The Problem of Colliding Networks and its Relation to Cell Fusion and Cancer

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ABSTRACT Cell fusion, a process that merges two or more cells into one, is required for normal development and has been explored as a tool for stem cell therapy. It has also been proposed that cell fusion causes cancer and contributes to its progression. These functions rely on a poorly understood ability of cell fusion to create new cell types. We suggest that this ability can be understood by considering cells as attractor networks whose basic property is to adopt a set of distinct, stable, self-maintaining states called attractors. According to this view, fusion of two cell types is a collision of two networks that have adopted distinct attractors. To learn how these networks reach a consensus, we model cell fusion computationally. To do so, we simulate patterns of gene activities using a formalism developed to simulate patterns of memory in neural networks. We find that the hybrid networks can assume attractors that are unrelated to parental attractors, implying that cell fusion can create new cell types by nearly instantaneously moving cells between attractors. We also show that hybrid networks are prone to assume spurious attractors, which are emergent and sporadic network states. This finding means that cell fusion can produce abnormal cell types, including cancerous types, by placing cells into normally inaccessible spurious states. Finally, we suggest that the problem of colliding networks has general significance in many processes represented by attractor networks, including biological, social, and political phenomena.

INTRODUCTION

Cell fusion is a process that combines two or more cells into one (1,2). The resulting cells are called heterokaryons (containing different nuclei) or, if the cells multiply, hybrids. Cell fusion has diverse functions, both as a physiological process and as a tool in therapy and research: it is required for normal development (2), has been implicated in cancer (3–5), and has been explored for stem cell therapy (6,7). For example, fusion of a sperm to an egg creates a hybrid that produces all cell types of our body (2); fusion of myoblasts produces skeletal muscles (8); fusion of monocytes creates osteoclasts, the cells that remodel bones (9); and fusion of macrophages, which is a part of the immune response, results in foreign-body giant cells (10). At the onset of human pregnancy, fusion of trophoblasts creates the syncytiotrophoblast, a giant cell that serves as the interface between the fetus and the mother and secretes a set of hormones, including chorionic gonadotropin, which is detected by pregnancy tests (11). Finally, fusion between circulating stem cells and resident cells of some organs can yield progenitor-like cells that can repopulate damaged tissues, a phenomenon that has been explored with a view to producing progenitors for stem cell therapy (see previous reviews (6,7,12).

These functions of cell fusion rely on its poorly understood ability to create new cell types. The potential danger of this ability may explain why cell fusion in the body is tightly controlled and is restricted only to certain cell types. For example, sperm fuses only to the egg, muscle precursors do not fuse to epithelial cells and epithelial cells do not fuse to each other (1,2). However, this regulation can be bypassed by molecules capable of fusing cell membranes (13). For example, viral fusogenic proteins function by fusing the viral envelope to the cell membrane, thus injecting the viral content into the target cell. Because these proteins can also fuse membranes of neighboring cells (13), infections with some viruses, such as herpes virus, measles, or HIV, are accompanied by accidental, indiscriminate cell fusion (4,14). The fate of the resulting cells is unknown.

We and others have proposed that accidental cell fusion can cause cancer and its progression (see previous reviews (4,5,15–17)). This model is based on several observations: 1), multinucleated tumor cells, whose origin is unknown, are common in cancers and precancerous lesions; 2), fusogenic proteins are often expressed in common cancers; 3), cell fusion causes conditions characteristic for cancer cells, such as chromosomal instability and epigenetic plasticity; 4), cell fusion can create new cell types or produce dedifferentiated cells; 5), fusion between cancerous and host cells has been demonstrated in experimental models of cancer; and 6), fusion of nontumorigenic cells can produce hybrids that form tumors in experimental animals.

Finally, a common trait of cancer cells and cell hybrids is the diversity of abnormal cell types and the heterogeneity of their populations (3–5). The phenotypic diversity of cancer cells is such that even experienced pathologists can disagree on identifying a particular cancer cell type (18), and the underlying molecular diversity has led to the view that each individual cancer is unique and thus requires personalized therapy (19). Genome-wide gene expression studies

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show that each cell hybrid is also unique (20–29). Some of this diversity can be ascribed to chromosomal instability, which is common in hybrids (4,30), but heterokaryons and hybrids that have stable chromosome complements also have unique gene expression patterns (23,31).

How does cell fusion produce new cell types? Cell fusion may combine properties of parental cells, thus creating a combination that is not found in other cell types. For example, hybridomas combine the ability of lymphocytes to produce a specific antibody with the ability of myeloma cells to proliferate indefinitely. Likewise, metastatic cells were proposed to arise by fusion of premalignant cells, which proliferate freely but do not spread, to cells that travel freely in the body, such as macrophages or circulating stem cells (5,15,32). However, simple blending of properties appears to be limited to fusion of closely related cell types (33) and does not explain how heterokaryons or hybrids acquire emergent properties (34). For example, in a mouse model of liver damage, fusion of bone-marrow-derived cells to hepatocytes produced cells that express a set of genes normally active in neuronal cells (34). Moreover, fusion of distinct cell types usually yields cells that fail to express cell-type-specific genes of each of the parents but retain expression of the housekeeping genes, a phenomenon called extinction (29,31,33,35). The mechanisms of extinction are largely unknown, and the functional properties of the resulting cells, which are of particular interest because the lack of a normal cell type identity is a common feature of cancerous cells, are poorly understood (35). The current view is that properties of hybrids are a result of interaction among a small set of parental cell-type-specific transcriptional regulators that somehow cancel each other (35).

We thought that the consequences of cell fusion could be understood better by considering the model of cells as attractor networks (36–38). A basic property of these networks is that they adopt a set of discreet, stable, and self-maintaining states called attractors. For example, a human cell is a network that interrelates tens of thousands of genes and an even larger inventory of their products, which implies an astronomical number of possible network configurations. However, each cell normally adopts only one of ~400 stable states, known as cell types (39).

The attractor model is often visualized by comparing a cell to a ball that rolls on a virtual landscape whose points represent all states that the cell can adopt (40). Once the ball rolls into a basin, it settles at the bottom, which is an attractor that corresponds to a cell type. According to this model, the number of attractors that the cellular network can adopt determines the number of existing cell types. Because a perturbed network returns to its attractor unless it is moved beyond the edge of the attractor's basin, the attractor model can explain why a differentiated cell retains its cell type despite perturbations and noise. The model also explains why switching a differentiated cell from one cell type to another is difficult, as this transition would require removing the cell from its current basin and transferring it to the desired basin across a hilly landscape that is filled with unrelated basins (38,40,41).

If cell types are network attractors, then understanding the consequences of fusing cells requires an understanding of the consequences of fusing attractor networks. Therefore, in this study, we represent cell types as attractors of artificial neural networks and model their fusion computationally. We find that hybrid networks can assume a new attractor state that is unrelated to either of the parental attractors and often has abnormal properties. Our findings explain how hybrids can acquire emergent properties and why properties specific to the parental cell types could be extinguished. Our findings also imply that even fusion of normal cells is prone to produce diverse abnormal hybrids with virtually unpredictable properties.

METHODS

Description of the model

To model the intracellular regulatory network, we use time-dependent network equations similar to the continuous Hopfield model (42). The equations describe the levels of expression of a set of genes. The levels of expression are defined by the concentration of gene products in the cell contained in variables x_i, where i is the gene number. Variable x_i is shown in Figs. 1–3 by colored square arrays. This variable defines the deviation of concentration of products of gene number i from the basal level observed in the absence of all other genes. Red, green, and white pixels in Fig. 1 *B* correspond to the values of variable x equal to 1, −1, and 0 respectively. The gene products are assumed to be created at the rate described by variable u_i and eliminated with time constant τ. The equation describing the dynamics of gene expression is therefore

$$\tau \frac{dx_i}{dt} = u_i - x_i. \quad (1)$$

The rate of gene-product creation is related to the concentration of other products by the network interaction matrix, $u_i = F(\sum_j W_{ij} x_j)$. Here, $F(a)$ is a nonlinear function that relates the rate of gene production to the concentrations of other gene products. We use the nonlinear function frequently employed in neural networks that form sparse representations (43,44). We assumed that $F(a) = \tanh[(a-\theta)/\lambda]$ for $a \geq \theta$, $F(a) = \tanh[(a+\theta)/\lambda]$ for $a \leq -\theta$, and zero otherwise ($\lambda = 0.1$, $\theta = 0.5$). This function has an interval of inputs within which it is zero. This property makes it possible for some genes to remain inactive, which means that gene activities are sparse (43,44). The function also saturates at large positive and negative values of input. We used $\theta = 0.5$ and $\lambda = 0.1$ in our simulations. However, we have verified that similar results can be obtained if these parameters and function F are reduced by up to 50%, which implies that our conclusions are robust with respect to the choice of parameters.

The network weight matrix contains information about the patterns of gene activities in embedded cell types:

$$W_{ij} = \sum_c \frac{\xi_i^c \xi_j^c}{N^c}. \quad (2)$$

Here, ξ_i^c is the gene activity pattern corresponding to cell type number c, N^c is the number of nonzero pixels in this pattern, and the sum is assumed over 90 embedded cell types. This expression is similar to the standard form used in the Hopfield model (45), with the normalization dependent on the number of active genes. The patterns were generated from a library of black