COMMENTARY

Cell–cell communication during collective migration

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Cell motility plays a critical role in many biological and medical processes, including wound healing, morphogenesis, and cancer metastasis (1). Often, this movement is guided by external chemical cues in the form of chemoattractant gradients. These cues consist of diffusive chemoattractant molecules that bind to surface receptors on the cell membrane and activate intracellular signaling pathways, resulting in a polarized asymmetric cell that chemotaxes in the direction of higher chemoattractant concentrations. How chemotaxis works on a single-cell level has been the subject of many detailed experimental and modeling studies (2, 3). In most physiologically relevant cases, however, cells do not move in isolation but, instead, move in groups. This collective motion is a process that is not yet well understood and may play a critical role in the spreading of cancer (4). In particular, it is not clear whether cells that move within a group communicate with each other and, if so, how this cell–cell communication affects the directionality of the group. In PNAS, Ellison et al. (5) performed experiments that suggest that cell–cell communication plays a critical role in branching morphogenesis of the epithelial tissue in mammary glands. Furthermore, in a companion PNAS study, they present a mathematical model of this communication and derive the fundamental limits of the precision of gradient sensing of this model (6).

The experiments by Ellison et al. (5) investigate the collective cellular response of epithelial branches in mammary glands using organoids, 3D in vitro organotypic cultures (7). When placed in a gradient of epidermal growth factor (EGF) Ellison et al. (5) find that the formation and extension of these branches exhibit a significant directional bias toward high EGF concentrations (Fig. 1). Without an EGF gradient, however, branch formation displays no directional bias, implying that the multicellular structure is guided by external EGF cues. Importantly, the EGF gradients are generated in mesoscopic fluidic devices and are stable for several days, allowing the quantification of the branching process over a prolonged period.

The simplest possible explanation of the observed collective guidance is that each cell is able to sense the chemoattractant gradient and polarizes, independently of its neighbors. The collective branching motion is then the result of the average motion of individual cells. Things are not that simple, however. Ellison et al. (5) clearly show that single cells, dissociated from the organoid, do not respond to EGF gradients and move around aimlessly (Fig. 1). This result is consistent with other multicell experiments that show that collective chemotaxis is possible in the absence of single-cell chemotaxis. For example, both lymphocytes and neural crest cell clusters have been shown to migrate directionally and to display a much higher chemotactic sensitivity than individual cells (8–10).

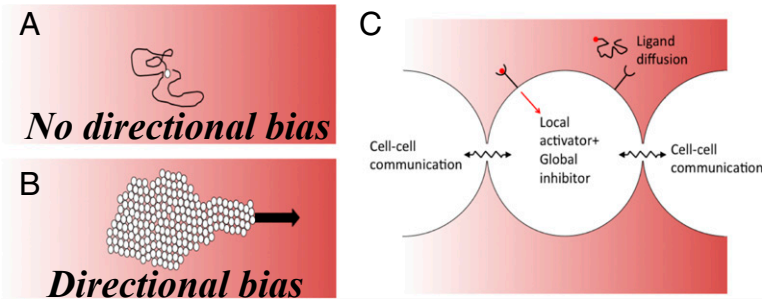

Fig. 1. Collective chemotaxis can be more effective than single cell chemotaxis. (*A*) Single mammary epithelial cells, dissociated from organoids, show little or no directional bias in the presence of an EGF gradient (schematically shown by the red background). (*B*) Fully intact organoids, however, display a clear bias of branching toward higher EGF concentrations. (*C*) The proposed model incorporates a local activator, activated by the diffusive and noisy chemoattractant concentration, and a global inhibitor that can by communicated from one cell to its neighbors. This cell–cell communication is noisy, schematically indicated by the squiggly line, resulting in an upper bound on the accuracy of gradient sensing.

Another possibility is that a multicellular cluster acts as a large "supercell" with concentration detectors at the front and back of the cluster. The achievable accuracy of gradient detection is then limited by the diffusive noise of the chemoattractant. Limits on the accuracy of concentration measurements were worked out for bacteria in a classic study by Berg and Purcell (11) and were recently extended to include gradient sensing (12–14). In the case of a cluster of size

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A and detector of size a, Ellison et al. (5) show that the signal-to-noise (SNR) ratio will scale as

$$\frac{1}{SNR} \approx \frac{\bar{c}_{1/2}}{a^3 (Ag)^2},$$

where g is the concentration gradient and where $\bar{c}_{1/2}$ represents the background concentration. This means that the accuracy is predicted to increase indefinitely for larger and larger cluster size. Through careful experimental quantification, Ellison et al. (5) find that the directional bias saturates for large sizes, clearly at odds with the above scaling law.

What could be limiting the directional bias for large cluster sizes? The above expression for the SNR only takes into account measurement noise and assumes perfect, noise-free communication between all cells. This is clearly not possible and Ellison et al. (5) examine what happens when one takes into account communication noise. They propose a new model for collective chemotaxis in which cell–cell communication is achieved by means of noisy, molecular diffusion and transport processes and show that noise from this cell-to-cell communication limits the possible accuracy of gradient detection. Specifically, they examine a multicellular version of the local excitation global inhibition (LEGI) framework (15, 16). The LEGI model postulates that the external chemoattractant concentration generates a local activator and a global, diffusive inhibitor and that the response of the cell is proportional to the difference of the activator and inhibitor levels: positive at the front of the cell and negative at the back. A key element in the LEGI model is adaptation, resulting in a response that is independent of the background concentration (17). Ellison et al. (5) extend this model to a multicellular cluster by assuming that each cell produces a local activator as well as an inhibitor (Fig. 1). This inhibitor can then be exchanged to the cell's neighbors, resulting in a positive/negative difference of activator and inhibitor levels at the front/back of the cluster. Importantly, this cell–cell communication is inherently noisy, and Ellison et al. (5) show that this noise results in the saturation of the precision of gradient sensing for large cluster sizes. Intuitively, this saturation can be understood by realizing that effective communication is only possible over a certain length scale n_0 that depends on the ratio of the exchange rate and the activator decay rates. Beyond this length scale, noise degrades the signal and sensing accuracy no longer increases with increasing cluster sizes. Importantly, the analytically derived expression for the SNR fits the experimental data quite well, illustrating the value of combined theoretical–experimental studies. Using this fit, the effective length scale is estimated to be around $n_0 \approx 3-4$ cell diameters. In other words, cells within the branch effectively communicate with roughly three to four neighbors. As a final step in their elegant study, Ellison et al. (5) probe possible biochemical candidates for the proposed cell–cell communication. Their results, obtained using a series of drug interventions, suggest that gap junctions and calcium release from intracellular stores are intimately involved in collective gradient sensing.

One major simplification in deriving the limits of gradient sensing in the study of Ellison et al. (5) is the assumption that measurements are taken instantaneously. In other words, temporal integration is ignored, even though increasing the time of measurement can potentially increase the accuracy of gradient sensing (12, 18). In the companion study, Mugler et al. (6) perform a rigorous theoretical study that derives the fundamental limits of the precision of gradient sensing in a multicellular system in the presence of cell–cell communication and temporal integration. They consider a one-dimensional array of immobile cells that obey the same multicellular LEGI model as Ellision et al. (5). In the limit of a measurement time that is much larger than the receptor equilibration timescale, the timescale of messenger turnover by degradation, and the timescale of messenger exchange from cell to cell they are able to derive analytical expressions for the precision of gradient sensing, which again saturates for large system sizes. Interestingly, they find that gradient sensing precision can be increased if the local activator is also exchanged between cells. The reason for this increase is that even though the local messenger exchange weakens the comparison between activator and inhibitor levels it also decreases the measurement noise. The latter can dominate, as long as the exchange occurs on a timescale that is slower than the inhibitor exchange. Whether this mechanism, which they term "regional excitation-global inhibition", is actually used by a biological system remains to be determined.

It should be noted that the proposed model of the two PNAS studies neglects several potentially important aspects of collective motility. First of all, the model is analyzed using the idealized geometry of immobile cells arranged on a line. Cell motion, resulting in cell rearrangement, and higher dimensionality may affect the gradient sensing precision. Furthermore, the model does not incorporate contact inhibition of locomotion during which a cell in contact with other cells attempts to move away from its neighbors (19). These aspects are incorporated into several recent modeling studies for collective chemotaxis (9, 20, 21) and their relative importance is currently unclear. It is likely, however, that combined theoretical and experimental studies, as presented by Ellison et al. (5) and Mugler et al. (6), will be instrumental in unraveling the fundamental mechanisms of collective cell motility.

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