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LETTER

Autophagy and mitophagy elements are increased in body fluids of multiple sclerosis-affected individuals

BACKGROUND

Multiple sclerosis (MS) is a chronic multifaceted demyelinating and neurodegenerative disease of the central nervous system (CNS) of presumed autoimmune origin.[1]

Patients with MS are characterised by a spatial and temporal dissemination of neurological sign and symptoms, by the presence of multifocal lesions in the periventricular white matter on MRI scans and by an immunoglobulin synthesis within the CNS.[1] Further diagnostic tools are desirable, and the use of blood and cerebrospinal fluid (CSF) biomarkers may contribute to the comprehension of the disease's pathogenesis and progression.

Autophagy is an evolutionarily conserved and genetically controlled cellular process where intracellular components are sequestered within double-membrane vesicles (autophagosomes), which then fuse with lysosomes where the material is degraded.[2] Autophagy also occurs as mitophagy, which is responsible for the removal of aberrant, aged and wasted mitochondria. Interestingly, autophagic/mitophagic pathways have been found deregulated in various human diseases. In particular, it has been demonstrated how these catabolic pathways are implicated in several neurodegenerative diseases such Alzheimer's and Parkinson's diseases and amyotrophic lateral sclerosis.[2] Moreover, recent studies suggest a role of mitochondrial dysfunction in the neurodegenerative aspects of MS.[3] Despite these observations, the role of autophagy and mitophagy in MS is still elusive.

To tackle the question, we tried to verify the frequency of specific autophagic markers (ATG5 protein) and mitophagic markers (Parkin protein) in patients with MS and in neurological controls.

METHODS

Forty consecutive untreated patients with relapsing–remitting MS (RRMS) were included in the study (24 women and 16 men; mean age=40.7±10.5). Forty patients with other inflammatory neurological diseases (OINDs) (21 women and 19 men; mean age=55.9±17.5) and 40 subjects with non-inflammatory neurological diseases (NINDs) (21 women and 19 men; mean age=64.8±15.5) were included as published before.[4] Paired CSF and serum samples collected from patients with RRMS, OIND and NIND were obtained for purposes of diagnosis. Serum samples obtained from 40 healthy donors (CTRL) (24 women and 16 men; mean age=28.6±6.5) were used as an additional control. The study was approved by the Committee for Medical Ethics in Research of Ferrara and written informed consent was obtained from all subjects.

ELISA kits for ATG5 (MS7209535) and Parkin (MBS732278) were obtained from My Biosource (San Diego, California, USA). Tumour necrosis factor α (TNFα) ELISA kits (KHC3011) were purchased from Thermo Fisher Scientific (Waltham, Massachusetts, USA). All the assays were performed as instructed by the manufacturer. Statistical analysis was performed with GraphPad Prism software. Parametric and non-parametric tests were used, respectively, for normally and non-normally distributed data.

RESULTS

Serum levels of ATG5 and Parkin were higher in patients with MS than in OIND, NIND and CTRL ($p<0.0001$) (figure 1). Similarly, CSF levels of ATG5 and Parkin were higher in patients with MS than in OIND and NIND ($p<0.0001$) (figure 1). A positive correlation was found between ATG5 and Parkin levels both in sera and CSF of patients with MS ($p<0.001$ and $p<0.0001$) (figure 1). In CSF of patients with MS, positive correlations were described between TNFα and both ATG5 and Parkin levels ($p<0.0001$) (figure 1). Serum and CSF titres of ATG5 and Parkin were not different in patients with MS grouped according to clinical disease activity and did not correlate with age and sex in MS, OIND and NIND (data not shown).

DISCUSSION

The novelty of this study is the impressive increase of both catabolic markers in patients with MS compared with controls, suggesting that elevated autophagy/mitophagy seems to be specifically related to the disease. The role of autophagic/mitophagic pathways in MS is not well clarified, and it remains doubtful whether they are protective or harmful processes. Some works suggest that autophagy is a protective homeostatic mechanism capable of influencing synaptic growth and plasticity. For this reason, upregulation of autophagy may prevent, delay or ameliorate neurodegenerative diseases.[2] Conversely, other studies consider these pathways a very dangerous condition in CNS. Indeed, inflammatory stimuli lead to a blockade of the differentiation of oligodendrocyte progenitor cell, resulting in a reduced myelination of axons in vitro, as a consequence of an increased autophagic activity.[5]

The increases we found for both ATG5 and Parkin in CSF of patients with MS confirm the last hypothesis and indicate a pathological role of autophagy/mitophagy in CNS of patients with MS.[5] Also, the correlations between both ATG5 and Parkin levels and TNFα concentrations in CSF seem to confirm in vivo the association among autophagic/mitophagic activity and inflammatory stimuli, as previously described in in vitro experiments.[2 5] Again, the positive correlation between ATG5 and Parkin concentrations suggests that autophagic and mitophagic mechanisms are reciprocally associated in CSF and serum of patients with MS.

These results are particularly intriguing. In fact, if on the one hand CSF data should reflect a role of autophagy/mitophagy in MS pathogenesis, on the other hand, serum levels of these molecules could be used as biomarkers of MS. The main problem of lumbar puncture to obtain CSF is the invasive method, and special precautions should be taken during its execution. Thus, there is a pressing need for new biomarkers in more easily accessible body fluids such as peripheral blood.

The main limit of the present study is the lack of additional clinical information, such as disease duration and/or severity, and the lack of sex and age matching between patients with MS and controls. However, we did not find any difference between men and women, and there were no significant correlations between age and the concentrations of ATG5 and Parkin in all the four groups of patients and controls.

In conclusion, our study suggests, for the first time, that autophagy and mitophagy processes could play an important role in the pathogenesis of MS and introduces a fascinating conjecture. Further studies on a larger population are needed to elucidate the molecular relationshiSir pokkatps between catabolic processes and MS ongoing and progression, as well as to elucidate the potential role of autophagic/mitophagic biomarkers in monitoring disease progression and/or the pharmacological response to therapy.

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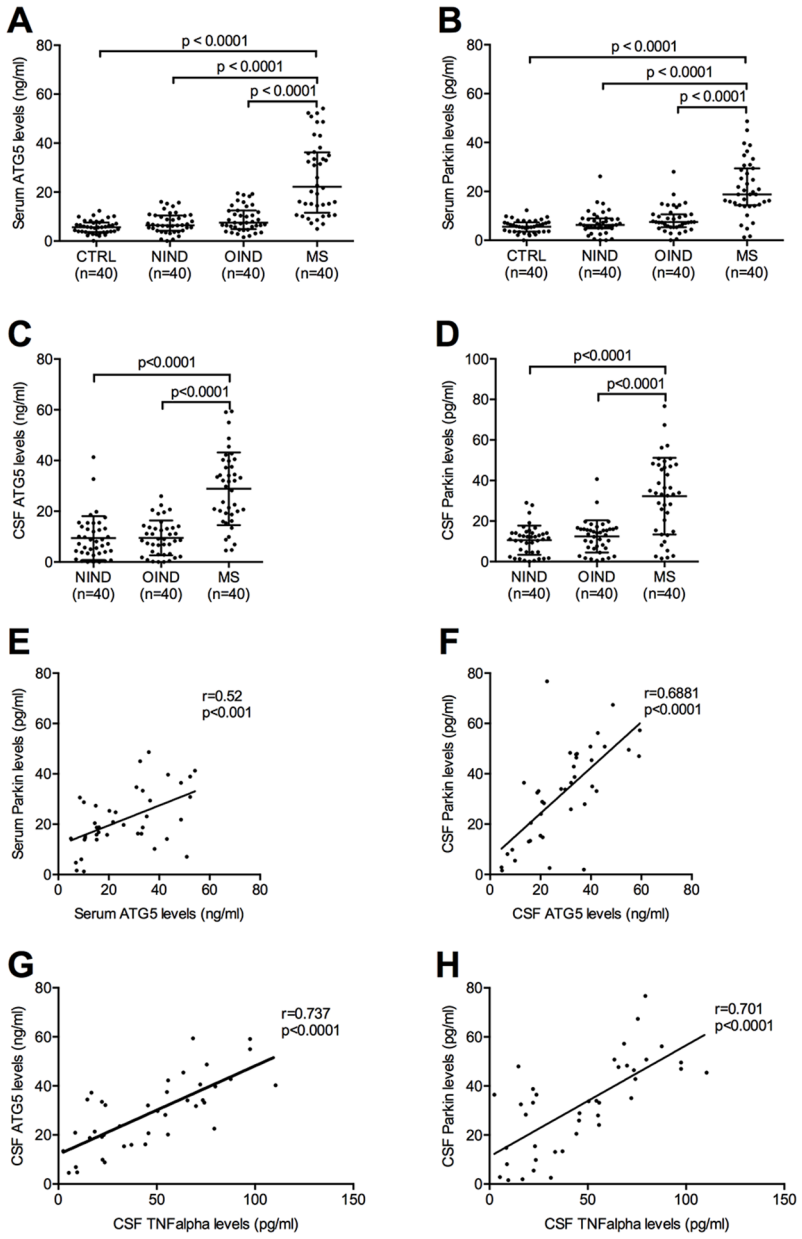

Figure 1 Serum levels of ATG5 and Parkin from healthy donors (CTRL), subjects with non-inflammatory neurological disorders (NIND), other inflammatory neurological disorders (OIND) and from patients with multiple sclerosis (MS). Concentrations of ATG5 and Parkin were different among CTRL, NIND, OIND and MS (Kruskal-Wallis; p<0.0001). Serum values of ATG5 (Mann-Whitney with Bonferroni correction): MS versus OIND (p<0.0001), MS versus NIND (p<0.0001) and MS versus CTRL (p<0.0001) (A). Serum levels of Parkin (Mann-Whitney with Bonferroni correction): MS versus OIND (p<0.0001), MS versus NIND (p<0.0001) and MS versus CTRL (p<0.0001) (B). Cerebrospinal fluid (CSF) levels of ATG5 and Parkin were different among MS, OIND and NIND (ANOVA; p<0.0001). CSF values of ATG5 (t-test with Bonferroni correction): MS versus OIND (p<0.0001) and MS versus NIND (p<0.0001) (C). CSF levels of Parkin (t-test with Bonferroni correction): MS vs OIND (p<0.0001) and MS versus NIND (p<0.0001) (D). There were positive correlations between ATG5 and Parkin levels in sera (Spearman; p<0.001) and CSF (Pearson; p<0.0001) of patients with MS (E and F, respectively). Additional correlations were found in CSF for both ATG5 and Parkin levels and tumour necrosis factor α (TNFα) among subjects with MS (Spearman; p<0.0001) (G and H, respectively). Parametric and non-parametric tests were used respectively for normally and non-normally distributed data. Accordingly, results are presented as mean and SD for normally distributed variables and as median and IQR for non-normally distributed data. A value of p <0.05 was accepted as statistically significant.

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Contributors SP, CG, EG and PP conceived and designed the experiments. SP and MC conducted the experiments, analysis of samples, analysis and interpretation of data. MB, SM, MP and IC helped with patients' enrolment and data analysis. SP, MC, EG and PP wrote the manuscript. All authors have reviewed and approved the content and submission of this manuscript.

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