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Epithelial cell homeostasis: a Piezo of the puzzle

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Summary

A recent study shows that upon stretching or wounding epithelia display a fast proliferative response. By generating quickly more cells they re-establish optimal cell density and seal the wound. This is induced by the stretch-activated channel Piezo1, through calcium signalling.

Main Text

Healthy epithelia in our bodies are exquisitely homeostatic and maintain a tight balance between cell loss and cell replacement. In particular, sensing and maintaining an optimal cell density is very important to epithelial tissue function. Thus mechanisms are in place to prevent tissues from becoming overcrowded. One key factor that prevents epithelial overcrowding is the membrane protein Piezo1, a mechanosensor that is activated upon cell crowding and induces cytoskeletal contractions that squeeze excess cells out of epithelial monolayers [1]. Because epithelia often form a physical barrier to the outside world or isolate the organs they encase, it is equally important that epithelial cell density does not drop, so that there are enough cells to form a continuous layer and maintain epithelial integrity. This is particularly challenging during injury or infection, which can lead to a sudden and drastic loss in cell number. How do epithelia respond and adapt to quickly reinstate cell density? A new study from the Rosenblatt group shows that the very molecule that ensures that epithelia are not too crowded, Piezo1, is also responsible for preventing or correcting a loss in epithelial cell density [2].

When epithelia undergo a sudden loss in cell number, such as during injury or wounding, rapid cell rearrangements and cell shape changes take place that allow cells to flatten and stretch so that they cover a wider surface, migrate towards the wound edge and rapidly seal holes [3]. It has long been known that cell stretching can drastically change the proliferative and survival properties of cells. For instance, two decades ago the Ingber group showed that when cells stretch over a wider surface this promotes their survival and proliferation [4]. More recent studies have shown that in fact cell stretching

leads to activation of the transcription factors YAP and TAZ, which in turn promote cell proliferation [5-7]. The proliferative response observed in these studies took place several hours after a stretch stimulus was applied [5-7].

In their new study, Gudipaty and colleagues from the Rosenblatt group discovered that epithelia also show a fast proliferative response to cell stretching with a 5-fold increase in mitotic figures as early as one hour from stretching [2]. How could cells proliferate so quickly if normally the cell cycle takes several hours? This tipped the authors off that cells recruited to proliferate must have been in a phase of the cell cycle close to mitosis, either G2 or M phase itself. Indeed their hypothesis was correct, as they observed an increase in cells transitioning from G2 to M phase upon stretching. This was clearly dependent on Piezo1 activity, as chemical or genetic inhibition of Piezo1 drastically abrogated the proliferative response.

Piezo1 is a stretch-activated calcium channel [8] and indeed the authors found that calcium signalling is key to the stretch-induced proliferative response, as it can be abrogated by calcium signalling inhibitors. So how does calcium signalling promote proliferation of stretched cells? Gudipaty and colleagues find that a key mediator is the Extracellular Signal Regulated Kinase 1 (ERK1), a calcium-activated kinase known to play a role in controlling the G2/M transition [9, 10]. The authors show that activated, phosphorylated ERK1 is detected within minutes after stretching and its inhibition is sufficient to block the rapid proliferative response.

Thus mechanical stretch via Piezo1 causes calcium dependent activation of ERK1, which promotes the G2/M transition, causing a rapid burst in cell divisions (Figure 1). Interestingly, the authors report a spike of calcium signalling in G2 cells just one hour prior to cell division. A tantalising hypothesis is therefore that calcium release from Piezo1 might provide a signal for G2 cells to enter mitosis. It would be interesting to test whether only cells in G2 undergo Piezo1-induced ERK1 activation or conversely, whether Piezo1 induces ERK1 activation across the cell cycle, but ERK1 can only promote cell cycle transition in G2.

An interesting question arising from these observations is: what is the nature of the G2 cells that proliferate upon stretch? Do confluent epithelia maintain a pool of cells arrested in G2 as a reservoir for rapid adaptive response to mechanical damage? This is an intriguing possibility. Alternatively these might simply be cells that were transitioning through G2 anyway and that a Piezo1-induced calcium spike quickly rushes into G2/M transition.

Epithelia therefore appear to have a two speed proliferative response to cell stretching: a fast response mediated by Piezo1 and a slower, perhaps more

sustained response, orchestrated by the mechanosensing pathways that control YAP and TAZ [5-7]. Are these at all linked or coordinated? Interestingly, Gudipaty and colleagues report that Piezo1 silencing drastically reduces proliferation also in confluent steady-state epithelia, where the coupling between density and proliferation is normally controlled by YAP and TAZ [11]. This suggests a more general role of Piezo1 in promoting proliferation and possibly a coordination between Piezo1 and YAP/TAZ activity. The authors point out that this hypothesis is consistent with the recent finding that tension-dependent YAP nuclear translocation requires Piezo1 in neuronal stem cells [12].

The unexpected picture emerging from this work is that remarkably Piezo1 is entrusted with two opposing functions in epithelial cells: to extrude excess cells in overcrowded epithelia and to generate new cells in undersupplied epithelia. What would be the advantage of employing the same molecule for these opposing tasks? One fascinating hypothesis suggested by the authors is that this would act to buffer the control of epithelial cell density from mutations, as changes in Piezo1 activity would affect density sensing both during crowding and during stretching resulting in a zero sum game.

From a mechanistic perspective, however it is not obvious how the same signal, calcium influx, can generate such opposing responses. Several explanations are possible and they are not mutually exclusive. Firstly, as the authors suggest, crowded epithelia may contain a lot of old and post-mitotic cells unable to proliferate in response to calcium. Proliferation might just not be an option for them. Secondly, the authors report that while artificially induced calcium signalling is sufficient to trigger mitotic entry, it is not sufficient to induce epithelial extrusion. Thus, in addition to calcium, other signals induced by Piezo1 or by other factors are necessary to trigger cell extrusion. Indeed upon crowding Piezo1 induces production of the lipid Sphyngosine 1-P, which by activating the GTP-ase Rho is responsible for acto-myosin contraction and cell squeezing [1]. This might be then the additional signal triggered by Piezo1 during cell extrusion.

In this scenario Piezo1 would signal differently in sparse versus compacted cells. This might seem odd at first. However additional data from Gudipaty and colleagues could be consistent with this model. The authors show that Piezo1 has a remarkably dynamic pattern of expression and localisation. In confluent cultures it accumulates in big intracellular structures, whereas upon stretching Piezo1 levels appear to drop substantially. In addition, the low levels of Piezo1 in sparse cultures redistribute from large intracellular blobs into finer granules as well as on the nuclear membrane and on the plasma membrane. Thus, changes in levels and/or intracellular localisation may modulate Piezo1 activity. Perhaps relevant to this is the observation that

another Piezo family member, Piezo 2, has recently been shown to require phosphoinositides to signal [13]. As these lipids have been suggested to form membrane microdomains [14, 15] a redistribution of Piezo1 may well be a way to modulate its activity.

In addition to epithelial cell homeostasis, the Piezo family of stretch-activated channels in recent years has emerged as being implicated in diverse physiological processes, from somatosensation, including light-touch sensing and nociception, to hearing and to shear stress, both in muscles and in the vasculature [16]. Understanding the complex cell biology of Piezo1 emerging from this work is no doubt going to be relevant to these other contexts as well.

Figure 1. Epithelial cell stretching causes opening of the stretch-activated Calcium channel Piezo1. Calcium release causes activation of the calcium-sensitive kinase ERK1. ERK1 activation in turn causes cells that were in the G2 phase of the cell cycle to enter mitosis. Through this mechanism epithelia react to stretching with a quick burst of cell divisions, which counters the decrease in cell density.

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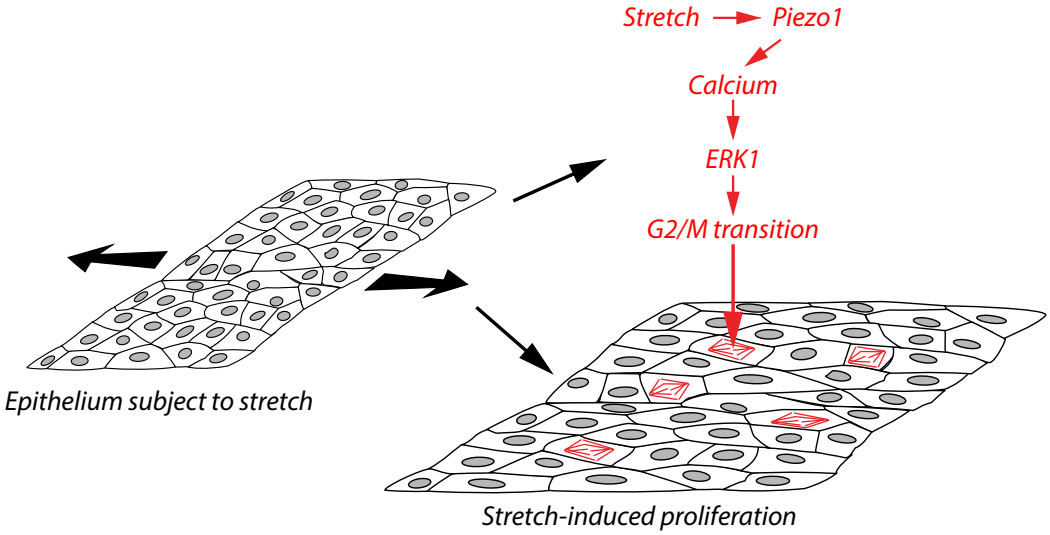
Stretch
Piezo1
Calcium
ERK1
G2/M transition
Epithelium subject to stretch
Stretch-induced proliferation