

## The pivotal role of astrocytes and their interaction with endothelial cells in blood-brain barrier formation and function

JACOPO J.V. BRANCA, FERDINANDO PATERNOSTRO, MASSIMO GULISANO, ALESSANDRA PACINI

Dept. Experimental and Clinical Medicine, Anatomy and Histology Section, University of Firenze, Firenze, Italy

The blood-brain barrier (BBB) is an essential cellular structure owning the role of strictly select the molecules that can enter the brain parenchyma, thus maintaining the homeostasis within the central nervous system keeping out toxic substances [1]. In order to comply with this physiological function, brain endothelial cells are closely sealed to each other by tight junctions (TJ). Moreover, other two cellular types, astrocytes and pericytes, key components of the neuro-vascular unit [2], are required to generate a structurally and functionally complete BBB.

In order to study the structural features of the BBB, various *in vitro* models have been used, even if little attention has been paid to the correct subcellular distribution of TJs. In order to study the morphological and molecular patterns of these proteins, in the present research we used two rat cell lines, brain endothelial cells (RBE4) and astrocytes (DITNC1), in order to highlight their role in the establishment of an intact BBB.

The cells were cultured from 3 to 7 days in different conditions. The RBE4 cells, when cultured alone, were stimulated with 1 and 5  $\mu\text{M}$  retinoic acid (RA), a well known molecule synthesized by astrocytes and pivotal for BBB development [3,4]. The RBE4 were cultured with the DITNC1, both through the interposition of a transwell or in direct contact. The expression and the correct localization of claudin 5 was examined.

The western blotting analysis clearly showed that, in the absence of RA, RBE4 express claudin 5 but the immunofluorescent analysis failed to evidence its subcellular distribution. On the other hand, when cells were treated with RA, immunofluorescence shows a scattered spot distribution, very different from the normal localization on the cellular perimeter.

Interestingly, only when the RBE4 cells were co-cultured with DITNC1 the claudin 5 was correctly expressed and distributed alongside the perimetral surface of the endothelial cells, distinctly in the contact co-cultures.

Even if the RBE4 cell line provides a validated *in vitro* BBB model, the presence of astrocytes plays a key role both in inducing the expression of the tight junction proteins such as claudin 5, and the correct distribution in the perimeter of the cells in order to seal each other, thus highlighting the capacity of DITNC1 astrocyte in the contribution of BBB components.

### References

- [1] Kadry H, Noorani B, Cucullo L. A blood–brain barrier overview on structure, function, impairment, and biomarkers of integrity. *Fluids Barriers CNS* 2020;17:69. <https://doi.org/10.1186/s12987-020-00230-3>.
- [2] Ahmad AA, Taboada CB, Gassmann M, Ogunshola OO. Astrocytes and Pericytes Differentially Modulate Blood–Brain Barrier Characteristics during Development and Hypoxic Insult. *J Cereb Blood Flow Metab* 2011;31:693–705. <https://doi.org/10.1038/jcbfm.2010.148>.
- [3] Shearer KD, Fragoso YD, Clagett-Dame M, McCaffery PJ. Astrocytes as a regulated source of retinoic acid for the brain. *Glia* 2012;60:1964–76. <https://doi.org/10.1002/glia.22412>.
- [4] Mizee MR, Wooldrik D, Lakeman KAM, Van Het Hof B, Drexhage JAR, Geerts D, et al. Retinoic Acid Induces Blood–Brain Barrier Development. *J Neurosci* 2013;33:1660–71. <https://doi.org/10.1523/JNEUROSCI.1338-12.2013>.

**Keywords:** astrocytes; brain endothelial cells; blood-brain barrier; tight junctions; ZO-1; claudin 5

# IJAE

## Italian Journal of Anatomy and Embryology

---

Official Organ of the Italian Society  
of Anatomy and Histology

**76° CONGRESSO  
della Società Italiana di Anatomia e Istologia**

Modena, 11-13 settembre 2023

**76<sup>TH</sup> MEETING  
of the Italian Society of Anatomy and Histology**

Modena, 11-13 September 2023

Vol. 127  
N. 1

**2023**  
Supplement

ISSN 1122-6714

  
FIRENZE  
UNIVERSITY  
PRESS

Founded by Giulio Chiarugi in 1901

**Editor-in-Chief**

Domenico Ribatti, University of Bari, Italy

**Managing Editor**

Ferdinando Paternostro, University of Firenze, Italy

**Editorial Board**

Gianfranco Alpini, Indiana University, USA  
Giuseppe Anastasi, University of Messina, Italy  
Juan Arechaga, University of Leioa, Spagna  
Erich Brenner, University of Innsbruck, Austria  
Marina Bentivoglio, University of Verona, Italy  
Anca M. Cimpean, University of Timisoara, Romania  
Lucio I. Cocco, University of Bologna, Italy  
Bruna Corradetti, Houston Methodist Hospital, USA  
Raffaele De Caro, University of Padova, Italy  
Valentin Djonov, University of Berne, Switzerland  
Amelio Dolfi, University of Pisa, Italy  
Roberto di Primio, University of Ancona, Italy  
Gustavo Egea, University of Barcellona, Spagna  
Antonio Filippini, University “La Sapienza”, Roma, Italy  
Eugenio Gaudio, University of Roma “La Sapienza”, Italy  
Paolo Mazzarello, University of Pavia, Italy  
Thimios Mitsiadis, University of Zurich, Switzerland  
John H. Martin, City University New York, USA  
Paolo Mignatti, New York University, USA  
Stefania Montagnani, University of Napoli, Italy  
Michele Papa, University of Napoli, Italia  
Jeroen Pasterkamp, University of Utrecht, The Netherlands  
Francesco Pezzella, University of Oxford, UK  
Marco Presta, University of Brescia, Italy  
Jose Sañudo, University of Madrid, Spain  
Gigliola Sica, University “Cattolica”, Roma, Italy  
Michail Sitkovsky, Harvard University, Boston, USA  
Carlo Tacchetti, University “Vita-Salute San Raffaele”, Milano, Italy  
Sandra Zecchi, University of Firenze, Italy

**Past-Editors**

I. Fazzari; E. Allara; G.C. Balboni; E. Brizzi; G. Gheri; P. Romagnoli

Journal e-mail: [ijae@unifi.it](mailto:ijae@unifi.it) – Web site: <http://www.fupress.com/ijae>

---



<b>Commemorazione della nascita di Gabriele Falloppio (Modena, 1523)</b>	<b>5</b>
<b>Invited lectures</b>	<b>9</b>
<b>Comunicazioni orali</b>	<b>13</b>
Neuroscienze	15
Cellule staminali, dalla biologia cellulare alle prospettive terapeutiche	41
Dalla morfologia alla patologia molecolare	51
Istogenesi, funzioni e disfunzioni dell'apparato muscolo-scheletrico	71
Tecnologie innovative, modelli 3D e organoidi per lo studio di patologie e drug discovery	81
Invecchiamento e patologie degenerative	93
Meccanismi molecolari di controllo della crescita cellulare	101
Anatomia e movimento	111
Morfologia, attività settoria e strategie didattiche	119
Medicina rigenerativa	135
Tessuti epiteliali e connettivi. Transizione epitelio mesenchima nell'organogenesi nella carcinogenesi	145
<b>Poster</b>	<b>159</b>
Neuroscienze	161
Cellule staminali, dalla biologia cellulare alle prospettive terapeutiche	183
Dalla morfologia alla patologia molecolare	193
Istogenesi, funzioni e disfunzioni dell'apparato muscolo-scheletrico	219
Tecnologie innovative, modelli 3D e organoidi per lo studio di patologie e drug discovery	237
Invecchiamento e patologie degenerative	253
Meccanismi molecolari di controllo della crescita cellulare	263
Anatomia e movimento	281
Morfologia, attività settoria e strategie didattiche	295
Medicina rigenerativa	305
Tessuti epiteliali e connettivi. Transizione epitelio mesenchima nell'organogenesi nella carcinogenesi	319
<b>Verbale della seduta amministrativa e dell'assemblea generale dei soci SIAI, 2022</b>	<b>329</b>