Bunina bodies

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Abstract

In 1961, Van Reeth and colleagues described the presence of intracellular inclusion bodies in the anterior horn cells of a patient with Pick dementia and atypical amyotrophic lateral sclerosis (ALS). A year later, Tat'yana Bunina, a neuropathologist from the USSR, described inclusion bodies with almost identical morphology, in the spinal cords and brain stems of two cases of familial ALS. She initially believed that they were a virus. However, electron microscopy and subsequent work involving intracerebral inoculation with material from various forms of ALS failed to demonstrate any signs of transmissibility. It is intriguing to speculate that Bunina’s original description of Bunina bodies may have been the original first clue on a path to unravelling the pathogenetic process in ALS.

Keywords

Bunina bodies; Amyotrophic lateral sclerosis; ALS.

Introduction

In 1961, Van Reeth and colleagues described the presence of intracellular inclusion bodies in the anterior horn cells of a patient with Pick’s dementia and atypical amyotrophic lateral sclerosis (ALS)[1]. A year later, Tat'yana Bunina, a neuropathologist from the USSR, described inclusion bodies with almost identical morphology, in the spinal cords and brain stems of two cases of familial ALS[2]. She initially believed that they were a virus. However, electron microscopy and subsequent work involving intracerebral inoculation with material from various forms of ALS failed to demonstrate any signs of transmissibility[3,4]. Rowland et al.[5] later confirmed the presence of the intracellular inclusion bodies and revealed that they were also present in patients with sporadic and Guamanian ALS. Their post mortem studies contributed to a growing body of evidence that suggested these structures were specific to patients with ALS. This was supported by Piao et al.[6], who found them in 88 out of 102 post mortem studies of patients with ALS. In his review of the neuropathology of ALS, Rowland et al. referred to these structures as Bunina bodies (BBs) and may have coined the term[2].

Following their discovery, BBs have been investigated by many researchers. Hart et al.[7] described their ultrastructure in 1977. More recently, Okamoto et al.[8] revealed that they were...
immunostained with anti-cystatin C serum and Mizuno et al.[9] found that transferrin localized within them.

### Distribution and appearance

BBs are present in most cases of ALS[2,5,6], with the exception of a subset of familial ALS with posterior column involvement and motor neuron disease with basophilic inclusions[2]. BBs have a predilection for the lumbar cord as opposed to thoracic and cervical sections and are present in greater numbers in the lower motor neurons of patients with an associated dementia[10,11]. They are most commonly found in the motor neurons of the spinal cord and in the brainstem nuclei[2]. Rarely, their presence has been reported in Betz cells[12], the oculomotor[13] and Onuf nuclei[14], the non-motor neurons of the Clarke column[15] and the medullary reticular formation[16].

At the microscopic level, they have been identified in the cytoplasm and dendrites[17] but not within the axoplasm[2]. They have a diameter of 3–5 μm and with haematoxylin and eosin staining, appear as bright pink, oval eosinophilic inclusions, occasionally showing clear areas in the centre and forming clusters[10,18,19]. Staining with phosphotungstic acid-hematoxylin causes them to appear purple; they are light blue on Kluver-Barrera staining and red on Masson trichrome stain. They do not stain with silver, periodic acid Schiff, Sudan black B or Congo red[2].

### Ultrastructural analysis

Many investigators have analysed BBs at the ultrastructural level. The general consensus is that they are formed of amorphous electron dense material and surrounded by tubular and vesicular structures. This was emphasized by Okamoto et al.[2], who examined serial sections of anterior horn cells using electron microscopy. They found that typical BBs consisted of amorphous material surrounded by tubular and vesicular structures with a central area containing 10-nm filaments and no limiting membrane. Sasaki et al.[20] observed both constricted and unconstricted bundles of filaments within the BBs, measuring 20–25 nm in width.

Ultrastructural properties of BBs have provided some clues regarding their origin. On observing BBs and laminated cytoplasmic bodies, Tomonaga et al.[18] concluded that they were very similar to the rough endoplasmic reticulum (rER). Trace element studies by Yoshida et al.[21] supported this view after confirming that aluminium (Al) binds strongly to BBs and the rER and less so with mitochondria and lipofuscin granules. They suggested that BB formation might be the result of nucleic acid dysmetabolism at the rER secondary to Al depletion.

Some have recorded the finding of bodies similar in structure to BBs in the Betz cells of non-ALS cases. Although the inclusions resembled the earliest stage of BB formation, it was deduced that they might be part of an age-related degeneration, because they were only present in the eldest portion of the cohort. This underscores the view that BB formation might be a facet of the degenerating process of a neuron[22].

### Immunohistochemical analysis

To date, only two proteins have been identified within BBs: transferrin and cystatin C. In their study of 12 patients with ALS, Mizuno et al.[23] found that antibodies to human transferrin localized within BBs and in some basophilic inclusions, whereas they did not localize in skein-like inclusions and Lewy body-like inclusions. They therefore hypothesised that transferrin may be involved in the formation of BBs.

Cystatin C, a lysosomal protease inhibitor, is produced by all nucleated cells and is present in various extracellular fluids in humans. Okamoto et al.[24] used a polyclonal rabbit antiserum against human cystatin C and demonstrated antibody localization in BBs. Furthermore, Lewy body-like inclusions and skein-like inclusions were not labelled with anti-cystatin C. Researchers have subsequently failed to show any genetic link between cystatin C and ALS, and have postulated that its localization within BBs may be related to abnormalities within proteins that interact with it[25]. Other components such as neurofilament, tau, alpha- and beta-tubulin, microtubule-associated proteins, actin, myosin, desmin, synaptophysin, amyloid precursor protein, glial fibrillary acidic protein, alpha-synuclein and p62 were not found within BBs[2,26].
Alongside BBs, other hallmarks of ALS include skein-like inclusion bodies and Lewy body-like inclusion bodies. They show positive immunoreactivities for ubiquitin and tdp-43 (a transcriptional repressor). The majority of investigators have failed to replicate these findings in BBs, although a small percentage have found some structures recognised by antiubiquitin antibodies in or around BBs[27].

Discussion

Since their characterisation by Bunina in 1962, our understanding of BBs has evolved. Initially labelled as a neurotrophic virus[4,28], these structures are now recognised as markers of neurodegeneration. We have arrived at this point following intense study by many researchers.

The Golgi complex, which is responsible for the processing of proteins, has been implicated in the formation of BBs. This idea was given impetus by the work of Stieber et al.[29], who found that Golgi bodies within motor neurons in ALS patients were usually fragmented and atrophied. Using immunohistochemical analysis with anti-MG160 antibody (staining for the membrane sialoglycoprotein of the medial cisternae of the Golgi body), Stieber et al. supported this finding, but also recognised that BBs themselves did not show staining for anti-MG160.

Yoshida et al.'s[21] suggestion that BBs may be related to nucleic acid dysmetabolism at the rER secondary to Al and magnesium depletion is appealing, with regard to recent attention on nucleic acid metabolism in the aetiology of ALS.

There is growing evidence that abnormalities in RNA processing might play a central role in the degeneration of motorneurons. This hypothesis has gained further credence following the discovery of tar DNA binding protein-43 (TDP-43) and fused-in liposarcoma (FUS) in ALS patients. In familial cases, mutations in genes coding for the proteins TDP-43 and FUS have been found to have a direct effect on all aspects of the RNA lifecycle, from gene transcription to translation and degradation, leading to defects in cell function[30]. TDP-43 and FUS have a very similar structure and function, underscoring the importance of nucleic acid dysmetabolism in the pathogenesis of ALS[31].

It is intriguing to speculate that Bunina's original description of BBs may have been the original first clue on a path to unravelling the pathogenetic process in ALS.

References