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Alternative Routes to Induced Pluripotent Stem Cells Revealed by Reprogramming of the Neural Lineage

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SUMMARY

During the reprogramming of mouse embryonic fibroblasts (MEFs) to induced pluripotent stem cells, the activation of pluripotency genes such as NANOG occurs after the mesenchymal to epithelial transition. Here we report that both adult stem cells (neural stem cells) and differentiated cells (astrocytes) of the neural lineage can activate NANOG in the absence of cadherin expression during reprogramming. Gene expression analysis revealed that only the NANOG+E-cadherin+ populations expressed stabilization markers, had upregulated several cell cycle genes; and were transgene independent. Inhibition of DOT1L activity enhanced both the numbers of NANOG+ and NANOG+E-cadherin+ colonies in neural stem cells. Expressing SOX2 in MEFs prior to reprogramming did not alter the ratio of NANOG colonies that express E-cadherin. Taken together these results provide a unique pathway for reprogramming taken by cells of the neural lineage.

INTRODUCTION

Overexpression of the four transcription factors, *Oct4, Sox2, Klf4*, and *c-Myc* (OSKM) is sufficient to reprogram somatic cells into induced pluripotent cells (iPSCs) (Jackson and Sridharan, 2013). The mechanism of reprogramming is incompletely elucidated due to the inefficiency of the process with about 5% of the cells reaching the iPSC state under standard serum or serum replacement culture conditions (Papp and Plath, 2013). While a variety of somatic cells have been used as a starting point for the reprogramming process (Hussein and Nagy, 2012), mechanistic studies have been largely limited to those using mouse embryonic fibroblasts (MEFs). Tracking reprogramming populations has delineated a series of events that take place in a timed manner such as the loss of somatic cell gene expression followed by mesenchymal to epithelial transition (MET) indicated primarily by the acquisition of the cell surface marker E-cadherin (Samavarchi-Tehrani et al., 2010; Li et al., 2010). This is followed by the gain of expression of pluripotency markers such as OCT4 and NANOG, by the appearance of stabilization markers such as DPPA4, and independence from exogenous reprogramming factor expression (Apostolou and Hochedlinger, 2013). Overlaid on these transitions, experiments on single cells have revealed an early stochastic phase of gene expression followed by a late hierarchical phase triggered by the activation of *Sox2* (Buganim et al., 2012). Therefore, we were interested in determining if cells that expressed endogenous SOX2 followed the same pathway as MEFs and focused on reprogramming both adult stem cells (neural stem cells [NSCs]) and differentiated cells (astrocytes) from the neural lineage.

Both human and mouse NSCs can be reprogrammed with the omission of exogenous *Sox2* in the reprogramming cocktail (Kim et al., 2008), and can even be reprogrammed with *Oct4* alone (Kim et al., 2009). NSCs can also be more readily reprogrammed to intermediate stages, called partially reprogrammed cells, than MEFs (Silva et al., 2008). Remarkably, we found that upon induction of reprograming, in both NSCs and astrocytes, NANOG expression preceded or was concomitant with E-cadherin expression and the expression of SSEA1, an intermediate marker of pluripotency.

Abrogation of E-cadherin expression through shRNA-mediated knockdown reduces reprogramming efficiency from MEFs and compromises the quality of iPSCs obtained (Chen et al., 2010), while MEFs lacking E-cadherin cannot form Nanog+ colonies (Redmer et al., 2011). E-cadherin can also replace *Oct4* in the reprogramming factor cocktail (Redmer et al., 2011). Truncations of E-cadherin in MEF reprogramming revealed the necessity of the extracellular domain (Chen et al., 2010). Interestingly, in the absence of E-cadherin in embryonic stem cells (ESCs), N-cadherin is able to functionally replace E-cadherin to maintain pluripotency (Hawkins et al., 2012).

We found that Nanog+ colonies from NSC reprogramming cultures can have N-cadherin, E-cadherin, or neither cadherin. However, colonies that expressed stabilization markers (Golipour et al., 2012), such as *Dppa4*, and that were transgene independent always co-expressed NANOG and E-cadherin. Gene expression analysis of populations sorted for expressing NANOG alone (N+) or NANOG and E-cadherin (N+E+) revealed that the N+E+ population expressed higher levels of cell cycle genes suggesting a greater

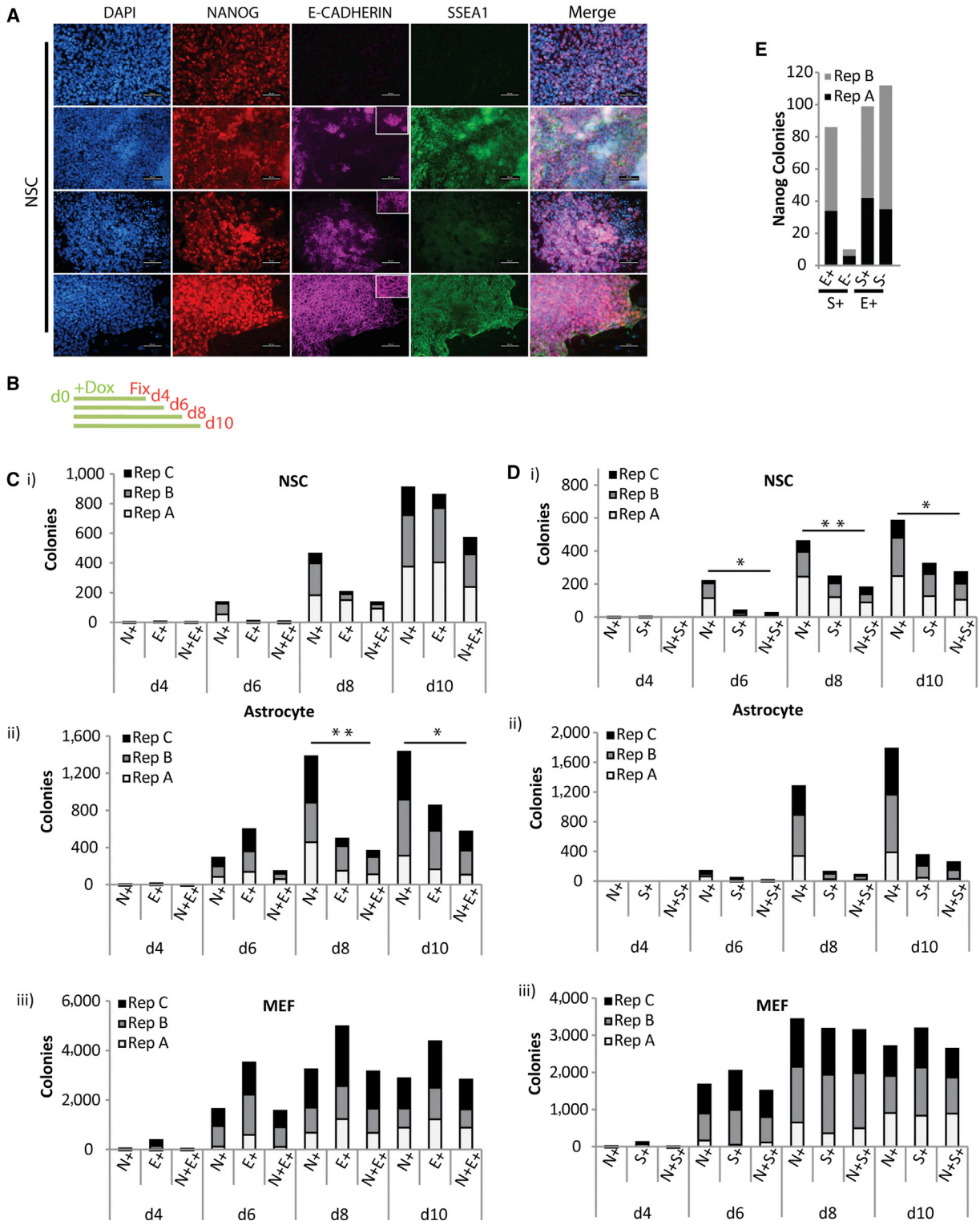

Figure 1. Nanog+ Colonies from Neural Stem Cell and Astrocyte Reprogramming Can Emerge Independent of E-Cadherin or SSEA1

(A) Immunofluorescence (IF) images of NANOG colonies on day 10 of reprogramming NSC with E-cadherin and/or SSEA1. Scale bar, 50 μm. Insets, magnification of field.

(B) Scheme of experiment presented in (C) and (D). Dox was added to cells on day 0 (d0) and reprogramming cultures were fixed on indicated days.

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