

A mathematical model quantifies proliferation and motility effects of TGF- β on cancer cells

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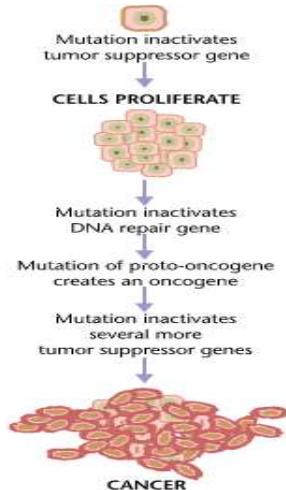
- ▶ Shizhen Wang, Nicole Bryce (Department of Cancer Biology, Vanderbilt University)
- ▶ Glenn F. Webb (Department of Mathematics, Vanderbilt University)

Overview of the talk

- ▶ Introduction to the biological background, in particular transforming growth factor (TGF) β
- ▶ Formulation of the mathematical model
- ▶ Experimental techniques
- ▶ Results
- ▶ Discussion/Conclusion

Biological background

- ▶ In normal organisms, the growth of cells is under tight regulation by growth factors.
- ▶ Disruption of this regulation is the most frequent cause of cancer diseases.



Transforming growth factor (TGF) β

- ▶ TGF- β is a a potent inhibitor of cell proliferation.
- ▶ On the other hand, TGF- β can accelerate cancer progression by enhancement of tumor cell motility, survival and increase in tumor angiogenesis.

Thus, TGF- β has properties of both a tumor suppressor and a tumor promoter.

Goals of our study

We want to study and separate quantitatively the effects of $\text{TGF-}\beta$ on cell proliferation and motility. We use computational simulation to understand the behavior of cells under $\text{TGF-}\beta$ exposure.

The Fisher–Kolmogorov equation

We work with a version of the Fisher–Kolmogorov equation (Fisher 1930, Kolmogorov *et al.* 1937). Let $u(x, t)$ denote the density of tumor cells, then

$$\frac{\partial}{\partial t} u(x, t) = D\Delta u(x, t) + \alpha u(1 - u),$$

with zero-flux boundary conditions on $\partial\Omega$. Denote the *normalized mass* by

$$U(t) = \frac{1}{|\Omega|} \int_{\Omega} u(x, t) \, dx.$$

History of the Fisher–Kolmogorov equation

- ▶ R. A. Fisher, 1930 (spread of an advantageous gene in a population)
- ▶ A. N. Kolmogorov, I. G. Petrovskii, N. S. Piskunov, 1937 (existence of solutions and traveling waves)

Models of cell growth based on Fisher–Kolmogorov equation (a **very** incomplete list)

- ▶ I. Prigogine, R. Lefever *et al.*, \approx 1980
- ▶ A. R. A. Anderson, M. A. J. Chaplain, 1998 (angiogenesis, cell migration)
- ▶ D. Drasdo, S. Höhme, 2003 (birth and death in avascular tumors, individual–based and continuum models)
- ▶ H. Enderling *et al.*, 2006 (breast cancer development, local treatment and recurrence)
- ▶ A. M. Stein *et al.*, 2007 (glioblastoma spheroid invasion)

Interpretation of the diffusion constant D

D has to account for at least three simultaneous processes.

- 1 Dividing cells occupy increasing spaces.
- 2 Individual cells plated on a Petri dish undergo a random walk.
- 3 Cells in a cluster may break lose from that cluster.

Thus, let

$$D = D_p + D_m,$$

where D_m accounts for effects 2 and 3.

Heuristics for the components of D

Let ℓ be the typical diameter of a cell and T_d the cell cycle time, then the dispersion due to proliferation should be

$$D_p = \kappa \frac{\ell^2}{T_d},$$

(Prigogine & Lefever 1980, Drasdo & Höhme 2003, Chaplain & Matzavinos 2006), where κ is a dimensionless factor (typically 2–4). For the random motility, the Einstein–Stokes equation has been suggested

$$D_m = \frac{k_B T}{3\pi\ell\eta},$$

where k_B is Boltzmann's constant, T the temperature, η the dynamic viscosity of the medium (Chaplain & Matzavinos 2006). But is $\eta = \eta_{H_2O} = 10^{-3} \text{ Pa s}$?

Numerical values for D

With $\kappa = 1$, $\ell = 10 \mu m$ and $T_d = 16 h$ we obtain

$$D_p = 6 \mu m^2 h^{-1}.$$

On the other hand,

$$D_m = 100 - 300 \mu m^2 h^{-1}$$

(Bray 1992, Chaplain & Matzavinos 2006).

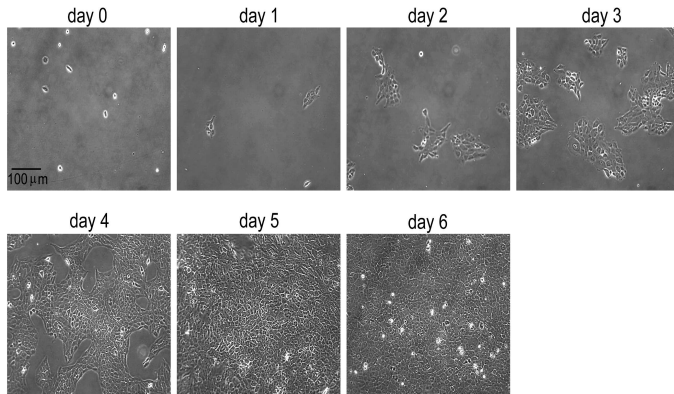
The modified Fisher–Kolmogorov equation

As cells become more densely packed, their random motility should decrease. Thus we suggest

$$\begin{aligned}\frac{\partial}{\partial t} u(x, t) &= \nabla \cdot (D(U) \nabla u(x, t)) + \alpha u(1 - u), \\ D(U) &= D_m(1 - U) + D_p, \\ U(t) &= \frac{1}{|\Omega|} \int_{\Omega} u(x, t) \, dx.\end{aligned}$$

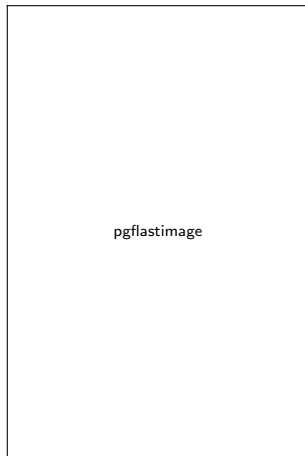
Experiments: growth assay

Seed MCF10A/HER2 cells in plates and count them periodically.



(Hinow, Wang *et al.* 2007)

Experiments: random motility of single cells



(Bryce *et al.* 2005)

Experiments: random motility of single cells

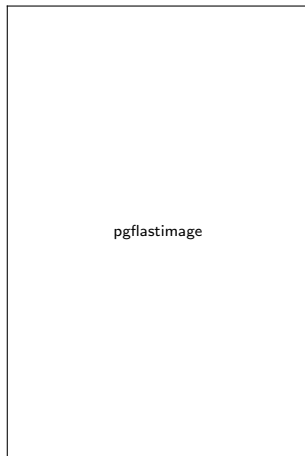
Positions $(x_i, y_i)_{i=1}^N$ of a single cell are recorded every $\Delta T = 5 \text{ min}$ over 5 h . The mean-squared displacement (MSD) is calculated according to

$$r^2(k\Delta T) = \frac{1}{N-k} \sum_{i=1}^{N-k} ((x_{i+k} - x_i)^2 + (y_{i+k} - y_i)^2). \quad (1)$$

To obtain the random motility of a single cell we fit this estimate with a linear function

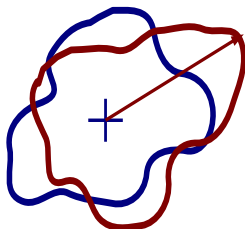
$$r^2(k\Delta T) = 4D_m k\Delta T. \quad (2)$$

Experiments: random motility of cell clusters



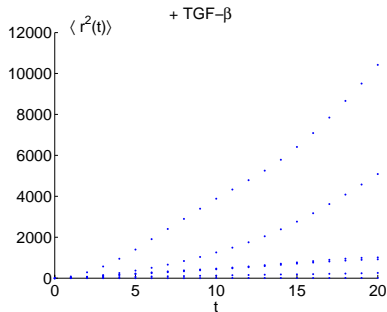
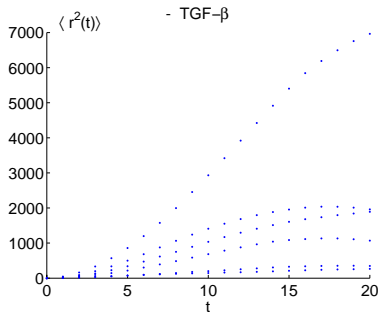
Experiments: random motility of cell clusters

On the initial frame of the movie the center of the cluster is identified. On each subsequent frame of the movie, a straight line is drawn to the point on the boundary of the cluster the farthest from the initial center.



We obtain a star of radii $(r_i)_{i=1}^N$.

Results: Random motility of single cells



Selected r^2 vs. k curves for untreated (left) cells and cells treated with $5 \mu M$ TGF- β (right). \implies The variation of the slopes is too big to unanimously define D_m .

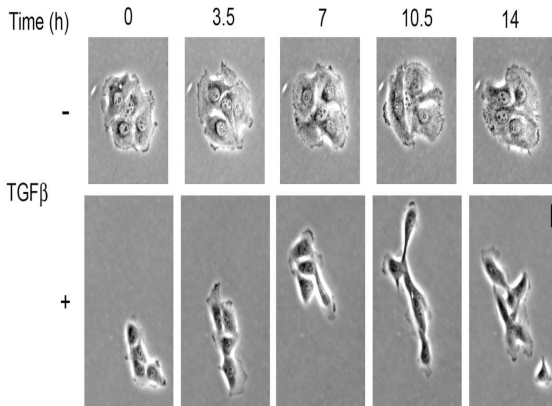
But: Percentage of mobile cells

Define a cell to be mobile if it moves outside of a $100 \mu m \times 100 \mu m$ square centered at the cell's original position.

$c \text{ (ng ml}^{-1}\text{)}$	motility (%)
0	33
0.5	45
1	49
2	51
5	56

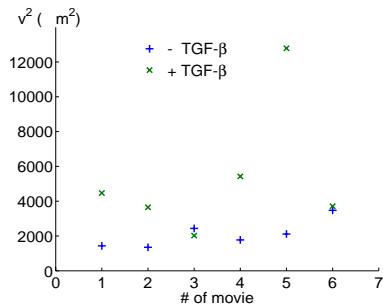
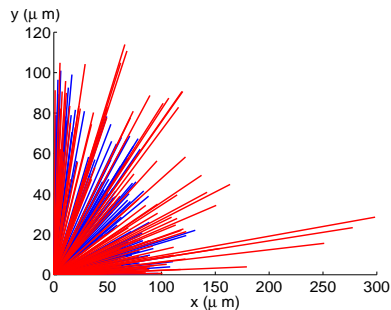
The percentage of mobile cells increases as with the concentration of TGF- β .

Results: Random motility of cell clusters



⇒ Clusters are more mobile and less cohesive in the presence of $\text{TGF-}\beta$.

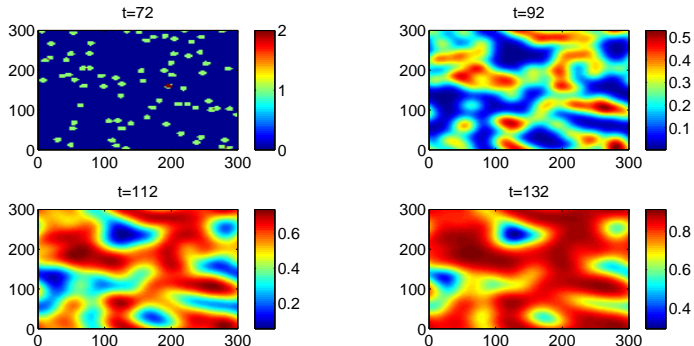
Results: Random motility of cell clusters



Coverage radii for cell clusters in absence (blue) and presence (10 μ M, red) of TGF- β (left). The variation of the squared radii (right) is computed according to

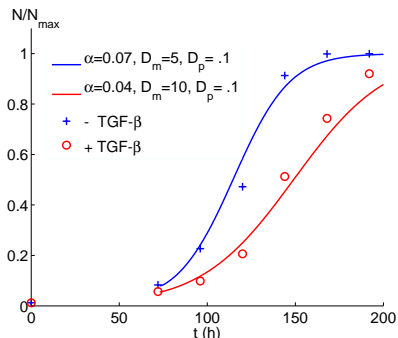
$$v^2 = \frac{1}{N-1} \sum_{i=1}^{N-1} |r_{i+1}^2 - r_i^2|.$$

Results: The modified Fisher–Kolmogorov equation



Simulation of cells at $t = 72 h$ (initial datum, upper left) and $t = 92, 112 h$ and $t = 132 h$. The parameters are $D = 5 \mu m^2 h^{-1}$, $D_p = 0.1 \mu m^2 h^{-1}$ and $\alpha = 0.07 h^{-1}$.

Results: Simulation of growth curves



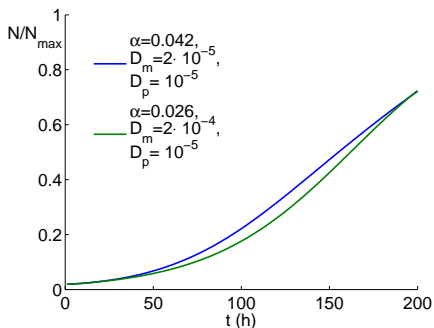
Shown are the untreated cells (control, +) and cells treated with $1 \mu M$ TGF- β (o).

TGF- β	$D_m (\mu m^2 h^{-1})$	$D_p (\mu m^2 h^{-1})$	$\alpha (h^{-1})$
-	5	0.1	0.07
+	10	0.1	0.04

Table: The parameter values used in the numerical simulations.

Discussion

The parameter-to-solution map given by the Fisher-Kolmogorov equation is not “injective”.



Conclusions

- ▶ We have developed a general spatial model of proliferating cell cultures *in vitro*, which allows quantification of the properties of proliferative capacity, cell mobility, and clustering as the population attains confluence.
- ▶ The novelty of our interpretation of the Fisher–Kolmogorov equation is that cells begin as isolated geometric regions corresponding to seeding.
- ▶ Our model has a relatively small number of parameters, D_m , D_p , α , and β .

Conclusions

- ▶ D_m 's calculated from the mean-squared displacement of single cells exhibit large variances for cells under the same conditions and little or no differences for cells under different TGF- β conditions.
- ▶ However, the fraction of mobile cells increases in the presence of TGF- β .
- ▶ Likewise, the cluster motility assay suggests that clusters are more mobile and/or less cohesive if TGF- β is present.

⇒ These are feasible approaches to parametrize the unbiased random cell migration in a large population of cells.

Outlook, open questions

- ▶ Experiments to determine D_m 's *in vivo* and *in vitro* need to be carried out, in two and three dimensions, with varying cell types.
- ▶ This becomes even more important once the tumor microenvironment is included into the mathematical model.
- ▶ We plan to build upon our model and include extracellular matrix, matrix degrading enzymes and chemo-/haptotaxis.

Acknowledgments

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S. Wang, P. Hinow, N. Bryce A. M. Weaver, L. Estrada, C. L. Arteaga and G. F. Webb. A mathematical model quantifies proliferation and motility effects of TGF- β on cancer cells. Available at [arXiv:0710.5665v1](https://arxiv.org/abs/0710.5665v1)

Thank you for your attention