Tracking movement and migration speed of collective cell migration

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Developmental biologists seek a better understanding of the methods used by cells to self-organize into appropriate three-dimensional arrangements. Such information could reveal the mechanisms behind certain diseases such as cancers that are characterized by or perhaps even caused by a disruption of tissue architecture. In addition, understanding how cells organize into organs and tissues is necessary for engineering artificial organs and tissues from living cells.

As cells organize into three-dimensional structures, they move in interconnected groups. This process is called 'collective cell migration'. Researchers from Johns Hopkins School of Medicine and the University of North Carolina at Chapel Hill sought to elucidate the role of the small GTPase Rac in the movement of Drosophila melanogaster border cells, as a model for studying collective cell migration. This group of 6-8 migratory cells are derived from epithelial follicle cells in the fly's ovary. Rac is known to be a contributor to the complex network of signals controlling border cell movement, however, its exact function is not known.

The researchers led by Dr. Denise Montell of Johns Hopkins, made use of new photoactivatable analogues of Rac to observe how locally activating Rac altered border cell movement. They generated transgenic flies expressing the photoactivatable form of Rac (PA-RacQ61L) tagged with the red fluorescent protein mCherry. A 458-nm laser provided photoactivation, and a confocal microscope was used to acquire time-lapse images. The photoactivation scan took ~25 s, and at 30 s the border cells were imaged with a wavelength of 568nm. Prior to, and after photoactivation, time-lapse images of 15 to 20 z-planes separated by 1.5 µm were acquired.

The researchers made use of the Imaris software for several functions in this experiment including, identifying and analyzing colocalization of two or more fluorescent markers and rendering 3-D reconstructions from the time-lapse images. Dr. Xiaobo Wang, a member of the research team, said that Imaris is very powerful for decreasing non-specific background while increasing contrast and sharpness.

A critical portion of the experiment was tracking the direction and distance that the border cells moved in order to calculate the speed of cell migration. To do this, researchers first identified the middle of the cluster by restricting the objects "size filter" to match the actual size of the border cell cluster. A spot object of a defined size was added at the calculated center (spot is 10% scale size in Figure 1). A time series analysis of the border cell cluster centers in each image produced a tracking line (Figure 2). Finally, Imaris calculated the cluster's speed by dividing the distance the cluster moved by the elapsed time (Figure 3). Dr. Wang said that the size of the filter is important because if it is too small, the software shows many spots (intensity centers) based on varying fluorescence levels of different regions of the cell cluster. Conversely, if it is much bigger than the cluster diameter, neighboring background fluorescence is included in the calculation, which shifts the location of the cluster's center.

Montell's team found that local Rac activation caused protrusion in the treated cell, as well as retraction of protrusions from the side and back cells. This effect resulted in the polarizing of the cluster, which caused movement in the direction of highest Rac activity. Inhibiting Rac in the lead cell caused other cells to protrude in all directions. These results suggest that Rac activation in the front of the cluster is key for guidance, and that this activation influences the migration direction for the whole group.

"The demonstration that photoactivatable Rac can steer groups of cells in a whole, live, developing 3-D organ provides insight into the basic science of social cell behaviors," said Dr. Montell. "In addition, it offers some, though perhaps distant, promise of using this technique therapeutically." The researchers have used this approach in human mesenchymal stem cells and are currently testing whether macroscopic light gradients can guide mesenchymal stem cells expressing photoactivatable Rac.

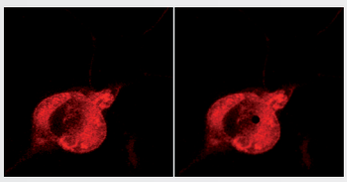

Figure1: The researchers studied a border cell cluster (left), using Imaris to find the center cluster's center (black circle on the right).

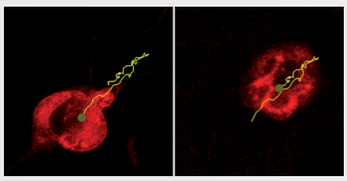

Figure 2: The border cell cluster travel path was tracked during forward and backward migration after Rac was photoactivated (beginning time point: left, final: right).

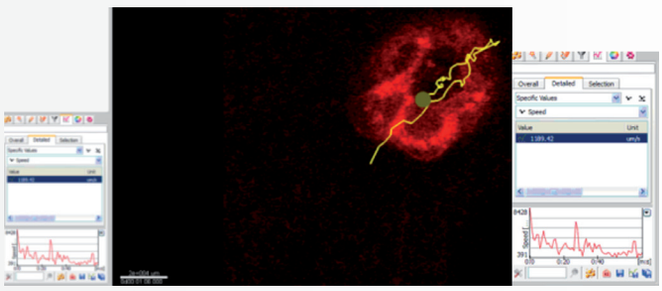

Figure 3: Imaris software can calculate the average and time-series speed during a time series of images. The calculated speed is not the actual speed because the scale is not the actual size (20,000 µm in Imaris is actually around 5 µm). Here we can see that the software calculates an average speed of 1189 µm /sec, which correlates to an actual speed of 0.3 µm /min.

Research Paper: Light-mediated activation reveals a key role for Rac in collective guidance of cell movement in vivo, Nature Cell Biology, Vol. 12, No. 6, DOI: 10.1038/ncb2061.