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Physical Forces Influence the Self-organization of the Leader Cell Formation During Collective Cell Migration

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***Abstract*—Many dynamic physiological processes, including embryo development and wound healing, involve collective cell migration. During this process, leader cells emerge in self-organization manner to guide other cells migration. The mechanisms of their formation, however, are not clear. We hypothesized that the physical forces among cells, and between cell and substrate influence the formation of leader cells. Here, we presented a computational model of cell monolayer migration based on the Glazier-Graner-Hogeweg (GGH) model. Our results indicated that the adhesion forces among cells and the cell-substrate adhesion forces regulate the formation of leader cells. Increasing cellular mobility also affected the characters of leader cells. In summary, our results provided evidence for that physical forces among cells and between cell and substrate, along with mobility of individual cells, affect the self-organization process of leader cell formation during collective cell migration.**

I. Introduction

Collective cell migration is a basic and complicated biological phenomenon, which occurs in morphological development, tissue repair, and wound healing processes. Previous research suggested that collective cell migration also has an important impact in pathological procedures such as cancer invasion and metastasis. Comparing with individual cell migration, cells in collective migration form cohesive groups with cell-cell adhesion to coordinate their movement [1]. This complex migration process is guided by chemical and mechanical cues during migration, which has been well studied. For example, the physical forces arising from the tensions in actomyosin rings transmitted to the matrix through focal adhesion. Actin stress fibers in cells at the migration frontiers protrude towards the migration direction to drive epithelial monolayer collective migration in *in vitro* wound healing [2].

In this process, the leader cells emerge to explore the tissue environment and interact mechanically with the follower cells to guide the other cells during collective cell migration [3-6]. Therefore, it is generally believed that leader cells make vital impacts in the migration process. Definitive evidence established that leader cells at the leading edge of cell monolayer would exert traction forces on their surroundings to drive collective cell migration [7].

Current studies have demonstrated that a variety of factors can regulate and control the emergence of leader cells. Nevertheless, most reports describing regulation and control of the formation of leader cells focused on biological and chemical signals, such as promoting the transmission of Notch1-DLL4 signal and diminishing activity of RhoA [8-11]. While mechanical forces have a significant impact on regulating the formation of leader cells. Reducing wound stress promoted leader cell formation during the migration of smooth muscle cells [12]. The organization of the actin cytoskeleton and its collective polarization also induced the formation of leader cells [13]. The mechanical interactions between the follower cells could effectively select the leader cell of the wound edge [14]. Although these findings reveal leader cell formation during collective cell migration, the role of adhesion forces in leader cell formation remains poorly understood. Remarkably, recent evidence has shown that the interactions of cell-cell and cell-substrate adhesion could affect collective cell migration [15]. However, if and how the adhesion forces might control the formation of leader cells at the margin is not clear.

We presented a computational model of cell monolayer migration using the Glazier-Graner-Hogeweg (GGH), whose results confirm that adhesion forces affected the formation of leader cells. We proved that without extra stimulation, varying adhesion energies by varying either one of E-cadherin–E-cadherin adhesion between cells, integrin–collagen adhesion between cells and substrate, density of adhesion molecules, and cell migration mobilities or all of them together will cause cells to migrate heterogeneously to emerge leader cells. In the simulation, we also observed that increasing cellular mobilities will make the movement of cell clusters more intense and easier to spread and transfer. In summary, our data provided evidence that the leader cell formation is affected by cell-cell adhesion, cell-substrate adhesion and cellular mobilities during collective cell migration.

II. Methods

The simulated cell behavior is based on the Glazier-Graner-Hogeweg (GGH) model, which could depict cellular activities and interplays, such as its shape, motility, adhesion, and response to extracellular signal by using an effective-energy formulation [16-17]. The GGH in this paper uses cell-substrate adhesion, cell-cell adhesion, volume restriction and surface area restriction to build the effective energy function governing cellular mobility. The boundary energy is defined to represent the variations of energy, which is due to adhesion between different types of cells.

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Considering the boundary energy, volume restriction, and surface area restriction together, the basic GGH effective energy can be expressed as:

$$H_{GGH} = \sum_{i,j} J(\sigma(i),\sigma(j))(1-\delta(\sigma(i),\sigma(j))) + \sum_{\sigma} \lambda_v (v_\sigma - V)^2 + \sum_{\sigma} \lambda_s (s_\sigma - S)^2 \quad (1)$$

Where, v_σ and s_σ denote volume and area. V and S denote the target of volume and surface area. λ_v and λ_s is defined separately as the elasticity parameter of volume and area. $\sigma(i)$ and $\sigma(j)$ are two different cells. $J(\sigma(i),\sigma(j))$ and $\delta(\sigma(i),\sigma(j))$ is defined separately as the adhesion energy and the usual Kronecker delta function.

In our model, we take into consideration two different cell types: cell and medium. Taking into account that contacts occur between cell-medium and cell-cell, the adhesion energy terms in (1) can be presented by cell-substrate and cell-cell adhesion energy terms respectively. In this model, a variety of adhesion molecules, which determine the adhesion energy at cell-cell and cell-substrate, are considered as the principal reasons [16-17]. Furthermore, adhesion energy is regulated by adhesion molecule densities and molecule binding affinities. So we can get the formulation of adhesion energy

$$W(\sigma(i),\sigma(j)) = -\sum_{m,n} L_{m,n} \cdot \min(X^m_{\sigma(i)}, X^n_{\sigma(j)}) \quad (2)$$

where, m and n denote different adhesion molecules. The binding affinity is defined by $L_{m,n}$, which is between a pair of adhesion molecules of cells. $X^m_{\sigma(i)}$ and $X^n_{\sigma(j)}$ denote the density of different adhesion molecules.

In this paper, the adhesion between cells is mediated by E-cadherins $X^{Ecad}_{\sigma(i)}$, and the adhesion between cell and substrate is mediated by integrins $X^{Int}_{\sigma(i)}$ and collagen $X^{Coll}_{\sigma(j)}$. The binding affinity of E-cadherins is defined by $L_{Ecad,Ecad}$. The binding affinity of integrins and collagen is defined by $L_{Int,Coll}$.

When we investigate that varying density of integrin and integrin-collagen binding affinity also affects simulation results, so we use the following formulation,

$$X^{Int}_{\sigma}(t+1) = \alpha \cdot x + X^{Int}_{\sigma}(t) \quad (3)$$

where, $x = 0.01$, α is linear coefficient. $X^{Int}_{\sigma}(t)$ denotes initial density of integrins. So we can get integrin expression of all cells increasing linearly.

Finally, combining all the above equations, the GGH effective energy can be expressed as,

$$H_{GGH} = -\sum_{i,j}[L_{Ecad,Ecad} \cdot \min(X^{Ecad}_{\sigma(i)}, X^{Ecad}_{\sigma(j)}) + L_{Int,Coll} \cdot \min(X^{Int}_{\sigma(i)}, X^{Coll}_{\sigma(j)})] \times (1-\delta(\sigma(i),\sigma(j))) + \sum_{\sigma} \lambda_v (v_\sigma - V)^2 + \sum_{\sigma} \lambda_s (s_\sigma - S)^2 \quad (4)$$

In our final formalism, six factors determining the effective energy are as following: E-cadherin–E-cadherin binding affinity, E-cadherin density, integrin–collagen binding affinity, integrin density, volume restriction, and surface restriction.

III. Results

We used CompuCell3D to implement the formulation of GGH described in the methods section and get the following results. During cell monolayer migration, leader cells and follower cells emerged at the wound frontier (Fig. 1a). We hypothesized that the intercellular adhesions and cell-substrate adhesions affect the leader cell formation. In our model, the intercellular adhesions were mediated by the expression and binding affinities of E-cadherin molecules. The cell-substrate adhesions were mediated by the expression of integrin molecules and its binding affinity with collagen molecules (Fig. 1b). We tested the effects of these factors under conditions with low and high cellular mobilities (Fig. 1c-d). But we found that compared with low cellular mobility, cell clusters are easier to spread and transfer under high cellular mobility (Fig. 1c-d).

First, we tested that how intercellular and cell-substrate binding forces regulate leader cell characters with constant integrin and E-cadherin expressions. In both low and high cellular mobilities, the density of the leader cells increased when the affinity between E-cadherin molecules reduced or the binding affinity between integrin–collagen increased (Fig. 2 a-b). Meanwhile, the density of leader cells was higher with low cellular mobility (Fig. 2 a-b). The size of leader cells formed a contour with specific patterns of E-cadherin and integrin-collagen binding affinities. Increasing the cellular mobility increased the size of leader cells (Fig. 2 c-d).

The expression of integrin may increase during wound healing process. We further tested how this affects the

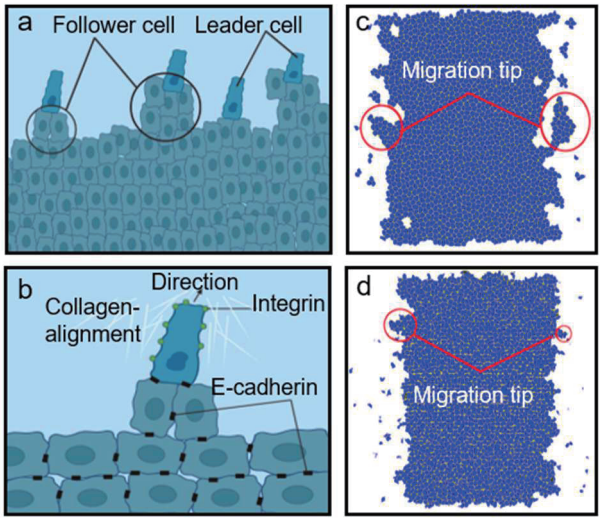

Figure 1. Leader cells in collective cell migration. (a) The formation of leader cells and follower cells during collective cell migration; (b) E-cadherin–E-cadherin and integrin–collagen binding forces in leader cell during collective cell migration; (c) simulation result of collective cell migration in low cellular mobility; (d) simulation result of collective cell migration in high cellular mobility.

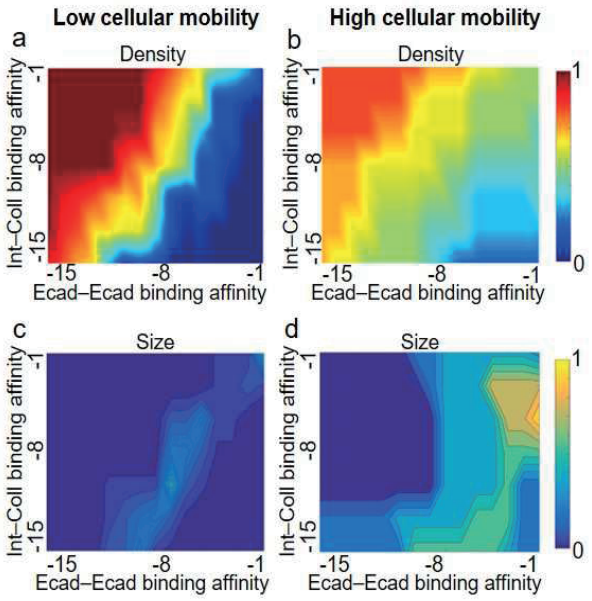

Figure 2. Intercellular and cell-substrate binding forces regulate leader cell characters with constant integrin and E-cadherin expressions. The density of leader cells with low cellular mobility (a) and with high cellular mobility (b); the size of leader cells with low cellular mobility (c) and with high cellular mobility (d). Ecad- Ecad: E-cadherin- E-cadherin. Int-Coll: integrin–collagen.

formation of leader cells. The binding affinities between E-cadherin molecules and between integrin and collagen molecules affected the density and size of leader cells in a similar manner as the case of constant E-cadherin expression during the wound healing process (Fig. 3 a-b). The leader cells were larger with the cellular expression of integrin linearly increasing compared with the constant integrin expression case (Fig. 3 c-d). But when the binding affinity between integrin and collagen molecules with linearly increasing integrin expression was bigger and the binding affinity between E-cadherin molecules was smaller, it formed larger leader cells (Fig. 3 c-d).

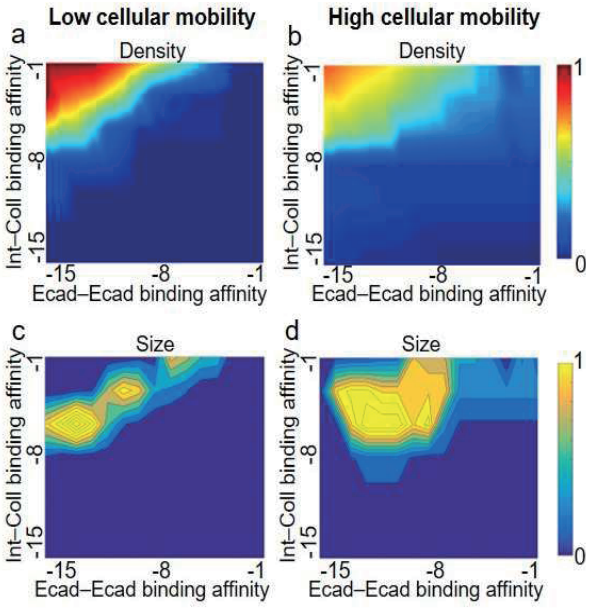

Figure 3. Intercellular and cell-substrate binding forces regulate leader cell characters with linearly increasing integrin expression and constant E-cadherin expression. The density of leader cells with low cellular mobility (a) and with high cellular mobility (b); the size of leader cells with low cellular mobility (c) and with high cellular mobility (d).

We evaluated the effect of the rate of integrin expression leader cell characters. Under low cellular mobility, the faster the integrin expression increases, the smaller the average density and size of leader cells (Fig. 4). The average density and size of leader cells had the maximum value at a medium rate of integrin expression with high cellular mobility (Fig. 4).

Adhesion forces not only correlate with the binding affinity between molecules, but also influence by the density of adhesion molecules. So we further evaluated the effect of the expression density of E-cadherin and integrin on the leader cell characters with constant integrin–collagen binding affinity and E-cadherin–E-cadherin binding affinity. In both low and high cellular mobility cases, increasing E-cadherin expression density and reducing integrin expression density increased the density of the leader cells (Fig. 5 a-b). But increasing the cellular mobility reduced the density of leader cells (Fig. 5 a-b). Increasing cellular mobility enlarged the size of the leader cells (Fig. 5 c-d). But when the density integrin and E-cadherin were smaller, the leader cells were larger compared with low cellular mobility (Fig. 5 c-d).

IV. Conclusion

In our research, we have built a simulation model of collective cell migration to evaluate the roles of cell-cell adhesion, cell-substrate adhesion, and cellular mobility in

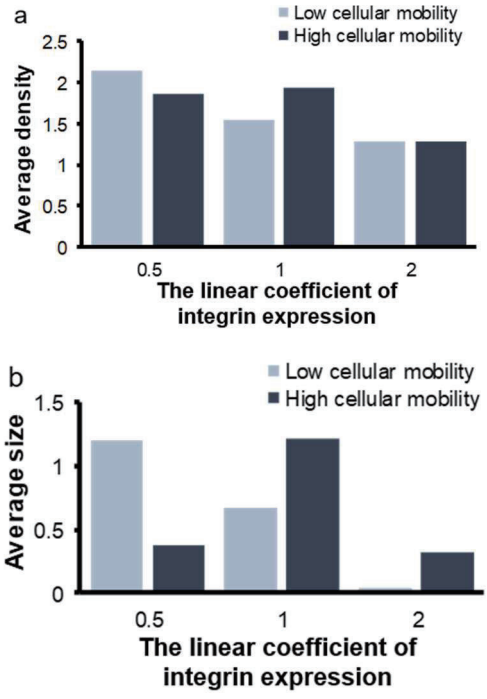

Figure 4. The linear coefficient of integrin expression affects the density (a) and size (b) of leader cells.

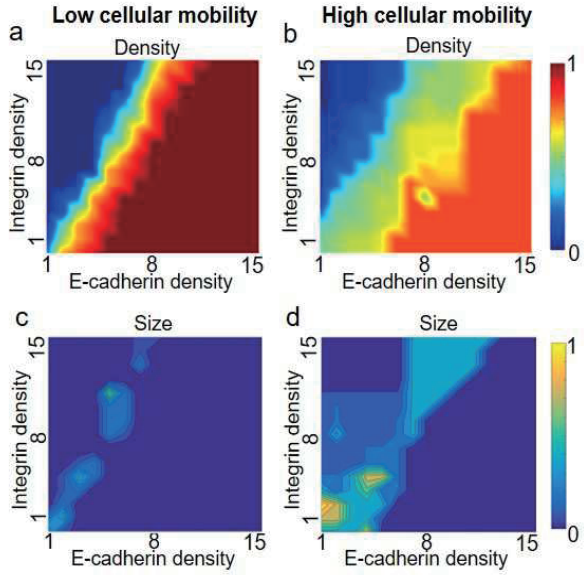

Figure 5. Intercellular and cell-substrate binding forces regulate leader cell characters with constant integrin–collagen binding affinity and E-cadherin–E-cadherin binding affinity. The density of leader cells with low cellular mobility (a) and with high cellular mobility (b); the size of leader cells with low cellular mobility (c) and with high cellular mobility (d).

the characteristic of leader cells. The density of leader cells increased as the binding forces among cells decreased and the cell-substrate binding forces increased. However, when the adhesion forces between cells were too small and cell-substrate adhesion forces were too large, cell clusters would tend to spread and transfer. The size of leader cells formed a contour with specific patterns of cell-cell adhesion and cell-substrate adhesion. The cellular mobility affected the role of these two types of binding forces in leader cell formation. These results indicated that the adhesion forces among cells, and between cell and substrate regulate the formation of leader cells. They thus affected the direction and speed of collective cell migration.

Via changing the adhesion forces of cell-cell and the cell -substrate adhesion force, it is possible to control the emergence of leader cells, which is beneficial to investigate the physiological and pathological processes involving collective cell migration, such as accelerating wound healing and reducing cancer metastasis. Furthermore, the work on the leader cells proposed here not only demonstrates the significance of physical forces in leader cell formation, but also provides a new mechanism framework for future research on the collective migration of cells in physiological and pathological aspects.

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