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Cell Death in Cortical Development: How Much? Why? So What?

Minireview

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Since Hamburger and Levi-Montalcini (1949) demonstrated that cell survival in the chick spinal cord could be regulated by the amount of neuronal target available, normal cell death has come to be an accepted feature of neuronal development (reviewed by Cowan et al., 1984; Clarke, 1990; Oppenheim, 1991; Raff et al., 1993). Cell death is an inherently difficult process to measure, however, so that for most regions of the nervous system the total amount of cell death that normally occurs is not yet clear.

This issue of how much cell death actually occurs has been brought to the fore rather dramatically in a recent paper by Blaschke et al. (1996), who report massive normal cell death in the developing neocortex of the mouse using a new highly sensitive assay. Their assay detects DNA fragmentation (which is characteristic of normal developmental cell death) using in situ end ligation (ISEL) that they have enhanced for optimal sensitivity. Their results show the presence of large numbers of dying cells in the postmitotic upper layers of the cortex as expected from previous studies and, more importantly, they also show large numbers of dying cells within the cortical ventricular zone, a proliferative region not previously associated with cell death. By their assay, over 50% of the cells in both the ventricular zone and in the postmitotic cortical plate appear to be undergoing cell death during much of the period of neurogenesis. Although dying cells have been reported previously in proliferative regions in the retina (Young, 1984), cortex (Ferrer et al., 1992), and spinal cord (Homma et al., 1994), the numbers of dying cells seen in those studies were too small to judge their significance. Blaschke et al. go to considerable lengths to demonstrate that the large numbers of dying cells they see in the proliferative zone are due to the increased sensitivity of their technique and not because their assay labels nondying cells as well.

The large numbers of dying cells that Blaschke et al. report in both proliferative and postmitotic regions of the embryonic cortex give rise to some interesting questions: How much total death does occur during the development of the cortex? Why do developing cells die? So what does this cell death mean for our understanding of cortical development, and in particular, how does it affect our interpretation of cortical cell lineage studies?

How Much?

To convert directly from histological counts of dying cells to the total number of cells that die, one must know how long dying cells remain detectable before they are cleared away by phagocytosis. This apparent clearance time is dependent on the detection method and is notoriously difficult to measure. A relatively insensitive method such as counting pyknotic nuclei using a histological stain like propidum iodide may have very rapid clearance times, whereas the more sensitive techniques are likely to have longer clearance times because they are capable of detecting cells earlier in the process of dying. Even for a single technique, the apparent clearance time may well vary from region to region because phagocytosis presumably depends to some extent on the density and nature of the neighboring cells within the tissue.

It is hard, therefore, to know how much total death occurs based on the report by Blaschke et al. of 50% or more of cells labeled in their cell death assay. Clearly, with over 50% of cells undergoing apoptosis in the ventricular zone, the clearance time must be considerably longer than the cell cycle time or there would be no net increase in cell number. The fractions of ISEL-labeled cells that Blaske et al. report in the upper layers of the cortex and the ganglion cell layer of the retina suggest that the ISEL clearance time is relatively long compared with the overall periods of cell death in those regions, because the peak fraction of ISEL labeling is comparable to the total cell loss previously reported to occur in those regions (based on counting cells before and after the cell death period). This would suggest that perhaps the 50%–70% fraction of labeled cells reported by Blaschke et al. is indeed close to the actual fraction of total cell loss.

Another way to determine the total amount of cell death that occurs in the proliferative zone would be to measure accurately the rate and time course of neurogenesis and then calculate the total number of cells that are generated, which could be compared to the number that survive to determine the total fraction of cells that die. Caviness et al. (1995) have reported data on the rate of neurogenesis for the developing mouse cortex in terms of the cell cycle times and the fraction of dividing cells throughout the period of neurogenesis. Although more measurements are needed to determine precisely the total number of cells generated, their data are consistent with 50%–70% total cell death during proliferation.

Why?

What causes cells to die in the cortical proliferative zone and why would the embryo bother to generate so many cells just to kill them off right away? The explanation that is usually given to explain normal cell death in nervous system development is that extra cells need to be produced during neurogenesis to allow for size matching between populations of nondividing cells during synaptogenesis. In the cortex, that explanation makes sense for the dying cells seen in the upper postmitotic layers, but it clearly does not make much sense for cell death that occurs within the proliferative zone.

Three possible reasons come immediately to mind for why cells might die during proliferation (Figure 1). The first is that cell death may act like Darwinian natural selection by weeding out unfit cells. Cells with errors in

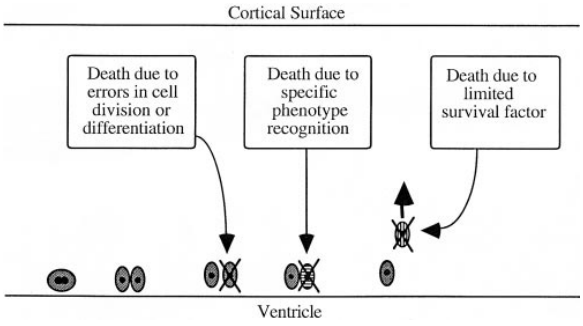

Figure 1. Examples of Possible Cell Death Mechanisms for Cells in the Proliferative Zone

DNA replication, for example, or errors in cell differentiation may somehow be signaled to die or may automatically trigger their cell death mechanism. Although conceivable, it seems unlikely that cells dividing in the cortical ventricular zone would be so much more error prone than other dividing cell populations. With more sensitive methods for detecting cell death, however, maybe there will turn out to be more proliferative death everywhere than was previously suspected.

The second explanation, favored by Blaschke et al., is that cell selection in the cortex may be similar to negative selection in the development of T cells in the thymus, involving some specific phenotype recognition mechanism. In the thymus, death does not appear to weed out unhealthy cells; instead, it plays a constructive role by allowing the generation of a huge number of cell phenotypes through active gene rearrangement, and then killing off the majority via phenotype specific selection. This is an intriguing possibility because it opens the door to far more neuronal phenotypic variability than is typically thought.

The third obvious possibility is that cell death in the proliferative zone is not cell specific, but instead is the result of an inadequate supply of exogenous survival factor(s). This would then be similar to the notion of target-derived trophic factor dependence believed to mediate the later wave of cell death associated with synapse formation. Such a survival factor dependence in a proliferating CNS population has been demonstrated in the generation of glial cells (Barres et al., 1992), where 50% of oligodendrocytes die soon after they leave the cell cycle and begin to differentiate. Death in this case seems to be a selective means of regulating cell numbers based on the need to match the number of oligodendrocytes to the size of the axon population to be myelinated. In this view, the survival of any particular cell would depend on its own changing trophic requirements and the local environment in which it finds itself. Competitive interactions with other similar cells may also play a role if the survival factor is in limited supply.

So What?

Although a lot more must clearly be learned about exactly how much cortical cell death occurs and what causes it, the simple fact that there seems to be a lot more death than was previously known is enough to warrant a reevaluation of our understanding of how different cortical cell types arise, particularly as has been revealed by cell lineage experiments. Lineage studies are worth singling out because they depend on the assumption that relatively little cell death occurs. By labeling cells with a heritable marker while they are dividing and then waiting until later in development or even until maturity to analyze the progeny in labeled clones, developmental cell death that occurs between labeling and analysis could have a significant impact on the results of such studies. The many lineage studies that have recently been undertaken in the nervous system (reviewed by McConnell, 1995), especially in the retina and cortex, have assumed that too little cell death occurs to have a significant role in shaping clone composition. If half or more of the cells produced in a region die during development, however, the composition of any observed labeled clone almost certainly provides an incomplete view of the cells originally generated in that clone.

My colleagues and I have explored the relationship between cell death and cell lineage in the developing rodent retina (Voyvodic et al., 1995). We found that the amount of cell death that normally occurs in the retina is sufficient to obscure lineage patterns by producing much more apparent clonal variability than might actually be the case (Figure 2). Lineage studies in the retina (Holt et al., 1988; Wetts and Fraser, 1988; Turner et al., 1990) have interpreted the high degree of variablity seen in clone composition to indicate that there is no underlying lineage pattern. This conclusion would imply that progenitor cells remain multipotent in terms of cell identity until they stop dividing, at which time each postmitotic cell differentiates independently depending on the signals it receives within its local microenvironment. Because our measurements in the retina only dealt with postmitotic cell death, it will be interesting, in the light of the results of Blaschke et al., to apply the more sensitive ISEL-labeling technique to see whether there is even more cell death occurring among the dividing cell population in the retina as well.

Whereas the results from retinal lineage studies have emphasized clonal heterogeneity and multipotential precursor cells, cortical lineage studies have resulted in more homogeneous clones that are thought to reflect restricted cell fate potential in the precursor cells. The observation that most of the cells produced in the cortex seem to die during development does not necessarily mean that any of the conclusions based on lineage studies are wrong, but it does suggest that we carefully consider how death might alter lineage interpretations.

There are two major reasons why cell death is a significant problem for interpreting cell lineage studies. The first is the simple fact that the observed clones are incomplete, so that one cannot conclude for any individual clone that the observed cells are as closely related as they might appear, nor that cell types that are not observed were not produced in that clone. A clone containing only two cells, for example, could be assumed to contain sister cells that result from a single cell division in the absence of cell death, but with cell death might conceivably represent distant cousins. Similarly, a clone with only a single type of cell might tell us something about the potential of the precursor cell in the absence of cell death, but with cell death there might originally have been other cell types that did not survive.

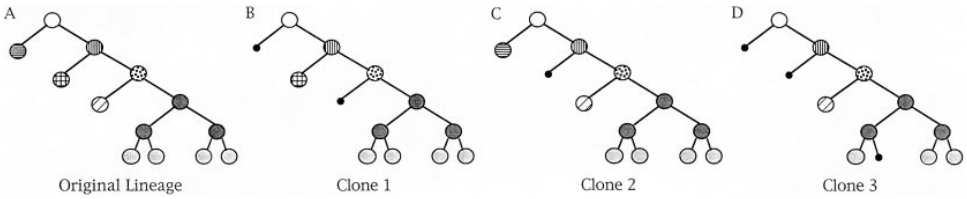

Figure 2. Examples of Clonal Variability Due to Cell Death

(A) A hypothetical retinal cell lineage that produces seven cells with four different phenotypes; in the example, a single cell is produced for three cell types, and four cells are produced for the fourth cell type (corresponding to rod photoreceptors, which are produced in relatively large numbers).

(B–D) Three examples of different clones that could result from the lineage in (A) with 50% cell death occurring in all cell types except rods. Dead cells are indicated with small black circles. The resulting clones look different even though the underlying lineages are identical. Variability in the availablility of mitogenic growth factors could provide further clonal variablility independent of either cell type induction or cell survival mechanisms.

The second reason why cell death complicates lineage analysis is the fact that we do not know why cells die. If cell death is random or occurs with uniform probability, then the effects of cell death on clone composition could be dealt with on a statistical basis given sufficient measurements. For example, if death is the result of random errors in DNA replication with a 50% probability that any particular cell would die, then the lineage results could simply take that survival rate into account when calculating clone sizes and assume that except for size the patterns of lineages observed over many clones accurately reflect the patterns that were originally generated. The same argument would hold if death is due to dependence on some nonspecific survival factor that is present at close to threshold levels, such that the survival of any individual cell is simply determined by whether its particular threshold requirement is satisfied.

But as soon as the survival probability for different cell types becomes nonuniform, or if the survival of one cell is affected by the survival of neighboring cells, as is the case when cells compete for limited survival factors, then incorporating cell death into a lineage analysis becomes considerably more complicated. Competition, for example, would act to select against similar cell types within a clone because the presence of a closely related competitor would tend to decrease a cell's own probability for survival. Of course, nonuniform survival probability would also affect the distribution of different cell types within clones, perhaps killing off many or all cells of some types while sparing cells of other types. Similarly, if the probabilities of survival change with time during development, then clone composition will appear to change even if the lineages originally generated are all the same. Differences in survival conditions between cell lineage analyses performed in vivo and in vitro could also lead to apparent differences in clone composition, which might have nothing to do with differences in cell potential. Finally, the possibility raised by Blaschke et al. that large scale death in neurogenesis might reflect cell-specific phenotype recognition mechanisms similar to those occurring in the thymus further complicates lineage interpretations by shifting the cell selection process from cell populations down to the level of individual cells. Even without the extreme degree of phenotypic heterogeneity produced by antigen-specific receptor gene rearrangements in the thymus, one could imagine a scenario where some significant degree of neuronal specificity could be generated by a similar combination of nonspecific cell diversification followed by phenotypic selection.

What conclusions can and cannot be safely drawn from cell lineage analysis without worrying about the impact of cell death on clone composition? One can conclude, for example, that cell types seen together in mixed clones share a common ancestor. One cannot conclude that different cell types arise from divergent cell lineages based on seeing only unmixed clones, unless multiple kinds of unmixed clones are consistently seen within a single age and location; in the latter case, it is hard to imagine cell type selection mechanisms that would result in clone-specific selection unless those clones themselves are somehow phenotypically different lineages. Finally, one cannot determine when cell identity decisions are made based on clonal variability, unless the amount of variability caused by selective survival can somehow be identified and factored out.

Clearly, without knowing how much cell death occurs in development or what causes it, one could speculate forever on cell selection schemes and their roles in generating any number of observed cell lineage patterns. By the same token, however, disregarding potentially large amounts of cell death when evaluating cell lineage data could lead to a similarly fanciful view of the underlying developmental mechanisms involved. Until more is known about cell death in regions like the developing cortex, it is perhaps best not to disregard selective survival as a potentially important mechanism in determining both the number and cell type of the neurons and glia that are found in the the mature nervous system.

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