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

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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 μM retinoic acid (RA), a well known molecule synthetized 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.

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