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Quiescent satellite glial cells of the adult trigeminal ganglion

Research Article

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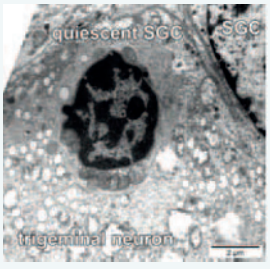
Abstract: Sensory ganglia comprise functional units built up by neurons and satellite glial cells (SGCs). In animal species there was proven the presence of neuronoglial progenitor cells in adult samples. Such neural crest-derived progenitors were found in immunohistochemistry (IHC). These findings were not previously documented in transmission electron microscopy (TEM). It was thus aimed to assess in TEM if cells of the human adult trigeminal ganglion indeed have ultrastructural features to qualify for a progenitor, or quiescent phenotype. Trigeminal ganglia were obtained from fifteen adult donor cadavers. In TEM, cells with heterochromatic nuclei, a pancytoplasmic content of free ribosomes, few perinuclear mitochondria, poor developed endoplasmic reticulum, lack of Golgi complexes and membrane trafficking specializations, were found included in the neuronal envelopes built-up by SGCs. The ultrastructural pattern was strongly suggestive for these cells being quiescent progenitors. However, further experiments should correlate the morphologic and immune phenotypes of such cells.



Keywords: *Sensory ganglion • Transmission electron microscopy • Satellite glial cells • Progenitor cells • Human trigeminal ganglion*

1. Introduction

The sensory ganglia enclose neurons with their projections, nerve fibers derived from extraganglionic sources and glial cells: Schwann cells and satellite glial cells (SGCs). The later envelop the sensory neurons to form neurono-glial functional units [1-3] in which the SGCs interact with neurons through gap junctions [4] and in a paracrine manner [5-7]. In TEM was found that the neuronal envelopes also include interstitial cells with morphologies allowing them to be considered telocytes [8].

The ultrastructure of SGCs includes polysomes, rough endoplasmic reticulum, Golgi apparatus,

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lysosomes, multivesicular bodies, autophagic vacuoles, microtubules and intermediate filaments [8].

Studies in experimental rat models demonstrated that also neural crest-derived progenitor cells (PCs) exist in sensory ganglia (dorsal root ganglia and trigeminal ganglia) [9-11]. Studies aimed at isolating and characterizing PCs have attempted to purify phenotypes and to generate pluripotent cell lines to increase the efficiency of therapies with grafts of neuronal precursors [12]. These PCs likely originate from SGCs [10]. In human and rat trigeminal ganglia SGCs were found positive for nestin, a marker of neuroepithelial stem cells [13-17]. Nestin expression in cells may indicate multi-potentiality and regenerative potential [18].

These findings were not previously documented in transmission electron microscopy (TEM). It was aimed to assess in TEM whether or not a subclass of SGCs could be ultrastructurally qualified for a progenitor, or quiescent phenotype, distinctive from the ultrastructural standard of SGCs.

2. Materials and method

For the present study, autopsy samples of trigeminal ganglia were dissected out in 15 adult cadavers (sudden cardiac or traumatic deaths), in the "Mina Minovici" Institute of Legal Medicine, Bucharest. Donor cases neither were diagnosed nor died from neurodegenerative disorder causes; the mean age was 62 years and the sex ratio was 3:2. Approval for the present study was granted by the Bioethics Committee of the "Mina Minovici" Institute of Legal Medicine, Bucharest, according to the generally accepted international standards and national laws.

For TEM examination, small tissue fragments about 1-2 mm^3 were prefixed in fresh ice-cold 4% glutaraldehyde in sodium cacodylate buffer, pH 7.4 for 4 hours at 4°C. After fixation, the tissues were 6x washed in 0.05 M sodium cacodylate buffer (pH 7.4) at 4°C, postfixed in 2% osmium tetroxide in 0.1 M sodium cacodylate at room temperature for 2.5 hours, stained *en bloc* with 0.5% aqueous uranyl acetate overnight at 4°C and washed with 0.05 M sodium cacodylate buffer. After dehydration in graded series of ethanol and infiltration with propylene oxide, specimens were embedded in Glycid ether (Epon 812-equivalent) and finally polymerized at 60°C for 48 hours. Semithin sections were stained with 1% toluidine blue for light microscopy. Ultrathin sections (80-100 nm) were cut using a diamond knife and collected on 200 mesh copper grids, and double counterstained with uranyl acetate and subsequently lead citrate. The grids were examined in a Philips electron microscope EM 208S operated at an acceleration voltage of 80 kV. An image acquisition system consisting of a video camera Veleta and the iTEM Olympus Soft Imaging System was used.

3. Results

In all samples the trigeminal ganglion histology was accurately identified, both on semithin and ultrathin cuts. Neurons were surrounded by, and in close contact with SGCs which were building in neuronal sheaths and presented characteristic large, rounded and euchromatic nuclei. There were seemingly no differences with respect to gender or age.

On semithin cuts, cells, different to SGCs, were found participating in the neuronal glial sheaths; these cells seemed smaller than the SGCs and presented heterochromatic nuclei (Figure 1). However, the semithin cuts were not suitable for an accurate diagnostic of these later cells.

In TEM, cells closely contacting neurons and belonging to the SGCs sheaths were identified, and were ultrastructurally different to the SGCs (Figure 2-4). These peculiar SGCs were characterized by a quiescent status, as suggested by a distinctive ultrastructural pattern: (a) smaller size than that of SGCs (Figure 3A); (b) small and heterochromatic nucleus, as compared with the larger euchromatic nucleus of the SGCs (Figure 2-4); (c) a huge amount of free ribosomes within the cytoplasm (Figure 2-4) – strings of ribosomes were also found coating the nuclear envelopes (Figure 4D); (d) few perinuclear mitochondria (Figure 2-4); (e) few cisterns, occasionally dilated, of rough endoplasmic reticulum (RER) (Figure 2); (f) absent Golgi complexes; (g) bundles of intermediate filaments were occasionally identified in perinuclear locations (Figure 4C). Peg-and-socket junctions between neurons and this distinctive subclass of SGCs were also encountered (Figure 3).

As referred to the ultrastructural pattern, partly differentiated SGCs were also assessed (Figure 5); in these SGCs the nuclear/cytoplasmic ratio was decreased, the nuclei preserved a heterochromatic pattern, cisterns of RER were enriching the pool of organelles which was observed in quiescent SGCs, and the amount of intermediate filaments was consistently represented. In differentiated states, the ultrastructure of SGCs had the complete ultrastructural pattern of an active cell (Figure 5), the nuclei being rather euchromatic and the amount of free ribosomes being consistently decreased (see also Introduction).

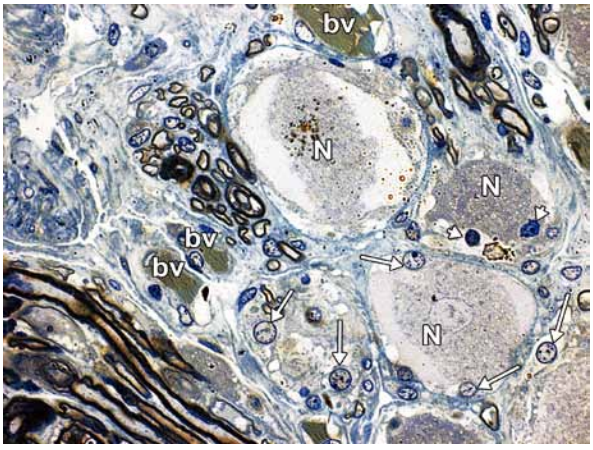

Figure 1. Semithin cut of a human adult trigeminal ganglion. Toluidine blue staining. Blood vessels (bv) are indicated. Neurons (N) are encapsulated by satellite glial cells with euchromatic nuclei (arrows) and by smaller cells with heterochromatic nuclei (arrowheads).

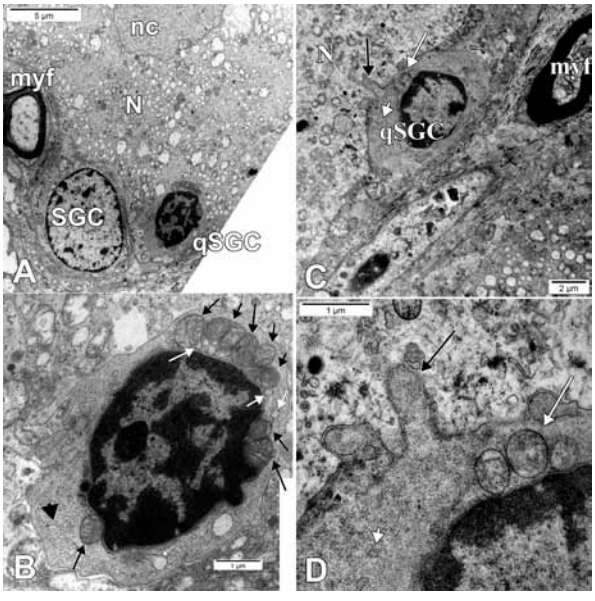

Figure 2. Ultrathin cuts of human adult trigeminal ganglia. A.General view. A trigeminal neuron (N) with its nucleus (nc) is identified, neared by a myelinated fiber (myf). A differentiated satellite glial cell (SGC) contacts the neuron and it is neared by a quiescent SGC (qSGC) with a progenitor phenotype, detailed in (B) at higher magnification. B.Digitally reconstructed image. The cytoplasmic content of the qSGC is uniquely represented by mitochondria (black arrow), abundant free ribosomes (black arrowhead) and scarce cisterns of rough endoplasmic reticulum (white arrows), occasionally dilated. C. A quiescent SGC (qSGC) with a progenitor phenotype contacts a trigeminal neuron (N) and form peg-and-socket junctions (black arrow). The qSGC has few mitochondria (white arrows) and abundant free ribosomes (white arrowhead). D. Detail of the neuronoglial junction in (C).

4. Discussion

Stem cells reside in stem cell niches, such as the muscle satellite cells niche, subepicardial stem cell niche, the

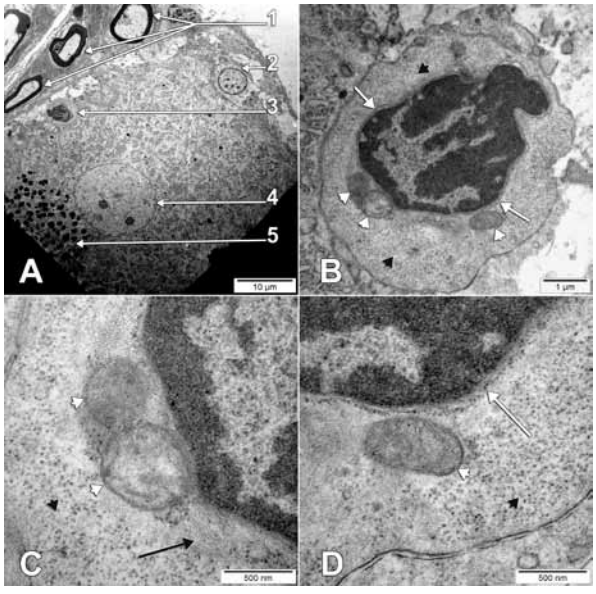

Figure 3. Ultrathin cut of human adult trigeminal ganglion. A.General view. 1.myelinated fibers; 2.satellite glial cell; 3.quiescent SGC; 4.nucleus (with two nucleoli); 5.lypofuscin. The quiescent SGC (detailed in B, C, and D) has a progenitor phenotype: heterochromatic nucleus, the nuclear envelope is coated by ribosomes (white arrows), contains a few perinuclear mitochondria (white arrowheads), intermediate filaments (black arrow) and the cytoplasm is filled with free ribosomes (black arrowheads).

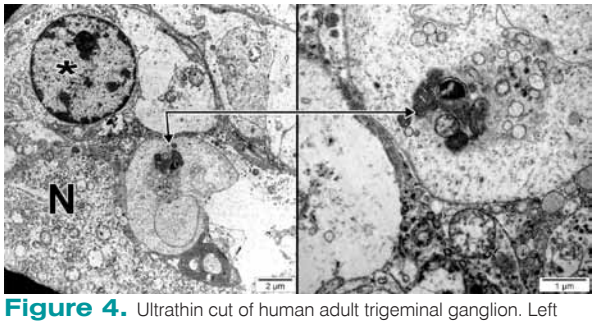

Figure 4. Ultrathin cut of human adult trigeminal ganglion. Left panel: general view, partly depicting a trigeminal neuron (N), a differentiated SGC (*), and a partly differentiated SGC (connector, detailed in the right panel).

CNS subventricular/subependymal zone, or the dental pulp stem cell niche [19-23]. In general, stem and PCs have been identified and characterized in terms of molecular markers [24]. In such niches a distinctive class of stromal cells, the telocytes, usually positive for CD117/c-kit, were found „*nursing*" the stem cells [20]. Such c-kit-positive interstitial cells were also described in the human adult trigeminal ganglion [25].

Seemingly, PCs indeed exist in human adult trigeminal ganglia. PCs do not synthesize and export products in the extracellular matrix, as supported by the ultrastructural features of the quiescent SGCs: (a) lack of nucleolus; (b) lack of membrane specializations for trafficking, such as caveolae or vesicles; (c) lack of Golgi

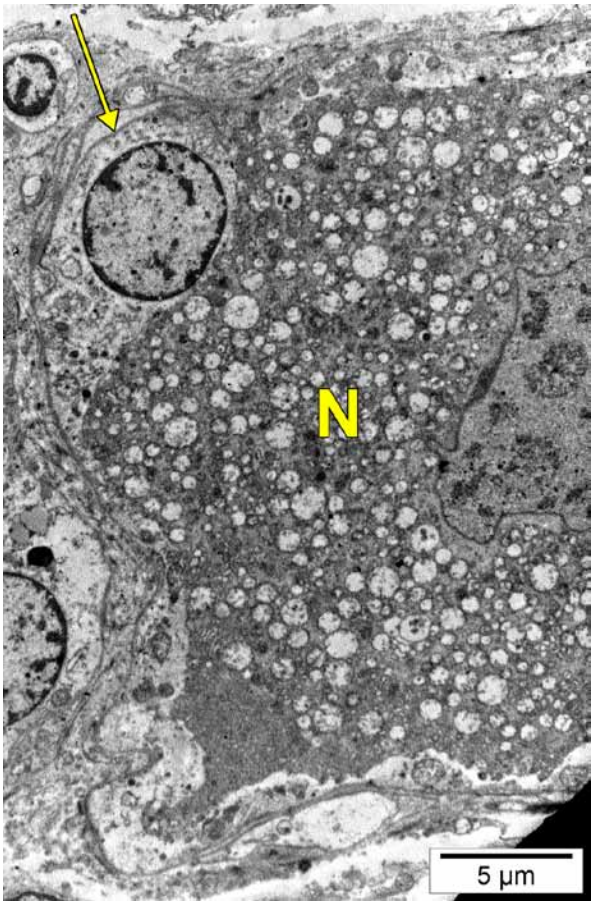

Figure 5. Ultrathin cut of human adult trigeminal ganglion. A trigeminal neuron is presented (N) contacting a differentiated SGC (arrow).

complexes. The evidence presented here completes previous evidence of nestin positive cells in the human adult trigeminal ganglion [17]; those cells were considered at that time, in light microscopy, as being SGCs, since they were participating in the neuronal sheaths. The present results suggest that distinctive morphological subclasses of SGCs should be considered, ranging from a quiescent to a differentiated phenotype, and that quiescent SGCs could be viewed as potential PCs.

Within the muscle stem cell niche, the myogenic satellite cells are characterized by their small size, high nuclear/cytoplasmic ratio, relative absence of cytoplasmic organelles and increased nuclear heterochromatin, which represent a quiescent state of those cells [26]. Similar morphologies were documented in this study for the quiescent SGCs which are known to undergo morphological changes and to proliferate upon mechanical injury to sensory neurons [2]. It is tempting to speculate that, from a morphological point of view, the quiescent SGCs found in the present study could be, similarly to the muscle stem cell niche, progenitors of a ganglionic niche of the trigeminal ganglion. Such quiescent SGCs could be activated by peripheral stimuli [27]. There is no satisfactory *in vivo* evidence of stem/ PCs within sensory ganglia at this time. *In vitro* studies did, however, confirm that, in mammals, postmigrating neural crest-derived stem cells in sensory ganglia preserve their multipotency [11]. Such an *in vitro* study demonstrated that progenitors originate from SGCs and a subpopulation of glial cells may be involved in neurogenesis after nerve injuries [10]. It is likely that the quiescent SGCs found here are those glial cells involved in neurogenesis. Further molecular studies are however needed to check whether or not markers of stemness, such as nestin and p75 neurotrophin receptor [10], are specific for the quiescent morphological phenotype of SGCs, in humans.

A limitation of this pilot study on human adult trigeminal ganglion *in situ* progenitors is the lack of a thorough characterization of putative progenitor cells, by immunoelectron microscopy on the same samples which would have been of significant interest to the scientific community. Therefore further studies should address this issue.

The general basic concept in which the trigeminal ganglion is defined as comprising neurons and glial cells [25] could be updated to include PCs. The morphological and immune phenotypes of these progenitor cells should be firmly correlated, in order to facilitate quantitative experiments.

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All the authors have equally contributed to this study.

Conflict of interest statement

Authors state no conflict of interest.

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