Role of paracrine signaling and cell movements in the self-organization of micropatterned human embryonic stem cell colonies

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Human embryonic stem cells (hESCs) offer a unique window into early stages of our own development. In a previous study, we showed that spatially confined hESCs treated with Bone Morphogenic Protein 4 (BMP4) ligand, self-organize to form spatial patterns of differentiation, thus recapitulating gastrulation *in vitro*. In the current study, we quantitatively examined the role of cell movements and cell communication through paracrine signals in this self-organization. Our results show that waves of paracrine signals, moving from colony edge inwards, are essential for hESCs self-organization. Based on experimental results, we propose a reaction-diffusion based mathematical model that recapitulates the signaling wave and correctly predicts the self-organized patterning of spatially confined hESCs.

Keywords **— Embryonic development, stem cells, self-organization**

Gastrulation is a stage in embryonic development when a homogeneous population of stem cells self-organizes into the three germ layers: endoderm, mesoderm, and ectoderm. These germ layers eventually form all the cells of the developed embryo. Despite its importance, the mechanisms underlying gastrulation are not completely understood. Genetic studies in mouse embryos have revealed the signaling pathways involved in gastrulation, however, we still do not understand how these signaling pathways function together to initiate differences in a homogeneous population of cells. The interplay between signaling and cell movement during gastrulation is also not well understood [1].

Human embryonic stem cells (hESCs) offer a good model to investigate the mechanisms underlying gastrulation. As an *in vitro* system, they can be used for quantitative studies, which are very difficult to perform in a developing mammalian embryo. In a previous study, we showed that when hESCs grown in circular micropatterned colonies are differentiated by Bone Morphogenic Protein 4 (BMP4) ligand, they self-organize to form robust spatial patterns of differentiation. These patterns comprise consecutive radial rings of differentiated cells, with cells in each ring representative of a distinct germ layer [2]. Thus, in response to minimal cues - spatial confinement and BMP4, hESCs undergo gastrulation-like events in vitro.

In the current study, we examined the role of cell movements and paracrine signaling to better understand this self-organized pattern formation. We computationally tracked sparsely labeled cells to determine their movement trajectories during differentiation. We also studied the temporal and spatial evolution of secondary paracrine signaling pathways, Wnt and Nodal, which are necessary for this self-organization. Comparing the signaling data with cell movement data, and coupling it with cell fates revealed that the spatial patterning is due to an expanding wave of paracrine signals than moves from the edge of the colony towards its center and not due to the movement of cells. Taking cues from experimental results, we formulated a simple mathematical model based on reaction-diffusion that correctly predicts the self-organized spatial patterning of hESCs in micropatterned colonies.

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