Preferential recruitment of ataxin-3 independent of expanded polyglutamine: an immunohistochemical study on Marinesco bodies

H Fujigasaki, T Uchihara, J Takahashi, H Matsushita, A Nakamura, S Koyano, K Iwabuchi, S Hirai, H Mizusawa

Abstract
In an immunohistochemical study of Marinesco bodies—a neuronal intranuclear inclusion often seen in neurons of the substantia nigra of patients with hepatic encephalopathy—it was shown that one of the polyglutamine proteins, ataxin-3, is preferentially recruited into this inclusion, whereas other polyglutamine proteins (ataxin-2 and TATA box-binding protein) are not. This suggests that recruitment of each of the polyglutamine proteins may be differently regulated. Because this nuclear inclusion is thought to be formed in response to cellular stress, as occurs in hepatic encephalopathy, even in the absence of an expanded CAG/polyglutamine repeat, recruitment of ataxin-3 and ubiquitin into Marinesco bodies may represent a cellular response to noxious external stimuli unrelated to expanded CAG/polyglutamine.

Keywords: ubiquitin; hepatic encephalopathy; neuronal intranuclear inclusion

Ataxin-3 is the product of the gene responsible for Machado-Joseph disease (MJD)/spinocerebellar ataxia (SCA) type 3, one of the dominantly inherited cerebellar ataxias. Abnormally expanded polyglutamine within ataxin-3 has been considered to be involved in the pathogenesis of this disease. Polyglutamine expansions are also responsible for other polyglutamine diseases: SCA1, 2, 6, and 7, Huntington’s disease, dentatorubral-pallidoluysian atrophy, and spinal and bulbar muscular atrophy. Recent studies have shown that the disease related proteins with abnormally expanded polyglutamine, except for the α-1A calcium channel, form neuronal intranuclear inclusions (NIIs) in the affected brains.8 9 Neuronal intranuclear inclusions also contain ubiquitin, chaperon proteins, and proteins carrying non-expanded polyglutamine.14 15 We have previously reported that Marinesco bodies, another example of ubiquitinated intranuclear inclusions found in pigmented neurons of the substantia nigra in normal people, also contain wild-type ataxin-3.4 5 This finding indicates that ataxin-3 is recruited into this intranuclear inclusion formed in the absence of expanded polyglutamine. The aim of this study is to extend this finding by looking for ataxin-3 and other polyglutamine proteins in Marinesco bodies that are abundant in brains from patients with hepatic encephalopathy.7

Materials and methods
Five midbrains from patients with hepatic encephalopathy, mean age (SD) 59.4 (9.7), and five age matched controls without neurological disorders, mean age (SD) 58.8 (8.6), were examined. Age at death and pathological diagnosis are summarised in table 1. Thick (6 μm) sections of formalin-fixed midbrain were stained with haematoxylin and eosin, or immunostained with antibodies against ubiquitin (1:200, DF2, IgM monoclonal, a generous gift from Dr H Mori, Osaka City University), ataxin-3 (1:1000), ataxin-2 (1:200, 15F6),2 6 and TATA box binding protein (TBP) (1:1000, Santa-Cruz),4 under the conditions described previously.4 6 7 The number of Marinesco bodies labelled by each staining was quantified by scanning a hemisubstantia nigra. The statistical significance of differences between the hepatic encephalopathy group and the control group were determined by the non-parametric Mann-Whitney U test. The results obtained with the different staining methods were compared by analysis of variance (ANOVA). A double immunofluorescence study was performed with the antib ubiquitin and antia ataxin-3 antibodies, visualised by antimouse IgM coupled with Cy-5 for the antiubiquitin antibody and antimouse IgG coupled with FITC for the antia ataxin-3 antibody. Confocal images were obtained with a Leica TCS-SP confocal laser microscope (Heidelberg, Germany).

Results
Many Marinesco bodies were seen on sections stained with haematoxylin and eosin (fig 1a), the antib ubiquitin antibody (fig 1b), and the antia ataxin-3 antibody (fig 1c). Marinesco bodies detected by these three methods were quantified. The mean number of Marinesco bodies in the hepatic encephalopathy group...
Ataxin-3 in Marinesco bodies

Table 1  Summary of patients and numbers of Marinesco bodies

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age at death</th>
<th>Pathological diagnosis</th>
<th>Numbers of Marinesco bodies</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>HE</td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>Oesophageal cancer</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Malignant lymphoma</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Lung cancer</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Cervical cancer</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Myocardial infarction</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>58.8</td>
<td>30.8</td>
</tr>
<tr>
<td>Hepatic</td>
<td>6</td>
<td>Liver cirrhosis</td>
<td>24</td>
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<tr>
<td>Encephalopathy</td>
<td>7</td>
<td>Hepatocellular carcinoma</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Liver cirrhosis</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Hepatocellular carcinoma</td>
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</tr>
<tr>
<td></td>
<td>10</td>
<td>Hepatocellular carcinoma</td>
<td>138</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>59.4</td>
<td>66.4</td>
</tr>
</tbody>
</table>

H&E=haematoxylin and eosin.

The number of Marinesco bodies visualised by haematoxylin and eosin, the antiataxin-3, or ubiquitin antibodies did not differ significantly either in the hepatic encephalopathy group or in the control group (p>0.9 ANOVA). The localisation of ubiquitin differed from that of Ataxin-3. Double immunofluorescence with the antiubiquitin and the antiataxin-3 antibodies showed that ataxin-3 was localised in the central core of the Marinesco bodies surrounded by ubiquitin which appears as a ring on the sections (fig 1 d, e, and f).

Discussion

Marinesco bodies are the eosinophilic intranuclear inclusions seen in pigmented neurons of the substantia nigra. It has been reported that these bodies increase in number during aging and in diseases such as hepatic encephalopathy. Although the pathological relevance of these inclusions has yet to be shown, their accumulation during aging or under pathological conditions such as hepatic encephalopathy suggests that formation of Marinesco bodies may be a cellular response to external stimuli. This hypothesis is supported by the fact that Marinesco bodies contain ubiquitin, known to be involved not only in stress responses but also in various neurodegenerative disorders. We previously showed that ataxin-3 is recruited into Marinesco bodies. The present study shows that almost all Marinesco bodies seen either in hepatic encephalopathy or in control groups contain ataxin-3 in common, indicating that it is one of the main components of this inclusion. Furthermore, neither ataxin-2 nor TBP were found in Marinesco bodies either in the hepatic encephalopathy group or in the control group, further indicating that Marinesco bodies are identical regardless of their pathological origin.

Because proteins with non-expanded polyglutamine can be recruited into nuclear inclusions formed in the presence of another protein with pathologically expanded polyglutamine, it has been postulated that an interaction between expanded polyglutamine and non-expanded polyglutamine may be a prerequisite for their nuclear translocation and the formation of NIIs. However, the consistent recruitment of wild-type ataxin-3 into Marinesco bodies indicates that another protein specific mechanism distinct from non-specific polyglutamine-polyglutamine interactions might mediate recruitment of wild type ataxin-3 into ubiquitinated intranuclear inclusions, as demonstrated previously. The absence of ataxin-2 and TBP in Marinesco bodies also supports the hypothesis that wild-type ataxin-3 may be recruited into ubiquitinated intranuclear inclusions by a protein specific mechanism, independent of a polyglutamine-polyglutamine interaction. Although the physiological and pathological functions of ataxin-3 remain to be elucidated, its preferential recruitment and colocalisation with ubiquitin in Marinesco bodies suggests that ataxin-3, like ubiquitin, is involved in cellular reactions to stress, as in hepatic encephalopathy or aging. The double immunofluorescence study clearly shows that ubiquitin almost always accumulated on the periphery of Marinesco bodies, whereas ataxin-3 was concentrated in the centre. This characteristic morphological feature is shared by NIIs of MJD/SCA3 brains as we reported elsewhere. The recruitment of wild-type ataxin-3 into NIIs followed by ubiquitin may be a common mechanism in the formation of these nuclear inclusions. Interestingly, a recent study showed that wild-type huntingtin can attenuate the cellular toxicity of huntingtin in vivo with expanded polyglutamine. The recruitment of wild-type ataxin-3 into these nuclear inclusions might therefore be one of the cellular reactions aimed at counteracting noxious stimuli of extracellular origin. We do not know yet whether recruitment of ataxin-3...
into NIIs triggered by the abnormal expansion of the CAG repeat in the ataxin-3 gene is mediated by the same mechanism as in Marinesco bodies. The consistent presence of wild-type ataxin-3 surrounded by ubiquitin in Marinesco bodies and in NIIs of MJD/SCA3 suggests that a process involving both ataxin-3 and ubiquitin may be shared at least partly by these two different conditions.

In conclusion, the present study showed the consistent recruitment of ataxin-3 into Marinesco bodies surrounded by ubiquitin as previously seen in SCA3/MJD, suggesting that these inclusions are formed to reduce cellular stress. The absence of other polyglutamine proteins in Marinesco bodies suggests that the mechanism of recruitment does not involve polyglutamine alone but also a specific feature of the ataxin-3.

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