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Report

Transient Accumulation of 5-Carboxylcytosine Indicates Involvement of Active Demethylation in Lineage Specification of Neural Stem Cells

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SUMMARY

5-Methylcytosine (5mC) is an epigenetic modification involved in regulation of gene activity during differentiation. Tet dioxygenases oxidize 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC). Both 5fC and 5caC can be excised from DNA by thymine-DNA glycosylase (TDG) followed by regeneration of unmodified cytosine via the base excision repair pathway. Despite evidence that this mechanism is operative in embryonic stem cells, the role of TDG-dependent demethylation in differentiation and development is currently unclear. Here, we demonstrate that widespread oxidation of 5hmC to 5caC occurs in postimplantation mouse embryos. We show that 5fC and 5caC are transiently accumulated during lineage specification of neural stem cells (NSCs) in culture and in vivo. Moreover, 5caC is enriched at the cell-type-specific promoters during differentiation of NSCs, and TDG knockdown leads to increased 5fC/5caC levels in differentiating NSCs. Our data suggest that active demethylation contributes to epigenetic reprogramming determining lineage specification in embryonic brain.

INTRODUCTION

5-Methylcytosine (5mC) is a DNA modification contributing to the regulation of gene activity during development and differentiation (Reik et al., 2001; Bird, 2002). Tet (Ten-eleven translocation) dioxygenases (TET1/2/3) can oxidize 5mC, generating 5-hydroxymethylcytosine (5hmC), which, according to a growing body of evidence, plays specific biological roles in embryonic stem cells (ESCs) and the mammalian brain (Tahiliani et al., 2009; Ficz et al., 2011; Lister et al., 2013). 5hmC can be enzymatically oxidized further to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) (Ito et al., 2011; He et al., 2011). Both 5fC and 5caC can be recognized and excised from DNA by thymine-DNA glycosylase (TDG) followed by subsequent regeneration of unmodified cytosine by the components of base excision repair (BER) pathway (He et al., 2011; Maiti and Drohat, 2011). Correspondingly, recent genome-wide analyses of 5fC/5caC distribution in mouse ESCs (mESCs) revealed a TDG-dependent accumulation of these marks in gene regulatory elements, suggesting that this mechanism of active demethylation is operative in mESCs (Shen et al., 2013; Song et al., 2013).

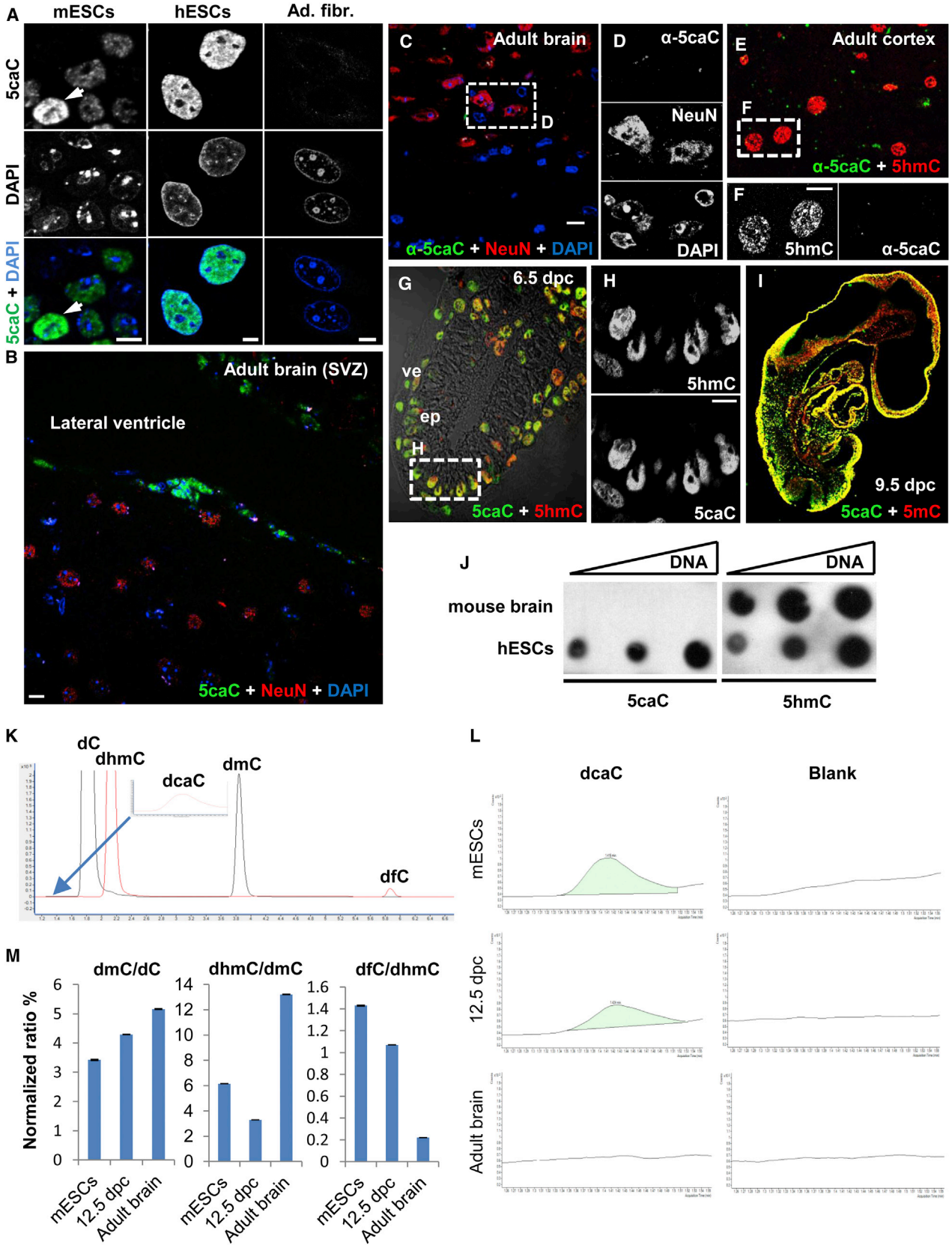
Paradoxically, contrary to the mESC-based results, recent studies imply that a passive replication-dependent demethylation mechanism is involved in epigenetic reprogramming during development of primordial germ cells (PGCs) (Seisenberger et al., 2012; Hackett et al., 2013). Likewise, although 5fC and 5caC are detectable in mouse zygotes, these marks are diluted in a replication-dependent manner during preimplantation development instead of being actively removed from DNA (Inoue et al., 2011). Thus, the role of BER-dependent demethylation in cellular differentiation and development is currently unclear.

In this study, we aimed to examine if a TDG/BER-dependent active DNA demethylation pathway is employed during mammalian embryogenesis and differentiation.

RESULTS

Widespread Oxidation of 5hmC to 5caC Occurs in ESCs and in Mouse Postimplantation Embryos

Since 5fC and 5caC can serve as intermediates in active demethylation, we checked whether these marks are detectable in ESCs and postimplantation embryos employing a sensitive immunostaining method based on the use of peroxidase-conjugated secondary antibody and tyramide amplification reagent, which we have previously applied to 5hmC detection (Ruzov



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