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Abstract

Background **Cooling is the single most effective treatment for acute neuronal injury. Understanding the molecular mechanisms that mediate cooling-induced neuroprotection might yield novel therapeutic targets for neurodegenerative disease. Many of these disorders involve modulation of tau, a protein that is enriched in neurons and becomes hyperphosphorylated in hypothermic, injury-resistant rodent brains as well as in Alzheimer's disease. We sought to establish a new model for exploring human tau physiology and its role in neuroprotection in the context of therapeutic hypothermia.**

Methods **Functional cortical neurons (hCNs) were differentiated from three independent human pluripotent stem-cell lines and validated for regional identity and tau status. Matched cultures were incubated at 37°C or clinically targeted temperatures for therapeutic hypothermia (32°C) and suspended animation (28°C). Effects of cooling on tau status and neuronal injury elicited by common neurotoxic stressors were established. Injury experiments were repeated while manipulating tau phosphorylation.**

Findings **hCN differentiation featured transitions in tau status, recapitulating transcriptional and post-translational human in-vivo cortical tau development. Key aspects of this development were reversed by cooling. Notably, cooling induced tau hyperphosphorylation via rapid inhibition of the major tau phosphatase, protein phosphatase 2A (PP2A). Multiplexed injury analysis confirmed that hypothermia robustly protected hCNs from oxidative (100 μM hydrogen peroxide) and excitotoxic (30 μM glutamate) stress (at 28°C, injury was reduced by 78% and 56%, respectively; p<0·0005). Selective block of a major tau kinase reduced hCN tau phosphorylation and abrogated hypothermic protection (p=0·001) against oxidative injury at 28°C (injury reduction diminished to 9% at 100 μM hydrogen peroxide), whereas pharmacological inhibition of PP2A mimicked cooling-induced tau phosphorylation and protected hCNs from oxidative injury at 37°C (up to 22% injury reduction [p=0·008], with no benefit under hypothermic conditions).**

Interpretation **To our knowledge, this is the first study of human neuronal tau physiology under hypothermic conditions. Although definitive experiments are needed, our findings support previous work linking phospho-tau to neuroprotection. Furthermore, cold-induced tau hyperphosphorylation is a potential trigger for proteostatic priming, a recently discovered mechanism of hypothermic preconditioning in hCNs. Exploiting these cryobiological effects might lead to new treatments for acute and chronic neuronal injury.**

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Contributors

NMR conceived the study, devised the methodology, conducted investigations, analysed the data, acquired funding and resources, and wrote the first draft of the abstract. PC devised the methodology, analysed the data, acquired funding and resources, and reviewed and edited the abstract. MRL conducted investigations, analysed the data, and reviewed and edited the abstract. RP, DS, and KB reviewed and edited the abstract. SB and DJAW acquired resources and reviewed and edited the abstract. GEH and SC acquired funding and resources, reviewed and edited the abstract, and supervised the study.

Declaration of interests

We declare no competing interests.