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Antibody meets the microbeam – or how to find neurofibrillary tangles

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Abstract

In biomedical research the distributions of physiologically or pathologically active elements around or in a certain structure (e.g. tangles, plaques or different cell types) are often of great interest. Therefore, μPIXE analyses are applied to yield quantitative and spatially resolved concentration images of the elements of interest. However, the localisation of the structures to be examined is sometimes scarcely practicable or even impossible. This paper proposes a method of localising the areas of interest for μPIXE analysis. The method is based on the application of a suitable antibody tagged with a single elemental marker (e.g. Ni, Co, Cd, Ag or Au). The antibody then binds selectively to the structures of interest. The elemental marker is detectable via μPIXE, thus, showing finally the structure of interest via the bound antibody. The versatility of the antibodies in combination with the easily applied marker facilitates the localisation of a variety of structures in both light microscopy and μPIXE-imaging. The method is demonstrated on several cellular and subcellular structures in the brain. The elemental concentrations of two consecutive slices, one stained with Ni-enhanced antibody, the other unstained control, are compared to show the feasibility of trace elemental analysis for particular elements in spite if immunohistochemical structure identification. It has to be stated that the proposed technique will not work for free diffusing elements (like Na, Cl, K and Ca) whose concentrations can be altered by wet sample preparation.

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1. Introduction

The metal metabolism in the brain is receiving growing interest, since it has been linked to Alzheimer's disease (AD), Parkinson's disease (PD) [1–4] and other neurodegenerative diseases. Metals supposed to be involved in these pathological processes are Al, Ca, Mn, Fe, Cu, Zn and Pb [2,5,6]. The associated mechanisms are supposed to be located in the oxidative potential and physiological imbalance of the involved metal ions [7,4]. Alterations in their homeostasis, redox activity and sequestration can result in an increased free radical production with profound cellular consequences, including cytotoxicity with subsequent apoptosis or necrosis. Additionally, recent findings enforce the evidence of neurodegenerative processes to oxidative stress caused by metal induced free radicals [8,9].

These results indicate a direct correlation between metal abnormalities and the increased oxidative damage found in neurodegenerative diseases. Therefore, it is of vital importance to gain precise information on the concentrations of metals on preferably cellular or even subcellular levels with an unambiguous correlation to the different cell types and structures.

For this exercise the technique of nuclear microscopy (μPIXE) is a versatile tool to investigate the concentration and distribution of different metals below the 100 μmol/l (∼1 μg/g) level [10–12]. However, the localisation of the structures to be examined is sometimes scarcely practicable or even impossible. Furthermore, the prior standard histochemical staining can lead to misinterpretations of the elemental concentrations due to overlapping elemental content [13].

We therefore combine the technique of nuclear microscopy (μPIXE) and the immunohistochemical (IHC) antibody marking with metal enhancement. In the IHC techniques an antibody is used to link a cellular antigen specifically to a stain that can be more readily seen with a light microscope (LM, Fig. 1). IHC provides a wide range of specimen source, antigen availability, antigen–antibody affinity, antibody type, and detection enhancement methods. The method is based on the application of a suitable antibody tagged with a single elemental marker (e.g. Ni, Co, Cd, Ag or Au). The marker is detectable via PIXE, thus, showing finally the structure of interest via the bound antibody (Ni-maps in Fig. 1). The versatility of the antibodies in combination with the easily applied marker facilitates the localisation of a variety of structures in both light microscopy and μPIXE-imaging.

2. Materials and methods

2.1. Sample preparation

Brains from patients with AD and PD were obtained at autopsy (Brain Bank of the University of Leipzig). The AD case met the criteria for definite diagnosis of Alzheimer's disease according to Ref. [14]. The diagnosis was based on the NIA-Reagan Institute Criteria for the neuropathological assessment of AD [15,16]. The Ethical Committee of the Leipzig University has approved the case recruitment. Brains are fixed by immersion in 4% formalin for 1 week or longer. Conventional protocols for paraffin embedding were used to cut sections of 6 μm thickness from the *frontal-*, *temporal-cortex* and *substantia nigra*.

2.2. Immunohistochemistry

The sections were treated according to standard IHC protocols for each antibody: preincubation, incubation with the primary antibody followed by the secondary antibody. Table 1 gives the antibodies together with the associated structures. The colour was developed using diaminobenzidine (DAB) as chromogen with nickel as enhancer. The sections were mounted on object-slides with Entellan® (Merck, Germany). The regions of interest were cut out and mounted as self-supporting target for ion beam analysis.

2.3. Ion beam analysis

The spatially resolved elemental analysis (μPIXE) was carried out at the Leipzig microprobe