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RESEARCH HIGHLIGHT

New mechanisms of neurite outgrowth and TrkA receptor activation/signaling

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The paper we recently published in PNAS, entitled "Neurite outgrowth induced by NGF or L1CAM via activation of the TrkA receptor is sustained also by the exocytosis of enlargeosomes" (Colombo *et al.*, 2014), reported studies carried out in clones isolated from the PC12 line, frequently employed as a neuronal model. Two original and integrated findings were obtained, concerning the vesicle traffic and fusion processes necessary for neurite outgrowth, and the activation by the adhesion protein L1CAM of the receptor TrkA. The latter tyrosine kinase was widely considered as a receptor activated exclusively by the neurotrophin NGF. Both these findings provide an explanation to results previously obtained in our and other laboratories.

Keywords: L1CAM; exocytosis of endosomes/enlargeosomes; neurite/axonal outgrowth; receptor binding; local signaling

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The outgrowth of axons and neurites taking place during differentiation requires profound changes to take place in neural cells. One of these change is the expansion of the plasma membrane. Previous studies by Thiery Galli and his group working with both PC12 cells and primary cultures of neurons, had identified surface expansion to be induced by the fusion of endosome organelles to the plasma membrane. Such fusion occurs by an exocytosis of endosome vesicles requiring VAMP7 as the vesicular components of the SNARE complex [4, 5].

Endosomes are ubiquitous organelles. Therefore their exocytosis could be involved in plasma membrane expansion not only in neurons but also in other types of cell. We did not question this possibility. Rather, the question we raised was whether, in neurons and neural cells, other exocytic organelles are also involved in the expansion process. In a previous study, carried out with a clone of PC12 spontaneously expressing high levels of REST, the transcription factor that represses many neural genes, we had found outgrowth to be sustained by the parallel exocytosis of at least two organelles, the endosome and the enlargeosome [7]. The enlargeosome is an exocytic organelle (vSNARE of its fusion= VAMP4) we had discovered in high REST cells, including high REST PC12 clones, which however was believed to lack in low REST neural and neuronal cells.

Now we have found that, in the low REST, wild-type PC12 (wtPC12) cells, long-term treatment with NGF, which induces neurite outgrowth upon a few days, drives the appearance and the exocytosis of enlargeosomes after only 6-12 hrs. The newly appeared enlargeosomes contribute, fifty-fifty with endosomes, to the outgrowth of long neurites (Fig. 1; [2]). Whether this occurs only in PC12 or also in neurons, is unknown. Since, however, the level of REST in neurons is not always low, but increases upon various types of stimulation [6], and also during aging [3], the expression of enlargeosomes and their participation in axon/dendrite

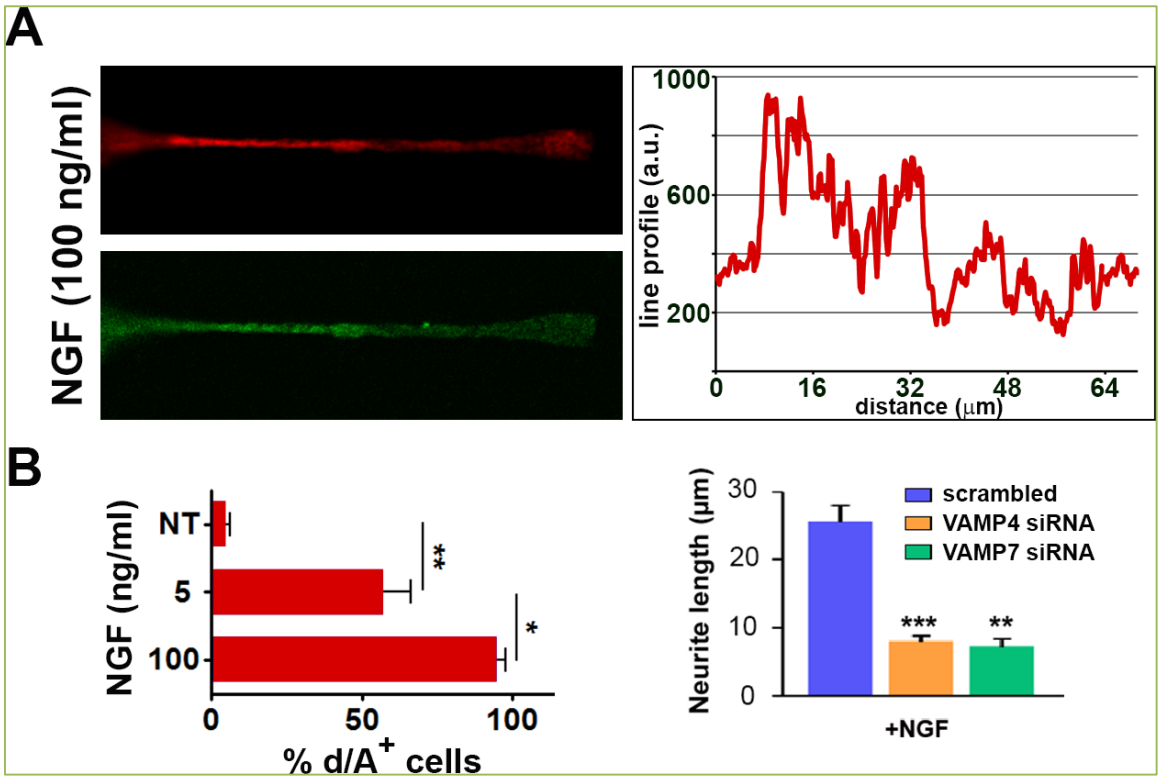

Figure 1. Enlargeosomes contribute to neurite outgrowth to an extent similar to endosomes. The top left panel shows the immunolabeling of a neurite in a wtPC12 cell stimulated with NGF for 72 hr. Red immunolabeling is the enlargeosome marker, green is β-tubulin, a cytoskeletal marker. The top panel to the right shows the distribution of the enlargeosome marker along the neurite shown on the left. On the bottom, the left panel shows the dose dependence of enlargeosomes induction by NGF in wtPC12 cells; the right panel shows the contribution of enlargeosomes and endosomes to neurite length in wtPC12 cells, revealed by down regulation of the vSNAREs specific of the exocytosis of the two organelles: VAMP4 (yellow, of enlargeosomes) and VAMP7 (green, of endosomes). Reprinted with permission [2].

outgrowth appear likely and deserve to be investigated. Axon/neurite outgrowth is interesting not only in physiology, but also in pathology and clinics. The identification of new mechanisms that sustain the process, such as the exocytosis of the two types of vesicles revealed by our investigation, could be the starting point for possible developments also in neurological areas.

Our second finding deals with the activation of TrkA in wtPC12 cells. This tyrosine kinase receptor is known to be activated by NGF and to generate at least 3 signaling cascades, those of PI3-kinase, ERK1 and ERK2, and PKC, respectively. On the other hand, the ectodomain of the adhesion protein L1CAM was known to bind not only other L1CAM ectodomains (homophilic binding) but also other molecules (heterophilic binding). We found that L1CAM, (as well as its soluble construct truncated of the transmembrane and intracellular domains) is able to bind TrkA; and that this binding is of high affinity and competes with the binding of NGF. Moreover, the function of L1CAM binding appears analogous to the function of NGF binding since it does induce the activation of TrkA; the generation of the three main cascades mentioned above; and the outgrowth of neurites. These effects appear in a cell expressing surface TrkA only when it is strictly co-incubated with other cells that express surface L1CAM (Fig. 2). This because activation of the receptor occurs only upon prolonged trans-binding of L1CAM [2].

The conclusion of the L1CAM/TrkA results is at least two fold. First, the receptor is not specific for only NGF, as it was generally believed, but is activated also by L1CAM, an adhesion protein which, in the brain, is exposed at the surface of neurons, concentrated at critical sites such as growth coness and synapses; second, the activation of TrkA by L1CAM can induce effects more localized than those induced by NGF. In fact, upon the binding of the neutrophin the receptor is redistributed into small areas of the plasma membrane that are internalized and transported along a pathway within the cell [1]. In this case, therefore, TrkA activation does induce effects spread to the whole cell or at least to large portions. In contrast, upon binding of L1CAM the receptor does not redistribute. Rather, it remains stack to the site where its activation is initiated. At variance with

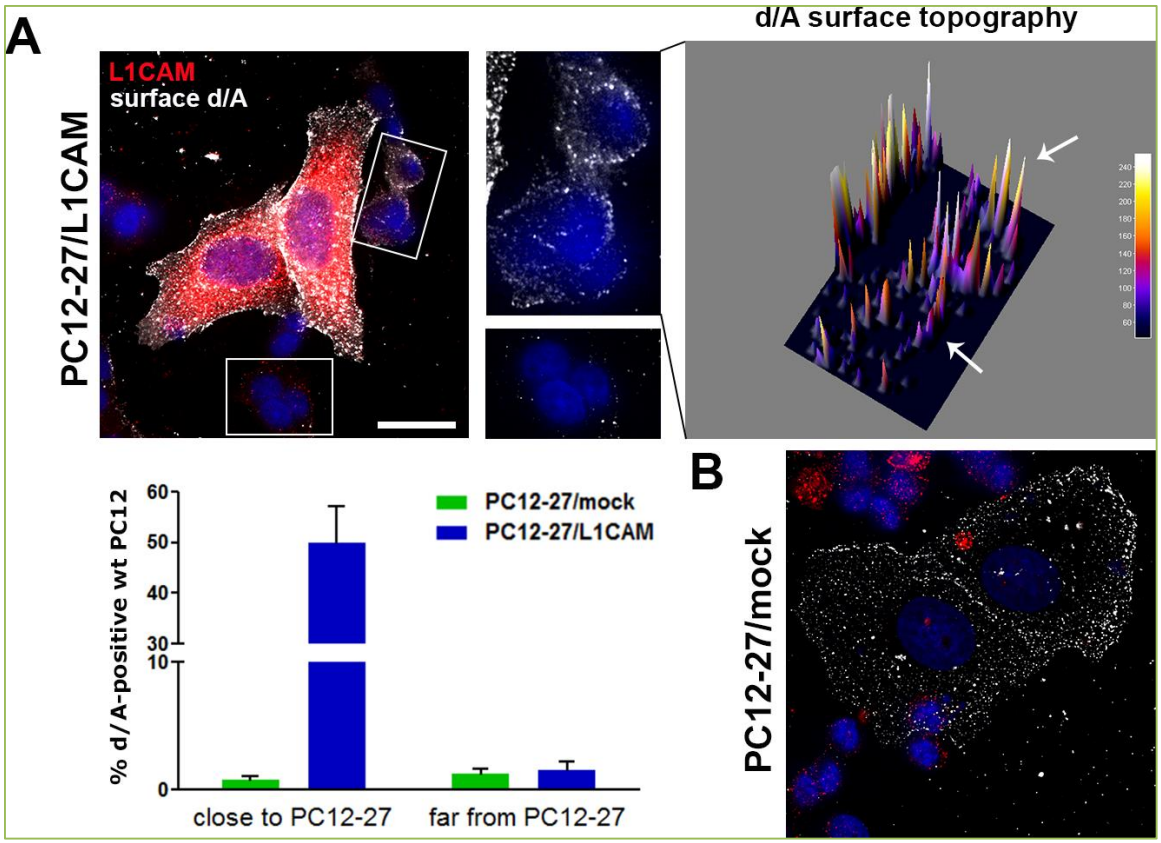

Figure 2. Local TrkA activation by L1CAM in PC12 cells. The two types of cells shown here come from clones of PC12 cells. The cells of the high REST clone, which are rich of enlargeosomes but very poor of TrkA, had been transfected with L1CAM (large flat cells); the cells of the wtPC12 clone (semi-spherical cells) express surface TrkA. In A the panels to the left show cells incubated together for 18 hr, then fixed and immunolabeled for L1CAM (red) and desmoyokin/Ahnak, a marker of enlargeosomes exocytized by wtPC12 only upon TrkA activation (white puncta). The activation of the vesicle exocytosis in the wtPC12 cells occurs due to the direct binding of their TrkA by the L1CAM of the high REST cells. In fact, the white puncta, revealed by surface immunolabeling, appears in the wtPC12 cells only when strictly adjacent (<10 μm) to a high REST cell, whereas the wtPC12 cells distributed far away from the high REST cell, remain all negative. The right panel shows a tridimentional model of the white puncta distribution in the high REST cells (green arrow) and two wtPC12 cell (blue and white arrows). The lower panel to the left shows a quantitative analysis of the wtPC12 cells close to L1CAM-transfected high REST cells (with white puncta), and far from the transfected high REST cells (without white puncta). The quantitative data of this panel were from a total of 120 cells. B shows two high REST cells rich of white puncta which however were mock transfected and therefore express no L1CAM. The wtPC12 cells are all devoid of white puncta, i.e. they did not have any TrkA activation. Reprinted with permission [2].

NGF, therefore, activation of TrkA by L1CAM can be considered as a local signaling. The relevance of this process in the general function of the brain and in pathological conditions, particularly in neurodegenerative diseases, remains to be investigated.

Conflicting interests

The authors have declared that no competing interests exist.

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