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Expression of L1 Cell Adhesion Molecule (L1CAM) in extracellular vesicles in the human spinal cord during development

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Abstract. – OBJECTIVE: L1 cell adhesion molecule (L1CAM) is a glycoprotein characterized by three components: an extracellular region, a transmembrane segment, and a cytoplasmic tail. L1CAM is expressed in multiple human cells, including neurons. The neural cell adhesion molecule L1 has been implicated in a variety of neurologic processes, including neuritogenesis and cerebellar cell migration. The presence of L1CAM on the surface of nerve cells allows the adhesion of neurons among them. Furthermore, when it is bound to itself or to other proteins, L1-CAM induces signals inside the cell. The aim of this work was to study L1CAM expression in the human spinal cord during development, at different gestational ages, through immunohistochemistry.

MATERIALS AND METHODS: Immunohistochemical analysis for L1CAM was performed in five human spinal cord samples, including three embryos and two fetuses of different gestational ages, ranging from 8 to 12 weeks.

RESULTS: L1CAM expression was detected in all 5 spinal cords examined in this study. The adhesion molecule was found in the vast majority of cells. The highest levels of immunoreactivity for L1CAM were detected at the periphery of the developing organs, in the spinal cord zones occupied by sensory and motor fibers. In the alar and basal columns, immunoreactivity for L1CAM was characterized by a reticular pattern, being mainly expressed in axons. Strong reactivity of L1CAM was also found in extracellular vesicles. This extracellular localization might indicate the ability of L1CAM to mediate the transduction of extracellular signals that support axon outgrowth.

CONCLUSIONS: The high reactivity of L1cam in the axons of developing neurons in the fetal spinal cord confirms previous studies on the ability of L1CAM to promote axon sprouting and branching in the developing nervous system. In this work, a new actor is reported to have a role in the complex field of human spinal cord development: L1CAM, whose expression is highly found in the developing neuronal and glial precursors.

Key Words:
L1CAM, Nervous system, Spinal cord, Development, Extracellular vesicles, Immunohistochemistry.

Introduction

L1 cell adhesion molecule (L1CAM) is a transmembrane protein expressed in multiple human

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cells, including neurons[1], Schwann cells[2], and renal collecting duct cells[3]. Recently, a pro-angiogenic role has been attributed to L1CAM produced by endothelial cells of tumor-associated vessels, supporting its potential as a therapeutic target[4,5]. L1CAM is a member of the L1 family, which includes also: CHL1 (Close Homolog of L1), NrCAM (neuronal cell adhesion molecule), and Neurofascin[6], all included in the immunoglobulin superfamily of cells adhesion molecules (Igcam)[7]. L1CAM is a glycoprotein characterized by three components: an extracellular region, a transmembrane segment, and a cytoplasmic tail[8]. The cytoplasmic tail can interact with multiple cytoskeletal proteins, including ankyrin, actin, and spectrin[9,10]. Moreover, the intracellular domain of L1CAM may be released and translocated into the nucleus[11]. The extracellular part of the protein may be processed by metalloproteinases and released into the extracellular space[12]. Interestingly, prenatal intra-peritoneal maternal administration of thymosin beta-4 (TB4), a small peptide with metal coordination property[13], has been shown to improve fetal development[14], suggesting a possible role for other glycoproteins, such as L1CAM, when administered during pregnancy. Extracellular L1CAM has been described in neuron-derived extracellular vesicles (NEVs)[15,16], including exosomes and microvesicles, which are thought to play a critical role in the central nervous system (CNS) development[17].

Originally detected in the post-mitotic neurons in the cerebral cortex[18], nowadays L1CAM is considered an important element in the developmental processes of the nervous system[19]. During development, exosomal L1CAM facilitates neuronal migration, differentiation, and axon guidance[20]. L1CAM expression has been recently reported in chandelier cells, a cell population that innervates pyramidal neurons (PYN), establishing and maintaining PYN innervation[21]. Moreover, L1CAM expression has been associated with epithelial-mesenchymal transition (EMT), a process involved both in development and in metastasis[22]. Mutations in the L1CAM gene are associated with multiple CNS malformations, collectively known as CRASH (Corpus callosum hypoplasia, Retardation, Adducted thumbs, Spastic paraplegia, and Hydrocephalus) syndrome[23] or, more recently, as L1 syndrome[24,25].

This work was aimed at analyzing, at the immunohistochemical level, L1CAM expression in the human spinal cord during the early phases of gestation, to shed light on the role of L1CAM during the initial spinal cord development, and morphogenesis.

Materials and Methods

Five human embryos were obtained during autopsy, at the Division of Pathology of the University Hospital Agency of Cagliari. Fetal ages were estimated from the gestational age, ranging from 8 up to 16 weeks.

Tissue samples were fixed in 10% phosphate-buffered formalin and embedded in paraffin (according to conventional techniques). Three micron-thick sections were stained with hematoxylin and eosin (H&E) and immunostained with a mouse monoclonal antibody (Sigma-Aldrich, clone UJ127) against L1CAM. The ultra-View Universal DAB Detection Kit was used for detecting primary antibodies. In brief, immunostaining was performed with an anti-L1CAM mouse monoclonal antibody (mouse IgG1 isotype), derived from the hybridoma UJ127.11. Slides were incubated for 20 minutes at room temperature at 1:100 dilution of the monoclonal anti-L1CAM primary antibody. Nervous structures were utilized as internal positive controls. As appropriate negative controls, spinal cord sections were processed omitting the primary antibody for L1CAM.

To obtain a semiquantitative evaluation of the degree of immunoreactivity for L1CAM, the following semiquantitative scoring system was applied: 0 = no reactivity; 1 = < 50% of immunoreactive cells; 2 = > 50 and < 75% of immunoreactive cells; 3 = > 75% of cells immunostained for L1CAM.

From clinical assessment, no evidence of genetic disease or malformations was present in the two embryos and three fetuses analyzed in this study.

Results

L1CAM expression was detected in all the 5 spinal cords examined in this study being expressed in the vast majority of cells (>75%; score 3). In all immunostained spinal cords, the highest levels of immunoreactivity for L1CAM were detected at the periphery of the developing spinal cords, where sensory and motor fibers are more frequent (Figures 1, 2). In the alar and basal columns, immunoreactivity for L1CAM was characterized by a reticular pattern, given to the high-

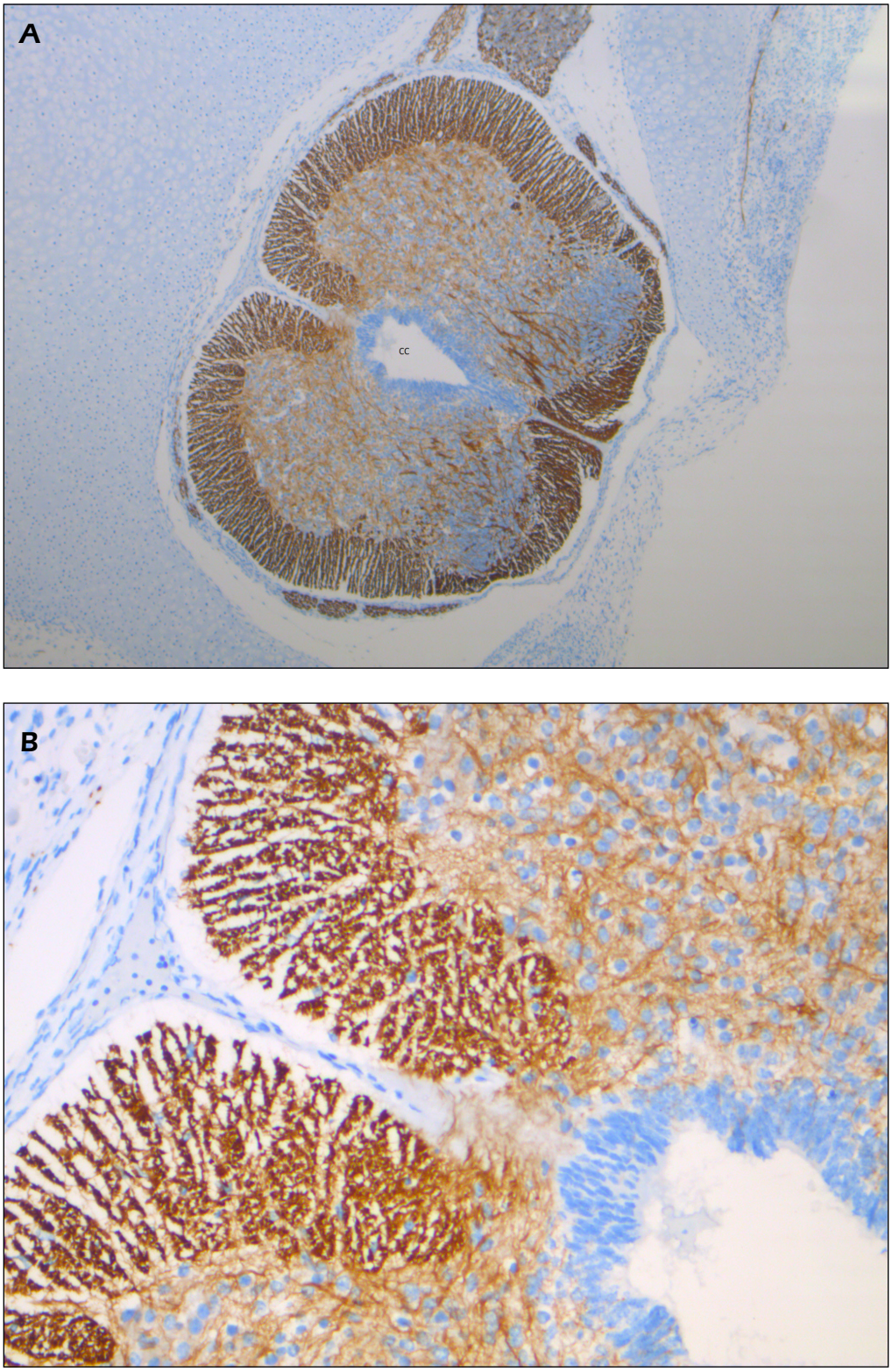

Figure 1. Spinal cord, 11 weeks of gestation. **A**, Immunostaining for L1CAM shows a strong immunoreactivity in the peripheral zones (marginal layers). In the intermediate layer and in the alar and basal plates, L1CAM shows a reticular pattern. No immunoreactivity is observed in the stem/progenitor cells of the ventricular layer surrounding the central canal (CC) of the spinal cord. Original magnification (50X). **B**, At higher power, L1CAM immunostaining shows a reticular pattern in the intermediate layer of the spinal cord, being mainly expressed by axons. Nuclei of neuronal and glial precursors are negative (100 X).

er L1CAM expression in axons (Figure 1A). No reactivity for L1CAM was observed in the stem/precursor glial and neuronal cells located near the central canal of the spinal cord (Figure 1B). In the longitudinal section of the spinal cord, immunoreactivity for L1CAM was stronger in the marginal layers (Figure 2A). In the intermediate areas, L1CAM appeared mainly expressed in the axons of the radial glia, extending from the marginal layers toward the central zones of the spinal cord (Figure 2B).

In all five spinal cords immunostained for L1CAM, the protein appeared mainly stored in the extracellular spaces, with localization inside exosomes and membrane-bound extracellular vesicles (EVs) (Figure 3A-3B). At higher power, EVs appeared of different size and shape, being localized in strict contact with the developing neuronal-glial progenitor cells (Figure 4A-4B)

No significant interindividual variability was found among the two embryos and three fetuses, regarding immunoreactivity for L1CAM in the developing spinal cord. The degree of L1CAM expression was not strictly correlated with the gestational age of embryos and fetuses: different degrees of immunoreactivity were detected in subjects with the same gestational age, whereas subjects with different gestational ages showed similar patterns of L1CAM expression.

Discussion

L1CAM is a cell adhesion molecule that plays a critical role in the development of the neuronal network, by regulating the formation of axon fascicles and promoting neurite outgrowth, through interaction with a wide spectrum of other cell adhesion molecules[26]. L1CAM functionally interacts with beta-1-integrins to potentiate neuronal migration through the extracellular matrix[27]. Our paper confirms the previous reports on the essential role played by L1CAM in human nervous system development[28,29]. In particular, our findings indicate a major role for L1CAM in the early phases of the development of the spinal cord.

A finding emerging from our study which deserves some consideration is the prominent extracellular expression of L1CAM in the developing spinal cord. In our study, L1CAM was not restricted to axons but was mainly observed inside membrane-bound vesicles localized among the neuronal and glial progenitors. Extracellular vesicles (EVs) include various types of membrane-bound structures released by cells, called exosomes, microvesicles, microparticles, ectosomes, or endosomes[30]. EVs transport proteins, microRNAs, messenger RNAs, and lipids among the cellular and the extracellular compartments[31]. Hem oxygenase-1 is associated with the vast majority of EVs in human biofluids[32]. Our data reveal that L1CAM is highly expressed in EVs in the developing spinal cord. This extracellular localization of L1CAM might be caused by the proteolytic cleavage of L1CAM and or exosome formation, resulting in extracellular soluble forms of this adhesion molecule. The precise localization of L1-CAM is important for establishing proper cell-cell contacts in neural circuits calling up the role in driving regulation and axon fasciculation, neuron migration, dendrite morphology, and synaptic plasticity[33]. Moreover, L1-CAM has been shown to regulate axonal and dendritic building formation[34].

In this context, a growing number of studies[35] have started considering exosome and cubosome as new strategies for drug delivery.

Our findings on the prevalent extracellular localization of L1CAM in the developing human spinal cord might shed light on the ability of this molecule to play a different role during human spinal cord development.

Another interesting finding is the absence of L1CAM expression in the premitotic neurons lining the spinal canal (Figure 3A). This finding suggests that, during spinal cord development, L1CAM expression characterizes the differentiation of stem/precursor cells toward a neuronal or glial fate, and confirms previous studies[36], in which L1CAM expression was restricted to the post-mitotic neurons in the central nervous system.

The observation of L1CAM expression in EVs in the spinal cord confirms previous studies[37] evidencing that many of the different functions of L1CAM are determined by homophilic and heterophilic interactions with other cell surface receptors. An emerging concept is that neural cell adhesion molecules (CAMs), including L1CAM, may act as coreceptors to assist in intracellular signal transduction and to provide the cytoskeletal link necessary for cell and growth cone motility, important for neuronal migration and guidance of the axons during development[38,39].

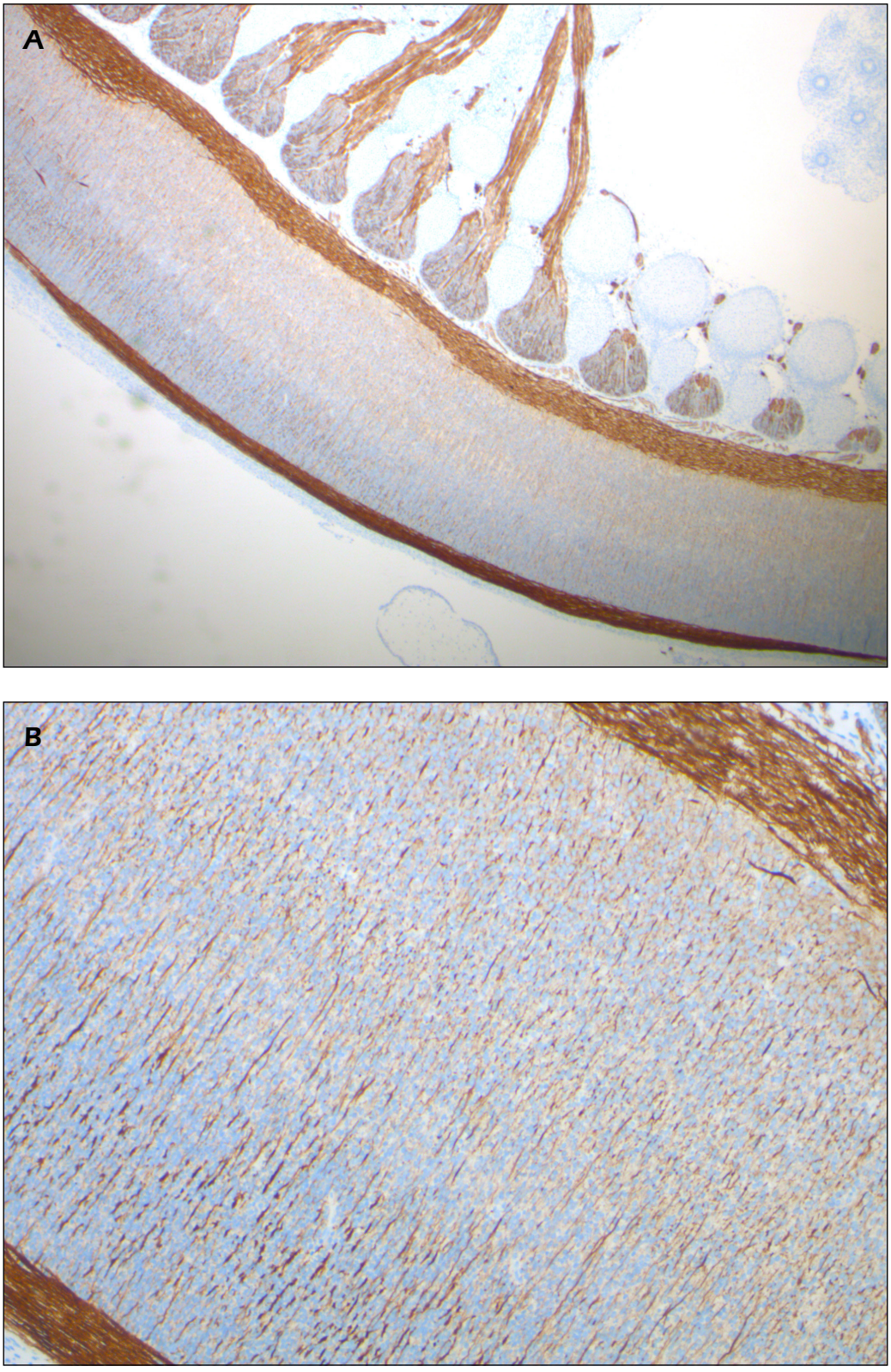

Figure 2. Spinal cord, 8 weeks of gestation. **A**, Longitudinal section of the spinal cord. Immunostaining for L1CAM shows strong immunoreactivity in the peripheral zone (marginal layers). L1CAM is also expressed in the spinal ganglia and in the nerve root (25X). **B**, At higher power, in the intermediate areas of the spinal cord, L1CAM marks axons of the radial glia, the principal organizer of the spinal cord development, extending from the marginal layers toward the central zones of the spinal cord (100X).

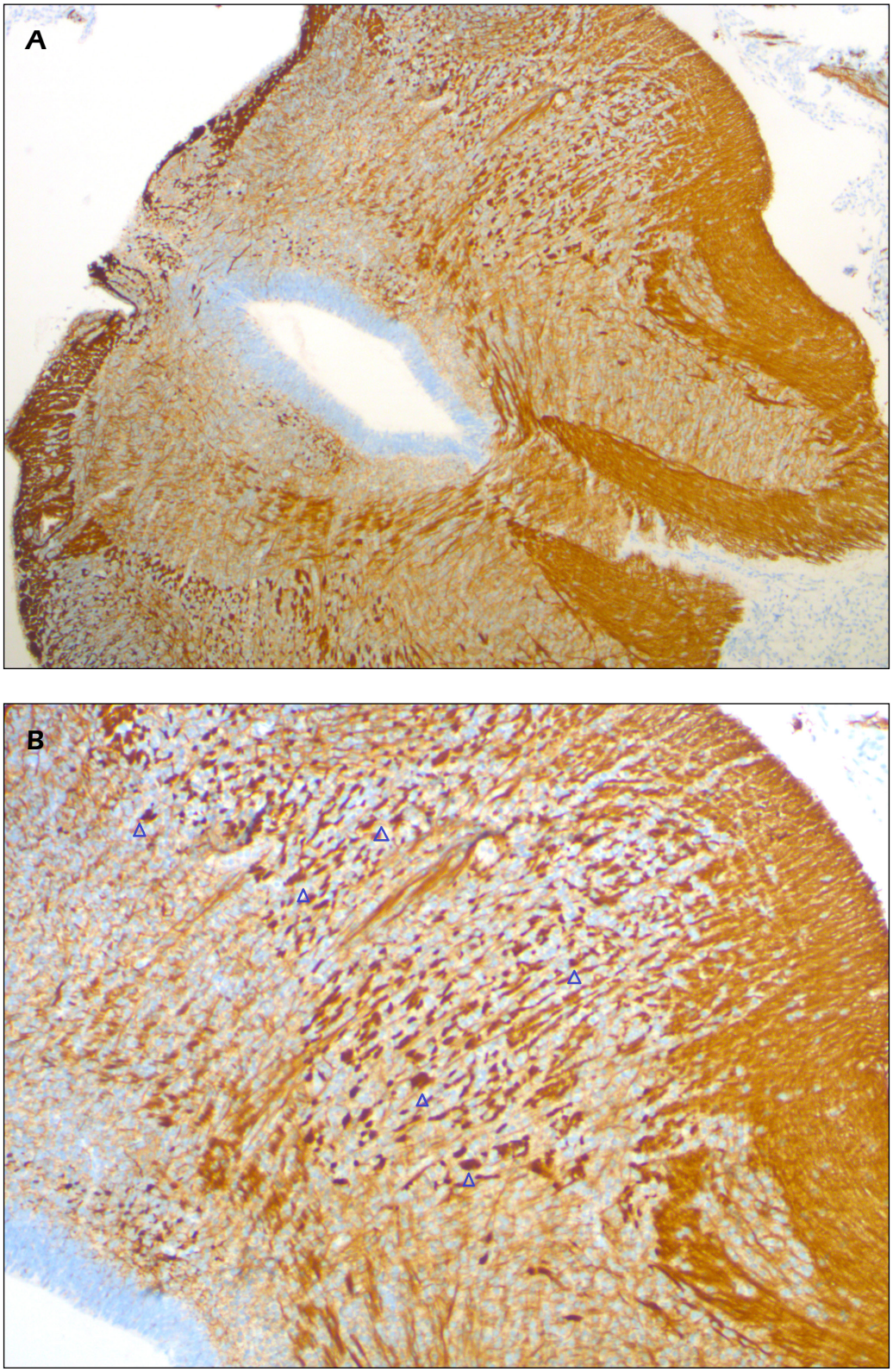

Figure 3. Spinal cord, 10 weeks of gestation. **A**, Radial glial cells in the human fetal spinal cord, immunostained with the antibody against L1CAM, appear strongly positive. The glial fibers run from the alar plate zone and the basal plate zone across the intermediate layer zone (50X). **B**, At higher power, L1CAM-negative migrating neurons are distinguishable from the L1CAM-positive axons. L1CAM is also expressed extracellularly, inside extracellular vesicles (EVs) (arrows, 100X).

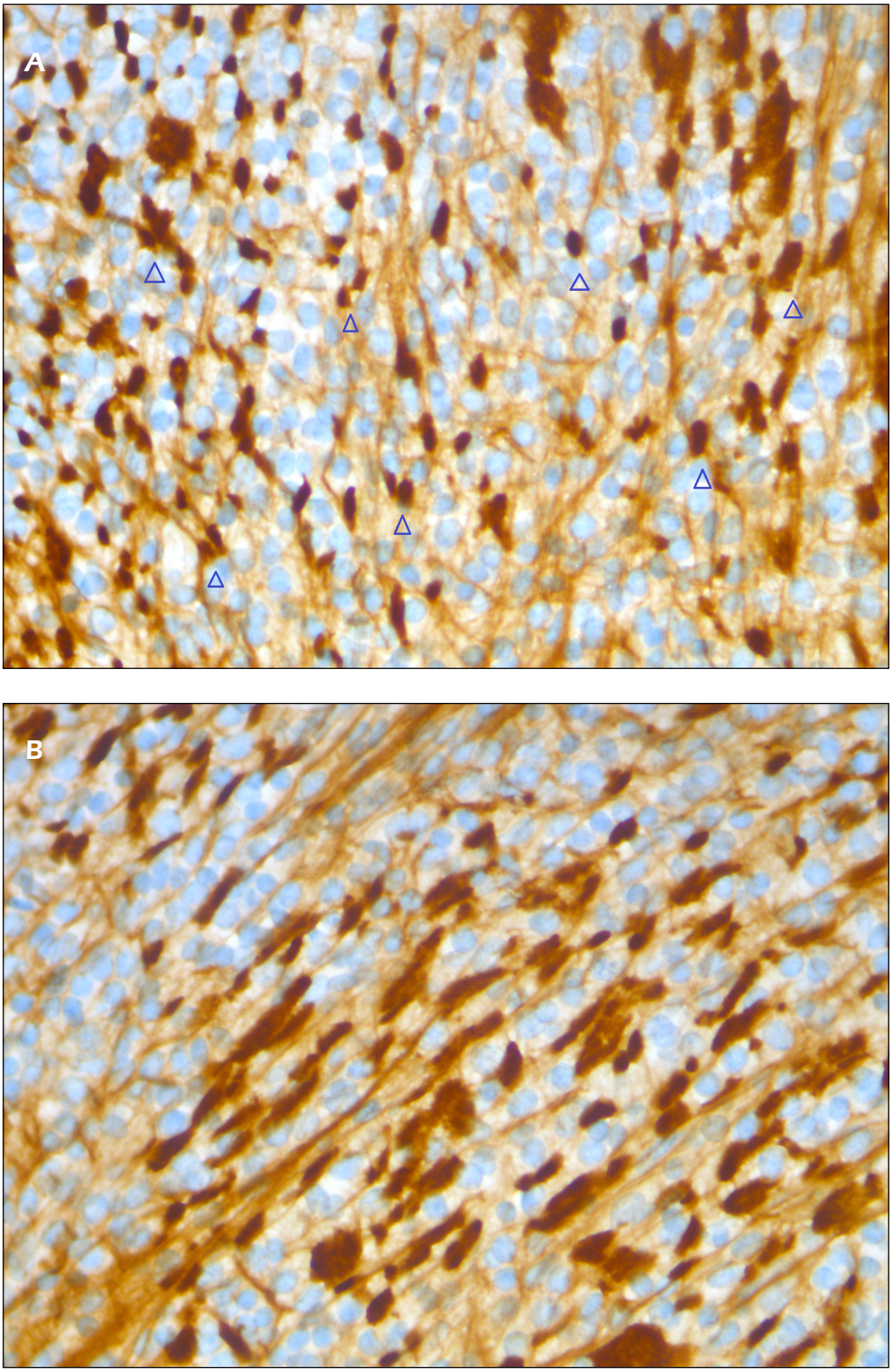

Figure 4. Spinal cord, 10 weeks of gestation. **A**, Neuronal precursor cells release L1CAM inside extracellular vesicles (EVs) which are localized in the interneuronal spaces (arrows, 400X). **B**, L1CAM-reactive extracellular vesicles show different sizes and shapes. In some fields, EVs represent the principal localization of L1CAM (400X).

Conclusions

In this study, we report L1CAM as an important player in the complex ontogenetic process of human spinal cord development. In all the developing spinal cords analyzed in this study, the expression of this transmembrane adhesion molecule was not restricted to developing neuron and glial precursor. L1CAM was also found, in some cases predominantly, inside extracellular vesicles and exosomes. This localization in EVs represents an interesting finding that deserves some consideration. Being consistent with previous literature[40], our findings confirm previous hypotheses on the ability of L1CAM to mediate the transduction of extracellular signals that support axon outgrowth. Our finding of an extracellular localization of L1CAM supports the hypothesis of a major role of this adhesion molecule in nervous system development, favoring the interactions between cells, axons, and the substrate[41]. The high reactivity of L1CAM in the axons of developing neurons in the fetal spinal cord confirms previous studies on the ability of L1CAM to promote axon sprouting and branching in the developing nervous system[42]. In sum, these findings suggest a link between L1CAM and the development of the SNC. Should our data be replicated in further studies, L1CAM might become a promising therapeutic target. Complexity and heterogeneity are frequently present during the development of normal and pathological mechanisms and biological systems of communication like microbiome and staminal cells start to be considered[43].

Conflict of Interest

The authors declared that they have no competing interests.

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Ethical Approval

All procedures were performed according to ethical national standards of the responsible committee on human experimentation and approved by the Ethic Human Studies Committee of the University Medical Center of Cagliari (N. PG/2020/10914).

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