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Distribution of lithostathine in the mouse lemur brain with aging and Alzheimer's-like pathology

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Abstract

We analyzed the cellular distribution of the pancreatic inflammatory protein lithostathine and its receptor EXTL3 in the brain of the lemurian primate *Microcebus murinus* which develops amyloid deposits along with aging. In adult animals (2–4.5 years old), lithostathine and EXTL3 immunoreactivities were largely distributed in the whole brain, and more intensively in almost all cortical layers and hippocampal formation. Lithostathine was observed in the perikarya and neurites of cortical neurons but also in glial cells in the border of the ventricle and the corpus callosum. In healthy aged animals (8–13 years old), highest densities of lithostathine-containing cells were observed, mainly in occipital and parietal cortex. In aged animals with Aβ deposits, the increase in lithostathine immunoreactivity was lower as compared with aged animals. Noteworthy, lithostathine-immunopositive cells did almost never colocalize with Aβ plaques. In conclusion, lithostathine immunoreactivity in adult *Microcebus murinus* appeared ubiquitous and particularly in visual, sensorial, and cognitive brain areas. Immunoreactivity increased with aging and appeared markedly affected in neuropathological conditions. Its possible neuroprotection or neurodegeneration role in Alzheimer pathology deserves therefore to be investigated.

Keywords: Lithostathine; Lemur; β-amyloid deposit; EXTL3; Reg1

1. Introduction

Many degenerative diseases result from the aberrant polymerization and accumulation of specific proteins (Walker et al., 2006). These conformational diseases or proteopathies include neurological disorders such as Alzheimer's, Parkinson's, Huntington's, and prion diseases, diverse systemic disorders, particularly amyloidoses including type II diabetes, light chain amyloidosis, and cystic fibrosis (Aigelsreiter et al., 2007). At least 40 different proteins forming deposits have been described so far. Disorders involving protein deposition include a major protein component that forms the core, and additional species, including metal ions, glycosaminoglycans, and glycoproteins. Many functions for these "pathological chaperones" have been reported, ranging from involvement in amyloidosis to a major role of stabilization of amyloid deposits. Their contribution to amyloid toxicity has been also investigated (Alexandrescu, 2005).

Among proteins that could contribute to amyloidosis, exocrine pancreatic protein named also lithostathine or Reg1 alpha or pancreatic thread protein (PTP), is an inflammatory protein that forms deposits in pancreatic ducts of patients in chronic calcifying pancreatitis (De Caro et al., 1979). Originally identified as a secretory protein produced in pancreas by acinar cells (Iovanna et al., 1991), lithostathine is very susceptible to self-proteolysis under specific pH conditions (Cerini et al., 1999). The cleavage produces a soluble N-terminal undecapeptide and a C-terminal part of 133 amino acids that precipitate and form protease-K-resistant fibrils at physiological pH (Gregoire et al., 2001). Although its physiological function in digestive organs re-

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mains debated (Bimmler et al., 1997; De Reggi et al., 1998), the protein tightly binds calcium carbonate crystals, suggesting an inhibitory activity on $CaCO_3$ crystal growth, thus preventing lithiasis (Bernard et al., 1992; Geider et al., 1996; Gerbaud et al., 2000; Lee et al., 2003). It has also been shown that acute pancreatitis induces Reg1 gene expression and protein production in the pancreas (Dusetti et al., 1996; Iovanna et al., 1991). This increase of Reg1 protein is associated with Reg1 receptor messenger RNA (Kobayashi et al., 2000) suggesting that an upregulation of the receptor expression may also be important in the proliferative response of pancreatic regeneration (Bluth et al., 2006). In gastric cells, the lithostathine protein was shown to be a regulator of gastric mucosal proliferation (Perfetti et al., 1994) and to function as a mitogenic and/or an antiapoptotic factor in the development of early gastric cancer (Sekikawa et al., 2005). Accordingly, Watanabe et al. (1994) suggested that Reg1 protein has a trophic effect on isolated islet cells. More recently, a potential role of lithostathine in normal and neoplastic germ cell proliferation has been also described (Mauro et al., 2008).

Interestingly, the presence of lithostathine was also evidenced in human brain (de la Monte et al., 1990; Ozturk et al., 1989). Protein expression depends on the developing human brain and the presence of neurofibrillary tangles in the pathogenesis of Alzheimer's disease (AD). More recently, accumulation of the protein was concomitant with the formation of Aβ and prion amyloid plaques in patients with AD and prion diseases (Duplan et al., 2001; Laurine et al., 2003). While the accumulation of the protein has been linked to the inflammatory process of the pathogenesis of AD (Duplan et al., 2001), little is known concerning the expression pattern of lithostathine in normal aging.

In the present investigation, we investigated the cellular distribution of lithostathine within the brain of a primate model of aging, *Microcebus murinus*. With age, loss of cholinergic neurons was evidenced in the basal telencephalon in some animals (Mestre and Bons, 1993). In addition, lemurian primate presents similar aging characteristics as those observed in humans, such as modifications of the biological rhythms (Perret and Aujard, 2006), cognitive alterations (Bons et al., 2006; Picq, 1995, 2007) and cortical atrophy (Dhenain et al., 2000; Kraska et al., 2009) and it spontaneously develops numerous extracellular β-amyloid deposits in the cortical parenchyma (Bons et al., 1994; Mestre-Frances et al., 1996, 2000). Rarely, neurofibrillary degeneration was observed in the cortical pyramidal neurons (Bons et al., 1991). In addition, accumulation of tau proteins was also observed into the neocortex of young and old mouse lemurs (Bons et al., 1995; Delacourte et al., 1995; Giannakopoulos et al., 1997; Mestre and Bons, 1993) but not correlated with the presence of Aβ plaques (Giannakopoulos et al., 1997).

To determine the impact of aging and related pathology on lithostathine and its receptor brain expression, we performed a comparative analysis of lithostathine distribution in the brain of *Microcebus murinus* as a function of age.

2. Methods

2.1. Brain tissue

The brains of 20 adult gray mouse lemurs *Microcebus murinus* aged from 1 to 13 years were collected. Three groups were considered: 8 adults (1–4.5 years old), 6 healthy aged (5–13 years old) (Perret, 1997), or 6 aged with amyloid plaques (5.5–13 years old). Gray mouse lemurs were all born and kept in captivity within our breeding colony (École Pratique des Hautes Etudes, license approval number 34-05-026-FS, France), according to the guidelines of the French Ethical Committee (Decree 87–848) and the European Community Directive (86/609/EEC). The animals were anesthetized with ketamine (150 mg/kg). Lemur brains were fixed by transcardial perfusion with 50 mL saline (0.9%) followed by 100 mL Antigenfix solution (Diapath, Martinego, Italy) or by immersion in Antigenfix solution for 24 hours. Brains were embedded in paraplast and sliced in 8 μm serial sagittal sections or cryoprotected (30% sucrose solution for 3 days), and quickly frozen into isopentane chilled in liquid nitrogen. Frozen brains were mounted on a cryostat (Leica, Nanterre, France) and serially cut into 10-μm sagittal sections.

2.2. Immunohistochemical procedures

2.2.1. Primary antibodies

The following antibodies were used to detect lithostathine immunopositive cells, its receptor, or to identify various cell phenotypes in the brain: (1) rabbit polyclonal antibody against human lithostathine protein (Litho-*romeo*, 1:100; Duplan et al., 2001); (2) goat IgG polyclonal antibody against lithostathine receptor (EXTL3, 1:100; R&D Systems, Abingdon, UK); (3) rabbit polyclonal antibody against amyloid peptide (1–42) (FCA3542, 1:1000; Calbiochem, Merck KGaA, Darmstadt, Germany); (4) mouse IgG monoclonal antibody against glial fibrillary acidic protein (GFAP, 1:1000; Sigma-Aldrich, Saint Quentin-Fallavier, France); (5) mouse IgG monoclonal antibody against human amyloid protein (1–42) (8G7, 1:100, Alexis Biochemicals, Nottingham, UK); (6) mouse IgG monoclonal antibody against β-tubulin protein (βtub, 1:250; Sigma-Aldrich); (7) mouse IgG monoclonal antibody against microtubule associated protein (MAP2, 1:500; Sigma-Aldrich); and (8) mouse IgG monoclonal antibody against neuronal nuclei (NeuN, 1:250; Chemicon, Millipore, Saint Quentin -en -Yvelines, France).

2.2.2. Single immunoperoxidase labeling

Paraffin sections were dewaxed and hydrated through toluene and ethanol gradient and were then washed in water followed by immersion in hydrogen peroxide 3% for 30 minutes, rinsed in Tris-buffered saline (TBS),