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

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Collaborators

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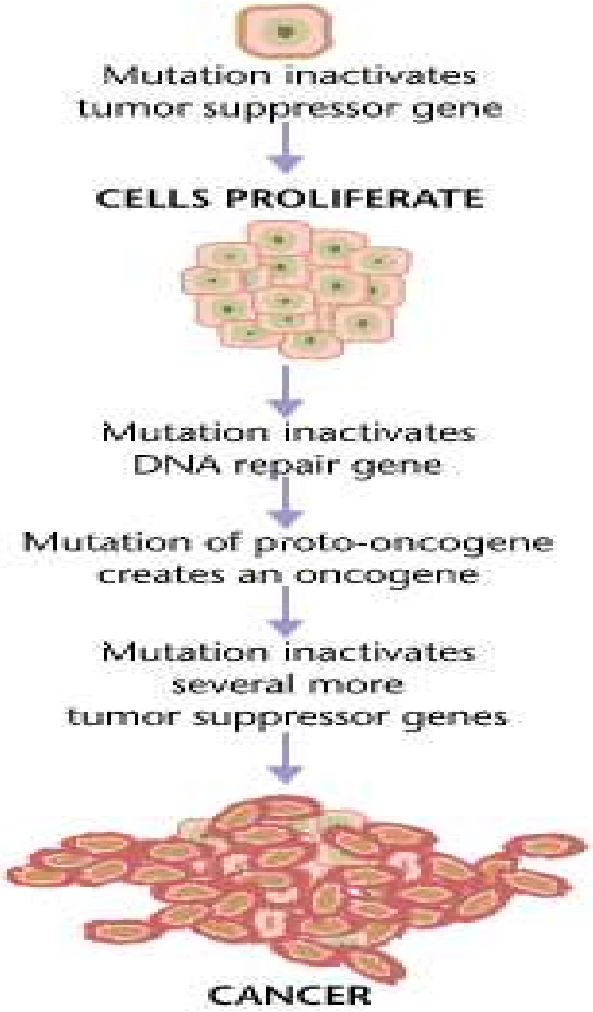
Overview of the talk

- 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 TGF–β on cell proliferation and motility. We use computational simulation to understand the behavior of cells under TGF–β 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

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

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 $\eta = \eta_{H_2O} = 10^{-3}\,Pa\,s$?

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).

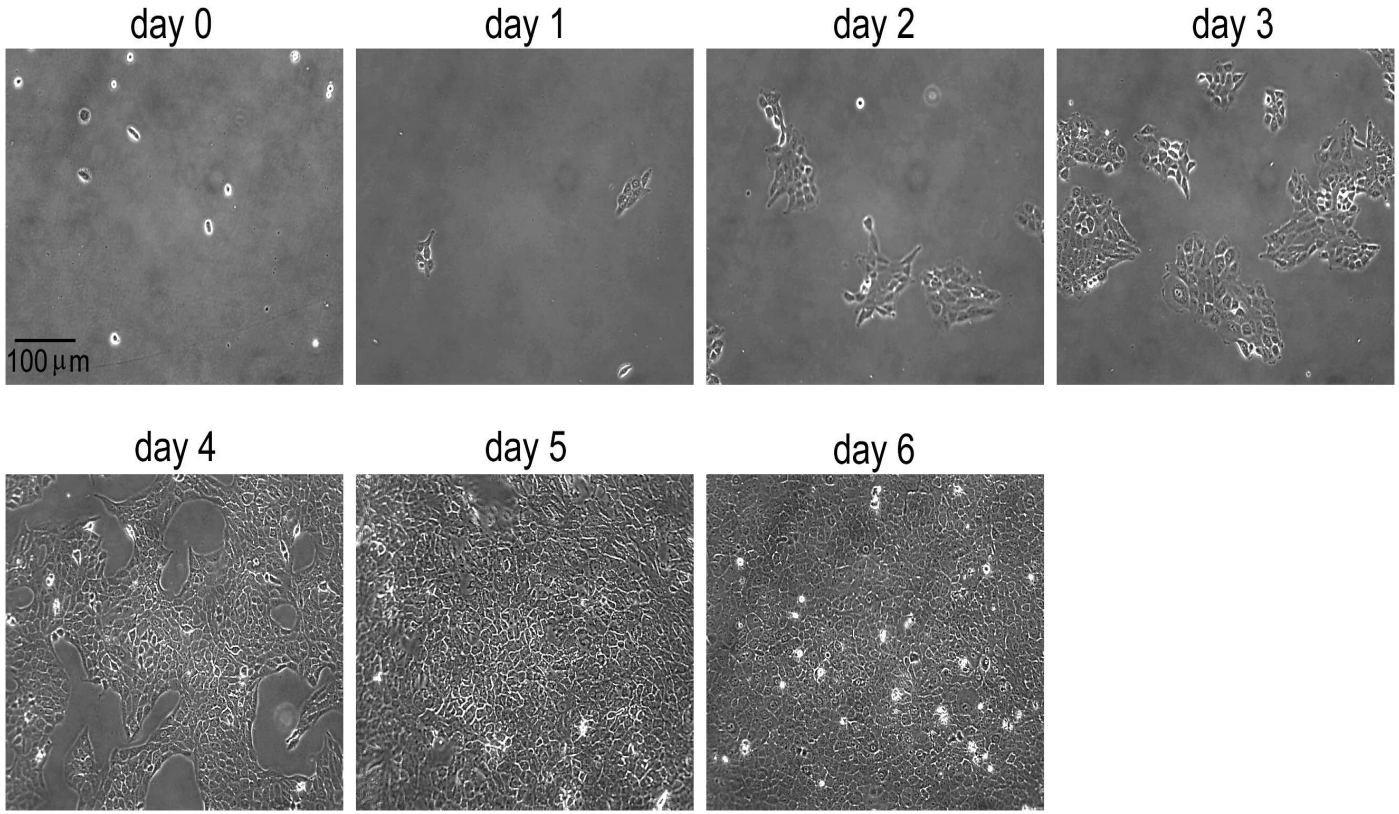
The modified Fisher–Kolmogorov equation

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

$$\frac{\partial}{\partial t}u(x,t) = \nabla \cdot (D(U)\nabla u(x,t)) + \alpha u(1-u),$$
$$D(U) = D_m(1-U) + D_p,$$
$$U(t) = \frac{1}{|\Omega|}\int_\Omega u(x,t)\,\mathrm{d}x.$$

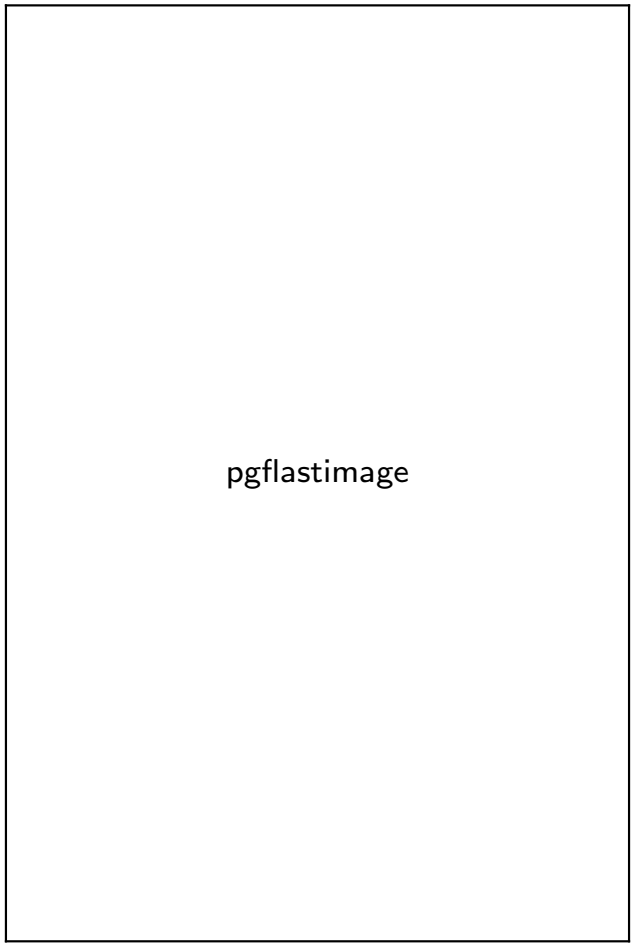
Experiments: growth assay

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

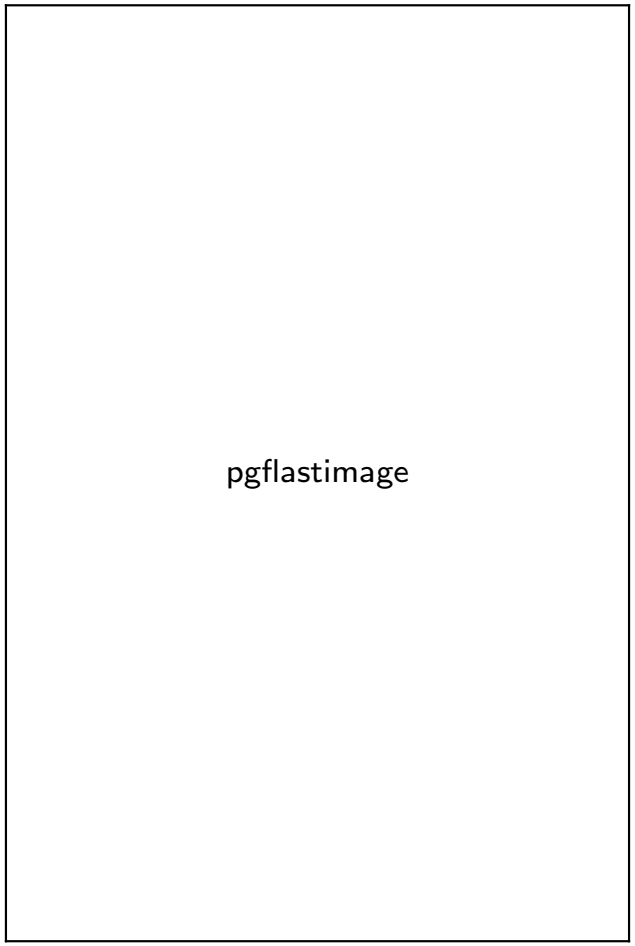
Positions $(x_i, y_i)_{i=1}^{N}$ of a single cell are recorded every $\Delta T = 5\,min$ over $5\,h$. The mean–squared displacement (MSD) is calculated according to

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

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

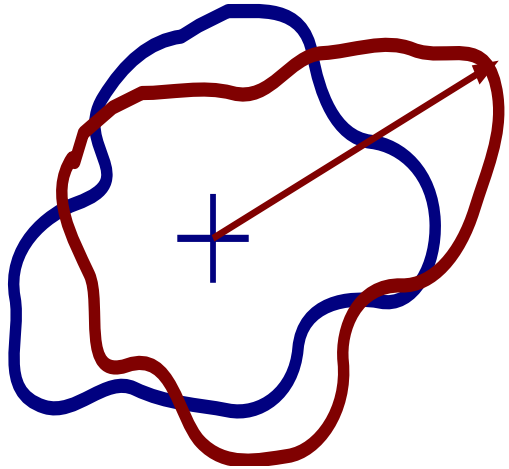
$$r^2(k\Delta T) = 4 D_m k \Delta T. \qquad (2)$$

Experiments: random motility of cell clusters



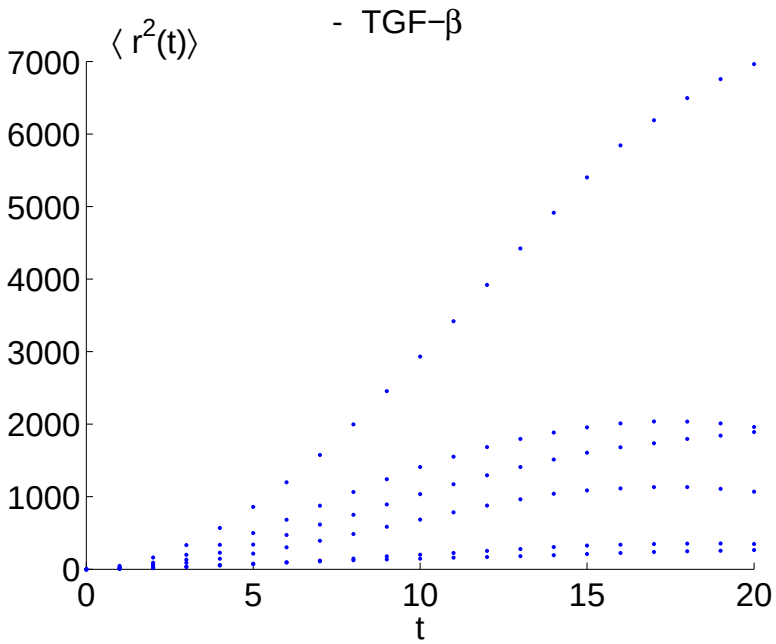
Experiments: random motility of cell clusters

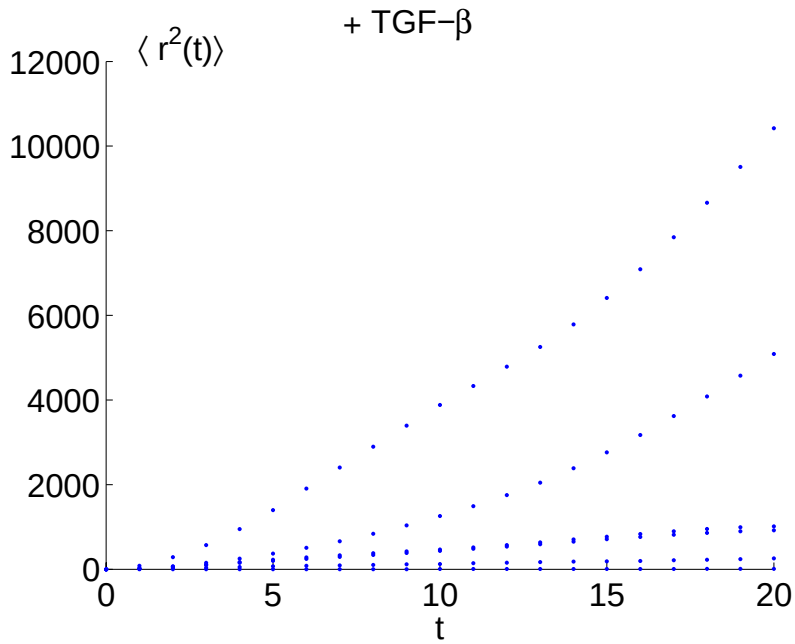
On the initial frame of the movie the center of the cluster is identified. On each subsequent frame of the movie, a straight line is drawn to the point on the boundary of the cluster the farthest from the initial center.



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 μM TGF–β (right). $\Longrightarrow$ The variation of the slopes is too big to unanimously define D_m.

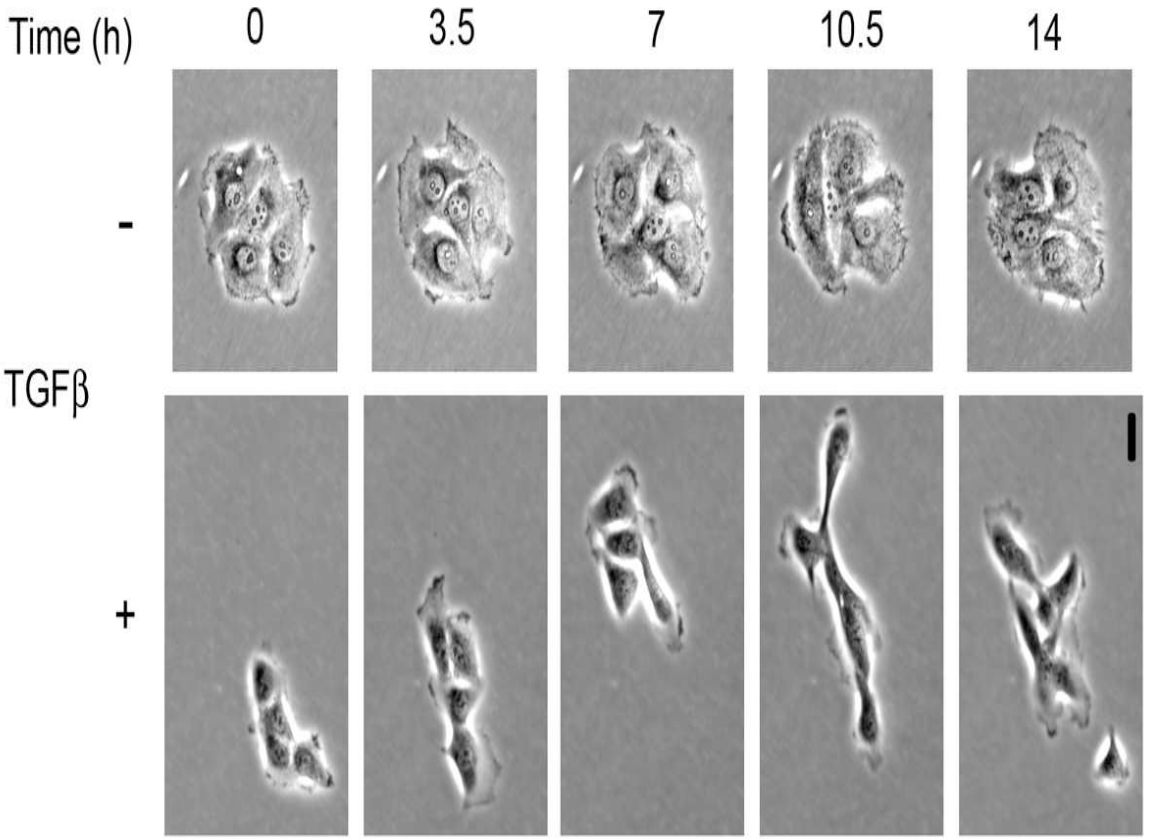
But: Percentage of mobile cells

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.

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

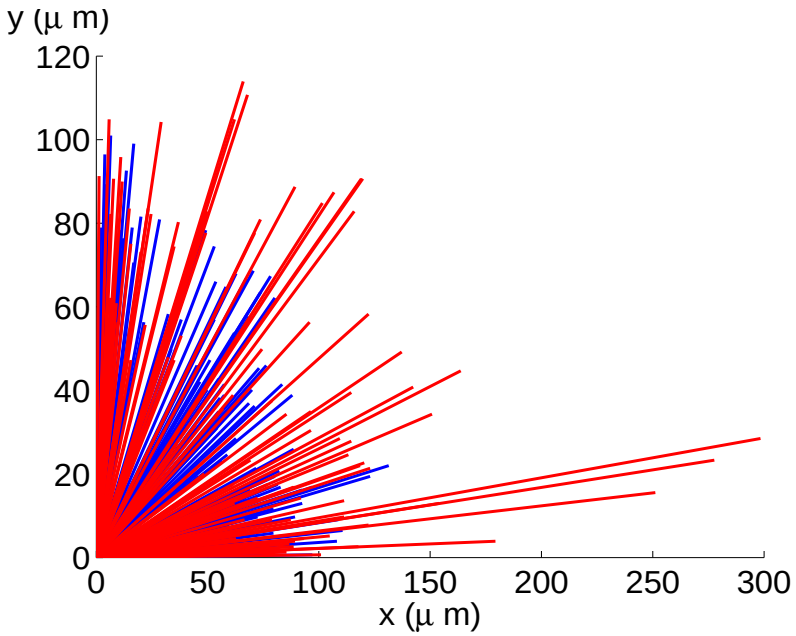
The percentage of mobile cells increases as with the concentration of TGF–β.

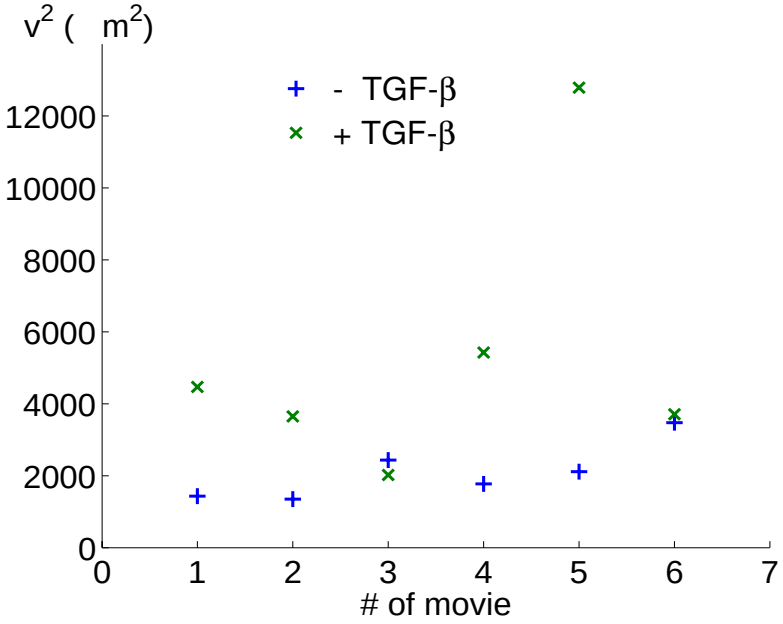
Results: Random motility of cell clusters



$\Longrightarrow$ Clusters are more mobile and less cohesive in the presence of TGF–β.

Results: Random motility of cell clusters

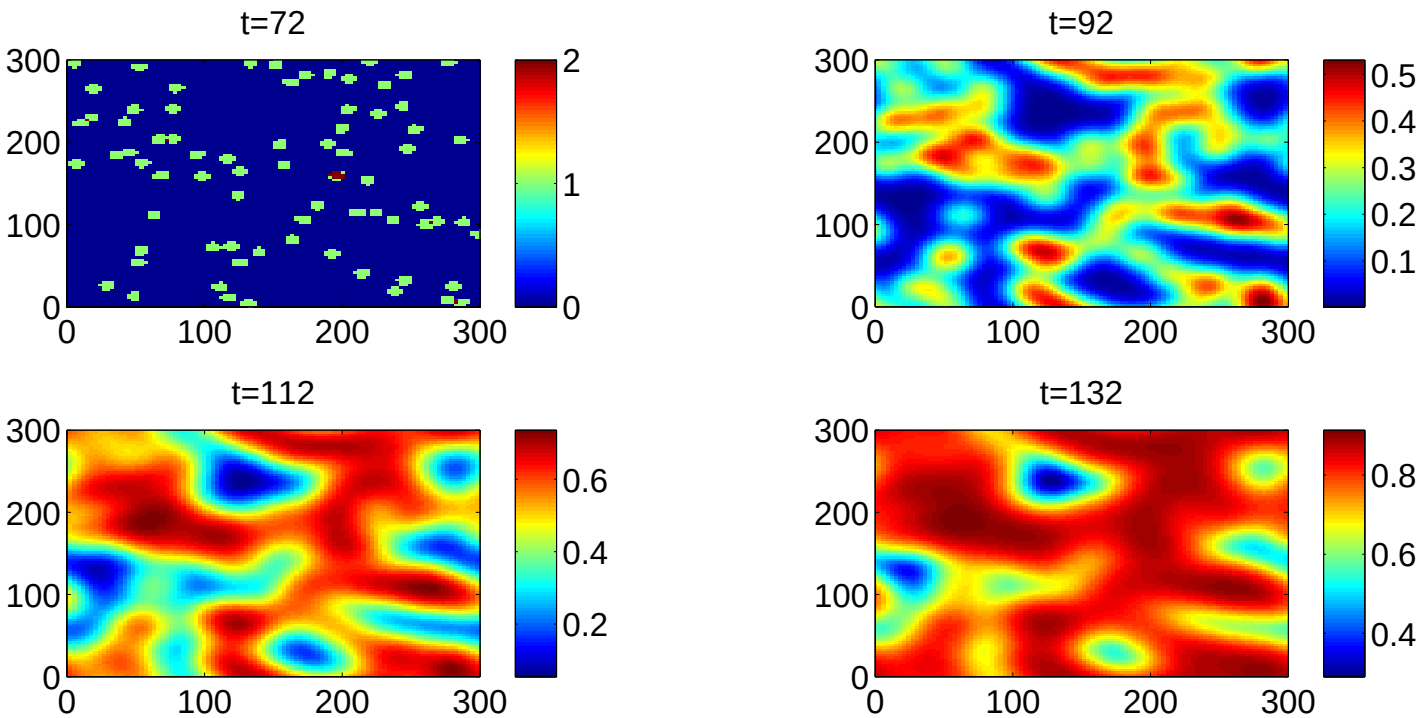




Coverage radii for cell clusters in absence (blue) and presence ($10\,\mu M$, red) of TGF–β (left). The variation of the squared radii (right) is computed according to

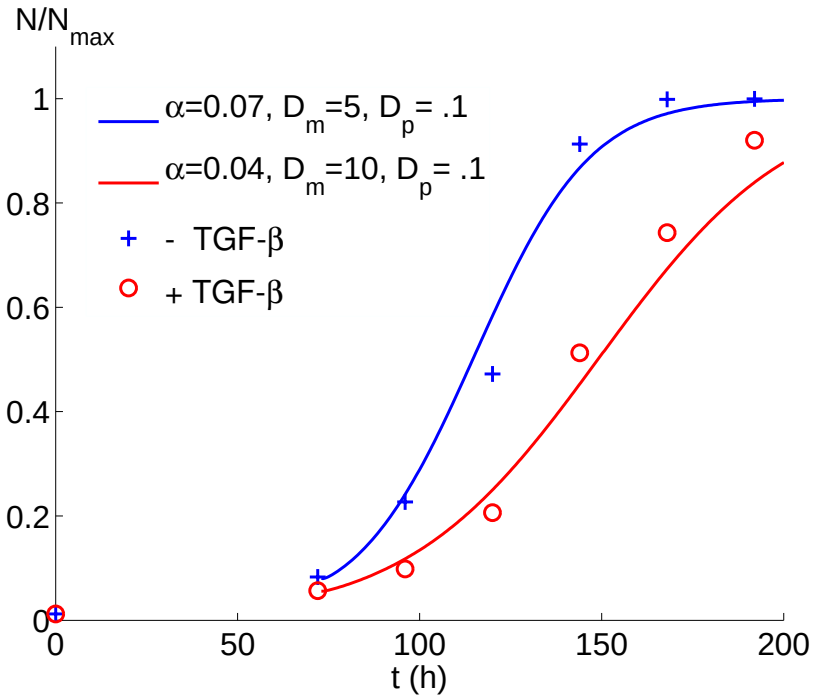
$$v^2 = \frac{1}{N-1} \sum_{i=1}^{N-1} |r_{i+1}^2 - r_i^2|.$$

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 ($\circ$).

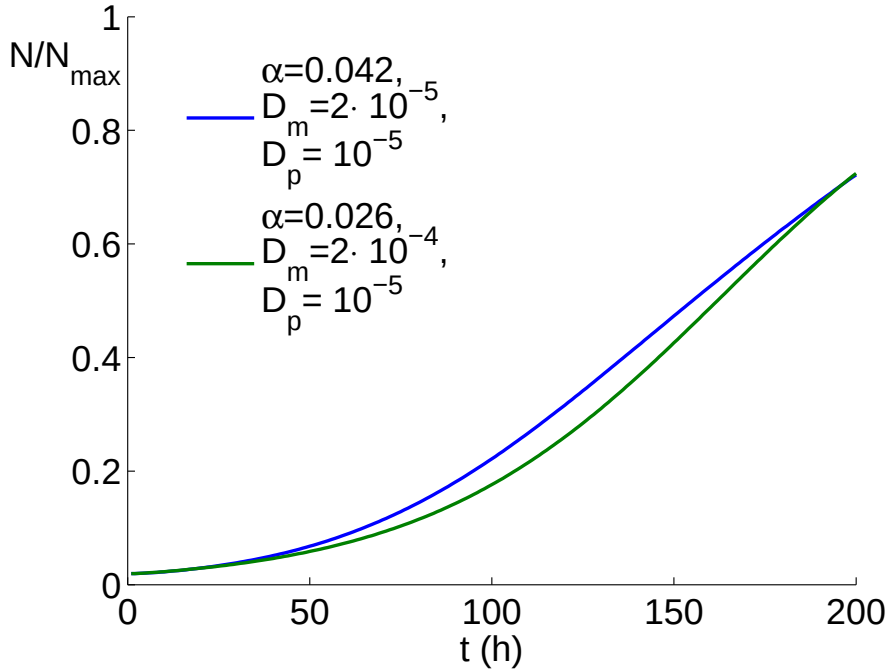
Results/Discussion

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.

$\Rightarrow$ 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`

Thank you for your attention