Cell competition: Bridging the scales through cell-based modeling

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Summary

Cell competition is a context-dependent cell elimination process which has been proposed to rely on several overlapping mechanisms. A new study combining cell-based modeling and quantitative microscopy data help to sort out the main contributors of mutant cell elimination.

Main text

Despite the high frequency of deleterious mutations which appear in adult epithelia, the initiation of tumours remains relatively rare[1]. This relies on the multiple mechanisms of intrinsic tumour suppression which prevents uncontrolled proliferation of pretumoural clones. One important mechanism which attracted a lot of attention in the past ten years is cell competition: a context-dependent cell elimination process which excludes miss-specified and abnormal cells from growing and homeostatic tissues. Cell competition is a widespread phenomenon occurring during early development and in adult tissues from *Drosophila* to Vertebrates[2]. Several mechanisms have been proposed to participate to cell recognition and elimination[3, 4]. Thus, one challenge of the field is to sort-out the relative contribution of each mechanism which often co-exist. For instance, cells mutant for the apico-basal polarity gene *scribble* are eliminated by apoptosis from *Drosophila* imaginal tissues and from MDCK cell layer (Madin-Darby Canine Kidney cell) when surrounded by wild type (WT) cells[5, 6]. This occurs through a combination of independent mechanisms: ligand-receptor interactions at the interfaces between the mutant and WT cells[7], secretion of factors inhibiting *scribble* mutants survival[8] or apoptosis induced by mutant cell compression[9, 10]. This illustrates the co-existence of multiple modes of elimination during competition despite similar genetic background. What is then the relative contribution of each phenomenon ?

Our ability to sort them is actually limited by the gap that remains between the two main metrics of cell competition: on the one hand the short term description of the distribution of cell death (often based on staining of apoptotic cells), and on the other hand the long term effect of cell competition on population size and survival. To fill this gap, theoretical frameworks are required to assess how these cellular mechanisms can indeed affect the long-term survival of a cell

population. These include continuous models based on differential equations and prey-predator interactions[11, 12], continuous models testing the effect of mechanical pressure on cell survival[13-15] or cell-based model testing the role of compensatory growth[16]. However, these frameworks either fail to integrate cellular scale information (e.g.: cell death distribution, cell shape, cell movements, local pressure, tension) and/or were not confronted quantitively with experimental data, most likely because quantitative datasets are still very scarce. The assessment of the contribution of each competition mechanism to the persistence or elimination of one cell population remains therefore very challenging.

This challenge has been elegantly tackle by Gradeci and colleagues in a recent article which bridges these gaps[17]. Focusing on the elimination of the *scribble*-silenced cells (Scribble^{KD}) in MDCK cells, the authors combined a new cell-based modeling framework with a highly quantitative dataset which was generated through long term live-imaging, segmentation and tracking[10]. Previously, the elimination of Scribble^{KD} cells was suggested to be driven by their lower homeostatic density[9, 10]. The homeostatic density is the density at which cell proliferation is perfectly compensated by cell death[14]. Above this value, death rate will be higher than proliferation, hence leading to population shrinkage, while below this value proliferation will be favored. Accordingly, Scribble^{KD} cells have higher basal p53 levels (a pro-apoptotic factor) which set their homeostatic density lower than WT cells[9]. Consequently, The WT cell population expands, brings Scribble^{KD} cell density above its homeostatic value, hence forcing their progressive disappearance. Yet, experimental data outlined other mechanisms contributing to Scribble^{KD} cell elimination, including active compaction of mutants cells through the convergent movements of WT cells toward them[9] as well as a local boost of proliferation of the WT cells neighbouring Scribble^{KD} cells[10]. As such, it remained unclear whether the difference in homeostatic density was sufficient to recapitulate the dynamics of Scribble^{KD} cell elimination. To test this hypothesis, Gradeci and colleagues developed a cell-based modeling approach (so called Cellular Potts Model) which integrates mechanical inputs (relative affinity between cells, adhesion to substrate, cell velocity and cell compressibility) as well as cell-decision automata controlling cell division and cell death (**Figure 1**). While the number of parameters are relatively large, many of them could be measured experimentally or constrained by the rich dataset previously generated by the authors, integrating data on cell shape, growth rate, number of neigbours and the relationship between density and death rate. Doing so, they could first recapitulate the dynamics of homogenous populations of WT or Scribble^{KD} cells including the density reached at confluency. Using the same parameters, they then implemented competition between WT and Scribble^{KD} cells. The model could recapitulate quantitatively the experimental evolution in time of cell number, death rate and division rate for the two populations. Altogether this suggested that the differences in mechanical properties

and homeostatic density are sufficient to recapitulate the dynamics of Scribble^{KD} cells elimination, independently of any sort of signaling between cells.

What are then the central regulators of this mechanical competition? Surprisingly, growth rate has little influence apart from delaying mutant cell elimination, in agreement with former predictions[14]. The outcome of competition here is mostly controlled by two parameters: the difference in homeostatic density between the two populations and their relative stiffness/compressibility (**Figure 1**). The homeostatic density is an emerging property of the model which relies on two parameters: the relationship between density and cell death as well as contact inhibition (in other words, the reduction of proliferation by crowding). Interestingly, while contact inhibition dominates the dynamics of WT cells, this is rather neglectable in Scribble^{KD} cells whose dynamics is dominated by apoptosis at high density. The second parameter, which was less expected, is the relative stiffness of cells. Scribble^{KD} are eliminated provided that their stiffness is lower than WT cells. This can be intuitively understood by the fact that cells can be compressed by their neighbours provided they are more deformable/less stiff. Altogether, this demonstrated that the difference in homeostatic density and stiffness are sufficient to recapitulate quantitatively the elimination of Scribble^{KD} cells.

To expand the application of their model, the authors implemented then another mode of competition which relies on the contact between the two cell types. Previously, it was shown that the elimination of cells with low Myc levels (an oncogene) correlated with the surface of contact shared with cells with high Myc levels[18]. As such, the authors implemented a sigmoid increase of the death rate of the “loser” population as a function of the surface of contact shared with the other cell type. Interestingly, the dominant parameters that govern the outcome of competition are here completely different from the mechanical competition described above (**Figure 1**). As expected, the degree of initial mixing (from a salt-and-pepper pattern to a fully sorted condition with two large groups) dramatically changed the outcome of competition, where sorting promoted cell survival. Alternatively, cell elimination could also be accelerated by stabilizing heterotypic contacts, hence facilitating the mixing between the two populations. These observations are in good agreement with experimental data showing the positive impact of cell mixing on cell competition[18]. Since colony size has a strong impact on the time required to eliminate loser cells, this also suggests that contact-dependent competition impose a race for elimination: once the clone reach a critical size, a huge amount of time (incompatible with the characteristic time of development) would be required for its elimination. This might be even more relevant for competition occurring in *Drosophila* imaginal tissues which is constrained by the restricted time window of larval growth.

To conclude, the combination of a cell-based modeling approach with the rich quantitative dataset previously generated by the authors established one of the most realistic modeling framework of cell competition. This is the first clear demonstration that differences in homeostatic density are sufficient to recapitulate quantitatively the dynamics of Scribble^{KD} cells elimination. Obviously, this does not exclude that other mechanisms may contribute to cell elimination. For instance, active compaction of mutant cells by directed migration of the WT cells toward the mutants was previously shown to accelerate compaction[9], and was more recently validated by the identification of a chemoattractant (FGF21) secreted by Scribble^{KD} cells[19]. While relatively intuitive, the role of stiffness differences in mechanical competition is one the most unexpected prediction of the model. This nicely fits recent data showing how bacterial infection of epithelial cells can trigger mechanical-driven cell elimination through a reduction of infected cell stiffness[20]. Given the increasing complexity of cell competition and the accumulation of pathways/mechanisms involved in cell elimination, such quantitative approaches will become more and more essential to comprehend the mechanisms that govern the long-term fate of cell populations.

Figure legends

Figure1: Cellular Potts Model and identification of the main parameters regulating mechanical competition and contact-dependent competition.

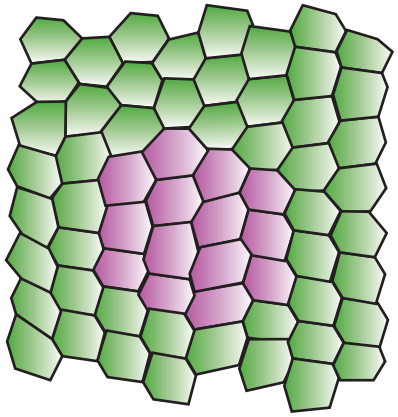
Left: Parameters of the cellular Potts Model, including mechanical parameters (J: surface energy, lower=stable junction), regulation of cell growth and division (progressive surface increase, division after a critical surface addition, so called “adder model”, and contact inhibition) and regulation of cell death and extrusion. Right: two models of competition simulated in the Cellular Potts Model, mechanical competition on the top and contact-dependent competition on the bottom. The conditions and the main parameters promoting “loser” cell elimination are outlined for each type of competition.

References

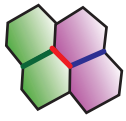
1. Kakiuchi, N., and Ogawa, S. (2021). Clonal expansion in non-cancer tissues. Nature Reviews Cancer *21*, 239-256.
2. Claveria, C., and Torres, M. (2016). Cell Competition: Mechanisms and Physiological Roles. Annu Rev Cell Dev Biol *32*, 411-439.
3. Merino, M.M., Levayer, R., and Moreno, E. (2016). Survival of the Fittest: Essential Roles of Cell Competition in Development, Aging, and Cancer. Trends Cell Biol.
4. Matamoro-Vidal, A., and Levayer, R. (2019). Multiple Influences of Mechanical Forces on Cell Competition. Curr Biol *29*, R762-R774.
5. Norman, M., Wisniewska, K.A., Lawrenson, K., Garcia-Miranda, P., Tada, M., Kajita, M., Mano, H., Ishikawa, S., Ikegawa, M., Shimada, T., et al. (2012). Loss of Scribble causes cell competition in mammalian cells. J Cell Sci *125*, 59-66.

6. Brumby, A.M., and Richardson, H.E. (2003). scribble mutants cooperate with oncogenic Ras or Notch to cause neoplastic overgrowth in Drosophila. EMBO J *22*, 5769-5779.
7. Yamamoto, M., Ohsawa, S., Kunimasa, K., and Igaki, T. (2017). The ligand Sas and its receptor PTP10D drive tumour-suppressive cell competition. Nature *542*, 246-250.
8. Katsukawa, M., Ohsawa, S., Zhang, L., Yan, Y., and Igaki, T. (2018). Serpin Facilitates Tumor-Suppressive Cell Competition by Blocking Toll-Mediated Yki Activation in Drosophila. Current Biology *28*, 1756-+.
9. Wagstaff, L., Goschorska, M., Kozyrska, K., Duclos, G., Kucinski, I., Chessel, A., Hampton-O'Neil, L., Bradshaw, C.R., Allen, G.E., Rawlins, E.L., et al. (2016). Mechanical cell competition kills cells via induction of lethal p53 levels. Nat Commun *7*, 11373.
10. Bove, A., Gradeci, D., Fujita, Y., Banerjee, S., Charras, G., and Lowe, A.R. (2017). Local cellular neighborhood controls proliferation in cell competition. Mol Biol Cell *28*, 3215-3228.
11. Nishikawa, S., Takamatsu, A., Ohsawa, S., and Igaki, T. (2016). Mathematical model for cell competition: Predator-prey interactions at the interface between two groups of cells in monolayer tissue. J Theor Biol *404*, 40-50.
12. Nishikawa, S., and Takamatsu, A. (2019). Effects of cell death-induced proliferation on a cell competition system. Math Biosci *316*.
13. Shraiman, B.I. (2005). Mechanical feedback as a possible regulator of tissue growth. Proc Natl Acad Sci U S A *102*, 3318-3323.
14. Basan, M., Risler, T., Joanny, J.F., Sastre-Garau, X., and Prost, J. (2009). Homeostatic competition drives tumor growth and metastasis nucleation. HFSP J *3*, 265-272.
15. Murphy, R.J., Buenzli, P.R., Baker, R.E., and Simpson, M.J. (2020). Mechanical Cell Competition in Heterogeneous Epithelial Tissues. B Math Biol *82*.
16. Tsuboi, A., Ohsawa, S., Umetsu, D., Sando, Y., Kuranaga, E., Igaki, T., and Fujimoto, K. (2018). Competition for Space Is Controlled by Apoptosis-Induced Change of Local Epithelial Topology. Curr Biol *28*, 2115-2128 e2115.
17. Gradeci, D., Bove, A., Vallardi, G., Lowe, A.R., Shiladitya, B., and Charras, G. (2021). Cell-scale biophysical determinants of cell competition in epithelia. elife.
18. Levayer, R., Hauert, B., and Moreno, E. (2015). Cell mixing induced by myc is required for competitive tissue invasion and destruction. Nature *524*, 476-480.
19. Ogawa, M., Kawarazaki, Y., Fujita, Y., Naguro, I., and Ichijo, H. (2021). FGF21 Induced by the ASK1-p38 Pathway Promotes Mechanical Cell Competition by Attracting Cells. Current Biology *31*.
20. Bastounis, E.E., Serrano-Alcalde, F., Radhakrishnan, P., Engstrom, P., Gomez-Benito, M.J., Oswald, M.S., Yeh, Y.T., Smith, J.G., Welch, M.D., Garcia-Aznar, J.M., et al. (2021). Mechanical competition triggered by innate immune signaling drives the collective extrusion of bacterially infected epithelial cells. Developmental Cell *56*, 443-+.

Cellular Potts Model



Cell mechanics:



Surface energy

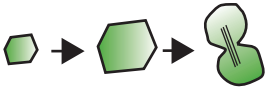
$J_{heterotypic}$
$J_{homotypic}$
$J_{homotypic}$

Substrate adhesion

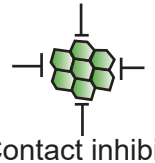
Active mobility

Stiffness/ compressibility

Cell growth and division:

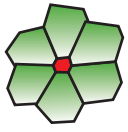


"adder" model

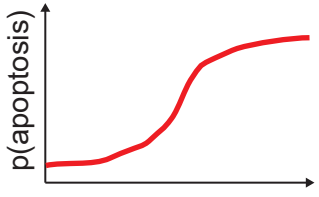


Contact inhibition

Cell death and extrusion:

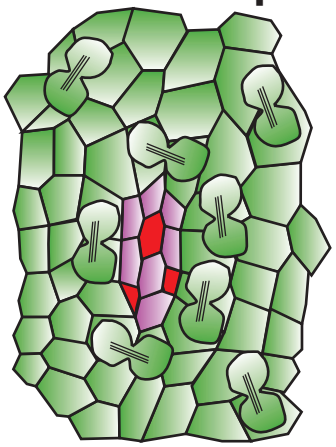


"live" extrusion
Area < Critical Area



Density OR contact -dependent death

Mechanical competition

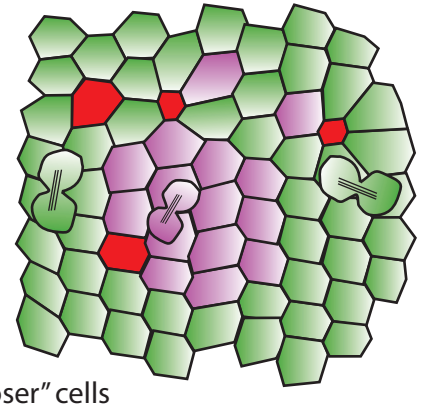


Mutant cells (Scribble^{KD})
WT cells
Apoptosis/extrusion

- Homeostatic density

- Stiffness/compressibility

Contact-dependent competition



"loser" cells
"winner" cells
Apoptosis/extrusion

- Initial conditions (mixed)
- $J_{heterotypic} < J_{homotypic}$