SHORT COMMUNICATION

Ultrastructural imaging reveals vascular remodeling in migraine patients

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Abstract

Migraine is a neurological disorder and one of the most common pain conditions worldwide. Despite its prevalence, the basic biology and underlying mechanisms contributing to the development of migraine are still poorly understood. It is still unclear, for instance, whether the vasculature, both extra and intracranial, plays a significant role in the generation of migraine pain. Neuroimaging data, indeed, have reported conflicting results on blood vessels abnormalities like vasodilation, while functional studies suggest that vessels dysfunction may extend beyond vasodilation. Here we combined light and electron microscopy imaging to investigate the fine structure of superficial temporal (STA) and occipital arteries (OA) from patients that underwent minimally invasive surgery for migraine. Using optical microscopy, we observed that both STA and OA vessels showed marked endothelial thickening and internal elastic lamina fragmentation. In the muscular layer, we found profound shape changes of vascular smooth muscle cells (VSMCs), abundant extracellular matrix, and the presence of clear extracellular vacuoles. The electron microscopy analysis confirmed putative VSMCs infiltrated within the intima layer and revealed a consistent shifting of VSMCs from contractile to a synthetically active phenotype. We also report the presence of (i) abundant extracellular vacuoles filled with fine granular material and membranes, (ii) multilamellar structures, (iii) endosome-like organelles, and (iv) bona fide extracellular vesicles in the matrix space surrounding synthetically active cells. As both the endothelial layer and VSMCs coordinate a variety of vascular functions, these results suggest that a significant vascular remodeling is occurring in STA and OA of migraine patients. Thus, this phenomenon may represent an important target for future investigation designed toward the development of new therapeutic approaches.

Keywords Migraine disorders · Pain · Phenotypic switching · Vascular remodeling · Microscopy · Vascular smooth muscle cell

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Introduction

Migraine is a complex neurological disorder with a strong genetic component and is one of the most common pain conditions worldwide (Ashina et al. 2021; Eigenbrodt et al. 2021). There are rare monogenic forms of migraine, as well as more common polygenic forms. Research into the genes involved in both types has provided insights into the role of both neural and vascular pathways (Sutherland et al. 2019). Numerous studies have also indicated that migraineurs have an increased risk of vascular diseases (Cavestro et al. 2021). For example, an association between migraine and ischemic stroke was earliest recognized (Øie et al. 2020). Other studies regarding arterial function in migraine pathogenesis have shown a greater stiffness or impaired compliance of the arterial system in migraine patients (Ripa et al. 2015). However,

the role played by the vasculature in migraine pathogenesis still remains controversial (Sacco et al. 2013; Mason and Russo 2018). The widely accepted vascular theory, indeed, based on the vasodilation of cerebral and meningeal arterial as the primary migraine trigger, has been recently refuted by a growing body of research.

Amin et al. (2013), for example, based on their negative correlation between migraine pain dilation of extracranial arteries, concluded that vasodilation does not primarily cause migraine symptoms.

While vasodilation itself may not contribute to migraine, it remains possible that vessels play a role in migraine even in the absence of vasodilation. Therefore, it appears that mechanisms underlying the association between migraine and altered arterial function are likely to be more complex and may involve both structural and functional changes in the arterial wall.

Blood vessels are dynamic structures constantly subjected to intense remodeling under different conditions. At the microscopic level, arteries and arterioles consist of an endothelium and basement membrane lining the inner vessel wall (tunica intima), a smooth muscle layer (tunica media), which mediates contraction and dilation, and a connective tissue layer (tunica adventitia) in contact with the surrounding tissue. The artery is innervated in two main layers: within the tunica media (myoneural synapses), and in the adventitial layer (sensory nerve endings).

Vascular smooth muscle cells (VSMCs) in the tunica media are essential for good performance of the vasculature (Rensen et al. 2007; Lacolley et al. 2012).

They regulate vascular tone primarily in response to sympathetic nervous system innervation (e.g., adrenergic receptor activation), nitric oxide (NO), as well as the local synthesis, uptake, and release of 5-HT. Many vascular diseases show microscopic abnormalities in this cell layer (Eid et al. 2012; Kapustin and Shanahan 2016; Petsophonsakul et al. 2019). For example, the shift of smooth muscle cell phenotype from contractile to synthetically active is one of the first signs of vascular injury. Under pathological conditions, VSMCs can dedifferentiate into a synthetically active phenotype, starting secretion of extracellular vesicles, cell proliferation, and migration to repair injury (Petsophonsakul et al. 2019). At the molecular level, this switching is regulated by different mechanisms, including autophagic activation (Salabei and Hill 2015). In addition to smooth muscle cells, endothelial cells might be strongly involved as well in the pathophysiology of migraine. These cells directly contact the circulating blood and also control vessel function. Endothelial cells are involved in the regulation of cell–cell barrier maintenance and, among others, vascular tone, vascular remodeling, immune surveillance, blood coagulation, and nutrient uptake. It is therefore clear that arteries do not simply provide a conduit for the movement of blood that can constrict and dilate, but rather they coordinate a much more intricate signaling between multiple cell types and in a bidirectional way with the nervous system. Thus, we could argue that dysregulation of any part of this specific vascular architecture and signaling process may contribute to migraine pathology (Jacobs and Dussor 2016).

Migraine surgery has been widely accepted as an effective surgical solution for chronic headaches refractory to medical and conservative treatment (Omranifard et al. 2016; Gfrerer et al. 2018). Four main trigger zones of migraine are amenable to surgical decompression: frontal (site I: supraorbital and supratrochlear nerves), temporal (site II: zygomatic-temporal branch of the trigeminal nerve), endonasal (site III: trigeminal end branches), and occipital (site IV: great occipital nerve) (Bertozzi et al. 2018).

Modern neuroimaging techniques, although very sensitive and informative, do not provide sufficient resolution to detect microscopic vessel wall alterations. Here, we combined light and electron microscopy imaging on fresh biopsies of the superficial temporal artery (STA) and occipital artery (OA) obtained from a cohort of patients that underwent minimally invasive surgery (MIS) to investigate vessel alterations and provide new evidence in support of the theory of a vasculature role in migraine pathology.

Materials and methods

The study was conducted in compliance with the Declaration of Helsinki and the Guidelines for Good Clinical Practice; all patients provided written informed consent. Fifteen patients (six male, nine female, mean age 43.5 years, range 37–50 years) were subjected to MIS for migraine (Gfrerer et al. 2018). Fifteen biopsies, ten of the STAs and five from OAs, were collected under the surgical procedure and immediately processed for optical and ultramicroscopic analysis.

Light and electron microscopy

All samples were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, postfixed in 1% osmium tetroxide, stained en bloc with 1% aqueous uranyl acetate, and dehydrated in graded alcohols and propylene oxide. Then samples were embedded in an Epoxy resin mixture and polymerized for 2 days at 60 °C. For light microscopy, 200 nm semithin sections were cut until the complete three layers of the vessel were detected and stained with 1% toluidine blue. Fifty-nanometer ultrathin sections were counterstained with 5% uranyl acetate in 50% ethanol and lead citrate. Electron micrographs from two levels of sectioning were acquired with a Hitachi HT77800 120 kV electron microscope (Hitachi, Tokyo, Japan) operating at 100 kV, equipped with a Megaview G3 digital camera and Radius software (EMSIS,

Muenster, Germany, version 2.0) using the snapshot and the multiple image alignment (MIA) montage tools.

Results and discussion

Light microscopy of STA and OA

Ten STA and five OA fresh biopsies from patients affected by migraine (median age of 46) were collected during a MIS procedure and immediately processed for light and electron microscopy analysis. In normal muscular arteries, the tunica intima (endothelial layer) consists of a single layer of endothelial cells surrounded by a connective tissue basement membrane with elastic fibers. The middle layer, the tunica media, is primarily composed of smooth muscle and is usually the thickest layer. We first analyzed 200 nm semithin sections stained with 1% toluidine blue of all STA and OA samples. Toluidine blue (TB) is a basic thiazine metachromatic dye with high affinity for acidic tissue components,

thereby staining tissues rich in DNA and RNA. In addition, being a cationic dye, TB staining visualizes well proteoglycans in the extracellular matrix (Sridharan and Shankar 2012). Alterations in the morphological structure of the vessel wall were observed in all STA and OA samples analyzed. The endothelial layer forms a continuous sheet of flattened and slightly polygonal cells around the lumen of the vessel, which appear narrowed and filled with red blood cells (RBCs) (Fig. 1a, b, e, f). Interestingly, we report intimal hyperplasia (Fig. 1a–c), fragmentation of the internal elastic lamina (IEL) with infiltration of polygonal cells with pale nuclei (Figs. 1c, d, 2b, 4a). Intimal hyperplasia was variable in its extent but present in all samples analyzed, as well as fragmentation of the IEL. These data are suggestive of an active process of vascular remodeling, which has already been reported occurring in distinct vascular pathologies (Renna et al. 2013; Kapustin and Shanahan 2016). The tunica media or muscular layer normally consists of a thick layer of concentrically arranged smooth muscle fibers. In STA and OA biopsies, we observed marked irregularity in

Fig. 1 Light microscopy of toluidine blue-stained 500 nm semithin sections from a representative superficial temporal artery biopsy from a female patient (#1). **a** Low-magnification toluidine blue-stained STA (magnification ×10). **b** Same image as in (**a**) with the three vessel layers depicted with different colors (magnification ×10), internal elastic lamina (IEL). **c** Intimal to tunica media border with internal elastic lamina (IEL) showing marked thickening (magnification ×20). **d** Inset box of image (**c**) showing internal elastic lamina (IEL) fragmentation (black arrowheads) and infiltrating polygonal cells (black arrows), vascular smooth muscle cells (VSMCs) magnification ×40. **e** Lumen of the vessel filled with erythrocytes (BRCs) and endothelial lining (magnification ×20). **f** Inset box of the image in (**e**) showing endothelial cells (EC, black arrowheads) and several polygonal cells with pale cytoplasm and heterochromatin poor nuclei underneath the endothelial layer (black arrows) (magnification ×40), red blood cells (RBCs). Scale bars: 200 μm (**a**, **b**), 50 μm (**c**, **e**), 20 μm (**d**, **f**)
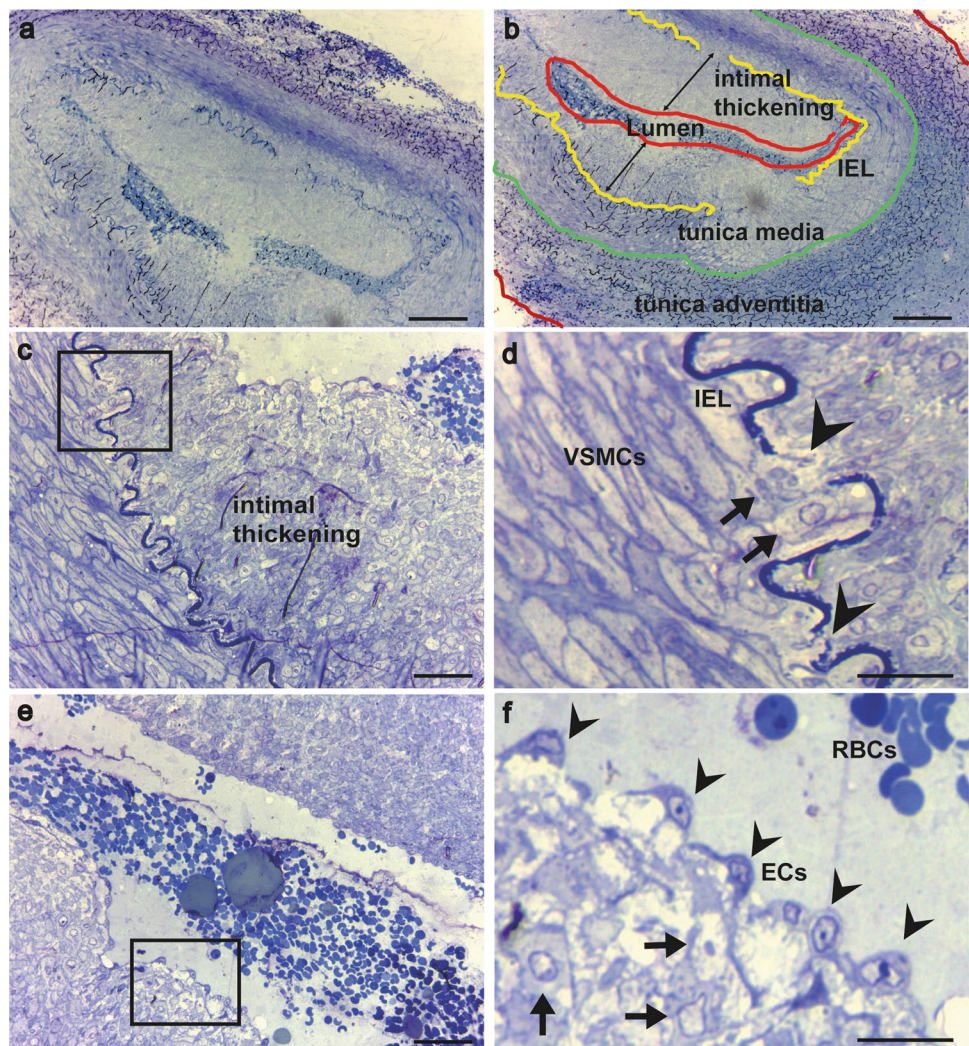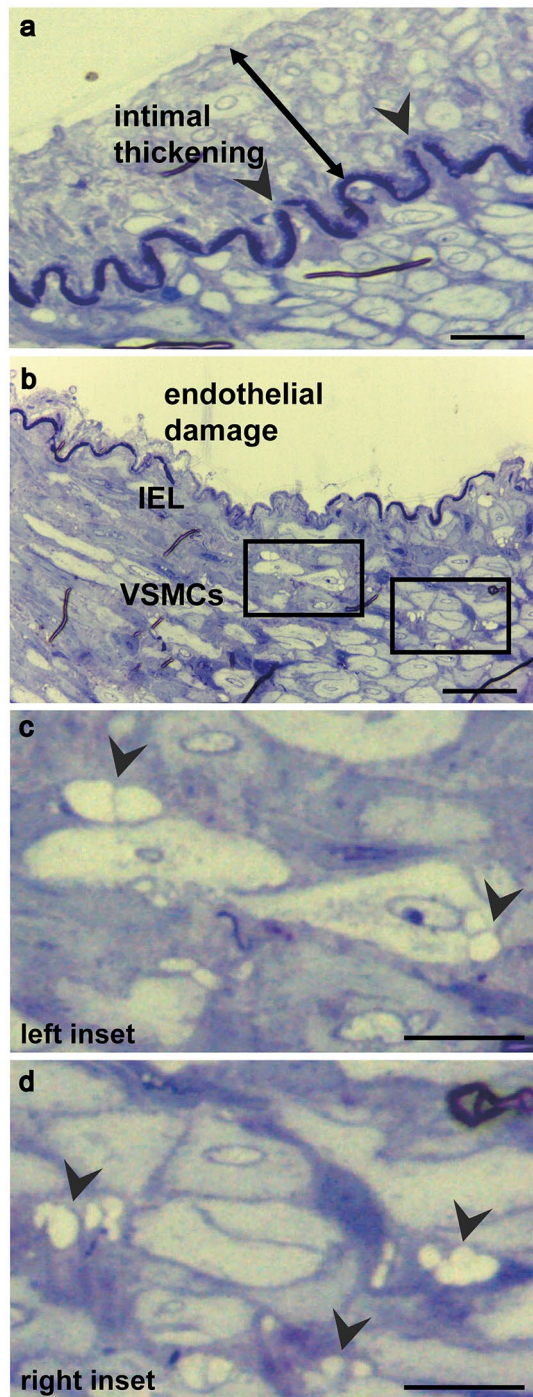
Fig. 2 Light microscopy of toluidine blue stained 200 nm semithin sections from two representative superficial temporal artery biopsies from two female patients (#2, #3). Representative images showing endothelial damage with IEL fragmentation (**a**), intimal hyperplasia (**b**), and limited metachromasia of VSMCs of different shapes (**a, b**). In (**b**) are also depicted two inset boxes enlarged in (**c, d**). Inset boxes from (**b**) showing VSMCs in the muscular layer surrounded by clear vacuoles (black arrowheads) and extracellular matrix (**c, d**). Scale bar: 50 μm (**a, b**), 20 μm (**c, d**)

the shape of VSMCs, different TB metachromasia of cells, separation of cells by abundant TB-stained extracellular matrix and clear vacuoles of different size (Fig. 2a, b). These results suggested a possible phenotype switching occurring in the VSMC layer (Petsophonsakul et al. 2019), consistent with vascular remodeling.

Ultrastructural changes in STA and OA

To investigate whether the cells in tunica media were effectively undergoing a phenotype switching, we performed ultrastructural analysis on 50 nm ultrathin sections of STA and OA samples. VSMCs are highly specialized cells that regulate vascular tone and participate in vessel remodeling in physiological and pathological conditions (Lacolley et al. 2012). It has been reported that VSMC phenotypic conversion from a contractile and quiescent to synthetically active state contributes to vascular pathologies including restenosis, atherosclerosis, and vascular calcification (Eid et al. 2012; Kapustin and Shanahan 2016; Pahk et al. 2017). In contrast to contractile cells, synthetically active cells proliferate, migrate, synthesize components of the extracellular matrix and actively release exosomes within the extracellular space (Kapustin and Shanahan 2016).

We observed, in both STA and OA samples, the presence of a heterogeneous population of VSMCs cells characterized by four distinct ultrastructural aspects. First, we show that the major fraction of synthetically active smooth muscle cells displayed a polygonal shape, irregular borders with migratory appearance, less packed myofilaments in the cytoplasm, reduced dense bodies, presence of lipofuscin, and abundant extracellular matrix (Fig. 3a). This population of cells coexisted with cells in a transitional state (Fig. 3b) and, to a lesser extent, with fully contractile cells (Fig. 3c). Many putative synthetically active cells, showing the ultrastructural morphology of VSMCs, were found in the intimal layer, suggesting migration of these cells from the tunica media and contributing to the thickening observed (Fig. 4a, b). Second, in the muscular layer, the interstitial spaces were considerably widened and filled with increased amounts of collagen and extracellular matrix (ECM). Third, bona fide extracellular vesicles (EVs) and multilamellar structures, as well as small endosome-like organelles, were observed embedded in the context of ECM (Fig. 5a, b). Fourth, several membrane-bound vacuoles of different sizes and filled with fine granular material were found in the extracellular space among synthetically active VSMCs. Occasionally, we also detected vesicular profiles budding inside the vacuoles (Fig. 5c, inset) and internal membranes (Fig. 5d), strongly suggestive of their endocytic nature. Interestingly, these structures were reminiscent of ghost bodies observed in early studies and are described as an exocytotic process in which cytoplasmic material is discarded by the cell in the
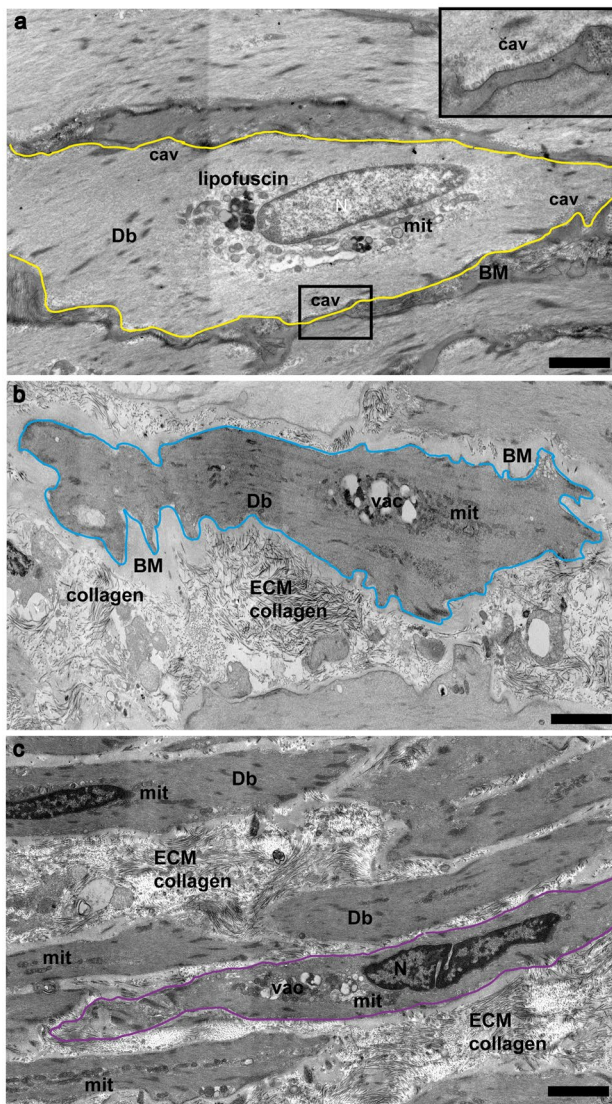
Fig. 3 Electron micrographs of the cellular phenotypes present in the muscular layer of occipital artery biopsies (patient #1 and #2). In (**a**) is shown a multiple image alignment (MIA) montage of a synthetically active cell (yellow borders) with clear cytoplasm, reduced filaments and dense bodies (Db), and presence of lipofuscin and mitochondria (mit) near the nuclear region. The *inset* shows the plasma membrane of the transitional cell decorated with abundant caveolae. In (**b**) is shown a MIA montage of smooth muscle cell with a transitional phenotype (border in light blue). Note the increased electron density compared to cells in (**a**), the irregular shape, presence of intracellular vacuoles (vac), mitochondria (mit), irregular basal membranes (BM), and of extracellular spaces filled with abundant collagen and extracellular matrix (ECM). MIA montage of smooth muscle cells with the contractile phenotype (border in violet) in (**c**). Note the fusiform shape, dense bodies (Db), nucleus (N), mitochondria (mit), intracellular vacuoles (vac), and extracellular matrix (ECM), mainly collagen fibers in the surrounding space. Scale bar: 2 μm (**a–c**)

Fig. 4 Light and electron microscopy of intimal thickening of representative STA. In (**a**) is shown a toluidine blue stained 200 nm semithin section from a superficial temporal artery biopsy (female patient #1) showing vessel lumen (lumen), a thickened endothelial layer comprising endothelial cells (EC, arrow) and polygonal cells with pale cytoplasm and heterochromatin-poor nuclei (intimal thickening), fragmented internal elastic lamina (IEL, arrowheads), and tunica media with VSMCs. In (**b**) are representative electron micrographs showing the lumen of the same vessel with a RBC, two adjacent endothelial cells (EC), and two putative VSMCs underneath ECs contributing to the intimal thickening. Scale bar: 30 μm (**a**), scale bar: 2 μm (**b**)

extracellular space (Trillo 1981). The nature of the granular material found within the vacuoles is currently unknown but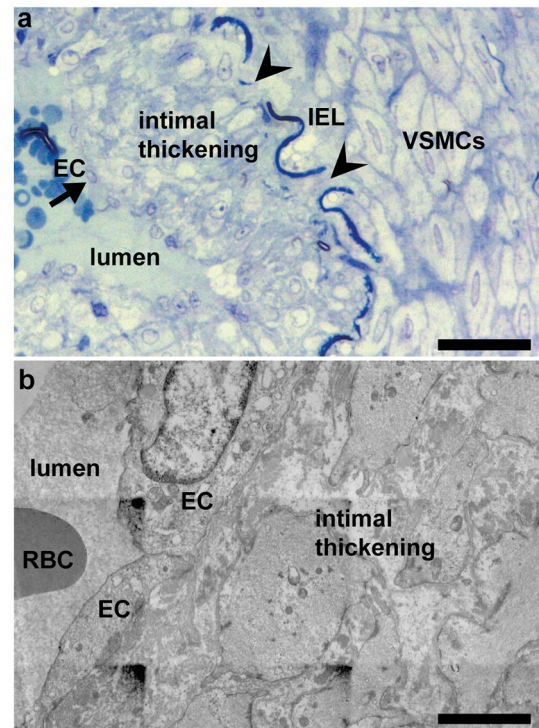 we might speculate that myofilaments and contractile machinery can be discarded from the cells as a result of phenotypical switching. Therefore, the presence of these extracellular vacuoles in all STA and OA samples analyzed might suggest that phenotypic switching is accompanied by a striking activation of degradative and/or autophagic processes (Fig. 5c, d).

Taken together, at the microscopic level, the modifications observed in the tunica intima and media of both STA and OA arteries support vascular injury and intense remodeling. Although these types of alterations cannot be considered specific for migraine as already described in different vascular disorders (e.g., hypertension and atherosclerosis) (Pahk et al. 2017), we should consider that in the history of our patients were not reported any of these latter pathologies. Therefore, we cannot exclude that vascular remodeling would represent a common early mechanism that is activated in different vascular pathologies. To our knowledge, this is the first report that describes vascular
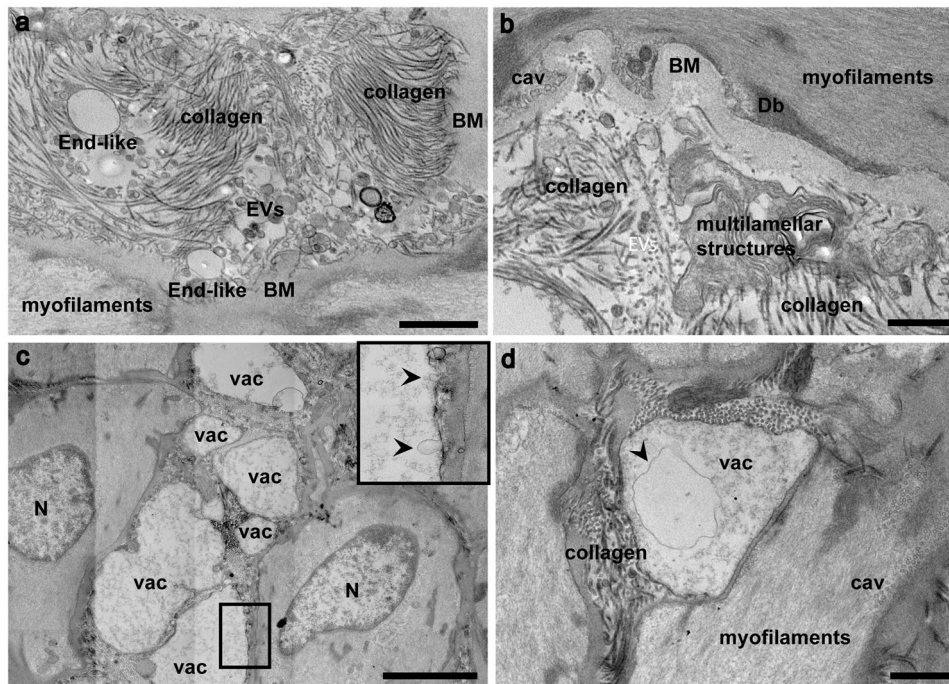
Fig. 5 Electron micrographs of extracellular matrix (ECM) among synthetically active cells of STA and OA biopsies. In (**a**) is depicted a representative image of a STA showing the basal membrane (BM) of a synthetically active cell, myofilaments, and extracellular matrix filled with collagen fibers (collagen), extracellular vesicles (EVs), and small endosome-like structures (endo-like). In (**b**) is shown a representative image of an OA showing the basal membrane of a synthetically active cell (BM), myofilaments, dense bodies (Db), caveolae (Cav), multilamellar structures, collagen fibers, and extracellular vesicles (EVs). Membrane-bound vacuoles (vac) filled with fine granular material immersed in the extracellular space surrounding synthetically active cells from STA are shown in (**c, d**). Note in the *inset* in (**c**) the presence of vesicular profiles budding within the vacuole (arrowheads), as well as the internal membrane (arrowhead) indicated in (**d**), suggestive of a likely endocytic nature of these structures. Scale bar: 1 μm (**a**), 500 nm (**b**), 2 μm (**c**), 500 nm (**d**)

microscopic abnormalities in chronic migraine patients and supports the theory that vascular remodeling, as either direct or indirect effect, might play an important role in migraine. Further molecular studies will be needed for better understanding the underlying mechanisms in the development of the observed ultrastructural changes.

Declarations

Conflict of interest The authors have declared that no competing interests exist.

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