Modelling Invasive Processes in Biology

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Outline

Epithelial sheets (rosettes)

Cranial neural crest cell invasion

Acid-mediated invasion hypothesis (cancer)
ROSETTES
Egg-cylinder stage mouse embryo: AVE cells migrate in a typical way to correctly orientate A-P axis
• The AVE then induces anterior pattern in the underlying epiblast by restricting expression of posterior markers to the opposite side of the epiblast cup

• In *Nodal* and *Cripto* mutants the AVE forms but does not migrate → severe gastrulation defects and failure to develop further
During AVE migration, multi-cellular rosettes form (discovered by Shankar Srinivas). Why?
Rosettes form by cellular rearrangement:

- time-lapse (AVE cells in green, expressing Hex-GFP). Scale bar 50 microns
Vertex Model (Oster and Weliky, Honda, Julicher, etc etc)
\[ P = \left[ C_A \frac{a_t - a}{(a_t - a)}^{n_1 + 1} \right] + C_H H + C_D \frac{\phi - \theta}{(\phi - \theta)}^{n_2 + 1} \right] \hat{P}, \]

\[ T = C_L \left( l_c \hat{T}_c + l_a \hat{T}_a \right) + C_P \left( \hat{T}_c + \hat{T}_a \right) p, \]

\[ \mu_i \frac{dx_i}{dt} = F_i, \]
Junctional rearrangements

- We allow vertices closer than a certain threshold distance to join together.

Vertex rearrangement known as a T1 swap
Cell growth and mitosis

- Cells are assigned a certain volume and which grows over time
- Cells can divide when they reach a certain volume
Induce Migration

Number of cells = 180
Comparison with experiment: I

- Reduction in mean polygon number in the Epi-VE (pre- and post-migration) agreeing with observations:
Comparison with experiment: II

• Similar trends observed – 6-sided cells relatively less frequent in Epi-VE, while 4-sided cells more frequent there
The Influence of Cell Mechanics, Cell-Cell Interactions, and Proliferation on Epithelial Packing

Reza Farhadifar,1,3 Jens-Christian Röper,2,3 Benoît Algouy,2 Suzanne Eaton,2,* and Frank Jülicher1,*

Introduction

The capacity of epithelial cells to modulate intercellular
Figure 2. Cell Division, Topology, and Morphology

(A) Cell loss in the whole model. The preferred area of a randomly chosen cell is increased, and the network is relaxed. A chance of losing by stochastic cell death is decreased. The loss of a cell is assigned from the potential area, and inhibitor nodes are random internal. The yellow line indicates the growth ratio of the network.

(B) Normalized energy per cell of a growing network as a function of the number of cells for parameter values corresponding to case 1 and case 3. Generation number is also indicated. The energy per node is what is measured as the growth of the network. The standard deviation, averaged over 280 individual trials, is indicated as a function of cell number in the trials.

(C) Normalized p0 of simulation as a function of generation number for the simulation of case 1. The slope of the line represents the characteristic generation number, at which the standard deviation decreases 1 (l/2).

(D) Normalized p0 of simulation as a function of generation number for the simulation of case 2.
Abnormal AVE migration in *ROSA26* mutant

- Has disrupted PCP [Planar Cell Polarity] and forms fewer rosettes
Migration without rosettes

Number of cells = 180
Migration with or without rosettes
Conclusions

• Our simple model qualitatively captures a lot of features observed in AVE migration
• Rosettes not essential for successful AVE migration
• Rosettes are essential for ordered migration observed *in vivo*

  • Trichas, Smith, White, Wilkins, Watanabe, Moore, Joyce, Sugnaseelan, Rodriguez, Kay, Baker, Maini, Srinivas, Multi-cellular rosettes in the mouse visceral endoderm facilitate the ordered migration of anterior visceral endoderm cells, PLoS Biology, 2012, 10(2), e1001256
MODELLING EPITHELIAL SHEETS
Cell-based modelling approaches

- Cell-centred
- Vertex Models (Oster, Honda, Davidson et etc)
- Potts Models (Glazier, Graner, Hogeweg)

- WHICH IS CORRECT??
• **Chaste** – Cancer, Heart And Soft Tissue Environment

• Modular

• Developed in a collaboration between Computer Science, Oxford and CMB – lead developers David Gavaghan, Joe Pitt-Francis, James Osborne and Alex Fletcher
Colonic crypt organization and tumorigenesis

Adam Humphries and Nicholas A. Wright
Movies  (Mirams, Fletcher, PKM and Byrne, JTB, 2012)
From a discrete to a continuum model of cell dynamics in one dimension

Philip J. Murray, Carina M. Edwards, Marcus J. Tindall, and Philip K. Maini

\[
\frac{\partial q}{\partial \tau} = \frac{\partial}{\partial r} \left( \frac{k}{\eta q^2} \frac{\partial q}{\partial r} \right),
\]

where \(k\) is the spring constant, \(\eta\) is the cell viscosity, \(\tau\) is time, and \(r\) is the spatial coordinate. We define the nonlinear diffusion coefficient \(D(q) = k/\eta q^2\).

• Individual cell movement at the **discrete scale** modelled using **nonlinear force laws** can be described by nonlinear **diffusion** coefficients on the continuum scale.

• Therefore, we can *(a)* relate different discrete models of cell behaviour; *(b)* derive discrete, inter-cell force laws from previously posed diffusion coefficients


• **Comparing vertex-based models (Fletcher, Osborne, PKM, Gavaghan, submitted)**
Movies  (Mirams, Fletcher, PKM and Byrne, JTB, 2012)
NEURAL CREST CELL MIGRATION
Migration of Cranial Neural Crest Cells

• Paul Kulesa, Rebecca McLennan, Katherine Prather, Jason Morrison
  [Stowers Institute, Kansas]

• Louise Dyson, Ruth Baker
1 Spicher and Michel (2007)
2 Kulesa et al. (2004)
3 Courtesy of P. M. Kulesa, Stowers Institute
• NC cell population is crucial for proper development of the heart and peripheral nervous systems

• Is the cellular origin of the highly aggressive cancers, melanoma and neuroblastoma
Fig. 1. NC cell direction is acquired after cells exit the neural tube and cells move faster than non-linear tissue growth. (A) Orientation angle measurements. (B-D) Typical projected images from 3D confocal z-stacks of transverse sections through the 4 NC migratory streams at 8, 16, and 24 hours after electroporation. (E) Average nuclear orientation angle with respect to distance along the migratory route from 8-24 hours. (F) Representative images of migratory NC cells. (G) Average cell body orientation angle with respect to distance along the migratory route for 8-24 hours. (H) Representative images of migrating NC cells. (I) Average length of the NC cell migratory domain at increasing developmental times. (J) Focal injection (arrowhead) of DiI into the lateral mesoderm prior to NC cell emigration. (K) Twenty-four hours after injection in J. Arrowhead indicates site of injection. (L) Average spread of DiI-labeled tissue. Scale bars: 100 μm. NC, neural crest; NT, neural tube.
Model and manipulation

- Can a chemoattractant (VEGF) produced by the overlying ectoderm be sufficient for robust invasion?
Cell invasion with one cell type with chemoattractant modelled by a reaction-diffusion equation
Cell invasion with “leaders” and “followers”
Model Prediction and Validation

• A single chemotactic gradient with a single cell type is not a feasible mechanism. There must be at least 2 cell types – one chemotactic, one not chemotactic.

• By FACS (flow cytometry analysis) and by LCM (laser capture microdissection) show significant differences in expression of 19 out of 84 genes.

• Leading NC cells have upregulated expression of cell guidance and navigation genes (cell guidance factor receptors [EphA4]; integrins [Itgb5]; MMPs [MMP2]; cadherins [Cdh7]). Trailing cells have upregulated expression of cadherins distinct from leading NC cells.
Loss of Trailing Cells

- Disrupts migration (maybe the effects of pushing are lost)
- No change – leaders forge ahead
- Affects movement of leaders through a global change in chemoattractant profile
Cells move more slowly due to a less directed VEGF gradient.
Transplant Trailing Cells to the Front
2D is NOT 3D!!

Fig. 4. Behavior and molecular profiles of trailing NC cells transplanted to the leading position of the migratory stream. (A-C) Experimental schematic.
(A) Transverse sections after transplantation. (B) Average nuclear orientation angles with respect to distance along the migratory route (72 host cells (blue), 128 donor cells (pink), 12 embryos). (D) Schematic representation of cell migration after transplantation. (E) Heat map of qPCR molecular profiles of LCM-isolated NC cells. (F) Model simulation: Leaders (yellow), trailers that are following others (white) and trailers that are not following others (red).
(G) Model simulation. Tissue transplant is half the width of the domain. Trailing cells given the ability to become leading cells. Scale bar: 50 µm. NC, neural crest cells; NT, neural tube; hr, hours.
Lead Cells Transplanted Proximally

- They maintain their ordering and migrate
- May overtake the host cells as, after all, they are the leaders
Model Prediction – nothing happens
C = cell density,  
N = cell number,  
d = fixed jump size  

**Exclusion principle**

Variable jump size (normally distributed)
References

• McLennan, Dyson, Prather, Morrison, Baker, Maini, Kulesa, Multiscale mechanisms of cell migration during development: theory and experiment, Development, 139, 2935-2944 (2012)

ACID-MEDIATED INVASION HYPOTHESIS
Acid-Mediated Invasion Hypothesis

• A bi-product of the glycolytic pathway is lactic acid – this lowers the extracellular pH so that it favours tumour cell proliferation AND it is toxic to normal cells.

Gatenby-Gawlinski Model

\[ \frac{\partial N_1}{\partial t} = r_1 N_1 (1 - N_1/K_1) - d_1 LN_1 \]

\[ \frac{\partial N_2}{\partial t} = r_2 N_2 (1 - N_2/K_2) + \frac{\partial}{\partial x} [D_2 (1 - N_1/K_1) \frac{\partial N_2}{\partial x}] \]

\[ \frac{\partial L}{\partial t} = r_3 N_2 - d_3 L + \frac{\partial^2 L}{\partial x^2} \]
Travelling waves of invasion
Experimental results
(Gatenby et al, Cancer Research, 66, 5216-5223, 2006)
Therapeutics

- Add bicarbonate to neutralise the acid
Metastatic Lesions (Robey et al, Cancer Res, 69(6), 2260-2268)
Acidity Generated by the Tumor Microenvironment Drives Local Invasion

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Figure 2. Intravital and ratiometric images of the pH environment (pHe) with SNARF-1. Data are shown for 1 tumor, which was representative of 4 control tumors. The pHe of HCT116-GFP tumors were measured using SNARF-1−free acid at day 7 (A) and day 14 (B). A total of 200 μL of 1 mmol/L of SNARF-1 solution was injected into the mice via tail vein injection. Images were captured using a ×1.25 lens, 50 minutes post-SNARF-1 injection. The pHe was measured and ratiometric images were converted to a pH image using calibration data as per Materials and Methods. Black arrows indicate the acidic environment toward which the tumor is growing. B, the day 14 ratiometric image was co-registered with its corresponding fluorescence image. The pHe was then measured 100 μm from the tumor edge (represented by the short red lines). pHe measurements were taken every 22.5° of arc and are located above red lines. Purple arrow indicates the region of strongest acidity and direction of tumor growth. C, tumor at day 14 was pseudo-colored red to superimpose the tumor image on day 4 (green) and measure tumor growth from tumor edge on day 4 to tumor edge on day 14. Radial lines designate angles and tumor growth was measured by pixels. D, tumor growth and pHe plotted as a function of angle.
Figure 4. Histology of the invasive edge. A, upregulation of NHE-1 exchangers and GLUT-1 are shown by a darker staining of the tumor cells at the invasive front, consistent with an increase in glycolysis and acidosis. CD31 staining is low, suggesting poor perfusion, and hence a potential for hypoxia. Images illustrate a region of the tumor edge (small thin arrows) invading into the peritumoral normal tissue (large arrows). B, expression of tumor markers GLUT-1, NHE-1, and CD31 were quantified on a per-cell basis (see Materials and Methods). Red indicates strong staining, orange is moderate, and yellow is weak.
• In the asymptotic limit (small parameter is tumour cell diffusion divided by lactic acid diffusion) this is Fisher’s eqn.

• Using asymptotics (some subtilies arise) -- determine parameter space in which gaps occur
  • McGillen, Martin, Robey, Gaffney, PKM, Riken Proceedings (Nishiura 60th birthday) in press
  • McGillen, Martin, Gaffney, PKM (J. Math. Biol. to appear)
FROM MOUSE TO HUMAN
Equivalent dose less effective in humans (Du Bois height-weight formula)
Results

• Previously unseen potentially dangerous elevation in blood pHe resulting from bicarbonate therapy in mice – confirmed by our in vivo expts

• Limited efficacy of bicarbonate in humans

• Martin, Robey, Gaffney, Gillies, Gatenby, PKM, Predicting the safety and efficacy of buffer therapy to raise tumour pHe: An intergrative modelling study, Brit. J. Cancer, 106,1280-1287 (2012)
Buffer therapy most effective

(a) in elderly patients with renal impairments

(b) in combination with proton production inhibitors (such as DCA), renal glomular filtration rate inhibitors (eg non-steroidal anti-inflammatory drugs and angiotensin-converting enzyme inhibitors) or

(c) with an alternative buffer reagent possessing an optimal pK of 7.1 – 7.2 (pK = -log(dissociation constant)) – does not elevate (to dangerous levels) blood pH in the same way that bicarbonate can!!
Conclusions

(a) Used a vertex-based model for AVE migration on the mouse ectoderm to investigate the role of rosettes
(b) Used a hybrid model to study neural crest cell invasion
(c) Used a PDE model to study tumour cell invasion
(d) Showed how many of these approaches lead to novel nonlinear diffusion type systems
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