Cell Cycle Features:

Do cells become homeless during neural tube closure?

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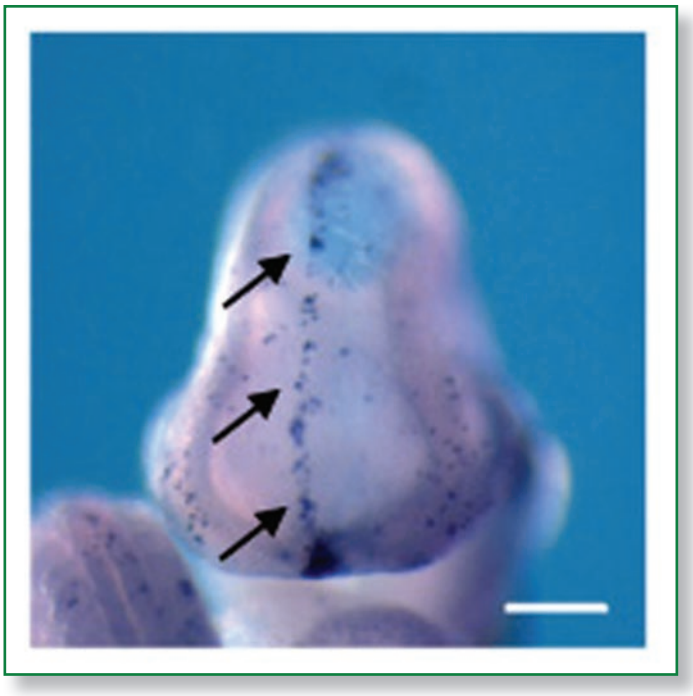

Figure 1. Front view of the mouse brain at embryonic day 9.5, after whole mount TdT-mediated dUTP nick end labelling (TUNEL) staining to reveal dying cells. Arrows indicate midline cell death, which corresponds to the site of remodelling of neuroepithelial and surface ectoderm cells, immediately following neural tube closure. Scale bar = 0.4 mm.

Programmed cell death is a physiological event of animal development, first described in 1951 by Glücksmann[1] as a normal component of developmental processes. Since then, the morphological events and signaling pathways that characterize apoptotic cell death, and distinguish it from inflammation-associated necrosis, have been investigated in great detail.[2] While apoptosis is the most studied and best characterized form of programmed cell death, a role in development for cell death with autophagy has also been suggested,[3] originally stimulated by studies in yeast. The requirement of apoptosis for cell removal in the developing interdigital region and in mammary tissue of male embryos is well established. Similarly, during nervous system development, programmed cell death is fundamental for regulating the number of differentiated neurons, with elimination of axonal misconnections. What is less clear is whether programmed cell death also plays a crucial role in morphogenetic tissue fusion events such as closure of the neural tube and fusion of the palatal shelves.

Building on previous work in chick neurulation,[4] we recently confirmed that dying cells are associated spatio-temporally with closure of the mouse neural tube (Fig. 1). We examined genetic mutants in which apoptosis is severely diminished and found that neural tube closure occurs apparently normally in the forebrain and spinal neural tube, although the hindbrain and caudal midbrain remain open. Most strikingly, when we inhibited apoptosis chemically in intact, cultured mouse embryos, we found that closure of the entire neural tube, including mid- and hindbrain, proceeded to completion.[5] Our findings indicate that apoptosis, while plentiful and strategically placed to participate in neurulation, is not actually required for completion of neural tube closure in mice.

In this context, it is interesting that controversy still surrounds a putative role for programmed cell death during fusion of palatal shelves. While cell death is abundant during this morphogenetic process, experimental inhibition of apoptosis during palatal shelf fusion has given conflicting results.[6,7] We suggest, therefore, that the occurrence of apoptosis at sites of morphogenetic tissue fusion may not necessarily indicate a functional role, but rather a secondary outcome.

The next question is: why do cells die during fusion of embryonic epithelia? Our study of the spatio-temporal distribution of dying cells during mouse neurulation revealed apoptosis predominantly associated with three main events: bending and fusion of the neural folds, post-fusion remodelling of the dorsal neural tube and surface ectoderm, and emigration of neural crest cells. In each of these embryonic events, cells undergo marked changes in shape and alteration in their association with the underlying extracellular matrix (ECM). For example, fusion and remodelling of the neural folds, to create tissue continuity across the dorsal midline, implies that some cells at the fold tips will alter their contacts with the adjacent cells and/or with ECM. Moreover, subsequent initiation of neural crest cell migration requires an epithelium to mesenchyme transition as cells detach from the neural folds and migrate away.

In the early 1990s, a new Greek-derived term—anoikis, i.e., "homelessness"—was coined to indicate apoptosis induced by lack of correct cell/cell or cell/ECM attachment. Signals from the ECM were found to be fundamental in preventing cells from starting the apoptotic intracellular program. Once initiated, however, anoikis did not differ from apoptosis either biochemically or morphologically, the term simply emphasising a particular stimulus for cell death.[8]

During neurulation, the basal surfaces of neuroepithelial cells contact extracellular matrix, which is interposed between neural plate and surface ectoderm dorsally, and between neuroepithelium and paraxial mesoderm or notochord, more ventrally. As the dorsal neuroepithelium bends inwards, to bring the neural folds together, a primitive basement membrane containing type IV collagen, fibronectin,

laminin and proteoglycans gradually extends in a proximo-distal direction along the neural plate/surface ectoderm interface. Only as neural tube closure nears completion does this matrix become organized into ultrastructurally distinct basal laminae, one associated with each epithelium.[9,10] Subsequently, these basal laminae are remodelled as the neural folds fuse and tissue continuity is established across the dorsal midline. Further ECM remodelling occurs as the neural crest cells emigrate from the spinal neural folds, although the earlier departure of neural crest cells in the cranial region, which occurs before neural fold fusion, may precede basal lamina formation. Hence, there is considerable evidence to expect cells at these sites of active neurulation morphogenesis to be at risk of losing ECM contact, detaching and undergoing anoikis.

In addition to contact with the ECM, there is also evidence for a role of altered cell adhesion leading to anoikis at the tips of the neural folds during neural tube closure. The Nf2 tumor suppressor (also called Merlin) regulates cell-cell adhesion during tissue fusion, by promoting the assembly and maintenance of apico-lateral junctional complexes. Studies of embryos mosaic for deletion of Merlin revealed fusion defects in a number of organs, including brain, heart, eye and palate.[11] The malformations were found to derive from ectopic cellular detachment during tissue fusion, owing to failure to maintain apico-lateral junctional complexes. In severely affected Merlin mutants, a more than 30-fold increase in apoptosis was detected at the tips of the neural folds where ectopic detachment was particularly marked. The authors suggested that anoikis may ensure that only epithelial cells forming stable cell-cell contacts can survive through morphogenetic tissue fusion events, to contribute to the subsequent development of the organ that is formed.

In conclusion, it is undoubted that embryonic tissue fusion events including neural tube closure are associated with plentiful apoptosis. Our findings demonstrate, however, that this programmed cell death is not essential for completion of the fusion process. The spatio-temporal association of apoptosis with neural tube closure suggests that cell death may be secondary to the cellular reorganizations that occur in such tissue fusions. It seems likely that this cell death is an example of anoikis, in which cells lose their essential attachments to the ECM and neighbouring cells, undergo detachment, and initiate the apoptotic signaling cascade. Presumably, this cell loss is the price the embryo pays for achieving such vital goals as closing the neural tube.

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