Solid stress, competition for space and cancer: The opposing roles of mechanical cell competition in tumour initiation and growth

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The coordination between cell proliferation, cell growth and cell death.

More recently, the contribution of cell shape and local tissue mechanics to this coordination has gained a lot of interest. This is in agreement with the impact of cell mechanics on several central regulators of cell growth and survival [2–4].

Mechanical forces experienced by tumour cells and neighbouring cells is part of those microenvironment e ects and were shown to a ect every step of tumour progression, from tumour initiation and growth to metastasis formation [9,10].

Cell competition is a process that triggers preferential elimination of one cell population by another through apoptosis [11–13].

other genetic modifications (*e.g.*: higher levels of the proto-oncogene Myc) were shown to generate “aggressive” clones that could eliminate and fully replace the neighbouring WT (wild tissue) cells without visible tissue defects [16,17]. This “supercompetition” was proposed to promote the early expansion of pretumoural cells and tumour initiation in a process akin to eld cancerization [18]. …this process is also conserved in mammals [19].

Yet, the mechanisms that trigger preferential elimination of one cell population are still actively debated. Three non-exclusive mechanisms have been proposed to participate to cell elimination: competition **for limiting extracellular survival factors**, competition driven by **contact-dependent death induction** and more recently competition **triggered by mechanical stress** [20–22].

cell compaction and differential sensitivity to mechanical stress could promote competitive interactions between cells [23,24] and could either

eliminate preferentially pretumoural cells [24] or promote the expansion of pretumoural clones [23,25,26].

In this review, I will describe the concept of mechanical cell com- petition by introducing the key parameters regulating such competitive interactions, and describe recent advances in the identification of the pathways regulating compaction-driven death. I will then describe ra- pidly the relationship between mechanical cell competition and other mechanisms of cell competition. I will then discuss the relevance of mechanical cell competition in tumour initiation and progression by documenting the effect of the mechanical environment associated with tumour growth, and the impact of residual stress (see Box 1) on tumour progression and intratumoural competition.

2.1. Initial hypothesis and theoretical grounds

In a seminal paper, Boris Shraiman proposed more than ten years ago that mechanical stress generated by differential growth could be at the basis of competitive interactions between cells [27].

tissues behave like solids which have little capacity to dissipate mechanical stress. In this environment, a relative increase of cell proliferation/growth in a subpopulation should push on the neighbouring cells, which will conversely resist to this pushing.

On long time scales, differential growth will build up pressure in the tissue, particularly in the fast growing population and its local environment (Fig. 1A–C). Intuitively, one would expect that such compression will strongly impair the capacity of the fast growing population to expand.

In questo caso sembra che il meccanismo di competizione cellulare (mediato dalla pressione residua generata dalla crescita differenziale) sia un meccanismo omeostatico che tende a riportare il tessuto in condizioni normali di pressione. La pressione residua è dissipata dall'attivazione di meccanismi apoptotici che inducono un rate di mortalità delle cellule dipendente dagli sforzi residui.

compression/negative strain (see Box 1) can significantly slow down cell proliferation and cell growth [28–35].

How then could this mechanical stress promote the expansion of one population

at the expanse of the other?

Here is where Boris Shraiman introduced the concept of differential sensitivity to mechanical stress: if the neighbouring slow-growing cells die at lower pressure compared to the fast-growing population, this will be sufficient to trigger preferential elimination of the neighbouring cells. This will lead to space and pressure release and further expansion of the fast growing population (Fig. 1A,D) [27].

Questo è lo stesso meccanismo di confinamento-competizione proposto nei sistemi di reazione-diffusione già a partire da Turing(1952) e Meinhardt(1975) per lo studio della morfogenesi dei tessuti. Vedi "Mathematical Biology" vol.1 (cap. 3, 11) e vol.2 (cap.2).

hypotheses of this model remained to be demonstrated ex- perimentally. First, there was at the time no clear evidence that compaction/compression could induce cell death *in vivo*. Secondly, it would require that different cell types have different sensitivity to mechanical stress, which remained to be demonstrated. Third, it required that stress is not dissipated through cell movements and neighbor exchanges.

Other theoretical frameworks also proposed a role for mechanics in competitive interactions between cells. This includes the concept of homeostatic pressure introduced by M. Basan et al. [41,42], which assumes the existence of a precise pressure at which cell proliferation and growth is perfectly compensated by cell death (Fig. 1E,F).

if one population has a higher homeostatic pressure than another, the former will always eventually eliminate the later, irrespective of the relative growth rate of the two populations in absence of mechanical constrains.

2.2. Tissue compaction can trigger cell elimination

Those models came again into light when it was shown later that compaction of epithelial cells could trigger their elimination (a key hypothesis of those two models)

a reduction of cell surface by changing the spacing of ECM (Extra Cellular

Matrix) spots is sufficient to trigger apoptosis in single endothelial cells

Questo effetto è interessante anche pensando alla fibrosi (epatica) dove la sovrapproduzione di ECM produce una "distruzione" della vascolarizzazione. L'effetto che si potrebbe considerare sarebbe non solo una compressione dei vasi, ma anche la perdita degli stessi dovuta all'apoptosi delle cellule epiteliali (si osserva che il volume del tessuto fibrotico non varia sensibilmente da quello sano).

Several groups suggest that compression is sufficient to trigger cell death and that different cell types have differential sensitivity to compression, in agreement with the hypothesis described in the previous section.

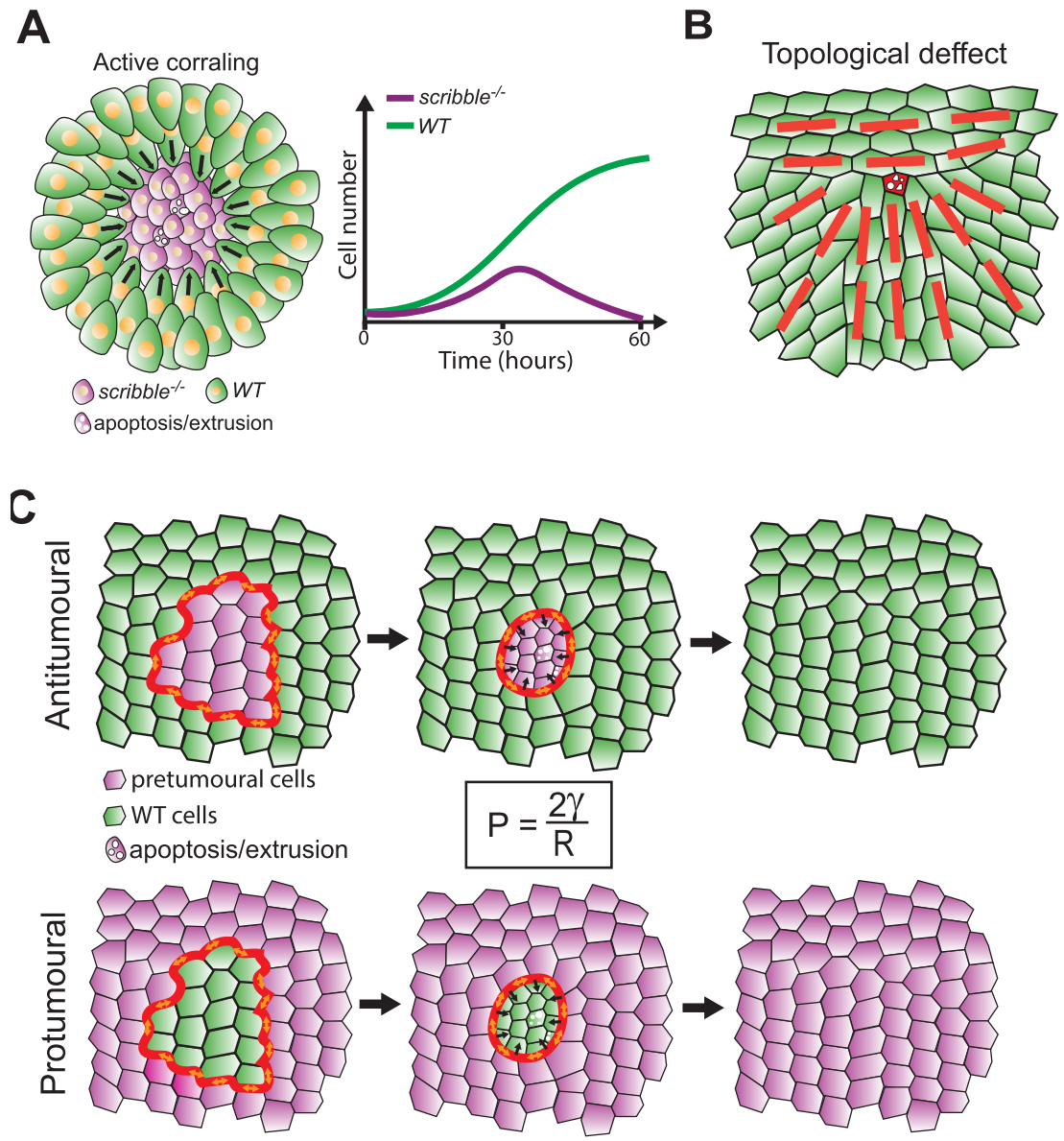
3. Mechanical cell competition triggered by cell migration and boundary conditions

compaction of *Scribble* mutant cells is also promoted by the active migration (*chemotaxis*) of WT cells toward the mutant cells [24] (Fig. 2A).

MDCK cells tend to align locally similar to nematic liquid crystals. However cell migration can generate spontaneous alignment defects (so called +1/2 nematic defects, comet like shape, Fig. 2B) which precede cell extrusion and cell death. Those defects correlated with a local increase of compressive forces (measured by traction force microscopy) and are sufficient to trigger caspase activation and cell extrusion [58].

Alternatively, upregulation of tension at the interface of the two cell populations can also generate compressive forces (Fig. 2C). The shape of cellular interfaces in epithelia can be well approximated by the distribution of surface tension

Laplace pressure will always exist at the interface, but its magnitude will vary depending on heterotypic adhesion strength (γ) and the curvature of the interface. This law for instance can explain the sorting and the formation of smooth boundaries between different cell lineages upon association [60].



More recently, Bielmeier and colleagues have shown that a large range of genetic mutations (activation of JAK-STAT or Ras, downregulation of polycomb proteins) in clones could generate cell sorting for large clones, cyst formation for intermediate size or cell elimination for small clones [62]. Those modifications were all associated with an enrichment of actin and the molecular motor Myosin II on the lateral surfaces of the cells at clone boundaries, leading to an increase of clone/WT cells interfacial tension. Increasing interfacial tension at clone boundaries in a 3D vertex model is sufficient to recapitulate the different observed morphologies (sorting for large clones, cyst formation for intermediate size).

The role of clone size can be elegantly explained by the Laplace law (see above and Fig. 2C): for a constant line tension, pressure will be inversely proportional to the radius of the clone. Hence small clones should experience much higher pressures than larger ones. While this was not formally tested, this suggests that misspecified cells in small clones will experience high pressure, which may cause their death and their elimination (Fig. 2C). Importantly, in this framework the elimination of cells does not rely so much on their genotype and cell intrinsic properties, but rather on the proportion of tissue occupied by the mutant cells and the WT cells, which set the shape/curvature of the interface [62]. …The relative sensitivity to compaction will only set the critical pressure, and therefore the critical clone radius, at which clones start to disappear.

Da confrontare con il moto per curvatura media che caratterizza l'evoluzione dei domini sulle lunghe scale di tempo per diffusione Allen-Cahn o Cahn-Hilliard.

In conclusion, I propose that the modulation of interfacial tension associated with cell misspecification can be at the basis of another mechanical cell competition. While the link between cell compaction, pressure and cell elimination was not formally tested in those conditions, it is very likely that the same compaction-driven death phenomenon will occur. Contrary to the first scenarios described above, this alternative mode of compaction does not rely on cell growth and could either eliminate pretumoural cells or promote their expansion depending on the proportion of tissue occupied by them.

4. Molecular pathways sensing compaction and triggering cell elimination

The first observation of compaction-driven extrusion in epithelia initially suggested that the process was **independent of caspases and apoptosis** [45,46].

In other systems, compaction-driven death has been associated with caspase dependent death and apoptosis. Cell volume reduction by osmotic stress triggers the activation of Caspases 3,8 and 9 and apoptosis [50]. Similarly, cell elimination induced at the center of compressed spheroid is both driven by necrosis and Bcl2 (an apoptosis inhibitor) dependent apoptosis [52]. Those results suggested that isotropic compaction can elicit the mitochondrial dependent apoptosis pathway and suggest that some sensing mechanisms must

relate compression to caspase activity.

The spontaneous extrusion of MDCK cells driven by topological defects can be blocked by the pan caspase inhibitor Z-VAD- FMK and is preceded by Caspase 3 activation (visualized with a live marker) [58].

Caspase activation is driven by the nuclear exclusion of YAP (Yes Associated Kinase) [58], the pro-survival transcriptional co-activator **inhibited by the Hippo pathway**. Hippo pathway is a central regulator of cell growth and cell survival and is one of the best documented examples of pathways modulated by cell shape and cell mechanics [3]. **Hippo pathway is inhibited by cell stretching [28,75,76], while it can be activated by contact inhibition and tissue densi cation [77]. Similarly, upregulation of cell proliferation/ growth in clones in *Drosophila* wing disc generates compressive forces that lead to an upregulation of Hippo pathway in the clones, which feedbacks negatively on clonal growth [31].** The same feedback may occur during normal development of the wing disc where a progressive upregulation of Hippo correlates with the progressive densi cation of the tissue [78].

Altogether, these results suggest that the compaction sensing me- chanisms and the routes used for cell elimination can be quite di erent depending on the tissue context, the amplitude and timescale of tissue deformations. Yet, they clearly show that several pro-survival and pro- apoptotic pathways can be modulated by changes in cell geometry or changes in tension.

5. Mechanical-based and biochemical-based competition: synergies and antagonisms

cell elimination during competition can also be driven by diffusive factors [83,84] or by contact-dependent apoptosis induction [40,68,69,85].

What is the relationship between those biochemical based eliminations and mechanical based eliminations?

While the relative contribution of those phenomenons was never clearly studied, one can already predict that they will not be favoured in the same conditions.

Indeed, contact-dependent cell elimination is enhanced by the high surface of contact between the two cell types [40] and the life-time of those contacts [68].

Overall, a high degree of cell mixing will favour contact-dependent mechanisms [40], while junction remodelings will prevent accumulation of mechanical stress driven by differential growth (see above). Alternatively, the high compactness of clones and the sorting behaviour associated with Ras activation [89] should reduce the contribution of contact-dependent elimination. The situations is even more complex for mechanical competition driven by boundary conditions. Here mechanical stress may act in the opposite direction compared to competitive elimination driven by biochemical cues. For instance, small activated Ras clones can be eliminated from *Drosophila* wing discs by Laplace pressure [62], while Ras should increase cell aggressiveness through the induction of Myc [87] and induce neighbouring cell elimination. Since the increase of line tension at clone boundaries promotes cell sorting and reduces the surface of contact between the two populations, contact-based elimination should be poorly effective in those conditions. Yet, the contribution of diffusive factors should still be significant and may alter the outcome of mechanical competition driven by boundary constraints.

In summary, the different modes of competition should coexist and may either act in synergy to eliminate one population, or may act antagonistically. The geometry of the interface between the two populations, the size of the clones as well as the mechanical properties of the surrounding tissue (*e.g.*: proportion of junction remodeling) will modulate the relative contribution of contact-based, diffusive-based and mechanical-based elimination. Assessing the relative contribution of each phenomenon remains very difficult at this stage as it would re- quire the characterization of unique molecular players that can completely shut down one type of competition and not the others.

6. Solid stress in tumor and the impact of mechanical cell competition on tumour progression

While the existence of mechanical cell competition has not been formally tested in real tumour, I will try to assess here whether the key ingredients required for such competition are indeed present in tumours and discuss its potential effect on tumour initiation and tumour progression.

Despite the high rate of proliferation of tumour cells, tumours do not grow exponentially. Many tumour growth curves are rather sigmoid and characterised by an early acceleration of volume expansion, followed by a slow-down and a

plateau phase [90,91].

While this halt of cell proliferation has been associated with a deprivation of nutrients and oxygen [93], other studies have proposed that mechanical constrains and compression may also halt tumour expansion [51,94].

The existence of solid stress *in vivo* was confirmed by several independent methods. Partial elimination of human tumour cells using Diphteria toxin could reduce cell density and was sufficient to increase the fraction of open blood and lymphatic vessels in human-tumour xenografts [95]. This is in agreement with a build-up of pressure in tumour generated by cell proliferation. Alternatively, the analysis of human tumour relaxation upon incision has been used to measure stored elastic energy. Residual stress generated by differential growth should lead to the isotropic compression of the internal tissue and an increase of tension tangential to the tumour mass [10] (Fig. 3).

Those correlations suggest that the compaction of the neighbouring tissue may be associated with worst prognosis and could be compatible with mechanical cell competition with the neighbouring cells.

6.3. Intratumoural competition for space

While mechanical cell competition between human tumours and the neighbouring healthy cells remains very hypothetical at this stage, there are currently more evidences for the existence of intratumoural competition (Fig. 4).

Intratumoural competition may also promote the emergence of drug resistant populations which will prevent tumour collapse upon treatment [110] (Fig. 4A).

On the other hand, competition for space may eliminate suboptimal cell populations which may not be very proliferative by themselves, but may globally promote tumour growth (Fig. 4B).

Accordingly tumour growth can be significantly reduced if this population is outcompeted by faster proliferating competitors. Finally, competition for space could prevent the emergence of drug resistant populations if drug-resistant cells have a lower homeostatic pressure and are outcompeted by non-resistant cells in the absence or at low doses of treatment (Fig. 4C).

6.4. Therapeutic avenues

Two strategies may be applied to overcome/alter spatial competition: modulating the mechanical environment of the tumour, or modulating the pathway involved in compaction sensing and death induction.

tumour cell elimination and a reduction of cell density [95]. While this may promote further tumour expansion through space release, this could also facilitate drug delivery through blood vessel opening [10].

Mechanical insulation of tumour cells through increased line tension at tumour boundary would be another efficient way to increase pressure within a tumour and to slow-down tumour expansion. However, there is to my knowledge no easy way to modulate specifically tumour/healty cells interface tension without affecting key regulators of cell survival/proliferation [89].

Alternatively, targeting the compaction sensing pathway may help to prevent neighbouring cell elimination and slow down tumour expansion.

The design of a successful treatment would probably rely on the capacity to precisely control in time and space the prevalence of mechanical cell competition.