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The linkage of neural progenitor cell cycle profiles between embryonic and adult stroke models: analytical approach II

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INTRODUCTION

In the adult brain, neural progenitor cells are present in the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone of the dentate gyrus (Luskin et al., 1997; varez-Buylla et al., 2000). Proliferation of neural progenitor cells is tightly controlled by cell cycle kinetics (Takahashi et al., 1993; Caviness, Jr. et al., 2003). Using cumulative and single pulse bromodeoxyuridine (BrdU) labeling methods, Nowakowski and his colleagues (1989) have determined the length of the cell cycle and established the cell cycle interval of mice during neocortical formation, the embryonic model. By adapting this approach, we found that stroke significantly increases the proliferating population of SVZ neural progenitor cells in rats subjected to 7 days of embolic middle cerebral artery occlusion (S7), which is associated with the reduction of the cell cycle length (T_c), especially the length in G_1 phase (T_{G1}) (Zhang et al., 2006). Using the enhanced analytic approach which permits the precision for cell cycle kinetic parameters, we further demonstrate that migration of neural progenitor cells out of the SVZ can be ignored in the estimation of the cell cycle parameters (Lu et al., 2007).

The cell cycle length is associated with neural progenitor cell progression from proliferation to neurogenic divisions during cortical neurogenesis (Caviness, Jr. et al., 1995; Caviness, Jr. et al., 2003). The proportion of neural progenitors that remain in (P fraction) and exit from (Q fraction) the cell cycle in the pseudostratified ventricular epithelium determines the degree of cortical neurogenesis during embryonic development 11 to 17 days after (E11 to E17, Takahashi et al., 1996a; Takahashi et al., 1996b). However, it remains to be determined whether the reduction of the cell cycle length of adult neural progenitor cells after stroke is related to P and Q fractions.

In the present study, we seek to establish the temporal profile of stroke neural progenitor cells by studying the cell kinetics and the cell behaviors over a period of 2 to 14 days after stroke. With analytical extension of Takahashi's previous work for the embryonic model (Takahashi et al., 1994; Takahashi et al., 1997), we tested the hypothesis that adult neural progenitor cells after stroke recapture embryonic cell kinetics. Mathematical modeling is used to study the cell

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length, T_c or T_{G1} , evolution over time, the correlation between T_c and T_{G1} , and the likelihood of the cells in the SVZ remained in or exited from the cycle. Given that stroke induces neurogenesis, we expect that after the stroke, more cells exit the cycle and the cell proliferation profile differs from the embryonic model.

METHODS

Experimental procedures were approved by the Institutional Animal Care and Use Committee of Henry Ford Hospital.

Animal model of stroke

Male Wistar rats (age 3–4 months) were employed. The middle cerebral artery (MCA) was occluded by placement of an embolus at the origin of the MCA (Zhang et al., 1997).

Experiments were initiated in rats at 2, 4, 7, and 14 days after stroke, corresponding to the maximum period of stroke induced neurogenesis (Zhang et al., 2001; Zhang et al., 2004a; Zhang et al., 2004b; Zhang et al., 2006).

Cumulative and single labeling experiments for cell cycle kinetics

BrdU labeling of stroke rats was performed by repeated intraperitoneal (i.p.) injection (2–3 h intervals; 50mg/kg, Sigma) over a total period of 18.5 hr, beginning at 8:00 A.M. Selected rats from 2, 4, 7 and 14 days after stroke were killed thirty minutes after each of the BrdU injections. Three to four animals per time point were sacrificed with a total of 10 time points. All neural progenitor cells passing through the S phase were labeled with BrdU until the cell proliferation reaches to the maximum. The number of BrdU labeled cells at S phase were counted in 4 sections per subject at each sacrifice time. The cumulative labeling experiment was used to estimate the total cycle length and the length in S phase.

Animals were injected with a *single dose* BrdU, 50mg/kg, and were sacrificed from 30 minutes (the minimum) until the time when 100% of the labeled cells were mitotic in the S phase. The percent of labeled cells in S phase was zero when cells were in G_2 phase, linearly increasing when cells entered in M phase, and were completely labeled when the first labeled mitotic cell appeared in S phase. Based on this experiment, the duration of T_{G2} and T_{G2+M} was determined. This experiment was completed in rats with 2, 4, 7 and 14 day after stroke for estimation of the cycle lengths in phases M and G_2 , respectively, therefore, to calculate the cycle length in G_1 phase (Lu et al., 2007).

Determining cell proliferation and postmitotic behaviors in the SVZ

Two separate tracers, 5-chloro-2'-deoxyuridine (CldU, 50mg/kg, Sigma) and 5-iodo-2'-deoxyuridine (IdU, 50mg/kg, Sigma) (Zink et al., 1998; Maslov et al., 2004) were used in two separate labeling protocols and were injected into two subsets of animals to distinguish the primary cohort of proliferating SVZ cells that exit the cycle (Q cells) from those which remain in the cycle (P cells) in a 2 hr interval (Takahashi et al., 1994; Takahashi et al., 1995a). N_{Q+P} is determined from Protocol 1 for one subset of animals and N_Q (the number of Q cells) is determined from Protocol 2 for the second subset of animals. The Q fraction was calculated as the ratio of N_Q to N_{Q+P} , and the P fraction is equal to $1 - Q$ fraction.

Histology evaluation

At the end of the experiments, animals were transcardially perfused with heparinized saline followed by 4% paraformaldehyde. Brains were removed and fixed in 4% paraformaldehyde. To analyze BrdU labeling, every 40th BrdU immunostained coronal brain section at the level of AP + 10.6 mm and AP + 9.2 mm (Paxinos and Watson 2nd ed. New York, NY, Academic

Press Inc. VIII. 1986) was digitized using computer imaging (Olympus BX40) via the MCID computer imaging analysis system (Imaging Research, St. Catharines, Canada). BrdU labeled and unlabeled cells or mitosis in each SVZ coronal section were presented as the number of the cells/section. Density for the 4 sections per subject was averaged to obtain a mean density value for each brain, according to published methods (Kuhn et al., 1996; Zhang et al., 2001).

Cell kinetic parameters and their interpretation

The total cell cycle length T_C , the length in S phase (T_S) and growth fraction (GF)—The number of BrdU labeled cells under the cumulative labeling experiment were measured at each time point per subject in rats with stroke at day 2, 4, 7 and day 14 after symptom onset and in rats without stroke. Concurrent linear regressions were used to fit the data through nonlinear interaction processes at each time point, with estimation of total cycle length, T_C , the length in S phase (T_S) and growth fraction (GF). Z-test statistic was used to compare the cycle parameter differences between different stroke models (e.g., 2-day stroke vs. 4 day stroke)(Lu et al., 2007).

The length in G_2 , M or G_1 phase—The labeled cells in the S phase under the single pulse labeling experiment were fitted into different concurrent linear regressions with the estimation of length both G_2 and M phases(T_{G_2+M}). The length of G_1 , T_{G_1} , and its standard error (SE) can be estimated based on the estimation of T_{C-S} and T_{G_2+M} from both the cumulative labeling and single pulse labeling experiments. Z-test statistic was used to compare cycle length differences between different stroke models.(Lu et al., 2007).

T_C as a function of time after stroke onset—In the embryonic study of the embryonic models at day 11 (E11) to day 16 (E16), T_C values ranged from 8.1h to 18.4h (Takahashi et al., 1995b) and were studied for T_C evolution over time. For the present study, we adapted Takahashi's approach using the regression analysis and also assessed the model fit, which are described in the following.

Assume t_i for $i = 1, 2 \dots M$ for some $M > 2$ are the time points after an event (e.g., days after stroke or embryonic), $T_C(t)$ can be fitted with either a linear regression model as

$$T_C(t) = \alpha + \beta t, \quad (1a)$$

where α (intercept) and β (slope) are unknown parameters, or otherwise a sigmoid regression model as

$$T_C(t) = \alpha + \frac{1 + me^{(-t/\tau)}}{1 + ne^{(-t/\tau)}}, \quad (1b)$$

where α , m , n and τ are unknown parameters.

Using Proc REG or Proc NLIN in SAS, the commercially available statistical computational package (SAS Institution, 1999), we estimated unknown parameters described either in Eq (1a) or in Eq(1b), respectively, based on estimated $T_C(t_i)$, for $i = 1, 2 \dots M$. The selection of the regression model depends on the model indentifiability and the model goodness-of-fit. Since only 4 onset time points (day 2, 4, 7 and day 14) were conducted for stroke models, the linear model would be fitted first into Eq (1a) because of the simplicity. Fitting Eq (1b) for the stroke model may be problematic for the model indentifiability.

Cycle length T_{G_1} evolution and Correlation of T_{G_1} with T_C —A similar mathematical discussion for $T_C(t)$ can be applied to the study of T_{G_1} evolution as a function of time after stroke onset. In addition, the relationship between T_C and T_{G_1} can be measured by calculating the correlation coefficient, r . The correlation coefficient (r) between two variables (e.g.,

variables Y and X), has a range of -1 to 1, where $|r|=1$ means a perfect correlation and 0 means no correlation. A positive correlation coefficient indicates that an increase in Y is correlated with an increase in X; a negative correlation coefficient, in contrast, indicates an increase in Y correlated with a decrease in X. The correlation coefficient can be tested against no correlation at all. The G₁ phase has been identified as the key regulation phase during neurogenesis in the developing brain (Takahashi et al., 1995b; Caviness, Jr. et al., 1999; Siegenthaler and Miller, 2005). The reduction of total cycle length led to the reduction in G₁ length (Takahashi et al., 1995b; Zhang et al., 2006), however, the report of the T_C and T_{G1} relationship was rather descriptive. In this study, the correlation coefficient was calculated for both the stroke model and the embryonic model (Takahashi et al., 1995b). Later, we show that T_C could be the surrogate of T_{G1} .

Comparison of T_C difference between embryonic and stroke models—Assuming the cycle length fits into the linear regression in Eq (1a), the slope β represents the rate of cell cycle evolution. Therefore, differences in slope between the stroke and embryonic models would indicate the cycle evolution difference between the two models. To test the slope difference, we use the single regression model proposed by Kleinbaum et al (1988)

$$T_C(t) = \eta + \beta_1 t + Z(\gamma + (\beta_2 - \beta_1))t, \quad (2)$$

where β_1 and β_2 are the slopes for the embryonic model and the stroke model, respectively, Z is the indicator variable of the stroke model, and η and γ are nuisance parameters. The same analysis approach can be used to test the difference in slopes between T_C and T_{G1} in the stroke model.

The number cell cycles from time t_a to time t_b (CC) ($t_a < t_b$)—From Eq(1a) or Eq(1b), we can see that T_C is evolving as the stroke onset time, t , increases, therefore, the number of cell cycles in 24 hours, in the early time period of event, e.g., E11–E12 as an example, with T_C in a range of 8.1 to 10.2 (Takahashi et al., 1995b), would differ from the number of cell cycles in a later time period (e.g., E15–E16 with T_C in a range of 17.5 to 18.4).

The concept of number of cell cycles was introduced (Takahashi et al., 1995a; Takahashi et al., 1995b), and their approach for cell cycle intervals (cell cycles as CC) E11 to E17 can be described in the following:

1. For each 24 hour period of E11 (e.g., at 9:00am of E11 to 9:00am of E12), a weighted T_C is calculated as $T_c^{w_{11-12}} = \frac{1}{24} \int_0^{24} (8.315 + 0.0825 t) dt$, where $T_C(t) = 8.315 + 0.0825t$ is the regression model from Eq (1) based on estimated T_C on day 11 to day 16.
2. To calculate cell cycles in time period E11–E12 as $CC^{11-12} = \frac{24}{T_c^{w_{11-12}}}$
3. To sum the CC^{11-12} to CC^{16-17} and obtain the CC^{11-16}

Using above methods, we could replicate the 11 CC over E11–E16 for embryonic models.

In addition, the above mentioned methods for CC calculation can be simplified using the following integral equation

$$CC^{t_a-t_b} = \int_{t_a}^{t_b} \frac{1}{T_c(t)} dt, \quad (3)$$

where $t_a < t_b$. Eq (3) is more accurate for CC estimation and can be used at any time period. Eq (3) was used to estimate CCs for both embryonic model and the stroke model.

The cell leaving the cycle (Q fraction)—The postmitotic behaviors of a 2-hr cohort of cells in the SVZ are monitored using two separate tracers based on two labeling protocols with measurements of N_Q and N_P and Q fraction. This method was proposed by (Takahashi et al., 1994) as the study of E14 model. Q behaviors had been studied extensively in the embryonic sequence E11 to E17 (Takahashi et al., 1996b) and in Q modeling (Lees et al., 2006)(Takahashi et al., 1997). Amplification of neurogenesis would occur if the number of cell cycles during Q fraction < 0.5 increased (Caviness, Jr. et al., 1995). With each successive cycle there is an increase in the fraction of postmitotic cells that left the cycle (the Q fraction) and also an increase in the length of the cell cycle due to an increase in the length of the G_1 phase of the cycle (Caviness, Jr. et al., 1999).

In addition, with respect to proliferative fate, cells could be classified as either “symmetric” when both daughter cells are either Q or P, or “asymmetric” when one daughter cell is Q and one is P (Rakic, 1988). Assuming the cells of Q or P obey a binomial distribution, the probability of the same fate daughter cells will be $P \times P$ (P&P symmetric) or $Q \times Q$ (Q&Q symmetric); and the probability of asymmetric daughter cells will be $2 \times P \times Q$, given by (Takahashi et al., 1996b). For this study, the cells exiting and the proliferative fate of cells were studied for a model of stroke. A one-way analysis of variance (ANOVA) was used to compare P/Q-fraction or the proliferation fate difference among the stroke groups. Analysis would begin testing for the group effect, followed by pair-wise group comparison if the group effect was detected at the level of 0.05. A polynomial regression model was used to model Q cells and the cell proliferation fates as a function of time after the onset, respectively.

The comparison between stroke and embryonic models was considered as a magnitude study for illustration of the mathematic modeling and approaches. We are aware of the possible biological differences between embryonic mice and embryonic rats; however, from the cell cycle kinetic point of view, they are compatible. T_C were 11 and 19 hrs for embryonic rat E12 and E18 (von and Jaensch, 1972), and 10.2 and 18.4 hrs for embryonic mice E12 and E16 (Takahashi et al., 1996b), respectively.

RESULTS

Cell kinetics for stroke models S2, S4, S7 and S14

Based on the above experiments, we estimated the cell cycle parameters at different time points of the stroke and the results are presented in Table 1 and Figure 1. $18.98(\pm 1.22)$ hrs of T_C and $12.63(\pm 0.49)$ of T_{G1} were previously reported in the non-stroke model (Lu et al., 2007). The total cell cycle lengths, T_C , in the stroke model, except 14d stroke, significantly decreased, compared to the non-stroke ($p < 0.05$). In the stroke model, T_C were significantly increased in the when the time of stroke onset was increased ($p < 0.01$), except the time between day 2 and day 4 of the stroke onsets ($p = 0.26$). Similar to the total cell cycle length, T_{G1} significantly increased in the stroke model when the time of stroke onset was increased ($p < 0.05$), except the time between day 4 and day 7 after stroke ($p = 0.96$).

Stroke T_C and T_{G1} evolution and correlation

The T_C estimations for S2, S4, S7 and S14 were fitted in Eq (2a) with a regression line of $T_C(t) = 11.428 + 0.029(t - 48)$ and the goodness-of-fit $R^2 = 0.994$ (Figure 2). T_{G1} progressed over time as $T_{G1}(t) = 7.351 + 0.020(t - 48)$ with $R^2 = 0.997$ (Figure 2). $T_{G1}(t)$ was strongly correlated with T_C (correlation coefficient $r = 0.99$ and $p < 0.01$ for testing no correlation) and no slope difference was detected between T_C and T_{G1} regressions ($p = 0.85$), indicating that T_C is the surrogate of T_{G1} . The same correlation pattern was observed for the embryonic model between T_C and T_{G1} using the data collected in Table 1 (Takahashi et al., 1995b) with $r = 0.99$ $p < 0.01$ for testing no correlation.

Comparison of Tc between the embryonic model and the stroke model

Fitting the estimated Tc values in Figure 1 (Takahashi et al., 1995b) of the embryonic model E11 to E16, one obtains the regression of $T_C(t) = 8.424 + 0.088(t - 246)$. Slight coefficient differences were observed, compared to $T_C(t) = 8.315 + 0.0825(t - 246)$, reported by (Takahashi et al., 1995b) and the difference is more likely be due to a rounding error.

Tc progression is slower in the stroke model (slope=0.029), compared to 0.088 progression in the embryonic model (p=0.025, in Figure 3), indicating a different dynamic change in cell cycle kinetics between the two models.

Cell cycle numbers (CC) from S2 to S14

The integration function of CC (the cell cycles) for stroke model is $34.483 \times \text{Log}(11.428 + 0.029 \times \text{time})$ using Eq (3). During the 12 days of a stroke period from day 2 to day 14 (S2–S14) a total of 19CC (actual 18.92) were completed (Figure 4) and the first 6 days of stroke period from day 2 was 11CC (actual 10.73). 11CC (actual 10.76) were accomplished during the 6 days of the embryonic period from day 11 to day 17 (E11–E17), while 8CC (actual 7.56) were calculated in 6 days of stroke period (S11–S17) based on the mathematical estimation.

Cells leaving the cycle

Q cell progression, $Q_{fraction} = 0.094 + 0.131 \times t - 0.013 \times t_2 + 0.001 \times t_3$ (Model-fit RRE=0.06 and MSE=0.004, where t is the day after the stroke onset) in Figure 5, was flatter than the progression in the embryonic model illustrated in Figure 7 by (Takahashi et al., 1996b), and the likelihood of the cell leaving the cycle in the stroke was much higher than in the embryonic model (e.g., 0.68 on S14 versus 0.34 on E14). Half of the cells were leaving the cycle (50% of Q cells) at day 6 (actual 5.5) for the stroke model (Figure 5) and at day 15 (actual at day 14.5) for the embryonic model.

The analysis shows that Q fractions were increased when time of stroke onset was increased, (p<0.05 for the pair-wise comparisons). 85% Q Fraction in the non-stroke model was observed and was significantly higher than those in the stroke models (p<0.05).

The symmetric Q+Q cells (both daughter cells leaving the cycle) significantly increased to $20 \pm 2\%$ at day 4, compared to $10 \pm 1\%$ at day 2, and increased consecutively at day 7, ($30 \pm 5\%$) and at day 14 ($48\% \pm 2\%$) with significant pair-wise group difference on any consecutive time points (p<0.03). The P and Q in relation to proliferation fates from S2 to S18 were estimated based on Q regression and presented in Figure 6. A similar proliferation fate propensity was observed in the stroke model, compared to the embryonic model, Figure 11 by (Takahashi et al., 1996b). However, the proliferation fates in stroke were quite different from the proliferation fate of the embryonic model (calculated based on Table 3 (Takahashi et al., 1996b)). At the onset of day 14, as an example, the distributions of P+P and Q+Q symmetric and P/Q asymmetric were 11%, 48% and 41% for stroke S14, 44%, 13% and 43% for embryonic E14, and 65%, 3%, and 32% for embryonic E13, and 17%, 35% and 48% for E15, respectively.

DISCUSSION

We extended analytical approaches for the cell cycle kinetics of the embryonic model to analyze neurogenesis in the adult stroke. This analytic algorithm is not only able to estimate the cell kinetic parameters of the interest with variation (e.g., SE) for stroke model, but also able to replicate the published results for embryonic models (Takahashi et al., 1995b; Takahashi et al., 1996b). The cell cycle kinetics of stroke models at day 2, 4, and day 14 seemed to fit into the cycle kinetics used for the embryonic models and the goodness-of-fit (SSR and MSE) are in same range as we have previously observed for the non-stroke model and the 7-day stroke

model (Lu et al., 2007). Therefore, we concluded that the embryonic cell cycle kinetics can be used for adult stroke. Although cell migration after stroke is an important factor in neurogenesis after stroke, the migration factor can be omitted in the estimation of cell kinetic parameters. Note that the cell death, as a part of neurogenesis process, has been omitted in analyzing the cell behaviors and cell cycles, but was previously discussed (Takahashi et al., 1995b).

Mathematical models of cell proliferation attempt to mimic the process with restrictions to simplify the biological situation. Therefore, the cell cycle parameter estimations should be viewed as reflecting principally the population proliferation patterns. Because of the heterogeneities in the proliferative cell population, these parameters are to be regarded as only an approximation of the true values or the average of cell proliferation from more than one proliferating cell type. This argument has been consistent with the discussion on embryonic models (Takahashi et al., 1993).

Proliferation of neural progenitor cells is tightly controlled by cell cycle kinetics (Takahashi et al., 1993; Caviness, Jr. et al., 2003). With proper mathematical modeling, we can analyze the behaviors of neural progenitor cells beyond our observation (experiments). The extended mathematical algorithm, proposed in the Methods Section, provides a useful tool to predict time points of cell behavior change (e.g., the time for 50% of Q fraction) based on the observed data. By extending the cell proliferation fates (symmetric and asymmetric cell divisions) for the stroke model up to 18 days after the stroke onset (S2–S18), we are able to see a similar tendency of stroke proliferation fate profiles, but with different paces, compared to the embryonic proliferations on E11–E17. However, the mathematical prediction needs to be validated by a biological experiment. There could be possible biological differences between embryonic mice and rats models. However, 11 to 19 hrs of the total cell cycle lengths for embryonic rat E12 and E18 were reported by Von and Jaensch (von and Jaensch, 1972) and 10.2 to 18.4hs were reported for embryonic mice E12 and E16 by (Takahashi et al., 1996b) suggesting that the two rodent models were compatible. Nevertheless, given the different rodents used in the stroke and in the embryonic models, cautious interpretation of the stroke and embryonic model comparison is warranted.

Estimating the length of G_1 requires the completion of both cumulative and single pulse labeling experiments, mathematical modeling and estimation of the cell cycle parameters and their variance. Throughout this cell kinetics reference study of the stroke model on the multiple onsets, we first demonstrated a superb correlation ($r=0.99$) between T_c and T_{G1} . Since T_c can be estimated only based on the *cumulative labeling experiment* alone, mathematically, T_{G1} can be quantified by T_c as $T_{G1}(t) = -0.31 + 0.67 T_c(t)$ for the stroke model and $T_{G1}(t) = -5.62 + 0.98 T_c(t)$ for the embryonic model, respectively. Therefore, we conclude that T_c can be used as the surrogate of T_{G1} .

Stroke induces neural progenitor cell proliferation by shortening the cell cycle length T_c or G_1 length in stroke up to 7 days after the stroke onset, compared to the non-stroke model. However, T_c evolution in stroke was 3 times slower (the proportion in slopes between the two models) than its evolution in the embryonic model (Figure 2 and $p=0.025$), suggesting possible different dynamic changes of P and Q fractions in the stroke model, compared to the embryonic model.

The mathematical model estimated that there were 11 and 8 cell cycles for the first and second 6 days, respectively, during 2 to 14 days after stroke, which is consistent with our previous findings that stroke augments neurogenesis by acutely expanding the neural progenitor pool during 2 to 7 days after stroke (Zhang et al., 2001; Zhang et al., 2006). Although the number of cell cycles is different between stroke-induced neurogenesis and embryonic cortical neurogenesis, rapid expansion of a progenitor pool is observed in the embryonic model,

suggesting that the adult progenitor cells after stroke recaptures the cell cycle kinetics of the embryonic progenitors.

Fitting the observed Q fraction data on a mathematical polynomial equation, illustrated in Figure 5, we could identify 5.5 days after stroke (with estimated Q fraction 50% in Figure 5) as the time point when more than 50% of neural progenitors start leaving the cycle of the SVZ, which is about 10 days earlier than its in the embryonic model in Figure 7 by (Takahashi et al., 1996b). At day 14 of the onset, the Q fraction for the stroke model is doubled, compared to the embryonic model, indicating that many the progenitor cells exit the cell cycle. In addition, extending stroke model up to 18 days after the stroke onset, we are able to see the similar propensity of the behaviors of cells remaining and exiting the cycle between the stroke and embryonic models, however, the differences between the two models are obvious (Figure 6 for the stroke versus Figure 11 (Takahashi et al., 1996b); a persistent and high proportion of Q+Q symmetric cells are observed in the stroke model with a longer duration starting earlier than day 2 at 9%, remains steadily increasing, and becomes 44% in between S13 and S14 while the Q+Q symmetric cells in the embryonic is not formed until day 12 (E12) and becomes 44% at E15. We conclude that cells leave the cycle earlier and more frequently after stroke compared to the embryonic model.

From this study, we have, first, established the temporal profile of the neural progenitor cell cycle kinetics and behaviors over a period of time after stroke. The profile includes the parameters for cell cycle evolution, cell cycle numbers at certain intervals, the time of the initial cell leaving and the cell leaving frequency. Information provided from this present study may possibly be used to design the stroke therapeutic treatment. All the analytic approaches proposed to compare the stroke model with the embryonic model, can be used to compare the stroke model with and without a treatment therapy.

In conclusion, the cell cycle kinetics for the embryonic model can be used for the stroke model with a goodness-of-fit. T_c is the surrogate measure of T_{G1} . Different cell proliferation profiles are observed between the stroke and embryonic models. T_c evolution is 3-fold slower but the cells leave the cycle earlier and more frequently in the stroke model, compared to those in the embryonic model.

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Reference List

- Caviness VS Jr, Goto T, Tarui T, Takahashi T, Bhide PG, Nowakowski RS. Cell output, cell cycle duration and neuronal specification: a model of integrated mechanisms of the neocortical proliferative process. *Cereb Cortex* 2003;13:592–598. [PubMed: 12764033]
- Caviness VS Jr, Takahashi T, Nowakowski RS. The G1 restriction point as critical regulator of neocortical neurogenesis. *Neurochem Res* 1999;24:497–506. [PubMed: 10227682]
- Caviness VS Jr, Takahashi T, Nowakowski RS. Numbers, time and neocortical neurogenesis: a general developmental and evolutionary model. *Trends Neurosci* 1995;18:379–383. [PubMed: 7482802]
- Kleinbaum, DG.; Kupper, LL.; Muller, KE. *Applied Regression Analysis and Other Multivariable Methods*. PWS-KENT; Boston: 1988.
- Kuhn HG, Dickinson-Anson H, Gage FH. Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *J Neurosci* 1996;16:2027–2033. [PubMed: 8604047]

- Lees KR, Zivin JA, Ashwood T, Davalos A, Davis SM, Diener HC, Grotta J, Lyden P, Shuaib A, Hardemark HG, Wasiewski WW. NXY-059 for acute ischemic stroke. *N Engl J Med* 2006;354:588–600. [PubMed: 16467546]
- Lu M, Zhang RL, Zhang ZG, Yang JJ, Chopp M. Linkage of cell cycle kinetics between embryonic and adult stroke models: An analytical approach. *J Neurosci Methods* 2007;161:323–330. [PubMed: 17141877]
- Luskin MB, Zigova T, Soteres BJ, Stewart RR. Neuronal progenitor cells derived from the anterior subventricular zone of the neonatal rat forebrain continue to proliferate in vitro and express a neuronal phenotype. *Mol Cell Neurosci* 1997;8:351–366. [PubMed: 9073397]
- Maslov AY, Barone TA, Plunkett RJ, Pruitt SC. Neural stem cell detection, characterization, and age-related changes in the subventricular zone of mice. *J Neurosci* 2004;24:1726–1733. [PubMed: 14973255]
- Nowakowski RS, Lewin SB, Miller MW. Bromodeoxyuridine immunohistochemical determination of the lengths of the cell cycle and the DNA-synthetic phase for an anatomically defined population. *J Neurocytol* 1989;18:311–318. [PubMed: 2746304]
- SAS Institution. SAS/STAT Software. SAS Institution; Cary (NC): 1999.
- Siegenthaler JA, Miller MW. Transforming growth factor beta1 promotes cell cycle exit through the cyclin-dependent kinase inhibitor p21 in the developing cerebral cortex. *J Neurosci* 2005;25:8627–8636. [PubMed: 16177030]
- Takahashi T, Nowakowski RS, Caviness VS Jr. Cell cycle parameters and patterns of nuclear movement in the neocortical proliferative zone of the fetal mouse. *J Neurosci* 1993;13:820–833. [PubMed: 8426239]
- Takahashi T, Nowakowski RS, Caviness VS Jr. The cell cycle of the pseudostratified ventricular epithelium of the embryonic murine cerebral wall. *J Neurosci* 1995b;15:6046–6057. [PubMed: 7666188]
- Takahashi T, Nowakowski RS, Caviness VS Jr. Interkinetic and migratory behavior of a cohort of neocortical neurons arising in the early embryonic murine cerebral wall. *J Neurosci* 1996a;16:5762–5776. [PubMed: 8795631]
- Takahashi T, Nowakowski RS, Caviness VS Jr. The leaving or Q fraction of the murine cerebral proliferative epithelium: a general model of neocortical neuronogenesis. *J Neurosci* 1996b;16:6183–6196. [PubMed: 8815900]
- Takahashi T, Nowakowski RS, Caviness VS Jr. The mathematics of neocortical neuronogenesis. *Dev Neurosci* 1997;19:17–22. [PubMed: 9078427]
- Takahashi T, Nowakowski RS, Caviness VS Jr. Mode of cell proliferation in the developing mouse neocortex. *Proc Natl Acad Sci U S A* 1994;91:375–379. [PubMed: 8278397]
- Takahashi T, Nowakowski RS, Caviness VS Jr. Early ontogeny of the secondary proliferative population of the embryonic murine cerebral wall. *J Neurosci* 1995a;15:6058–6068. [PubMed: 7666189]
- varez-Buylla A, Herrera DG, Wichterle H. The subventricular zone: source of neuronal precursors for brain repair. *Prog Brain Res* 2000;127:1–11. [PubMed: 11142024]
- von WR, Jaensch B. Generation times of the matrix cells during embryonic brain development: an autoradiographic study in rats. *Brain Res* 1972;46:235–250. [PubMed: 4635365]
- Yamashita T, Ninomiya M, Hernandez AP, Garcia-Verdugo JM, Sunabori T, Sakaguchi M, Adachi K, Kojima T, Hirota Y, Kawase T, Araki N, Abe K, Okano H, Sawamoto K. Subventricular zone-derived neuroblasts migrate and differentiate into mature neurons in the post-stroke adult striatum. *J Neurosci* 2006;26:6627–6636. [PubMed: 16775151]
- Zhang R, Zhang Z, Wang L, Wang Y, Goussev A, Zhang L, Ho KL, Morshead C, Chopp M. Activated neural stem cells contribute to stroke-induced neurogenesis and neuroblast migration toward the infarct boundary in adult rats. *J Cereb Blood Flow Metab* 2004a;24:441–448. [PubMed: 15087713]
- Zhang R, Zhang Z, Zhang C, Zhang L, Robin A, Wang Y, Lu M, Chopp M. Stroke transiently increases subventricular zone cell division from asymmetric to symmetric and increases neuronal differentiation in the adult rat. *J Neurosci* 2004b;24:5810–5815. [PubMed: 15215303]
- Zhang RL, Chopp M, Zhang ZG, Jiang Q, Ewing JR. A rat model of focal embolic cerebral ischemia. *Brain Res* 1997;766:83–92. [PubMed: 9359590]

- Zhang RL, Zhang ZG, Lu M, Wang Y, Yang JJ, Chopp M. Reduction of the cell cycle length by decreasing G(1) phase and cell cycle reentry expand neuronal progenitor cells in the subventricular zone of adult rat after stroke. *J Cereb Blood Flow Metab.* 2006
- Zhang RL, Zhang ZG, Zhang L, Chopp M. Proliferation and differentiation of progenitor cells in the cortex and the subventricular zone in the adult rat after focal cerebral ischemia. *Neuroscience* 2001;105:33–41. [PubMed: 11483298]
- Zink D, Cremer T, Saffrich R, Fischer R, Trendelenburg MF, Ansorge W, Stelzer EH. Structure and dynamics of human interphase chromosome territories in vivo. *Hum Genet* 1998;102:241–251. [PubMed: 9521598]

Figure 1.

Concurrent linear regressions were used to fit the data through nonlinear interaction processes at each stroke time point (Day 2, 4, 7 and Day14), respectively with estimation of total cycle length, T_C and the length in S phase. T_{G1} , was be estimated based on the estimation of T_{C-S} and T_{G2+M} from both the cumulative labeling and single pulse labeling experiments at each time after the stroke onset, respectively. T_{G1} significantly increased in the stroke model when the time of stroke onset was increased ($p < 0.05$), except time between day 4 and day 7 after stroke ($p = 0.96$).

Figure 2.

Based on T_C estimation for S2, S4, S7 and S14, T_C progresses as $T_C(t) = 11.428 + 0.029(t - 48)$ with the goodness-of-fit $R^2 = 0.994$. T_{G1} obeys $T_{G1}(t) = 7.351 + 0.020(t - 48)$ and $R^2 = 0.997$. increased as the stroke onset time T_C or T_{G1} increased, and T_{G1} was strongly correlated with T_C ($r=0.99$) There is no slope difference between T_C and T_{G1} regressions ($p=0.85$) based on Eq (2).

Figure 3.

A significant slope difference was detected between the stroke and the embryonic models on T_c progression ($p=0.025$), where T_c progression is significantly slower in the stroke model (slope=0.029), compared to 0.088 progressions in the embryonic model, indicating that cells in the stroke model were less active than the cells in the embryonic model.

Figure 4.

The number of cell cycles on a daily period. During the 12 days of a stroke period from day 2 to day 14, the cumulated cell cycles were 19 cc (actual 18.92cc) in the stroke model. 11CC (actual 10.76) were estimated in 6 days of the embryonic period from day 11 to 17. At the same 1 day period of the onset, e.g. day 11 to 12, cell cycled 2.6 intervals in the embryonic model, compared 1.33 cc in the stroke model.

Figure 5.

Q fraction regression, $Q\ Fraction = 0.094 + 0.131 \times Day - 0.013 \times Day^2 + 0.001 \times Day^3$ and its 95% confidence intervals over time after stroke onset with model-fit RRE=0.06 and MSE=0.004. Half of the cells were leaving the cycle (50% of Q cells) at day 6 (actual at day 5.5) after the stroke onset.

Figure 6.

The P and Q in relation to proliferate fates from S2 to S17, estimated based on Q regression, where the solid brown area is P+Q or Q+P asymmetric, the light brown area is Q+Q symmetric and the shaded line area is P+P symmetric. With the theoretical extension to S17, a similar proliferation fate propensity was observed in the stroke model, compared to embryonic model (Figure 11 (Takahashi et al., 1996b)). However, different proliferation fates were observed in 6 day period of day 11 to day 17 after the onset between the two models. At the onset of day 14, as an example, the distributions of P+P and Q+Q symmetric and P/Q asymmetric were 11%, 48% and 41% for stroke, 44%, 13% and 43% for embryonic E14, and 65%, 3%, and 32% for embryonic E13, and 17%, 35% and 48% for E15, respectively.

SVZ Cell cycle parameter estimation (SE) in non-stroke and stroke rats

Table 1

Parameters	Non-stroke	2d stroke	4d Stroke	7d Stroke	14d stroke
Cumulated labeling (N)	36	22	40	50	34
GF	20.06 (0.38)	23.55 (0.39)	30.17 (0.29)	31.05 (0.32)	24.14 (0.69)
T_C^*	18.98 (1.22)	11.49 (0.66)	12.51 (0.62)	15.28 (0.59)	19.74 (0.99)
T_S	4.56 (0.70)	2.25 (0.41)	2.40 (0.36)	3.94 (0.37)	4.78 (0.60)
T_{CS}	14.42 (0.67)	9.24 (0.36)	10.12 (0.34)	11.34 (0.30)	14.96 (0.65)
Model Fit: SSR/MSE	53/1.6	29/1.52	75/2.03	94/2.00	58/1.88
Single Pulse labeling (N)	32	27	24	27	27
T_{G2}	0.57 (0.02)	0.57 (0.02)	0.54 (0.02)	0.53 (0.02)	0.55(0.02)
T_M	1.22 (0.05)	1.22 (0.05)	1.26 (0.04)	1.24 (0.05)	1.24 (0.05)
T_{G2+M}	1.79 (0.03)	1.79 (0.04)	1.80 (0.03)	1.78 (0.03)	1.79 (0.03)
Model Fit: SSR/MSE	515/17.2	438/17.5	239/10.8	368/14.7	348/13.9
T_{GI}^{**}	12.63 (0.49)	7.45(0.24)	8.32 (0.27)	9.56 (0.24)	13.17 (0.49)

* T_C difference ($p<0.01$) between any two stroke onsets, except stroke onset 2 day versus 4 days ($p=0.26$);

** G1 length difference ($p<0.05$) between any two stroke onsets, except stroke onset 4 day versus 7 days ($p=0.95$).