Annotations for the paper:

Insulin signaling modulates border cell movement in Drosophila oogenesis

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Introduction

Cell migration is an indispensable process required for the development and maintenance of multicellular organisms. It is extensively employed during embryonic development to build complex tissue layers, while in adults, immune cells migrate to impart body immunity. However, instances of improper cell migration can lead to developmental defects and cancer metastasis. Therefore, understanding when, why, and how cells acquire the ability to migrate is central to understanding cell migration itself.

Drosophila, the common fruit fly, has emerged as an excellent model system to study various biological events over the years. The migration of border cells is one such process that occurs in the ovaries of flies and has helped provide insights into crucial factors regulating collective cell migration. During *Drosophila* oogenesis, the stem cell niche in a structure called the germarium gives rise to egg chambers. These egg chambers subsequently go through 14 stages of development generating a mature egg, thus the ovary functions as an assembly line to manufacture eggs. Each developing egg chamber comprises an outer somatic follicle cell layer encapsulating the inner 15 nurse cells and oocyte, which are germline cells. During stage 8 of oogenesis, around 6-10 epithelial cells on the anterior end of the egg chamber acquire the ability to migrate upon ligand-receptor JAK/STAT signal activation, resulting in partial epithelial to mesenchymal transformation (EMT[#]). This poised-to-migrate cohort of cells is known as the border cell cluster. The cluster then rounds up, detaches from the epithelia and undertakes directed migration towards the oocyte to form the micropyle, a channel required for sperm entry and fertilisation of the mature egg. **Thus, the border cell migration system provides a powerful model to study collective cell migration within a tissue niche.**

How did we arrive at the idea that insulin signaling may be regulating the process of border cell migration?

The formation of an egg requires the deposition of yolk and other materials necessary for vitellogenesis. It was previously observed that fly mutants for Insulin signaling components like Insulin receptor(InR), Insulin Receptor Substrate lacked vitellogenic egg chambers. We noted that vitellogenesis occurs between stages 8-10, and so coincides with the migration of border cells. Armed with this observation, we set out to test our hypothesis that Insulin signaling modulates the migration of border cells. The fly has 8 insulin-like peptides or Ilps, but only one

Insulin Receptor. To test our hypothesis, we assessed the migration efficiency of a moving cluster - a parameter to assess complete or incomplete migration, as the cluster reaches the oocyte border at the end Stage 10, marking a successful migration event. Perturbing InR by targeted knock-down rendered a significant proportion of border cell clusters with incomplete migration or migration defects[#] (35%), compared to wild-type control (5%). Additionally, to validate the above approach, we generated InR knockout border cells using a popular technique routinely used by geneticists, Mosaic Analysis using a Repressible Cell Marker or MARCM[#]. These mutant clusters also exhibited defective migration, further solidifying and establishing the role of insulin signaling in modulating border cell migration.

Subsequent steps

With the first line of evidence that border cell migration requires insulin signaling, we next set out to discern how this works. During the MARCM analysis, when a few cells at the anterior end and either a few or all the border cells were mutant for InR, the mutant clusters frequently failed to detach from the anterior epithelia, suggesting that insulin signaling might be required for border cells to detach, in addition to controlling migration. Past research identified Par-1 kinase as a regulator of border cell detachment, such that Par-1 mutant clusters failed to detach from the epithelia. It was shown that Par-1-mediated modulation of actomyosin dynamics is required for border cell detachment and subsequent protrusion formation. Actin-rich protrusion and myosin activity together form actomyosin contractility networks, which are essential components of migratory cells or, in this case, border cells.

To delve deeper into the intricacies of the insulin signaling in border cells, we performed live imaging and analysis of migrating border cell clusters depleted of InR function. We observed that the clusters not only struggled to detach from the epithelia, but also had small and fewer protrusions, the finger-like projections necessary for cell movement. The live protrusion dynamics correlated with fewer F-actin filaments and more F-actin puncta in InR-depleted clusters than in controls, suggesting that Par-1 and actomyosin dynamics are potential downstream components affected upon InR signaling perturbation.

To confirm that Par-1 and actomyosin are effectors of insulin signaling, we conducted genetic interaction tests to find out whether insulin signaling and Par-1/Actomyosin function independently or as part of a linear pathway. First, knocking down Par-1 in a heterozygous *inr* mutant background resulted in a synergistic increase in detachment defects, rather than when each component was used separately. Second, expression of activated myosin component rescued the migration defects observed on targeted knock-down of InR. Third, inhibiting an inhibitor of actomyosin dynamics, Mbs, in the background of InR knock-down also rescued the migration defects to a similar extent. These three genetic interaction tests supported the idea that Par-1 and Actomyosin dynamics were under the control of Insulin signaling to modulate the migration of border cells.

In a nutshell, the study sheds light on how insulin signaling modulates border cell migration, a vibrant cell migration prototype. The wound healing process is also similar to the migration of border cells, where **stationary** cells at the edge of the wound undergo epithelial to

mesenchymal transition to acquire **migratory** fate, and undertake migration to fill up the wound gap. If one looks at a broader significance of the study, there is an intriguing parallel observed in impaired wound healing of diabetic patients. In diabetic patients, caused either due to the lack of insulin or peripheral insulin resistance, the healing of wounds is significantly delayed such that it becomes a menacing problem causing wounds to bleed for a longer time, thereby also increasing the risk of infection. Similarly, perturbing insulin signaling in the border cells of fruit flies also results in incomplete cell migration. Such similarity and parallels between flies and humans, shows the evolutionarily conserved mechanisms with which organisms are built. We hope that the system of border cell migration will one day be utilised in drug discovery screens, to uncover potential pharmacological components not only to aid wound healing, but also to treat tumour metastases.

- **by Sudipta Halder[1] and Aditi Sharma[1].**

A guide to unconventional terms(#):

EMT: Epithelial to Mesenchymal transition occurs when stationary epithelial cells undergo changes in gene expression, allowing them to discard/suppress epithelial character and acquire gene expression profiles resulting in transformation into a migratory population. This occurs regularly during tissue and organismal development and also during wound healing. EMT is the cause of tumors undergoing metastasis and invading other tissue via circulation.

Migration Defect: A method to quantitate border cell migration. The border cells complete migration when they reach the border between the oocyte and nurse cells at sStage 10. The migration defect quantitates the percentage of egg chambers where migration is not completed, i.e., the border cells fail to reach the oocyte border at the end of sStage 10.

MARCM: Loss of function analysis is classically used to decipher the role of unknown genes. Often, such loss of gene function is lethal for the development of the embryo and hence it is not possible to investigate gene function in post-embryonic stages. Mosaic analysis, of which MARCM is a variation, is an ingenious recombination-based genetic technique, using which homozygous mutant cells can be obtained, identified and observed in an organism heterozygous for the mutant allele. Such a homozygous mutant cell, upon duplication, also yields more mutant cells, thus generating a clone of homozygous mutant cells. This genetic recombination event can be designed to occur throughout the organism or in a tissue-specific manner, thus allowing the complete development of the organism, albeit a heterozygote.

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