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Accumulation of tumor-suppressor PTEN in Alzheimer neurofibrillary tangles

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Abstract

The phosphatase and tensin homologue deleted on chromosome 10 (PTEN) negatively regulates intracellular levels of PIP3 and antagonizes the PI3K signaling pathway important for cell survival. The present study determined whether altered distribution of PTEN occurs in Alzheimer's disease (AD) brains. We investigated a possible role for PTEN in postmortem brain tissues from elderly controls and patients with AD using immunoblotting and microscopic analyses. Intense immunolabeling was found in the large neurons such as pyramidal cells. In normal neurons, PTEN was located in the nucleus, the cytoplasm of cell bodies and the proximal portion of apical dendrites. Reduced expression and redistribution of PTEN was seen in the remaining neurons in AD. In addition, PTEN was redistributed in damaged neurons from the nucleus and cytoplasm to neuritic pathology such as intracellular neurofibrillary tangles (NFTs), neuropil threads and dystrophic neurites within senile plaques in AD hippocampus, subiculum, entorhinal cortex and angular gyrus. Furthermore, double immunofluorescence staining showed dual labeling of intracellular NFTs for PTEN and tau, labeling of some axons for PTEN and phosphorylated neurofilament, and weak labeling of a few reactive astrocytes around senile plaques for PTEN and GFAP. Double labeling of NFTs was observed in a subset of tangle bearing neurons either for PTEN and GSK3β or for PTEN and MEK. Thus our results suggest that PTEN delocalized from the nucleus to the cytoplasm and to intracellular NFTs may cause a deregulation of PI3K pathway in the cytoplasm and may induce the nuclear dysfunction of PTEN in AD degenerating neurons.

Key words: PTEN
phosphatase
PI3K
tau
neurofibrillary tangles
Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disorder defined by progressive memory loss and cognitive impairments and characterized at the pathological level by intracellular neurofibrillary tangles (NFTs), extracellular senile plaques (SPs), neuronal loss, and activation of glia [4, 15]. The number of NFTs correlates directly with the severity of dementia [1]. These tangles are composed of straight and paired helical filaments, with a major component being an aberrantly hyperphosphorylated form of the microtubule-associated protein tau [3, 5, 11]. The tau protein is normally expressed in the cytoplasm of cell bodies and neurites and distributed to axons, where it binds to and stabilizes microtubules. This function of tau can be modulated by its phosphorylation at some serine, threonine or tyrosine residues [5, 9, 14], and abnormal hyperphosphorylation of tau in AD brains has been suggested to result from an altered tau kinase and phosphatase balance [10].

The phosphatase and tensin homologue deleted on chromosome 10 (PTEN, also named as MMAC1 or TEP-1) functions both as a protein tyrosine phosphatase and as a lipid phosphatase, negatively regulating the phosphatidylinositol 3-kinase (PI3K) signaling pathway through the down regulation of intracellular level of phosphatidylinositol-3,4,5-triphosphate. PTEN is also known to be an important tumor suppressor, modulating cell migration, growth, survival and apoptosis [6, 24]. PTEN deficiency in brain causes defects in synaptic structure, transmission and plasticity in the hippocampus [7]. The PI3K signaling pathway is activated by extracellular signals and regulates crucial biological processes with its opposing partner PTEN. In human AD brain, recent studies have shown decreased levels and altered distribution of PTEN along with activated PI3K signaling, suggesting that a loss of PTEN or PTEN-containing neurons contributes to neurodegeneration in AD [8, 22, 26]. Although the localization in NFTs of some downstream components of the PI3K signaling pathway, such as Akt [20], GSK-3β [18], (phosphorylated) ERK1/2 and (phosphorylated) MEK1/2 [19], has been reported, precise localization of PTEN has not yet been clarified in AD brains. In this study, we investigated the localization of a PTEN in human brains from elderly controls and AD patients using immunoblotting and light and confocal laser microscopy.

Postmortem human brain tissue from 12 control cases without neurological disorders (7 males and 5 females; age range: 56-89 years; mean age±SD, 74.5±12.3 years) and 13 sporadic AD cases (6 males and 7 females; age range: 58-90 years; mean age±SD,

76.8±9.4 years) was used in this study. No significant difference was seen in postmortem delay between control (mean±SD, 8.7±7.1 h) and AD cases (9.1±6.4 h). All the procedures in this study were performed strictly according to the local Ethics Committee's Clinical Study Guidelines and were approved by the internal review board. Cytoplasmic extracts were prepared from the angular cortices in postmortem brains of control and AD patients. The brain tissues were homogenized with a polytron in 10 vol of ice-cold 20mM Tris-HCl, pH 7.4 containing 5mM EDTA, 5mM EGTA, 10mg/ml leupeptin, 10 mg/ml pepstatin, and 2mM phenylmethyl sulfonate. Then samples were processed for immunoblotting as described previously [16]. The fixation and processing of human brain tissues, including cerebellum, thalamus, striatum, hippocampus, entorhinal cortex, amygdale, superior/middle/inferior frontal gyrus, pre/postcentral gyrus, angular gyrus, substantia nigra, caudate, putamen, visual cortex, and the immunohistochemical examinations were performed as described previously [12]. The primary antibodies used in this study, their sources and dilutions are listed in Table 1. Double immunostaining for PTEN (purple) and tau (brown) was also carried out on the hippocampi. In addition, for the double immunofluorescence labeling of neuritic pathology for PTEN and either Tau, pTau, GSK3β, phosphorylated neurofilament, astrocytic filament [glial fibrillary acidic protein (GFA)] or complement protein (C4d), the sections were incubated with each combination of the primary antibodies. After immunostaining followed by incubation with appropriate secondary antibodies coupled either to fluorescein or rhodamine isothiocyanate (Millipore), sections were analyzed by a confocal scanning laser microscope (OLYMPUS, FLUOVIEW FV 500).

Immunoblot analysis showed that the PTEN antibody detected a single band at 55 kD in control and AD brains (Fig. 1A). Localization of PTEN in human brains was immunohistochemically examined at the light microscopic levels. PTEN immunoreactivity was localized to neurons (Fig. 1B-1E) in control brains. Intense immunolabeling was found in the large neurons such as pyramidal cells and Purkinje cells. In normal neurons, PTEN immunoreactivity was located in the cytoplasm, in the proximal portion of apical dendrites and in the nucleus. Cytoplasmic staining was thick in pyramidal neurons in the subiculum but weak in the CA1-3 subfields in control hippocampus (Fig. 1B and 1C). PTEN was redistributed from the nucleus to the cytoplasm in the remaining neurons in AD vulnerable regions such as hippocampus and entorhinal cortex (Fig. 1F-1H and 1J-1M), whereas

nuclear PTEN was seen in normal-appearing neurons in other brain regions of AD patients. In addition, numerous intracellular NFTs were strongly stained for PTEN in AD hippocampus, entorhinal cortex, amygdala, and frontal and parietal cortices. A few intracellular NFTs and threads were immunostained for PTEN in the substantia nigra in AD patients (Fig. 2G). Interestingly, cytoplasmic and nuclear PTEN immunoreactivities were decreased in tangle-bearing neurons (Fig. 1F-1L), while staining profiles were not changed in the neurons known to be resistant to AD pathology such as Purkinje cells. Double immunostaining analysis revealed that 90.8% of HT7-positive tangles were doubly immunostained for PTEN in the hippocampus, entorhinal cortex, angular gyrus, and superior frontal gyrus in a patient with mild dementia. Neuritic pathology such as neuropil threads and dystrophic neurites within SPs were also labeled for PTEN in AD brains (Fig. 1H and 1L). Figures 1M and 1N show serial sections stained for PTEN (Fig. 1M) and for MEKpS222 (Fig. 1N). Colocalization of PTEN in NFTs with active MEK was seen in a few neurons. The majority of PTEN-positive NFTs were not labeled with MEKpS222 in AD amygdala. Double immunofluorescence staining showed dual labeling of intracellular NFTs and dystrophic neurites within SPs for PTEN and tau detected with HT-7 (Fig. 2A) or AT-8 antibodies (Fig. 2B). Similar results were obtained in the cell bodies and the proximal processes of astrocytes or in axons for the combination either of PTEN and GFA (Fig. 2C) or of PTEN and phosphorylated neurofilament (Fig. 2D), however the expression of PTEN in astrocytes was low and rarely seen around senile plaques. By contrast, PTEN-positive intracellular NFTs were clearly distinguished from extracellular NFTs labeled for complement C4d (Fig. 2E). GSK3β colocalized with PTEN in some intracellular NFTs (arrows in Fig. 2F), but many of the intracellular NFTs labeled for PTEN were not doubly stained for GSK3β.

Immunoblot data showed that the Anti-PTEN antibody recognized a single band at 55 kD in the total homogenates of human brains (Fig. 1A). Recent studies reported a significant loss or no significant changes of PTEN protein levels in AD brains [8, 22]. PTEN function enters an off state when phosphorylated at three residues (Ser^{380}, Thr^{382} and Thr^{383}) in the C-terminal domain whereas dephosphorylated PTEN is an active form [25] and the level of PTEN phosphorylated at Ser380 was significantly decreased in AD [22]. Thus, the quantitative expression and the phosphorylation state of PTEN remain to be further studied in more human brains from control and AD cases, since our data was

obtained from one area of one normal and one AD cases.

Immunohistochemical study revealed that the intracellular distribution of PTEN was altered in AD neurons and it was highly accumulated in intracellular NFTs, degenerative neuritis, neuropil threads and that a few reactive astroglia were weakly immunostained around senile plaques. PTEN, normally localized in the nucleus and the cytoplasmic compartment [21, 24], was redistributed in AD degenerating neurons predominantly to NFTs in cell bodies or apical dendrites as well as to degenerative neurites and neuropil threads. The number of neurons with their cytoplasm and nuclei positive for PTEN was remarkably decreased in the vulnerable regions to tau pathology, whereas the nuclei of non-tangled neurons remained positive for PTEN in other regions of AD brains. Cytoplasmic PTEN has a role as a negative regulator of the PI3K pathway, while nuclear PTEN plays a role in chromosome stability, DNA repair, cell cycle arrest and cellular stability. The altered distribution of PTEN may cause reduced PTEN levels in the nucleus and the cytoplasm and may lead to neuronal degeneration via mechanisms including unregulated activation of downstream components of the PI3K signaling pathway in AD brains. These results are consistent with previous studies [8, 22]. In the neurites and astrocytic processes, PTEN was colocalized respectively with phosphorylated neurofilament proteins and GFAP, filament formation of which is known to be associated with some kinases in the PI3K signaling pathway.

In the PI3K cascade pathway, active PTEN inactivates phosphoinositide-dependent kinase 1 (PDK1), leading to the activation of GSK3β or the inactivation of mitogen-activated protein kinases (MAPKs/ERKs), both of which phosphorylate tau, through the downregulation of Akt or MAPK kinase (MEK) activities [23] respectively, whereas inactive PTEN upregulates PDK1 activity, leading to the inverse responses of GSK3β or MEK-MAPK. We therefore investigated the association of PTEN with GSK3β or MEK in intracellular NFTs. As seen in Fig. 2F, GSK3β was colocalized with PTEN in a small subset of intracellular NFTs but may function to phosphorylate and inactivate PTEN as well as to phosphorylate tau [2]. Activated MEK1/2 was also colocalized with PTEN in some neurons (Fig. 1M and 1N), in accordance with the work of Kerr et al. reporting that overexpression of PTEN reduces tau phosphorylation via the downregulation of ERK1/2 activity [13].

Human tau has five tyrosines, three residues of which have been shown to be

phosphorylated in AD brains by tyrosine kinases including Fyn, c-Abl, Syk and TTBK1 [14]. Emerging evidences suggest that tau tyrosine phosphorylation is an early and critical event in the neurodegenerative process in tauopathies, including AD. Since PTEN is a dual specificity phosphatase, PTEN may directly dephosphorylate phospho-tyrosine residues on aberrantly hyperphosphorylated tau in intracellular NFTs.

In summary, PTEN was localized to neurons in human control brains, while PTEN was delocalized in degenerating neurons and PTEN expression was additionally found in reactive astrocytes in AD brains. In AD brains, PTEN was accumulated in intracellular NFTs, neuropil threads, degenerative neurites and reactive astrocytes around SPs. Furthermore, PTEN was colocalized with abnormal tau and phosphorylated neurofilament proteins in neurons and with GFAP in a few reactive astrocytes. Thus, abnormal redistribution of PTEN to neuritic pathology may be associated with the formation of tau pathology and may lead to deregulation of PI3K signaling and nuclear dysfunction of PTEN in AD brains. The role of PTEN in AD pathology awaits further investigation of brain tissues from patients with tauopathies, including AD.

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Table 1. Antibodies used with their types and dilutions

Antigen	Antibody	Source	Type	Dilution
PTEN		R&D	Rabbit polyclonal[1]	1:3000 (IB) 1:10000 (IHC)
Tau	HT-7	Innogenetics	Mouse monoclonal[2]	1:5000
pTau	AT8	Innogenetics	Mouse monoclonal[2]	1:10000
MEKpS222		Biosource	Rabbit polyclonal[1]	1:2000
GSK-3β		Sigma	Mouse monoclonal[3]	1:500
pNF-H	SMI-31	Calbiochem	Mouse monoclonal[3]	1:5000
GFAP	GA5	Millipore	Mouse monoclonal[2]	1:5000
C4d		Quidel	Mouse monoclonal[3]	1:500

[1]Rabbit polyclonal antibody (affinity-purified)

[2]Mouse monoclonal antibody (supernatant)

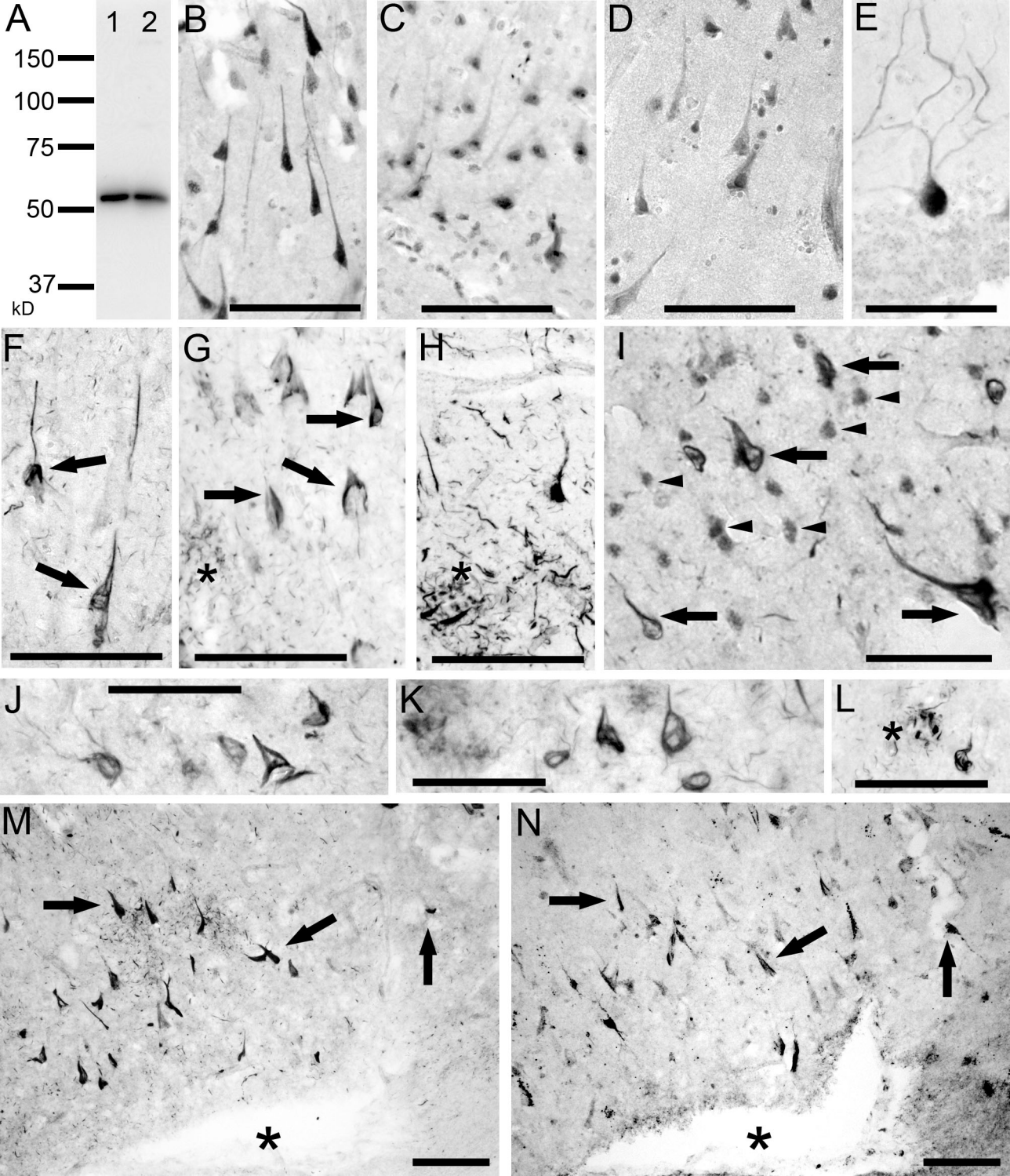
[3]Mouse monoclonal antibody (purified immunoglobulin from ascites)

PTEN, phosphatase and tensin homolog deleted on chromosome 10; pTau, phosphorylated tau at serine 199, serine 202 and threonine 205; MEKpS222, phosphorylated MEK1/2 at serine 222; pNF-H, phosphorylated neurofilament-H; GFAP, glial fibrillary acidic protein; C4d, complement 4d; IB, immunoblot; IHC, immunohistochemistry

Figure legends

Figure 1
Immunoblot (A) and immunohistochemistry (B-N) for PTEN (B-M) or MEK phosphorylated at serine 222 (N) in control (B-E) and AD brains (F-N). A, total homogenates (50 μg protein / lane) of angular cortices from control (lane 1) and AD cases (lane 2) were subjected to SDS-PAGE followed by immunoblotting. The PTEN antibody detected a single band at 55kDa. B (subiculum), C (hippocampal CA1), D (layers 5-6 of superior temporal gyrus) and E (cerebellum), neuronal expression of PTEN was observed in all brain regions examined. Strong immunostaining was seen in large neurons such as pyramidal cells (B-D) and Purkinje cells (E). Cytoplasmic staining was thicker in hippocampal pyramidal neurons in the subiculum than in CA1-3 subfields. F (subiculum) and G (hippocampal CA1), PTEN was redistributed from nuclei to cytoplasm and accumulated to intracellular NFTs (arrows) in AD vulnerable neurons. NFTs, degenerative neurites within senile plaques (asterisk) and neuropil threads were strongly immunolabeled for PTEN in the layers 5-6 (H), whereas PTEN immunoreactivity remained in the nuclei (arrowheads) in normal-appearing neurons but not in tangled neurons (arrows) in the layer 3 of middle temporal gyrus (I). Additionally, numerous intracellular NFTs and pretangled neurons were observed in the superficial layer of entorhinal cortex (J) and angular gyrus (K). Small amounts of PTEN-positive intracellular NFTs and degenerative neurites in senile plaques (asterisk) were seen in AD putamen (L). Serial sections of AD amygdala immunostained for PTEN (M) and for MEK1/2 phosphorylated at serine 222 (N). Different patterns of immunoreactivity can be seen, but some NFTs were doubly labeled (arrows). Asterisks mark the same vessel. Scale bars in B-H and L-N, 100μm; and in I-K, 50μm.

Figure2
Double immunofluorescence staining of the hippocampi (A, B, E and F) and middle temporal gyri (C and D), and simple PTEN immunohistochemistry in the substantia nigra (G) from AD brains. A, PTEN (red) was colocalized with HT-7-positive tau (green) in intracellular NFTs (yellow, indicated by thick arrows) and in dystrophic neurites (thin arrow) within a senile plaque (SP, asterisk). B, dystrophic neurites (thin arrow) in or around the SP (asterisk) and intracellular NFTs (thick arrows) were doubly labeled for PTEN (red) and AT-8-positive phosphorylated tau (green). C, glial filaments (yellow) in some astrocytes were doubly immunolabeled with anti-PTEN (red) and anti-GFA antibodies (green). D, PTEN (red) was colocalized in axons (yellow, indicated by arrowheads) with phosphorylated neurofilaments (green). E, PTEN (red) was not localized to extracellular tangles labeled for complement C4d (green, thin arrow) but to intracellular tangles (thick arrow). Processes of complement-activated oligodendrocytes [15] were seen (arrowheads). F, GSK3β (green) was colocalized in intracellular NFTs (yellow, arrows) stained for PTEN (red) in a few degenerating neurons, whereas many NFTs were labeled only for PTEN. Some degenerative neurites within a SP (asterisk) were labeled doubly or only for PTEN. G, PTEN accumulated in intracellular NFTs within neuromelanin-containing neurons in the AD substantia nigra. Scale bars, 50 μm.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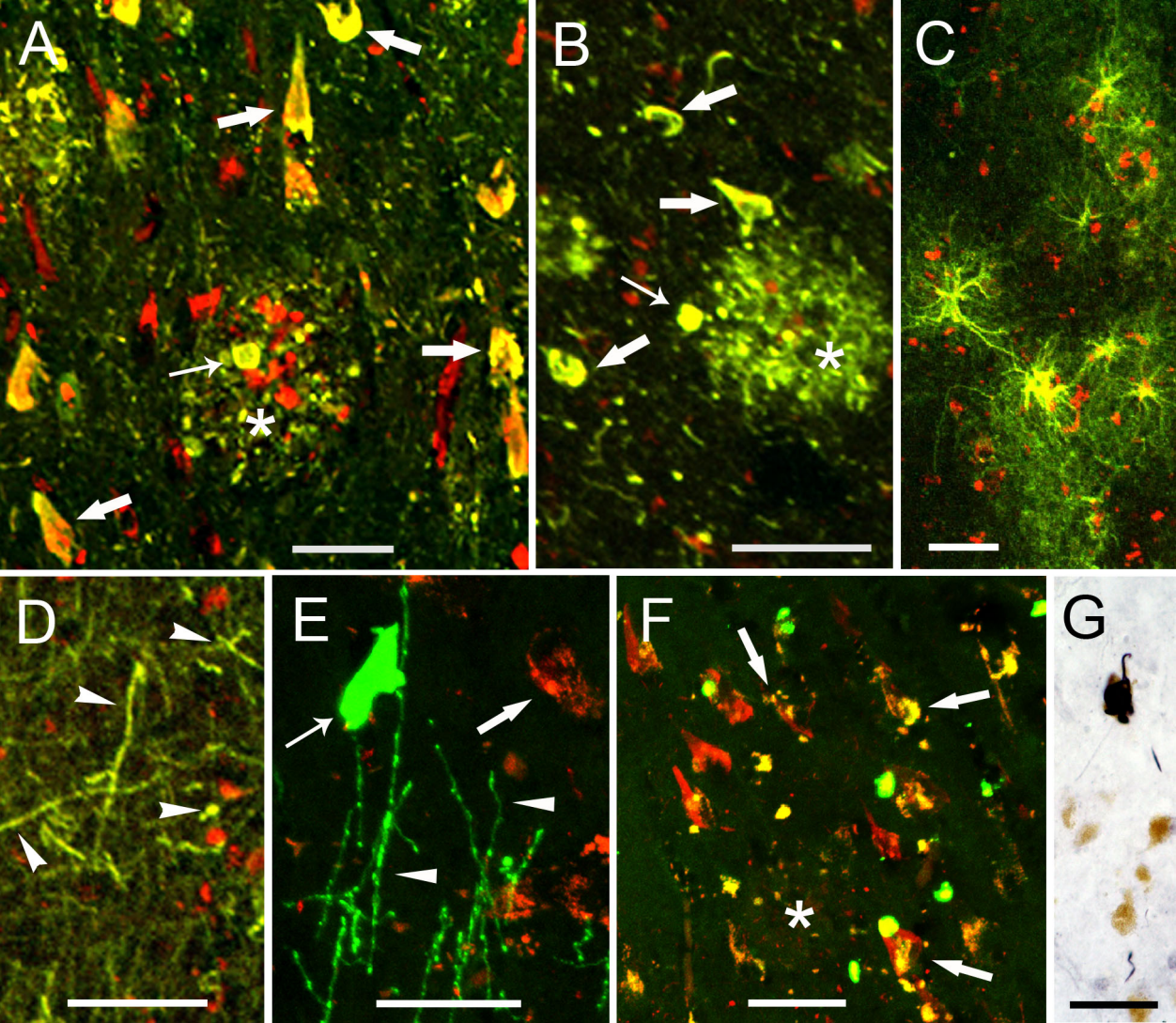
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