. (2010). Isolation of adult progenitor cells with neuronal potential from rabbit corneal epithelial cells in serum- and feeder layer-free culture conditions. Molecular Vision, 16, 1712–1719.
Müller, J., Ossig, C., Greiner, J. F., Hauser, S., Fauser, M., Widera, D., Kaltschmidt, C., Storch, A., & Kaltschmidt, B. (2015). Intrastriatal transplantation of adult human neural crest-derived stem cells improves functional outcome in parkinsonian rats. Stem Cells Translational Medicine, 4(1), 31–43.
Nagoshi, N., Shibata, S., Kubota, Y., Nakamura, M., Nagai, Y., Satoh, E., Morikawa, S., Okada, Y., Mabuchi, Y., Katoh, H., Okada, S., Fukuda, K., Suda, T., Matsuzaki, Y., Toyama, Y., & Okano, H. (2008). Ontogeny and multipotency of neural crest-derived stem cells in mouse bone marrow, dorsal root ganglia, and whisker pad. Cell Stem Cell, 2(4), 392–403.
Namiot, E. D., Niemi, J. V. L., Chubarev, V. N., Tarasov, V. V., & Schiöth, H. B. (2022). Stem cells in clinical trials on neurological disorders: Trends in stem cells origins, indications, and status of the clinical trials. International Journal of Molecular Sciences, 23(19), 11453.
Namsi, A., Nury, T., Hamdouni, H., Yammine, A., Vejux, A., Vervandier-Fasseur, D., Latruffe, N., Masmoudi-Kouki, O., & Lizard, G. (2018). Induction of neuronal differentiation of murine N2a cells by two polyphenols present in the Mediterranean diet mimicking neurotrophins activities: Resveratrol and apigenin. Diseases, 6(3), 67.
Pastrana, E., Silva-Vargas, V., & Doetsch, F. (2011). Eyes wide open: A critical review of sphere-formation as an assay for stem cells. Cell Stem Cell, 8(5), 486–498.
Petrou, P., Gothelf, Y., Argov, Z., Gotkine, M., Levy, Y. S., Kassis, I., Vaknin-Dembinsky, A., Ben-Hur, T., Offen, D., Abramsky, O., Melamed, E., & Karussis, D. (2016). Safety and clinical effects of mesenchymal stem cells secreting neurotrophic factor transplantation in patients with amyotrophic lateral sclerosis: Results of phase 1/2 and 2a clinical trials. JAMA Neurology, 73(3), 337–344.
Plaksina, E. M., Sidorenko, O. S., & Bozhok, G. A. (2015). Morphological and phenotypic peculiarities of cytospheres forming in adrenal cell cultures of neonatal piglets in normal and low adhesive conditions. Problems of Cryobiology and Cryomedicine, 25(2), 190.
Plaksina, K. M., Sidorenko, O. S., Legach, Y. I., Kovalenko, I. F., Bozhok, G. A. (2017). Ekspressiya β-III-tubulina v kul'ture kletok neonatal'nykh nadpochechnikov: Sravnenie monosloynogo i 3D-kul'tivirovaniya [Expression of β-III-tubulin in the neonatal adrenal cell culture: comparison of monolayer and 3D-culture]. The Journal of V. N. Karazin Kharkiv National University, Series Biology, 28(1), 76–86.
Rahman, A. A., Ohkura, T., Bhave, S., Pan, W., Ohishi, K., Ott, L., Han, C., Leavitt, A., Stavely, R., Burns, A. J., Goldstein, A. M., & Hotta, R. (2024). Enteric neural stem cell transplant restores gut motility in mice with Hirschsprung disease. JCI Insight, 9(17), e179755.
Santana, M. M., Chung, K. F., Vukicevic, V., Rosmaninho-Salgado, J., Kanczkowski, W., Cortez, V., Hackmann, K., Bastos, C. A., Mota, A., Schrock, E., Bornstein, S. R., Cavadas, C., & Ehrhart-Bornstein, M. (2012). Isolation, characterization, and differentiation of progenitor cells from human adult adrenal medulla. Stem Cells Translational Medicine, 1(11), 783–791.
Schizas, N., König, N., Andersson, B., Vasylovska, S., Hoeber, J., Kozlova, E. N., & Hailer, N. P. (2018). Neural crest stem cells protect spinal cord neurons from excitotoxic damage and inhibit glial activation by secretion of brain-derived neurotrophic factor. Cell and Tissue Research, 372(3), 493–505.
Shamsnajafabadi, H., Soheili, Z. S., Samiee, S., Ahmadieh, H., Pirmardan, E. R., & Haghighi, M. (2022). Neural differentiation of human retinal pigment epithelial cells on alginate/gelatin substrate. Molecular Vision, 28, 412–431.
Sidorenko, O. S., Bozhok, G. A., Legach, E. I., Gurina, T. M. (2011). Study of possibility to obtain and cryopreserve adrenal cell primary culture of newborn piglets. Problems of Cryobiology and Cryomedicine, 21(1), 58–67.
Soto, J., Ding, X., Wang, A., & Li, S. (2021). Neural crest-like stem cells for tissue regeneration. Stem Cells Translational Medicine, 10(5), 681–693.
Studer L. (2017). Strategies for bringing stem cell-derived dopamine neurons to the clinic-The NYSTEM trial. Progress in Brain Research, 230, 191–212.
Tesch, R. S., Takamori, E. R., Menezes, K., Carias, R. B. V., Rebelatto, C. L. K., Senegaglia, A. C., Daga, D. R., Fracaro, L., Robert, A. W., Pinheiro, C. B. R., Aguiar, M. F., Blanco, P. J., Zilves, E. G., Brofman, P. R. S., & Borojevic, R. (2024). Nasal septum-derived chondroprogenitor cells control mandibular condylar resorption consequent to orthognathic surgery: A clinical trial. Stem Cells Translational Medicine, 13(7), 593–605.
Tremblay, R. G., Sikorska, M., Sandhu, J. K., Lanthier, P., Ribecco-Lutkiewicz, M., & Bani-Yaghoub, M. (2010). Differentiation of mouse Neuro 2A cells into dopamine neurons. Journal of Neuroscience Methods, 186(1), 60–67.
Vega, F. M., Colmenero-Repiso, A., Gómez-Muñoz, M. A., Rodríguez-Prieto, I., Aguilar-Morante, D., Ramírez, G., Márquez, C., Cabello, R., & Pardal, R. (2019). CD44-high neural crest stem-like cells are associated with tumour aggressiveness and poor survival in neuroblastoma tumours. EBio Medicine, 49, 82–95.
Wu, X., Nelson, M., Basu, M., Srinivasan, P., Lazarski, C., Zhang, P., Zheng, P., & Sandler, A. D. (2021). MYC oncogene is associated with suppression of tumor immunity and targeting Myc induces tumor cell immunogenicity for therapeutic whole cell vaccination. Journal for Immunotherapy of Cancer, 9(3), e001388.
Xu, C., Ye, Z., Jiang, W., Wang, S., & Zhang, H. (2023). Cyclosporine A alleviates colitis by inhibiting the formation of neutrophil extracellular traps via the regulating pentose phosphate pathway. Molecular Medicine, 29(1), 169.
Xu, L., Zhang, M., Shi, L., Yang, X., Chen, L., Cao, N., Lei, A., & Cao, Y. (2021). Neural stemness contributes to cell tumorigenicity. Cell and Bioscience, 11(1), 21.
Yogi, K., Thorat, R., & Shirsat, N. (2022). *In vivo* tumorigenicity assays using subcutaneous and orthotopic xenografts in immunodeficient mice. Methods in Molecular Biology, 2423, 153–164.
Zavan, B., Michelotto, L., Lancerotto, L., Della Puppa, A., D'Avella, D., Abatangelo, G., Vindigni, V., & Cortivo, R. (2010). Neural potential of a stem cell population in the adipose and cutaneous tissues. Neurological Research, 32(1), 47–54.
Zhang, K., Cui, X., Zhang, B., Song, X., Liu, Q., & Yang, S. (2020). Multipotent stem cells with neural crest stem cells characteristics exist in bovine adipose tissue. Biochemical and Biophysical Research Communications, 522(4), 819–825.