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Stochastic cellular automata model of neurosphere growth: Roles of proliferative potential, contact inhibition, cell death, and phagocytosis

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ABSTRACT

Neural stem and progenitor cells isolated from the central nervous system form, under specific culture conditions, clonal cell clusters known as neurospheres. The neurosphere assay has proven to be a powerful in vitro system to study the behavior of such cells and the development of their progeny. However, the theory of neurosphere growth has remained poorly understood. To overcome this limitation, we have, in the present paper, developed a cellular automata model, with which we examined the effects of proliferative potential, contact inhibition, cell death, and clearance of dead cells on growth rate, final size, and composition of neurospheres. Simulations based on this model indicated that the proliferative potential of the founder cell and its progenitors has a major influence on neurosphere size. On the other hand, contact inhibition of proliferation limits the final size, and reduces the growth rate, of neurospheres. The effect of this inhibition is particularly dramatic when a stem cell becomes encapsulated by differentiated or other non-proliferating cells, thereby suppressing any further mitotic division – despite the existing proliferative potential of the stem cell. Conversely, clearance of dead cells through phagocytosis is predicted to accelerate growth by reducing contact inhibition. A surprising prediction derived from our model is that cell death, while resulting in a decrease in growth rate and final size of neurospheres, increases the degree of differentiation of neurosphere cells. It is likely that the cellular automata model developed as part of the present investigation is applicable to the study of tissue growth in a wide range of systems.

1. Introduction

Most of the twentieth century was dominated by the notion that the generation of new neurons ceases in the mammalian central nervous system (CNS) shortly after birth (see, for example, Brain and Walton, 1969). This 'no-new-neuron' dogma was gradually replaced when from the 1960s onwards evidence mounted that neurogenesis continues in two areas of the adult mammalian brain – the subventricular zone of the lateral ventricles in the forebrain, from where the young cells migrate anteriorly along the rostral migratory stream into the olfactory bulb to differentiate into interneurons; and the subgranular zone in the dentate gyrus of the hippocampus, where the adult-born cells develop into granule neurons (for review, see Ming and Song, 2005). As a seminal step towards the establishment of the concept of adult neurogenesis, two studies reported, in 1992, the isolation of multipotent, self-renewing cells, including *bona fide* stem cells, from the adult mammalian CNS (Reynolds and Weiss, 1992; Richards et al., 1992). Collectively, these cells are referred to as neural stem/progenitor cells (NSPCs).

Under serum-free culture conditions, single NSPCs proliferate to form a cluster of undifferentiated cells when epidermal growth factor (EGF), fibroblast growth factor 2 (FGF-2), or both are used to stimulate cell division. The two most frequently used in vitro systems to study the formation of such clusters are the neurosphere assay, a suspension system (Reynolds and Weiss, 1992); and the neural colony-forming cell assay, a system based on a collagen-containing semisolid matrix (Louis et al., 2008). The clonal clusters formed in these assays are commonly referred to as neurospheres and neural colonies, respectively. Both neurospheres and neural colonies can be dissociated to give rise to secondary spheres, and be induced to differentiate, by growth factor withdrawal and addition of serum to the culture medium, into the three major types of the cells of the CNS — neurons, astrocytes, and oligodendrocytes. Despite some limitations (for critical reviews, see Deleyrolle et al., 2008; Reynolds and Rietze, 2005), these assays have proven to be powerful in vitro systems to study some of the properties of NSPCs.

Although the neurosphere assay and the neural colony-forming cell assay have been employed in well over one thousand published cell biological investigations, the theory of the formation of

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the cellular aggregates in these in vitro systems is rather poorly understood. For example, it has remained elusive why the size of neurospheres and neural colonies is typically limited to a few hundred micrometers, although stem cell(s) within the cellular aggregates are thought to have unlimited proliferative potential. Similarly, a comprehensive understanding is lacking regarding the enormous heterogeneity in the terminal size of the neurospheres, even when they are derived from the same tissue sample. To address these gaps in theoretical understanding, we have in the present study simulated the growth of such cellular clusters by developing a cellular automata (CA) model.

Previous modeling attempts have provided proof of concept that certain features of neurosphere development can be simulated in congruence with biological observations. These features include the appearance of a necrotic core when neurospheres exceed a certain size, due to shortage of oxygen and lack of sufficient glucose supply (Liu et al., 2006); and the marked variability in neurosphere-size distribution, and the heterogenous distribution of various cell types inside spheres (Zhdanov and Kasemo, 2004).

To mimic the biological growth process as closely as possible to biological reality, we have expanded the number of parameters incorporated into our model, compared to these previous studies, and tested their effects in simulated growth of neurospheres. Based on cell biology observations, we expected that the proliferative potential of the NSPCs, contact inhibition of proliferation, cell death, and clearance of dead cells through phagocytosis play key roles in the formation of cellular aggregates, which will in the following simply be referred to as 'neurospheres'.

We have chosen the CA approach for modeling because it is particularly well suited to adequately capture the discrete nature of individual cells in tissue, and relevant if-conditions and probabilities (for reviews, see Jones and Chapman, 2012; Newgreen et al., 2013). CA modeling has been successfully applied to the study of cell proliferation and migration in several biological systems, including mesenchymal stem cells (Pérez and Prendergast, 2007), neural crest cells (Landman et al., 2011; Simpson et al., 2007), and adult muscle satellite cells (Garijo et al., 2012).

2. Model development

2.1. General structure of the model

We developed a CA model to simulate the growth of neurospheres based on the proliferation of NSPCs. Specifically, this model was constructed on the (x, y) plane, with dimensions determined by integers $L_x \times L_y$ where $1 \le x \le L_x$ and $1 \le y \le L_y$. The plane was then divided into squared lattices of unity edge length, yielding a total of $L_x \times L_y$ lattices. In this setting, each lattice can either be empty or occupied by one cell only. To allow each lattice location (x, y) to have four neighboring lattices at $(x+1, y)$, $(x-1, y)$, $(x, y+1)$, $(x, y-1)$, spatial limits where cell growth takes place are given by $2 \le x \le L_x - 1$ and $2 \le y \le L_y - 1$. Model rules were then programmed in Matlab ver. 2013a. Cell proliferation, cell death, and phagocytosis conditions were expressed accordingly using if-conditions and probabilities. Given appropriate initial conditions, the simulation model was executed for a certain number of discrete time steps, from $t = 1$ to $t = t_{final}$.

In the following, we describe the types of cells of the neurosphere, represented by different agents in the model's lattice; the cellular processes considered to contribute to the growth of the neurosphere; and the parameters used in our model to implement the rules governing these processes. These rules were motivated by cell biological observations of NSPCs in vivo, in situ, and in vitro detailed in the following sections. We assumed that the mitotic activity of an NSPC is regulated by its intrinsic proliferative potential, as well as by the density of the cells in its immediate vicinity through contact inhibition (see 2.2, 'Founder cell of the neurosphere colony and progenitor staging' and 2.3, Control of cell cycle progression and cell division'). We, furthermore, assumed that this local cell density also determines the probability with which the progeny of a mitotically active NSPC survive or die (see 2.4, 'Cell death'). Since a dead cell can be removed through phagocytosis, thereby reducing the mechanical strain on cells in its vicinity, we specifically investigated how variation in the interval between cell death and removal of cellular debris through phagocytosis ('clearance time', t_{clear}) affects cell proliferation (see 2.5, 'Phagocytosis').

2.2. Founder cell of the neurosphere colony and progenitor staging

We assumed that all cells within a neurosphere are the progeny of a single founder cell. We did not consider the possibility that neurospheres are formed by incompletely dissociated cells from the harvested tissue, by integration of free cells, or through fusion with other spheres – conditions that lead to chimeric, instead of clonal, structures (Mori et al., 2006; Singec et al., 2006).

Deleyrolle et al. (2008) have estimated that fewer than 10% of spheres in the neurosphere assay are derived from *bona fide* neural stem cells. This notion is consistent with the findings of single-cell mRNA profiling that revealed a very low percentage of stem cells among neurosphere cells (Narayanan et al., 2012). To accommodate for these two scenarios in our CA model, the founder cell was either a *bona fide* neural stem cell with unlimited capacity for self-renewal; or a progenitor cell with a limited ability to self-renew up to a maximum number of mitotic divisions (d_{max}). The d_{max} value was compared with the actual number of divisions of each progenitor. The MATLAB code kept track of the latter number and stored it in an array. This information was updated at the end of each time step.

Although the value of d_{max} of progenitor cells in the adult central nervous system is unknown, quantitative analysis of the proliferation dynamics of the neural progenitors in the subventricular zone of the adult mammalian brain has shown that after the initial division of the stem cell a progenitor cell divides on average 4–5 times (Ponti et al., 2013). Similar analysis in the adult cerebellum of teleost fish revealed an average of two mitotic divisions after the progenitor cells have left the stem cell niches to migrate to their target regions (Sîrbulescu et al., 2015). It is important to note that these numbers reflect the actual divisions observed, but not necessarily the maximum number of potentially possible divisions.

In our model, a *bona fide* stem cell undergoes either symmetric cell divisions (resulting in two daughter stem cells identical to the mother stem cell) or asymmetric cell divisions (self-renewing itself and producing one progenitor cell with a limited ability to self-renew). The progenitor cell and its progeny perform symmetric amplifying divisions. Although the progeny have been shown to exhibit intra-clonal cell-lineage heterogeneity, reflecting different states of neural developmental commitment (Suslov et al., 2002), they all are capable of cell proliferation to amplify themselves. The final symmetric division of the progenitor cell produces two differentiated cells, which subsequently neither divide nor migrate.

2.3. Control of cell cycle progression and cell division

The decision of both the *bona fide* stem cells and the progenitor cells regarding cell cycle progression is made, in our model, by assuming a mechanosensitive cell cycle checkpoint at the G_1-S interphase that senses the local cell density in the surrounding neighborhood. This checkpoint prevents the cells from entering the S-phase if the cell density, and thus the space available for newly generated cells, is below a certain threshold. Above this threshold, a decrease in cell density in the immediate vicinity leads to stretching of the tissue, which, in turn, is sensed as a regulatory