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Regulation of pluripotent cell differentiation by a small molecule, staurosporine

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ABSTRACT

Research in the embryo and in culture has resulted in a sophisticated understanding of many regulators of pluripotent cell differentiation. As a consequence, protocols for the differentiation of pluripotent cells generally rely on a combination of exogenous growth factors and endogenous signalling. Little consideration has been given to manipulating other pathways to achieve pluripotent cell differentiation. The integrity of cell:cell contacts has been shown to influence lineage choice during pluripotent cell differentiation, with disruption of cell:cell contacts promoting mesendoderm formation and maintenance of cell:cell contacts resulting in the preferential formation of neurectoderm. Staurosporine is a broad spectrum inhibitor of serine/threonine kinases which has several effects on cell function, including interruption of cell:cell contacts, decreasing focal contact size, inducing epithelial to mesenchyme transition (EMT) and promoting cell differentiation. The possibility that staurosporine could influence lineage choice from pluripotent cells in culture was investigated. The addition of staurosporine to differentiating mouse EPL resulted in preferential formation of mesendoderm and mesoderm populations, and inhibited the formation of neurectoderm. Addition of staurosporine to human ES cells similarly induced primitive streak marker gene expression. These data demonstrate the ability of staurosporine to influence lineage choice during pluripotent cell differentiation and to mimic the effect of disrupting cell:cell contacts. Staurosporine induced mesendoderm in the absence of known inducers of formation, such as serum and BMP4. Staurosporine induced the expression of mesendoderm markers, including markers that were not induced by BMP4, suggesting it acted as a broad spectrum inducer of molecular gastrulation. This approach has identified a small molecule regulator of lineage choice with potential applications in the commercial development of ES cell derivatives, specifically as a method for forming mesendoderm progenitors or as a culture adjunct to prevent the formation of ectoderm progenitors during pluripotent cell differentiation.

1. Introduction

Directing the differentiation of pluripotent cells in culture into specific cell populations has been a long-term challenge. The ability to impose a single, predetermined cellular outcome during differentiation at the expense of other outcomes is predicted to have many applications; it will provide specific cell populations for research, populations of cells that can be developed for commercial applications, such as for drug screening or cell-based devices, and ultimately cells for clinical use. Moreover, understanding the processes regulating directed differentiation in culture will give unique insights into the molecular regulation of these processes during embryogenesis.

In culture mouse ES cells can be directed to form a second pluripotent cell population, early primitive ectoderm-like (EPL) cells, in response to a medium conditioned by the human hepatocellular cell line, HepG2, and more specifically by the amino acid L-proline (Casalino et al., 2011; Lake et al., 2000; Rathjen et al., 1999; Tan et al., 2011). EPL cells share many properties with the

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embryonic primitive ectoderm, including morphology, gene expression, differentiation potential and cytokine responses (Lake et al., 2000; Pelton et al., 2002; Rathjen et al., 2002, 1999). In culture, EPL cell differentiation within embryoid bodies (EPLEBs) recapitulates molecular gastrulation and provides a sensitive model for identifying and understanding the regulatory pathways that determine lineage choice during differentiation (Hughes et al., 2009a, 2009b; Lake et al., 2000; Rathjen et al., 2002).

We have shown previously a critical role for cell:cell contacts in lineage choice during EPL cell differentiation (Hughes et al., 2009b), with disruption of cell:cell contacts resulting in the preferential formation of primitive streak-like intermediates, or mesendoderm, while maintenance of cell:cell contacts promoted the establishment of ectoderm. Maintaining E-cadherin integrity during differentiation, through suppression of γ-secretase activity with DAPT, prevented formation of primitive streak-like intermediates and results in the preferential formation of ectoderm (Hughes et al., 2009a). This outcome can be reversed by destabilising E-cadherin through addition of a neutralising E-cadherin antibody (Hughes et al., 2009a). A chemical screen looking for compounds able to promote pluripotency has identified a role for E-cadherin stability in hES cell survival and self-renewal (Xu et al., 2010), and in mouse ES cells E-cadherin has been linked to the maintenance of pluripotency (Hawkins et al., 2012). These data support a role for E-cadherin integrity, and cell:cell junction integrity, in pluripotent cell maintenance and pluripotent cell differentiation in culture and raise the possibility that chemicals which modulate junction integrity may be able to modulate differentiation and/or lineage choice from pluripotent cells.

Staurosporine is a broad spectrum serine/threonine kinase inhibitor (Tamaoki et al., 1986), which can affect the activity of a range of kinases including PKC, PKA, PKG and CaM (Ruegg and Burgess, 1989; Tamaoki et al., 1986; Yanagihara et al., 1991). At high concentrations staurosporine induces apoptosis in a large number of cell populations (Bertrand et al., 1994; Falcieri et al., 1993; Koh et al., 1995; Weil et al., 1996), including differentiating ES cells (Buschke et al., 2012). At lower concentrations, typically between 20 and 25 nM, staurosporine interrupts the formation of, or destabilises established, cell:cell contacts (Denisenko et al., 1994; Ratcliffe et al., 1997), decreases focal contact size (Hugo et al., 2009), induces epithelial to mesenchymal transition (EMT) (Newgreen and Minichiello, 1995, 1996) and has been shown to induce cell differentiation (Thompson and Levin, 2010; Zhang et al., 2005; Schumacher et al., 2003). Treatment of cells with lower concentrations of staurosporine has been suggested to target atypical PKCs in the cellular adherens junction and disrupt the association between the actin cytoskeleton and junction (Minichiello et al., 1999; Newgreen and Minichiello, 1996). It has also been shown to result in hyperphosphorylation of p100/p120, cellular adherens junction-associated proteins; this has similarly been postulated to modulate intracellular junction function (Ratcliffe et al., 1997).

Here, we examine the effect on lineage choice of adding low concentrations of staurosporine during EPL cell differentiation. The addition of staurosporine resulted in the preferential formation of mesendoderm and mesoderm, with a reduction in the formation of neurectoderm and neurons. Addition of staurosporine to human ES cells similarly promoted differentiation and induced the expression of primitive streak markers. Staurosporine affected lineage choice in conditions that have been shown to suppress primitive streak formation, including in the presence of the BMP4 antagonist noggin, and in serum-free medium conditions, suggesting that activity was independent of known

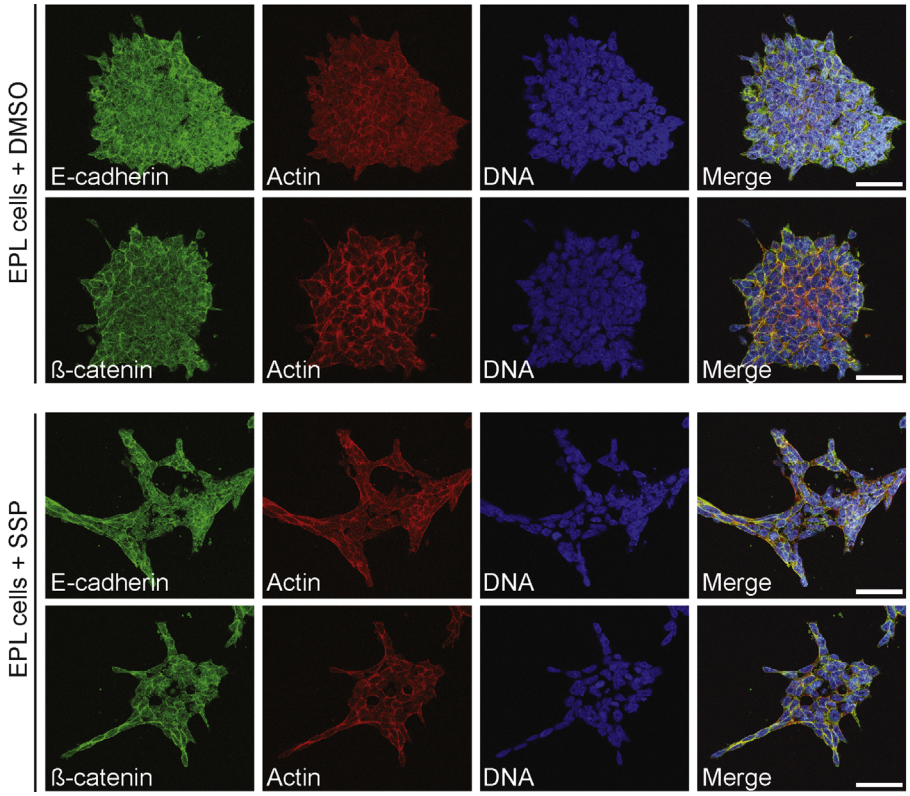

Fig. 1. Staurosporine treated EPL cell colonies retain E-cadherin, β-catenin and F-actin at cell borders. ES cells, grown in 50% MEDII for 3 days to form EPL cells, were transferred to 50% MEDII supplemented with staurosporine (lower panel) or DMSO (upper panel) and cultured for a further 24 h. Immunofluorescence shows the position of β-Catenin and E-Cadherin within the cell; rhodamine conjugated phalloidin was included to detect F-actin. DAPI was used to visualize cellular DNA. The distribution of β-catenin, E-Cadherin and F-actin was analysed by Laser Scanning Confocal microscopy. Size bar represents 50 μm.