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Extracellular volume regulation and growth

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Summary We have formalized extracellular and intracellular volume interaction with each other and the influence of these processes on the type of cell growth. The linearized model was verified by stereo metric solution and the results were compared with experimental data. Two theoretical solutions were found: Solution 1, extracellular volume (ECV) was calculated to be about 23% of total body volume (TV). Stereo metric solution suggested the cubic cell cluster formed by 8-cells. This hypothesis (Solution l) explains the ECV to be compatible with the widely accepted value (about 23% of TV). In addition, the 8-cell cluster hypothesis explains the existence of ECV oscillation with the period of about seven days. This hypothesis probably describes the dominant type of growth in humans. Solution 2, in this type of growth, ECV fills about 77% of TV. Instead of the 8-cell cube, in this type of proliferation 4-cells could form a tetrahedron. This type of growth could be beneficial in processes where free space in tissue or organ must be filled for example in peptic ulcer healing and namely in repopulating of free space in a bone after high dose chemotherapy.

Introduction

Earlier studies of cell polarity have mostly emphasised the importance of cell polarity. Many observations later have shown that eukariotic cells can polarise spontaneously in the absence of pre-established asymmetric clues. Modeling work, as well as recent experimental data from several organisms suggests that a combination of local positive feedback loops and global inhibitors could result in robust cell symmetry breaking through amplification of minute, stochastic variations [1]. Although the molecular connections emphasise a stepwise development of cell polarity starting from recognition of pre-existing spatial structures, a competing view has now emerged that cells have an intrinsic ability to break symmetry that is perhaps dependent solely on their internal biochemical states [2]. Cell polarity is regulated by an

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independent pathway [3]. Though, in the mammalian body, relatively homogenous populations of cells are present and the allocation of metabolic energy between the maintenance of existing tissue and the production of biomass. That is why the biomass production must be a closely controlled process that balances allocation of metabolic energy against the production of new biomass [4]. In addition, the proportion between intracellular volume (ICV) and extrtracellular volume (ECV) must be preserved.

Regulation of proportionality between intracellular and extracellular volumes

Extracellular volume is regulated by sodium balance in humans and many animal species. The sodium balance is controlled by the renin–angiotensin–aldosterone system in blood (systemic RAS). The substantial fraction of ECV is represented by blood. When EVC decreases, the kidney blood flow (perfusion) decreases as well. The decreased perfusion stimulates the sodium resorbtion in a kidney and increases blood osmolarity. The increased osmolarity stimulates water resorbtion in a kidney and thirst. This leads to the increase of extracellular volume. The RAS in organs (organ RAS) stimulates cell proliferation in the organs, for example in blood marrow and in the retina. Therefore, organ and systemic RASes increase both the intracellular and extracellular volumes. The key component of RAS is angiotensin I converting enzyme (ACE) which, as a component of systemic RAS, increases sodium retention in the kidney and probably, as an integral part of bone marrow RAS, increases cell proliferation in bone marrow [5].

The purpose of the study was to formulate the model of organ and systemic RAS interaction in proliferation control and compare it with experimental data.

Model of extracellular volume response to intracellular volume changes

Formulation of Model

Let x and y be changes of ICV and ECV, respectively. The change of total body volume V is:

$$\Delta V = \Delta\mathrm{ICV} + \Delta\mathrm{ECV} = x + y.$$

Let $x \neq 0$. Then the change of total body volume $\mathrm{d}V/V$ will be the sum of x and y. To make the equations dimensionless, we will express ICV in a cell volume which is the number of cells times a constant (single cell volume) and by analogy ECV in volume units.

$$\mathrm{d}V/V = (y/x + 1)\,\mathrm{d}(y) + (x/y + 1)\,\mathrm{d}(x). \quad (1)$$

The volume V obtained from Eq. (1) by integration is:

$$V = y^2/2x + y + C_1 + x^2/2y + x + C_2. \quad (2)$$

Since the total body volume V is the sum of x and y only, if $x = y = 0 \Rightarrow V = 0$, thus $C_1 = C_2 = 0$.

Assume that intracellular (x) and extracellular volume (y) change to maintain the total body volume V constant, y compensates x changes:

$$x + y(x) = 0. \quad (3)$$

Eq. (3) is trivially satisfied for stable solution $y = -x$.

In a normal growth or involution y changes proportionally with x.

Assume a slight x change of Δx and V maintained constant. Then the approximation of $\mathrm{d}V/V$ change by linearized model gives:

$$\begin{aligned}\Delta(\mathrm{d}V/V) &= \mathrm{d}(x^2/2y + x + y^2/2x)/\mathrm{d}x\ \mathrm{d}y(x)\Delta x - \Delta x \\ &= y(2x^3 + 2x^2 - y^3)/x(x^3 - 2xy^2 - 2y^3) - 1 \\ &= 0. \quad (4)\end{aligned}$$

Eq. (4) gives four solutions:

$$\begin{aligned}y_1 &= 1/2[x + 7^{1/2}x - (2(2 + 7^{1/2})x)] \approx 0.299x, \\ y_2 &= 1/2[x + 7^{1/2}x - (2(2 + 7^{1/2})x)] \approx 3.347x, \\ y_3 &= 1/2[x - 7^{1/2}x - 2^{1/2}(2x^2 - 7^{1/2}x^2)^{1/2}]y_3 \notin \boldsymbol{R}, \\ y_4 &= 1/2[x - 7^{1/2}x - 2^{1/2}(2x^2 - 7^{1/2}x^2)^{1/2}]y_4 \notin \boldsymbol{R}.\end{aligned}$$

In medicine the ECV is usually determined relative to the value of total body volume (TV):

$$\mathrm{ECV} = y/(x + y). \quad (5)$$

Then from Eq. (4), $\mathrm{ECV}_1 \approx 0.230 \times \mathrm{TV}$, $\mathrm{ECV}_2 \approx 0.770 \times \mathrm{TV}$.

These real solutions are not stable.

Geometric approach to problem

Assume an exponential growth and the same exposition of all cells to diffusing growth and differential factors. Then the cells will form convex polyhedra that are vertex, edge and face-uniform (Platonic solids). Let these polyhedra be formed by populations of spherical cells in exponential growth. Only two of Platonic solids can be easily formed by n cells, where $n = 2^k$ and $k \in \boldsymbol{N}$, terahedron and cube.

Eq. (5) gives for cube: $ECV_{n=8} = 1 - \pi/6 \approx 0.476$, for tetrahedron: $ECV_{k=4} = 1 - \pi/8\sqrt{2} \approx 0.722$. If a different cell shape from the rounded one is expected, the ECV is smaller. For example, $ECV_{n=8} = 0$ for cubic cells. The $ECV_{n=4}$ is close to ECV_2. These suggest that cubic cells form 8-cell clusters, the round ones shape tetrahedra.

Experimental evidence

The ratio between the changes of ECV and ICV can be estimated as the ratio between the excretion changes of sodium and potassium in 24 h as function $F=\Delta Na/\Delta K$. It is widely accepted that in humans, the ECV represents about 20% of the total body volume [6]. That is why, the normal growth can be fit by the equation: $y_1 \approx 0.299 \times x$ (Fig. 1, 1st quadrant: $x > 0$, $y > 0$) and the function F oscillates. Under physiological conditions, the period of the function F is not very different from seven days, the function F reveals a so-called circaseptan rhythm. Circaseptan rhythm is probably produced on an 8-cell population cluster since it is body size independent. It was proved even in beetles [7].

The cell production correlates with the frequency of the function F amplitude maximum. Cytotoxic treatment causes its dramatic decrease (Fig. 1, 2nd quadrant: $x < 0$, $y > 0$, $y = -x$). If there is a lack of proliferating cells in bone marrow, bone marrow ACE supports sodium retention and ECV expansion since sodium retention increases retention of water namely in an extracellular space. This mechanism stabilises the circulation, however, on the cost of blood dilution. Again, when bone marrow function recovers, the increase of sodium excretion precedes the increase of granulocytes in blood, which indicates the recovery of bone marrow function (Fig. 2) [5,8].

Probably also low $x > 0$ and $y_1 \approx 0.299 \times x$ may represent pathology, since the difference between y_1 and $y = x$ is low and, in addition, y_1 oscillates. This could increase the y_1 from $y_1 \approx 0.299 \times x$ to $y_1 = x$. ECV will increase from $ECV_1 \approx 0.23 \times TV$ to $ECV \approx 0.500 \times TV$ (Eqs. (4) and (5)). The calculated ECV is in agreement with preliminary results, suggesting that the number of capillary loops in retina ''capillary endings'' and, consequently, ECV increases about twice as much in comparison with health controls in patients with proliferative retinopathy [9].

The solution $y_2 \approx 3.347 \times x$ for $x > 0$ could be feasible if rapid bone marrow expansion is essential. This is typical after a high dose of chemotherapy and consecutive bone marrow transplantation. In this status, the function F period ranges from 2 to 3 days [8]. The cell cycle time [10] that is limited makes generation of tetrahedrons composed of 4-cells more probable.

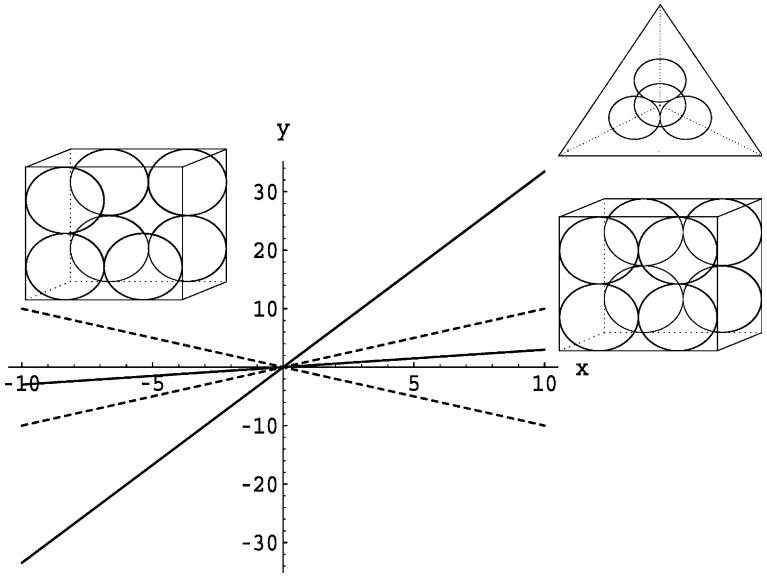

Figure 1 The response of extracellular volume y to intracellular volume change x. Full lines denote $y_1 \approx 0.299 \times x$ and $y_1 \approx 3.347 \times x$, dashed lines are graphs of functions $y = x$ and $y = -x$. In 1st quadrant, the 8-cell cube and the 4-cell tetrahedron show the corresponding stereometric solutions to y_1 and y_2. In 2nd qadrant, the graph of function $y = -x$ and the 7-cell cube depicts the increase of extracellular volume caused by the destruction of cells.

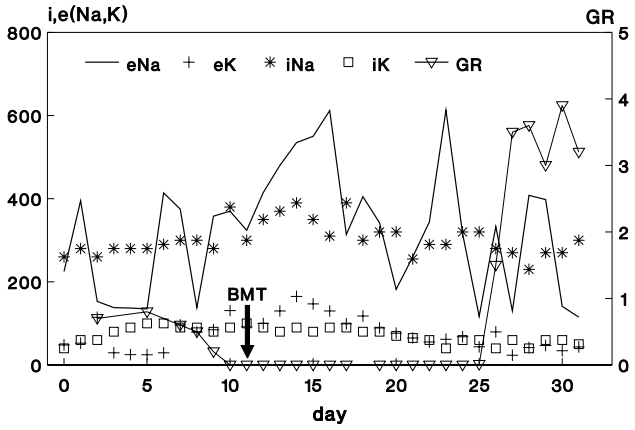

Figure 2 The influence of bone marrow cell transplantation on electrolyte balance and granulocytes in peripheral blood. Sodium (Na) and potassium (K) are intakes (i) excretions (e) in mmol/24 h. BMT indicates the day of transplantation of bone marrow cells. Granulocytes in peripheral blood (GR) are given in [$l0^6$/l].

Conclusions

Hypothetically the renin—angiotenin system regulates the ratio between ECV and ICV.

The circaseptan (of about 7-day period) rhythm is caused by generation of cubic structures, which are characterised by low ECV. The calculated value $ECV_1 \approx 0.23$ is in agreement with experimental data. This type of growth is probably dominant under physiological conditions.

Under extreme conditions, where tissue expansion is essential (e.g., bone marrow transplantation), the tetrahedral growth is possible.

Acknowledgements

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References

[1] Williams GH. Genetic approaches to understanding the pathophysiology of complex human traits. Kidney Int 1994;46:1550—3.
[2] Kirschner M, Gerhart J, Mitchison T. Molecular vitalism. Cell 2000;100:79—88.
[3] Harwood A, Braga VM. Cdc42, GSK-3: signals at the crossroads. Nat Cell Biol 2003;5:275—7.
[4] West GB, Brown JH, Enquist BJ. A general model for ontogenetic growth. Nature 2001;413:628—31.
[5] Hajek D, Tomiska M, Krahulcova E et al. I/D ACE gene polymorphism in survival of leukemia patients — hypothesis and pilot study. Med Hypotheses 2003;62:80—5.
[6] Lentner C, Lentner C, Wink A. Geigy scientific tables 1. Basel: Ciba-Geigy Limited; 1981. p. 55—9.
[7] Meyer-Rochow VB, Brown PJ. Possible natural circaseptan rhythm in the beach beetle *Chaerodes trachyscelides* white. Acta Neurobiol Exp (Warsz) 1998;58:287. p. 287—90.
[8] Hajek D, Kubesova H, Vyzula R et al. Bone marrow as multidimensional orbit oscillator after autologous bone marrow transplantation. Stem Cells 1999;17:25—30.
[9] Starha P, Hajek D, Karaskova J, et al. The identification of capilary endings in proliferative retinopathy. CAFIA 2000. Available from: http//www.cafia.org/cafia2000/public_html/index.html2003.
[10] Israels ED, Israels LG. The cell cycle. Oncologist 2002;5:510—3.