SUPPLEMENTAL MATERIAL

Supplement 1: network model of serotonergic signaling downstream of voltage

A feed-forward network can represent a wide variety of signaling relationships between initial conditions (DR2 representing 5-HT receptor 2 antagonist presence; C5HT, initial 5-HT concentration; D5HT, ivermectin; DR1, receptor 1 antagonist; DR5, receptor 5 antagonist) determining serotonin concentration (5HT), several serotonin receptors (R1, R2, and R5), and the final output of melanocyte state (HP). Nodes are activated with a sigmoid function, and each connecting arrow bears a weight whose value (between -10.0 and 10.0) represents the normalized strength with which it up- or down-regulates the next node to which it is connected. For example, w_{12} represents the strength with which R1 activity can induce or repress 5HTR5, while w_{11} represents the bias value of the activation function of R5. A set of values for the weights of each arrow represents (unambiguously specifies) a model for one possible relationship among these components. Then, each experiment (consisting of a set of treatments, which can perturb voltage and perhaps block one of the receptors, and a hyperpigmented or normal outcome of the experiment) can be compared to the prediction of any model: given a set of treatments and initial 5-HT concentration, what degree of hyperpigmentation phenotype is expected? Computing the output of the network and using a suitable formalism for capturing our experimental results in a database, a model can be ranked based upon how well its predictions match the outcomes of all our experiments. A simulated annealing search algorithm was used to identify networks whose predictions best match the results; such networks represent good models that can make new predictions for experimental conditions that have not yet been attempted.

Model description

Inputs:

$$x = \begin{bmatrix} c^{5HT} \\ d^{5HT} \\ d^{R1} \\ d^{R2} \\ d^{R5} \end{bmatrix}$$

d^{5HT}, d^{R1}, d^{R2}, d^{R5}: quantitative effect of drugs on extracellular serotonin level (d^{5HT}) and serotonin receptors sensitivity (d^{R1}, d^{R2}, and d^{R5}). Depending on the treatment, values are set to 0 (block), 1 (normal level), or 2 (enhance).

c^{5HT}: initial concentration (value between 1 and 2; 100 values tested during training).

Parameters:

$$\theta^{5HT} = \begin{bmatrix} w_1 \\ w_2 \\ w_3 \end{bmatrix} \quad \theta^{R1} = \begin{bmatrix} w_4 \\ w_5 \\ w_6 \end{bmatrix} \quad \theta^{R2} = \begin{bmatrix} w_7 \\ w_8 \\ w_9 \\ w_{10} \end{bmatrix} \quad \theta^{R5} = \begin{bmatrix} w_{11} \\ w_{12} \\ w_{13} \\ w_{14} \end{bmatrix} \quad \theta^{HP} = \begin{bmatrix} w_{15} \\ w_{16} \\ w_{17} \end{bmatrix} \quad \theta = \begin{bmatrix} \theta^{5HT} \\ \theta^{R1} \\ \theta^{R2} \\ \theta^{R5} \\ \theta^{HP} \end{bmatrix}$$

$$\begin{cases} 0 < w_i < 10 & if\ \ i = \{1,2,3,5,6,8,9,13,14\} \\ -10 < w_i < 10 & otherwise \end{cases}$$

Output:

$$a^{5HT} = [1\ c^{5HT}\ d^{5HT}\ ]\ \theta^{5HT}$$
$$a^{R1} = sigmoid(\ [1\ a^{5HT}\ d^{R1}]\ \theta^{R1})$$
$$a^{R2} = sigmoid(\ [1\ d^{R2}\ a^{5HT}\ a^{R1}]\ \theta^{R2})$$
$$a^{R5} = sigmoid(\ [1\ a^{R1}\ a^{5HT}\ d^{R5}]\ \theta^{R5})$$
$$h_\theta(x) = a^{HP} = sigmoid([1\ a^{R2}\ a^{R5}]\ \theta^{HP})$$
$$sigmoid(z) = \frac{1}{1+e^{-z}}$$

Penetrance:

$$x_d = \begin{bmatrix} d^{5HT} \\ d^{R1} \\ d^{R2} \\ d^{RX} \end{bmatrix}$$
$$P_\theta(x_d) = \frac{1}{100} \sum_{c^{5HT}=1,1.01,\ldots,2} step\left(h_\theta\left(\begin{bmatrix} c^{5HT} \\ x_d \end{bmatrix}\right)\right)$$
$$step(z) = \begin{cases} 1 & if\ z \geq 0.5 \\ 0 & otherwise \end{cases}$$

Bistability:

$$B_\theta(x_d) = \frac{1}{100} \sum_{c^{5HT}=1,1.01,\ldots,2} gauss\left(h_\theta\left(\begin{bmatrix} c^{5HT} \\ x_d \end{bmatrix}\right)\right)$$
$$gauss(z) = e^{\frac{-(z-0.5)^2}{2\cdot 0.1^2}}$$

Cost function (error):

$$J(\theta) = \frac{1}{m}\sum_{i=1}^{m}\left(P_\theta\left(x_d^{(i)}\right) - y^{(i)}\right)^2 + 0.1 \cdot B_\theta\left(x_d^{(i)}\right)$$

Best model found by simulated annealing search (error = 0.0538)

Parameters:

Parameter	w_1	w_2	w_3	w_4	w_5	w_6	w_7	w_8	w_9
Value	3.8	1.0	3.7	-8.4	8.4	2.3	-7.8	1.2	1.5
Parameter	w_{10}	w_{11}	w_{12}	w_{13}	w_{14}	w_{15}	w_{16}	w_{17}	
Value	-8.0	-3.7	9.9	9.7	3.1	0.7	8.2	-4.3	

Predictions:

TREATMENT:	5HT	R1	R2	R5		Exp. result	Model result
WT	1	1	1	1		0%	33%
IVM	2	2	2	2		100%	100%
IVM + R1 block	2	0	2	2		84%	100%
R1 block	1	0	1	1		22%	33%
IVM + R2 block	2	2	0	2		71%	100%
R2 Block	1	1	0	1		0%	0%
IVM + R5 block	2	2	2	0		100%	100%
R5 block	1	1	1	0		30%	33%
IVM + MTP	2	0	0	0		74%	100%
MTP	1	0	0	0		66%	0%
IVM + Fluoxetine	0	1	1	1		0%	0%

Supplement 2: movie of blood flow in chemically-induced tumor

This movie shows blood flowing through ectopic blood vessels that have grown into a 4NQO-induced ITLS.

Editor-in-Chief's report

The paper by Lobiken et al presents an original perspective on cancer, albeit in the model organism Xenopus. I am not in any way an expert in the field of membrane potentials, therefore my grasp of the significance of some of the paper's results is tenuous. I am impressed however by the wealth of data in this paper indicating the robust role of membrane depolarisation in creating cancer-like phenotypes. I have a number of comments:

We thank the editor-in-chief for his positive assessment and thoughtful comments:

2. In abstract, Vmem is not defined before use

Done. We now define it before use.

2. The introduction presents a rather stark epigenetic slant on cancer. The role of genetic mutations cannot be ignored altogether! After all, cancer cells in human patients certainly contain significant numbers of genetic mutations, and large-scale karyotypic disruption.

In this one primary manuscript, we did not intend to enter the complex debate regarding genetic vs. epigenetic causes of cancer. Our purpose was simply to remind the reader that in addition to the mainstream genetic paradigm, additional causes have been studied (since most readers are likely to be very familiar with the standard model of cancer as due to genetics). We have now revised the introduction to make this explicit. It should be noted however, that though there is not room to discuss this issue in this paper, it has been shown that host signals during regeneration and embryogenesis can fully normalize cells bearing many mutations and karyotypic disruptions, suggesting that such genetic events may accompany cancer but are not always sufficient for tumorigenic behavior. This is an interesting issue that has been discussed in several recent reviews. Regardless of the evolution of this debate, neither paradigm negates our primary data on the role of biophysical factors in oncogenesis.

3. In first paragraph "mechanistic dissection of these pathways..." - please define what pathways.

Done.

4. p.4, first paragraph - please define "physiological networks"

Done.

5. 1.3: it would be helpful to include a schematic figure defining "depolarisation", since "polarisation" has a number of meanings depending on whether the reader is a physicist or a cell biologist

Done, this has been added to Fig. 1A.

6. 1.3: "a number of ion channels, ..., are now recognised as bona-fide oncogenes... " - possible confusion between oncogene (as a gene), and that protein which is expressed by that gene

Done. We have now made this clear (p. 4).

7. 1.4, 1st paragraph second line from end: please define "currents" - currents of ions between cells perhaps?

Done (this was referring to currents across the cell's plasma membrane).

8. last line of p. 5 has typo (I)

Fixed.

9. p.6 bottom - please explain Goldman equation for non-specialists

Done.

10. 3.7 - how many embryos used here, since percentages given to 3 significant figures!

We now state the N of embryos, and limit the percentages to 2 significant figures.

11. 4.1 please define "instructor cells"

Done.

12. 4.2 for the physics audience can the authors make clear whether "bioelectricity" is an electric field disturbance linking distant cells, or located at the cell membrane, and changes thereof lead to transport of different signalling molecules

Done, we have now clarified this.

13. 4.5 - good to add "in frog embryos" after the phrase "...reduce tumour incidence"

Done.

A more major comment - the results from the computational study need to be more transparent here - after all, physicists like theory and computation. For example, how does the computational model explain the bimodality of the hyper pigmentation response?

We have now added text to clarify this.

<u>First referee's report</u>

This paper gives a fascinating view of the role of bioelectric polarization in triggering a cancer-like phenotype in frog embryos. The key experimental tool is the use of invermectin plus salt concentration to depolarize a sub population of "instructor cells". Controls are presented that make a good casefor depolarization, and not the invermectin, as causal in the cancer-like phenotype. In particular, the use of hyper sensitive receptors in a subpopulation of the cells, to generate a response at much lower drug concentrations supports the main argument. These are important results. The finding that drugs that cause hyperpolarization may revert the phenotype may even have an impact on treatment. This paper should be published as is.

We thank the reviewer for their highly positive comments and helpful suggestions.

My only qualification is that I found the opposing roles of different types of serotonin receptors confusing (as do the authors). I wonder, therefore, if tadpoles might be a confusing model for adult cancers because of the role of serotonin in development. Could the authors add some discussion of this point?

Done. We have now included some discussion of this issue in section 4.3 on page 19, mentioning the several aspects of adult physiology that are likewise regulated by serotonin (e.g., mitogenic activity). Serotonin signaling in *Xenopus* melanocyte metastatic transformation recapitulates for example phenotypes of several human cancers that involve altered serotonin physiology. Thus, the serotonin-regulated pathways acting in *Xenopus* development represent a useful window on similar functions that occur throughout lifespan.

of typos that need to be caught in proof reading. Just in case the authors/editors miss it, bottom of page 5 “subsequently results in the development…”

Fixed. We have now gone even more carefully over the manuscript and corrected the remaining typos.