New insight on nuclear superoxide dismutase 1 in Amyotrophic Lateral Sclerosis

L Diamanti[1,2], O Pansarasa[3], M Bordoni[1,3], V Crippa[3], D Sproviero[3], M Dell'Orco[3], S Gagliardi[3], A Poletti[4], M Ceroni[1,2], C Cereda[3]

1 Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy.
2 General Neurology Unit, C. Mondino National Institute of Neurology Foundation, IRCCS, Pavia, Italy.
3 Center of Genomic and post-Genomic, C. Mondino National Institute of Neurology Foundation, IRCCS, via Mondino 2, 27100 Pavia, Italy.
4 Center of Excellence on Neurodegenerative Diseases and Department of Pharmacological and Biomolecular Sciences, University of Milan

Introduction

In 2010 we found an over-expression of SOD1 mRNA in PBMCs of patients, in contrast with the unchanged cytoplasmic level of the protein (Gagliardi et al., 2010). We suppose that the "missing" protein re-localize in the nucleus. In fact recent study showed that, in PBMCs of sALS patients, SOD1 translocates from the cytoplasmic compartment to the nucleus (Cereda et al., 2013). To support this hypothesis Tsang and colleagues suggested a new function of SOD1 in oxydative stress response in yeast (Tsang et al., 2014).
The aim of this work is to demonstrate nuclear SOD1 translocation in our cellular models, to explain how SOD1 transolcates and a possible new function of the protein in the nucleus.

Materials and Methods

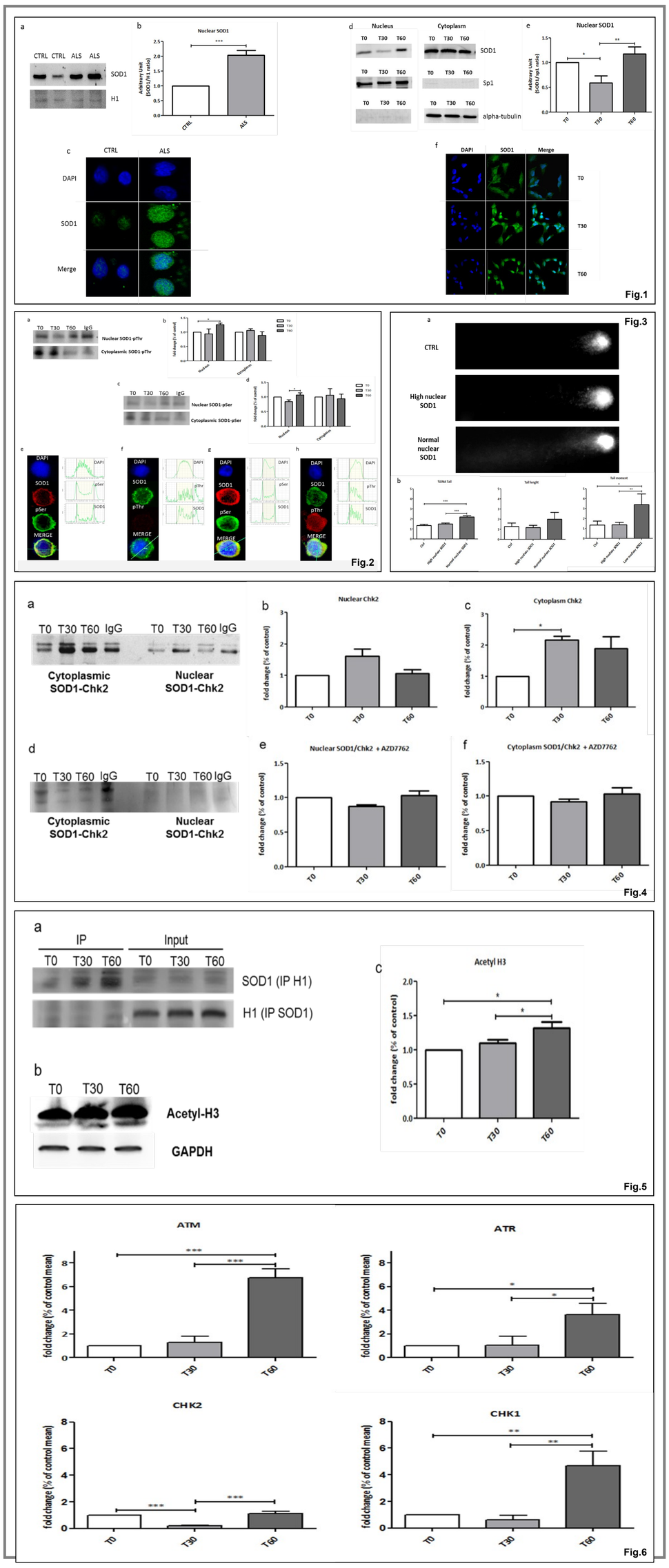
We studied by both western blot (WB) and immunofluorescence (IF) SOD1 localization in sALS patient and in SH-SY5Y untreated and treated with 1mM H_2O_2 for 30 and 60 minutes. By means Mass Spectrometry (MS) we searched for post-translational modifications that permit SOD1 re-localization. We studied DNA damage in patients' PBMCs using Comet Assay. Binding protein involved in regulation of SOD1 localization was identified by immunoprecipitation (IP). We analyzed level of histones acetylation by WB. We also analyzed mRNA expression of ATM, ATR, CHK1 and CHK2 by RT-qPCR.

Results

Fig.1 We confirmed by WB an increase in nuclear localization in patients' PBMCs. This data was confirmed by IF. The same was done with SH-SY5Y treated with 1mM H_2O_2 for 60 minutes. **Fig.2** As MS data suggests us, we found in SH-SY5Y treated for 60 minutes an increase of pThr and pSer in nucleus by IP of SOD1. We confirmed phosphorylation in patients' PBMC by IF. **Fig.3** With comet assay we found that an higher nuclear SOD1 seems to protect DNA from oxidative stress damage. **Fig.4** We supposed that phosphorylation of SOD1 is carried out by Chk2, so we confirmed the interaction after oxidative stress between Chk2 and nuclear SOD1 by SOD1 IP. We confirmed also that inhibition of Chk2 with AZD 7762 5nM prevent this interaction. **Fig.5** We supposed that SOD1 could act as transcription factor, in fact we found by IP that SOD1 interact with chromatin and by WB we saw an increase of Acetyl H3 after treatment with H_2O_2. No difference statistically significant were found in acetylation of other histones. **Fig.6** RT-qPCR in SH-SY5Y treated with 1 mM H_2O_2 for 30 and 60 min showed that ATM/Chk2 and ATR/Chk1 are actively transcribed after 60 min of oxidative stress.

Conclusions

We demonstrated that under oxidative stress SOD1, that re-locates at nuclear compartment probably thanks to its phosphorylation by Chk2, is involved in the protection against DNA damage. More interestingly, nuclear SOD1 seems to be involved in the regulation of gene transcription, mainly of those protecting the cell from oxidative stress damage.



References

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