

Proceedings of the 17th International Congress of Histochemistry and Cytochemistry

*August 27-30, 2025
PalaCongressi of Rimini, Italy*

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European Journal of Histochemistry
a journal of functional cytology

ISSN 1121-760X
volume 69/supplement 2
2025

ejh

under the auspices of
the University of Pavia, Italy



Published by PAGEPress, Pavia, Italy

Editorial Office:

PAGEPress s.r.l.

via A. Cavagna Sangiuliani 5, 27100 Pavia, Italy

Phone: +39.0382.1549020 - Fax: +39.0382.1727454

E-mail: info@pagepress.org

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Reg. Tribunale di Pavia n. 289/23.2.1984.

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The European Journal of Histochemistry was founded in 1954 by Maffo Vialli and published till 1979 under the title of *Rivista di Istochimica Normale e Patologica*, from 1980 to 1990 as *Basic and Applied Histochemistry* and in 1991 as *European Journal of Basic and Applied Histochemistry*. It is now published under the auspices of the University of Pavia, Italy.

The European Journal of Histochemistry is the official organ of the Italian Society of Histochemistry and a member of the journal subcommittee of the International Federation of Societies for Histochemistry and Cytochemistry (IFSHC), and has been an influential cytology journal for over 60 years, publishing research articles on functional cytology and histology in animals and plants.

The Journal publishes Original Papers, Technical Reports, Reviews, Brief Reports, Letters to the Editor, Views and Comments, and Book Reviews concerning investigations by histochemical and immunohistochemical methods, and performed with the aid of light, super-resolution and electron microscopy, cytometry and imaging techniques; attention is also given to articles on newly developed or originally applied histochemical and microscopical techniques.

Coverage extends to:

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- cell-cell interaction and molecular trafficking;
- biology of cell development and senescence;
- nerve and muscle cell biology;
- cellular basis of diseases.

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2024 Journal Citation Reports® (Clarivate): 2.1

P77**MEMBRANE SKELETAL PROTEIN, MEMBRANE PALMITOYLATED PROTEIN 6 (MPP6), IN MOUSE CEREBRAL SYNAPSES**

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A membrane skeletal protein, membrane palmitoylated protein 6 (MPP6) that interacts with protein 4.1G in PNS, is also expressed in the CNS. In this study, we investigated the localization of MPP6 and its associated protein complexes in the mouse cerebrum, as well as its effects on behaviour using MPP6 protein-deficient (KO) mice. MPP6 was detected in mouse cerebral lysates and synaptic membrane fractions, where it formed protein complexes with other MPP family members, including MPP1, MPP2, and a signal protein calcium/calmodulin-dependent serine protein kinase (CASK). However, the amounts of these complexes did not differ between KO and wild-type (WT) mice. Immunohistochemistry revealed that MPP6 was localized at synapses throughout the cerebrum, particularly in the postsynaptic regions. Ultrastructural analysis showed that synaptic cleft distances and postsynaptic density thickness were slightly reduced in KO mice compared to WT mice. In the elevated plus-maze test, a KO mouse exhibited unusual behaviour not observed in WT mice, although there was no statistically significant difference in the time spent in the open and closed arms between the two groups. Locomotor activity measurements revealed that KO mice were more active at midnight and less active from morning to noon than WT mice, implying alterations in sleep-wake regulation. These findings suggest that MPP6 plays a role in synaptic function by forming protein complexes with other MPP family members and signal proteins.

P78**THE ROLE OF EXTRACELLULAR VESICLES DERIVED FROM MESENCHYMAL STROMAL CELLS IN MYOFIBER REGENERATION**

A. Longhin¹, V. Gatta², G. Teti², C. Sassoli³, F. Chellini³, A. Tani³, M. Parigi³, R. Garella³, F. Palmieri³, R. Squecco³, M. Mattioli Belmonte⁴, C. Licini⁴, A. La Contana⁴, S. Zecchi Orlandini³, M. Falconi¹

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Traumatic injuries can severely impair skeletal muscle, leading to limited regeneration, muscle loss, fibrosis, and compromised function. Despite the exploration of various therapeutic approaches, no fully effective treatment has yet been established. Mesenchymal stem cells (MSCs) have emerged as a promising strategy for muscle repair, largely due to their paracrine signaling and immunomodulatory properties mediated by extracellular vesicles (EVs). Although the cellular and molecular processes involved in skeletal muscle regeneration are well understood, the specific role of EVs in mediating intercellular communication during myofiber repair remains under active investigation. This study aims to clarify the contribution of MSC-derived EVs to muscle repair and regeneration. EVs were isolated from murine myoblasts using a polyethylene glycol (PEG)-based liquid exchange precipitation protocol. EVs from control myoblasts, differentiated myoblasts, and MSCs were characterized in terms of size, surface markers, and myokine content using electron microscopy, western blotting, and ProQuantum immunoassays. These EVs were subsequently added to cultures of damaged, differentiated myoblasts to assess their regenerative potential. Myotube formation was evaluated via inverted light microscopy, while the expression of key myogenic markers was assessed through western blot analysis. The results confirmed the presence of EVs in both myoblasts and MSCs. The expression of EV markers varied depending on the experimental conditions used to simulate muscle damage. Injured myoblasts released EVs containing inflammatory factors, whereas MSC-derived EVs attenuated the inflammatory environment, enhanced myogenic repair, and promoted muscle regeneration. In conclusion, our findings highlight the critical role of MSC-derived extracellular vesicles in regulating myogenic differentiation and supporting the regeneration of skeletal muscle following injury.

Funding: PRIN 2022 programme (2022P2NAJ; CUP I53D23003030006) (funded by the European Union - Next Generation EU)

P79**AN INNOVATIVE FUNCTIONAL COOKIE MODULATES THE MUCOSA AND MYENTERIC PLEXUS IN THE GUT OF 3xTg-AD MOUSE MODEL OF ALZHEIMER'S DISEASE**

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Neurodegenerative diseases are characterized by the gradual degeneration of the neuronal populations, leading to Alzheimer's disease (AD) with the progressive onset of dementia, making this pathology one of the costliest and deadliest diseases of the current century. Patients with AD frequently exhibit reduced gut microbial diversity, suggesting a significant involvement of gut microbiota in influencing the pathogenesis and progression of AD. It has been proposed that gut dysbiosis, dysfunction of the intestinal epithelial barrier, and vascular deposition of amyloid-beta (A β) in the gut may precede the cerebral deposition of A β in a transgenic mouse

model of AD.¹ A hypocaloric cookie with prebiotic-rich ingredients (red lentils) coated with a multi-strain probiotic (SLAB51®) enriched chocolate² was tested on 3xTg-AD mice, a murine model of AD, to evaluate the protective effects on the mucosa and ENS of the ileum and colon. 8-week-old 3xTg-AD gender-balanced mice were divided into five groups and organized for the supplementation of functional cookies. The morphology of the ileum and colonic wall, the mucus secretion, the intestinal barrier integrity, and neurodegeneration of the myenteric plexus were assessed with histochemical and immunochemical approaches. All the experimental animals showed a well-conserved morphology of the intestinal wall without fibrosis. The SLAB51®, administered alone or with the functional prebiotic enriched cookies, red lentils based appeared to be useful to reduce mucus secretion. The intestinal barrier integrity seems to be enhanced by the cookie supplementation, related to a decrease in inflammatory pathways. Moreover, in the ENS, gliosis was decreased in functional cookies supplemented mice without a clear modulation of enteric cholinergic and nitrergic neurons. This innovative symbiotic cookie could represent an innovative nutritional approach to prevent the onset of AD-related gut alterations and promote healthy aging.

Funding: Next Generation EU - program "MUR-Fondo Promozione e Sviluppo - D.M. 737/2021.

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MITOCHONDRIA-LYSOSOME INTERACTIONS: EMERGING PLAYERS IN GLIOMA

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Despite research efforts, the results obtained in the therapy against glioma have improved but only slightly. For this reason, identifying new targets remains an important and necessary goal. The role of mitochondria, as potential target, has attracted considerable attention in recent times. Mitochondria and lysosomes communicate with each other through specialized membrane contact sites to regulate cellular metabolism and signaling pathways, impacting tumor cell survival and response to therapy¹. Mitochondria dynamics play a crucial role in glioma pathophysiology, contributing to metabolic reprogramming, fast proliferation, invasion and drug resistance. It has been recently demonstrated that the mitochondrial structure and functions are regulated by the TRPML1 lysosomal channel by enabling the calcium flux from lysosomes to mitochondria². To better investigate mitochondria in glioma progression, a potent TRPML1 agonist, ML-SA5 was used to stimulate Ca²⁺ influx into mitochondria in glioma cells. The activation of the lysosomal channel TRPML1 induces the production of mitochondrial reactive oxygen species, hyperpolarization of the mitochondrial membrane and cellular redistribution of these organelles. These changes are also associated with the activation of the mitochondrial enzyme P5Cs, the enhancement in collagen synthesis and increase of glioma migrating abilities. Overall, these

preliminary results suggest that the interaction between lysosomes and mitochondria via TRPML1 represents a promising avenue to target mitochondrial dynamics for glioma therapeutic strategies.

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AN IN VITRO CYTOTOXIC, PROAPOPTOTIC AND ANTITUMOR ACTIVITY ASSESSMENT FOR CERAMIDASE INHIBITORS ON HUMAN LARYNGEAL CANCER CELLS

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Laryngeal cancer is diagnosed approximately 20% of head and neck cancers worldwide¹. Among the potential sphingolipid-based treatment methods for the treatment of these fatal cancers² acid ceramidase inhibition is indicated as a beneficial treatment option³. In this study, the cytotoxic, proapoptotic and antitumor effects of recently developed human acid ceramidase inhibitors ceranib-2 and carmofur were investigated in human laryngeal cancer cells using MTT, Annexin V and colony formation assay. It was found that ceranib-2 and carmofur caused cytotoxicity in monotherapy and in combination at low doses. Proapoptotic effect was detected both in monotherapy and in combination therapy at the IC₅₀ concentrations (54.18, 51.94 and 27.15 µM for carmofur, ceranib-2 and their combination, respectively) after 24 h of exposure. The highest apoptosis rate was detected after the combination treatment. Inhibition of colony formation, which indicates antitumor effect, was most clearly seen in the combination group, and a significant decrease in colony number was also recorded in the monotherapy groups.

This study was supported by Scientific and Technological Research Council of Turkey (TUBITAK) under the Grant Number 123Z615. The authors thank to TUBITAK for their supports.

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