Quantitative insight into proliferation and differentiation of oligodendrocyte type 2 astrocyte progenitor cells in vitro

ANDREJ Y. YAKOVLEV†, KENNETH BOUCHER, MARGOT MAYER-PROSCHEL, AND MARK NOBLE

Huntsman Cancer Institute, Department of Oncological Sciences, University of Utah, 546 Chipeta Way, Suite 1100, Salt Lake City, UT 84108

Communicated by Raymond L. White, University of Utah, Salt Lake City, UT, October 5, 1998 (received for review July 12, 1998)

ABSTRACT As part of our attempts at understanding fundamental principles that underlie the generation of nondividing terminally differentiated progeny from dividing precursor cells, we have developed approaches to a quantitative analysis of proliferation and differentiation of oligodendrocyte type 2 astrocyte (O-2A) progenitor cells at the clonal level. Owing to extensive previous studies of clonal differentiation in this lineage, O-2A progenitor cells represent an excellent system for such an analysis. Previous studies have resulted in two competing hypotheses; one of them suggests that progenitor cell differentiation is symmetric, the other hypothesis introduces an asymmetric process of differentiation. We propose a general model that incorporates both such extreme hypotheses as special cases. Our analysis of experimental data has shown, however, that neither of these extreme cases completely explains the observed kinetics of O-2A progenitor cell proliferation and oligodendrocyte generation in vitro. Instead, our results indicate that O-2A progenitor cells become competent for differentiation after they complete a certain number of critical mitotic cycles that represent a period of symmetric development. This number varies from clone to clone and may be thought of as a random variable; its probability distribution was estimated from experimental data. Those O-2A cells that have undergone the critical divisions then may differentiate into an oligodendrocyte in each of the subsequent mitotic cycles with a certain probability, thereby exhibiting the asymmetric type of differentiation.

Abbreviation: O-2A, oligodendrocyte type 2 astrocyte.

†To whom reprint requests should be addressed. e-mail: yak@hci.utah.edu.

© 1998 by The National Academy of Sciences

PNAS is available online at www.pnas.org.
up to 100% (in clones of <15 cells). According to these results, a process that allows for a substantial degree of asymmetric differentiation within clones of cells regulates the initial generation of oligodendrocytes. (Asymmetric differentiation refers to situations in which a progenitor cell can, with a certain probability, either produce two further progenitor cells or differentiate, the probabilistic situation continuing as long as there are any progenitor cells in the clone.) The second component is associated with the continued self-renewal of dividing progenitor cells with a probability that is modulated by environmental signals, with some signals promoting differentiation and other factors promoting self-renewal. The third component is associated with eventual differentiation of all cells within a clone, mostly into oligodendrocytes but occasionally into adult O-2A progenitor cells with markedly different biological properties (3). This last component can be overridden by appropriate combinations of growth factors (5) or by certain classes of oncogenes (6).

It can be seen from the above that the development of a quantitative model of differentiation even within this well-studied biological system faces significant challenges. First, depending on environmental factors, the pattern of differentiation observed in families of dividing O-2A progenitor cells in vitro may appear to be more or less clonal, thus making quantitative inferences about the development of O-2A progenitor cells particularly challenging. In addition, one of the important complex features of both patterns of differentiation is that the probability of progenitor cell self-renewal clearly changes dramatically after a particular time point. To illustrate this point, suppose that every progenitor cell either gives rise to two progenitor cells with probability \( P \), or it differentiates into one oligodendrocyte with probability \( 1 - P \). In the symmetric model, \( P \) would go from 1 to 0 for all clonally related cells just before the occurrence of differentiation, whereas in a more general model \( P \) would decrease from 1 to 0 to a value less than or equal to 0.5 (to allow asymmetric division in association with eventual clonal extinction). Based on observations of clonal behavior of O-2A progenitor cells in vitro, it would appear that the number of mitotic cycles for which \( P = 1 \) (which we term the critical cycles) varies randomly from clone to clone, probably depending on the history of the founder cell of the clone. The actual number of critical mitotic cycles, however, cannot be observed directly; it should be thought of as a random variable that varies from zero to some unknown value. Nonetheless, as we demonstrate in the present paper, it is possible to estimate this quantity through the use of appropriate quantitative analysis.

In an earlier paper (7), we suggested representing the process of oligodendrocyte generation in vitro as a multitype Bellman-Harris branching stochastic process. When applied to experimental data on clonal growth and differentiation of purified O-2A progenitor cells from optic nerves of 7-day-old rats, the model provides a good fit to the mean number of progenitors and oligodendrocytes per clone at various points in time (7, 8). However, this model was inadequate in other key respects, including prediction of the variance values between clones and description of the underlying distribution of the number of critical mitotic cycles (8). Thus, our previous work did not provide a comprehensive mathematical model of differentiation of clonal families of O-2A progenitor cells into oligodendrocytes.

In the present paper, by relaxing constraints on our previous model so as to allow for an arbitrary distribution of the number of critical cycles, we have been able to provide a realistic quantitative theory for proliferation and differentiation of O-2A progenitor cells. This approach enables reliable estimation of the maximum number of critical cycles, the probability of progenitor cell renewal in the subsequent mitotic cycles, and the basic numerical characteristics (mean and variance) of the mitotic cycle duration.

Materials and Methods

All clonal data used in this analysis have been reported in detail (4). Data were obtained by analyzing (on days 3–6 after plating) the composition of clones of purified O-2A progenitor cells derived from optic nerves of 7-day-old rats. Only clones consisting of a single cell at day 1 after plating were analyzed. Cells were grown in chemically defined medium, supplemented with platelet-derived growth factor, in the presence or absence of thyroid hormone (T3, which promotes oligodendrocyte generation). Cell types were identified visually by the bipolar morphology of O-2A progenitor cells and the multipolar morphology of oligodendrocytes. The accuracy of this categorization is supported by histological data and also was confirmed at the end of each of these experiments by immunofluorescent analysis with cell-type-specific antibodies. The number of clones examined at each time after plating varied from 106 to 131.

Theoretical Basis for the Proposed Model

Any theory requires that certain underlying assumptions be made. The following observationally based assumptions are incorporated into the present analysis:

- The process begins with a single progenitor cell cultured at time \( t = 0 \). At the end of the mitotic cycle, every progenitor cell either gives rise to two progenitor cells with probability \( P \), or it differentiates into one oligodendrocyte with probability \( 1 - P \). We have seen that the founder cell and its immediate descendants appear to have a very high survival rate, and thus the model assumes that these particular cells do not die during the time of the experiment.
- As we have observed that in the conditions of our experiments oligodendrocytes do not divide and their survival is virtually complete for the first week after plating, we assume that oligodendrocytes neither divide nor die during the time of the experiment.

The founding progenitor cell acquires the competence for differentiation only after it undergoes a certain number, \( N \), of critical mitotic divisions. In other words, it is assumed that \( P = 1 \) for the first \( N \) mitotic cycles. As most clones tend to eventual differentiation of all members under the conditions analyzed in these experiments, we further assume that \( P = P_c \approx 0.5 \) for those cycles occurring once the critical cycles have been completed. The parameter \( N \) is treated as a non-negative random variable, and its distribution is to be estimated from experimental data.

The lengths of the mitotic cycle of the founder cell and its descendants of the same type are independent and identically distributed non-negative random variables with a common cumulative distribution function \( F(t) \) such that \( F(0) = 0 \).

Because of the manner in which the experiments are conducted, the progenitor cells, the only migratory cells in the population, do not migrate out of the field of observation. In addition to the above, the usual independence assumptions regarding the evolution of age-dependent branching processes (9) are adopted.

Mathematical Formulation of the Proposed Model

The model specified above is a special case of the Bellman-Harris age-dependent branching process with \( N + 2 \) types of cells; its properties for \( N = 0 \) were studied in detail by Jagers (10, 11). Let \( Z(t) \) be the number of progenitor cells and \( Z(t) \) the number of oligodendrocytes in a clone at time \( t \). By using general methods of the theory of branching processes it is possible to obtain integral equations for the expected values, \( M(t) = E(Z(t)) \), of the stochastic processes \( Z(t), t = 1, 2 \) (see ref. 8 for details). Given \( N \), these equations can be solved analytically to give

\[
M_1(t) = 1 - F(t) + \sum_{n=1}^{N-1} 2^n \left[ F^{n+1}(t) - F^{n+2}(t) \right]
\]

\[+ 2 \sum_{n=0}^{\infty} (2P_c)^n [F^*(N+n+1)(t) - F^*(N+n+2)(t)] \quad \text{[1]} \]

\[
M_2(t) = 2^n(1 - P_c) \sum_{n=0}^{\infty} (2P_c)^n F^*(N+n+1)(t), \quad \text{[2]}
\]

where \( F^*(t) \) is the cumulative distribution function of the lengths of the mitotic cycle of the founder cell and its descendants of the same type.
where $F^{k}$ is the $k$-fold convolution of the interdivision time distribution $F$ with itself. A solution for the corresponding variances can be obtained (8) along similar lines, but the resultant formulas are quite cumbersome and for this reason are not displayed here. If $P_0 = 0$, then all clonally related cells would differentiate in the $(N + 1)$th mitotic cycle. Mathematically, this special case is the equivalent of the original model of synchronous and symmetric differentiation by entire clones of cells.

We allow for an arbitrary distribution of $N$:

$$
\pi_0 = Pr(N = 0), \quad \pi_1 = Pr(N = 1), \ldots, \quad \pi_c = Pr(N = c),
$$

with a finite discrete support $(0, 1, \ldots, c)$, where $c$ is the maximum number of critical mitotic cycles. This compounding distribution is to be used for randomizing the parameter $N$ in formulas 1 and 2, as well as in expressions for the variances. The parameters $\pi_0, \ldots, \pi_c$ and $c$ are included in an estimation procedure (see below); this formulation makes our inference regarding the number of critical cycles essentially non-parametric.

**Results of Data Analysis**

To ensure computationally convenient formulas for the mean values and variances, the function $F(t)$ was specified as the Erlang distribution with shape parameter $\alpha$ and scale parameter $\beta$. This distribution is the most popular choice in cell kinetics studies (12), because the gamma family is quite flexible and reflects a multistage structure of the cell cycle. Furthermore, in this case the functions $M_i(t)$ and $M'_i(t)$, given by formulas 1 and 2, can be found in an explicit form. We estimated numerical parameters by fitting the model through data on the sample mean number of both types of cells per colony at different time intervals from the start of experiment. The weighted least-squares method was used to find the best fit.

Our preliminary studies (7, 8) indicated that thyroid hormone, T3, does not exert any significant effect on the number of critical mitotic divisions (see also Discussion). On this basis we required the estimates of $\pi_0, \ldots, \pi_c$ and $c$ to be equal for experiments with and without thyroid hormone, thereby reducing the number of unknown parameters; we found $c = 2$ for our present data. Because the model describing the development of O-2A progenitor cells in vitro contains three extra parameters ($\alpha$, $\beta$, and $P$) for each of the two experimental settings (with and without thyroid hormone), we ended up with nine adjustable parameters to simultaneously fit four (two curves for each setting) sets of experimental data. The variances for both types of cells, computed with the estimated parameter values, then were compared with their sample counterparts. This test is a stringent one for the agreement with the above values obtained by our estimation procedure. As to the process of oligodendrocyte generation in the presence of thyroid hormone, the analysis resulted in the following estimates: $\hat{P}_0 = 0.35$, $\hat{\alpha} = 5.0$, and $\hat{\beta} = 0.23$, when we have $\hat{\gamma} = 21.3$ h and $\hat{\sigma} = 9.5$ h. Our model thus suggests that the effect of thyroid hormone is $2$-fold. As proposed earlier (4), thyroid hormone decreases the probability for an O-2A progenitor cell to undergo self-renewal, thereby increasing the probability of oligodendrocyte generation. The estimated values of model parameters also suggest that the mean duration of the mitotic cycle for O-2A progenitor cells tends to be shorter in the presence of thyroid hormone.

**Discussion**

We have developed a mathematical model of clonal differentiation of O-2A progenitor cells into oligodendrocytes that provides a good description of our data, yielding estimated values of such biologically meaningful parameters as the probability of differentiation, cell cycle parameters, and the distribution of the number of critical cycles undergone before a progenitor cell becomes competent to differentiate.

The outcome of our analyses is highly surprising in respect to our hopes of rigorously distinguishing between the competing hypotheses of symmetric vs. asymmetric generation of oligodendrocytes from dividing O-2A progenitor cells, as discussed in the Introduction. These hypotheses represent special cases of our more general model, and acceptance of either hypothesis was a possible outcome of data analysis. What we have seen, however, is that the data do not drive the model to either of these extreme cases. Instead, our quantitative analysis reveals the need for both types of processes to quantitatively describe experimental observations. Specifically, we found that O-2A progenitor cells undergo a period of symmetric development up to a certain point (i.e., up to completion of the critical cycles), after which point an asymmetric differentiation process manifests itself. The concept of critical divisions that emerges from our analyses cannot be directly discerned through the narrative analytical method traditionally used in biological studies. The implication of this aspect of the generalized clock model presented in this paper is that every progenitor cell, isolated from optic nerves of postnatal rats and grown in the conditions of our experiments, divides not more than twice ($c = 2$) before it becomes competent for differentiation into an oligodendrocyte under in vitro conditions. Experimental data reported by Gao and colleagues (13) provide indirect...
The hypothesis that embryonic precursor cells undergo a longer sequence of critical cycles before the commencement of oligodendrocyte generation than do their postnatal counterparts. The number of critical cycles is likely to be the prehistory of the progenitor cells and oligodendrocytes, but explicit analytic expressions necessary for this purpose do not seem feasible within the framework of our stochastic model. However, our computer simulations, conducted with the use of GPSS/H (Wolverine Software, Annandale, VA) have shown that the least-squares estimation procedure yields accurate estimates of the parameters $c, P$ and the mean mitotic cycle duration that are robust to moderate perturbations in values of the parameters $\pi_0, \ldots, \pi_c$ (data not shown).

Given a realistic quantitative description of clonal differentiation, a number of important biological questions become approachable. For example, only a quantitative analysis can rigorously distinguish between the various models of O-2A progenitor differentiation that have been suggested by narrative biological analyses (1, 4, 14). Thus, it will be important to know whether the model we propose is consistent with clonal data on the development of O-2A progenitor cells isolated from prenatal animals of different ages, or grown in different conditions, and thus provides a truly general model. In other words, do the specific values of the probability of differentiation, cell cycle parameters, etc., change without leading to a change in the model structure itself? On a deeper level, the derivation of a quantitative model should allow determination of whether the specific inferences obtained from the analysis of O-2A progenitor cells provide general principles that apply more broadly to other cellular lineages.

An alternative to the concept of critical cycles is that the division probability $P$ gradually decreases with cycle number. No previous information on this dependency is available in the existing experimental data, so finding a pertinent form is the main challenge in developing the corresponding mathematical model.