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News and Commentary

Shaping developing tissues by apoptosis

HE Abud*,[1]

[1] Ludwig Institute for Cancer Research, PO Box 2008, Royal Melbourne Hospital, Parkville, Victoria 3050, Australia
* Corresponding author: HE Abud. Tel: +61 3 9341 3155; Fax: +61 3 9341 3104; E-mail: helen.abud@ludwig.edu.au

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Apoptosis plays an important role in sculpting the overall shape and organisation of organs during development.[1–5] Specific populations of cells are eliminated by programmed cell death at different stages of embryogenesis and also in adult tissues such as the mammary gland. The morphogenesis of organs typically involves the coordination of several cellular processes including migration,[6] proliferation, apoptosis[1–5] and changes in cell shape or polarity.[7] These changes are critical for specifying the structure and function of individual cell layers and whole organs. The mechanisms that regulate the survival of specific cells and the connections between cell death and other cellular processes at different times during embryonic development are largely unknown. This is partly due to the difficulties in studying tissues *in vivo* that are largely inaccessible for observation and cell biological or biochemical experimentation. Recently developed techniques have improved the culture of whole organs and allowed the maintenance in culture of tissue architecture in three dimensions. Some of these systems mimic remodelling processes *in vivo* and are beginning to be used to elucidate the events that control the initiation of cell death and the identification of underlying molecular pathways and growth factors that contribute to developmental apoptosis.[8–10]

Apoptosis and cavitation in early mouse embryos

In the mouse blastocyst, the inner cell mass (ICM), which gives rise to all cells within the embryo, consists of a solid mass of ectodermal cells covered by a layer of primitive endoderm. Following implantation, the ICM rapidly converts to a structure known as the egg cylinder by a process termed cavitation.[11] Apoptosis has a central role in transforming the core of ectoderm cells into a cavity (the pro-amniotic cavity) lined with columnar epithelium. Sectioning of embryos fixed at implantation has revealed the presence of embryonic cells in the forming pro-amniotic cavity, which exhibit all the hallmarks of apoptosis, including cell shrinkage and pyknotic nuclei.[11] These studies suggest that this cavity forms by selective apoptosis of cells in the centre of the mass of ectoderm cells. Cavitation can also occur during the differentiation of embryoid bodies *in vitro* and this has proved to be a useful system to investigate the mechanisms underlying the process of cavitation.[11] Studies in this system suggest that cavitation results from the interplay of two opposing signals: one signal is secreted from the visceral endoderm and induces apoptosis of the inner ectodermal cells, while the other is a survival signal located in the basement membrane underlying the ectodermal cells.[11] The interaction between these two opposing signals results in the formation of a monolayer as only those cells in contact with basement membrane escape the death signal and survive. The innermost ectodermal cells die by apoptosis.[11] Further studies have shown that BMP signalling is capable of reproducing the activity of the death signal during the differentiation of embryoid bodies. Blocking BMP activity prevents cavitation and addition of BMP protein to cultures promotes cavitation. Both *Bmp2* and *Bmp4* are expressed in early mouse embryos in a pattern that is consistent with them having a role in the cavitation of embryonic ectoderm *in vivo*;[12] however, the identity of the survival signal in the early embryo is still unknown.

Apoptosis and epithelial remodelling during gut development

The primitive gut tube is initially formed following a series of dynamic movements that convert the definitive endoderm cell layer to a closed tube surrounded by a layer mesoderm.[13] From E13.5 of mouse development, the endoderm cell layer begins remodelling, with the conversion of a pseudostratified cell layer into a differentiated epithelial monolayer.[14] Morphological remodelling of the epithelium proceeds in a proximal to distal direction. The innermost cells lining the lumen undergo apoptosis and exfoliate during this process, while cells adjacent to the basement membrane become polarised and survive. This ultimately results in the generation of a polarised epithelial monolayer along the length of the intestine.[10,14,15] The molecules controlling this process are completely unknown, and until recently this process has been difficult to study due to an inability to culture gut intestinal cells in a manner that resembles growth *in vivo*. Culture of segments of embryonic gut in suspension (catenary culture) preserves the tubular structure of the intestine[16] and differentiation of the epithelium mimics several key aspects of intestinal development, including the expression of markers of differentiation, cell polarisation and apoptosis of cells lining the lumen.[10] Future work in this system should help elucidate signals controlling cell survival and epithelial remodelling during intestinal development.[10]

Apoptosis and the separation of digits

Cell death during limb morphogenesis has been a subject of research for many years. The complex shape of the limb and the separation of the digits are dependent on highly coordinated phases of cell proliferation and cell death.[1] Limb buds first emerge from the flank of developing embryos as

undifferentiated protrusions consisting of an outer layer of ectoderm and an inner core of mesenchymal cells. These primitive structures undergo many elaborate changes in shape as the limb buds develop to produce the different structures present in the mature limb. One of the most striking changes is the molding of digits in the paddle-shaped limb bud. Separation of the cartilaginous condensations destined to become digits depends on the death of the intervening mesenchymal cells. *Bmp2* and *Bmp4* are specifically expressed in the interdigital regions where programmed cell death occurs.[17] Chick limb buds are accessible for manipulation '*in ovo*' and can be cultured *in vitro.* Overexpression of dominant-negative BMP receptors can suppress apoptosis[17,18] and results in webbing between the digits, while overexpression of BMP2 or BMP4 promotes apoptosis within limb buds.[19] Furthermore, expression of the BMP antagonist noggin within the limb buds of transgenic mice causes a reduction in the level of apoptosis and extensive syndactyly of the digits.[20] All of these data support a role for the BMP signalling pathway in interdigital apoptosis. Several lines of evidence implicate *Msx2* and *Dickkopf (Dkk-1)* as downstream targets of BMP-induced apoptosis in the developing limb.[21,22] Upregulation of both Msx2 and Dkk-1 is observed in sites of apoptosis upon stimulation with BMP. A corresponding downregulation of expression of these genes is observed when BMP signalling is blocked, and in mutants exhibiting limb malformations.

Essential roles of apoptosis in mammary gland development

The morphogenesis of ducts in the development of the mammary gland is dependent on the selective death of epithelial cells to form mammary acini, in which a hollow tube lined with polarised mammary epithelial cells is formed.[4,23] The establishment and maintenance of overall tissue structure is essential for the function and homeostasis of mammary epithelium. The development of techniques for culturing mammary epithelial acini in three-dimensional matrices has provided an opportunity to investigate the mechanisms underlying the development of normal glandular structure. MCF-10A cells placed in three-dimensional culture form mammary acini and undergo selective apoptosis.[9] In this model, apoptosis is confined to cells in the luminal space, while the cells in the periphery attached to the matrix survive. The Akt survival pathway is specifically activated in these peripheral cells, while cells occupying the lumen lack this survival signal.[9] Interestingly, inhibiting apoptosis by the introduction of the antiapoptotic proteins Bcl-2 and Bcl-X delays, but does not inhibit, the formation of a lumen in MCF-10A mammary acini. Expression of a proliferative oncogene, such as ErbB2[8] in combination with these antiapoptotic proteins, however, results in the filling of the luminal space.

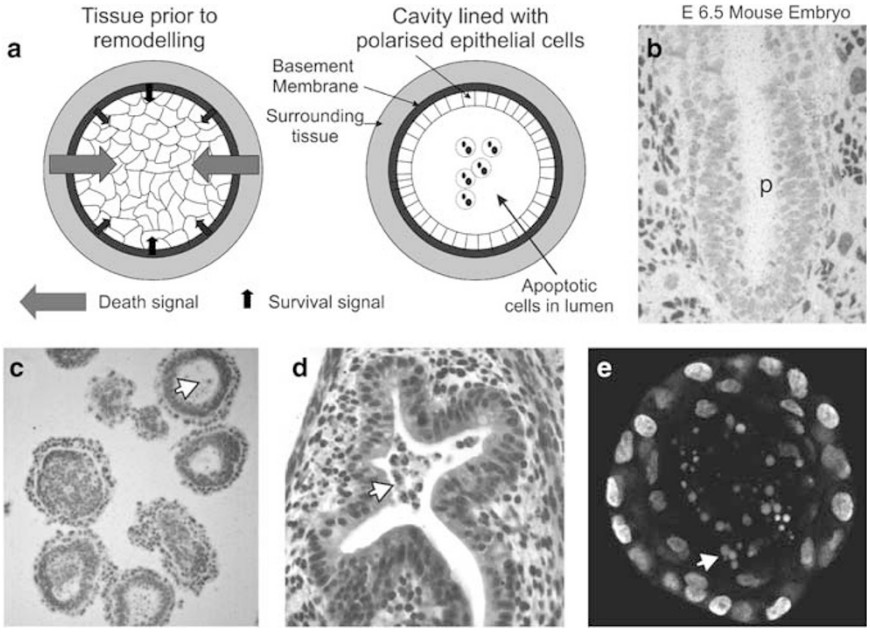

Figure 1 Apoptosis and the formation of cavities lined with epithelial cells during tissue development. A similar pattern of apoptosis is observed during the formation of cavities lined with epithelial monolayers in the egg cylinder, intestinal epithelium and mammary acini. Similar mechanisms may be involved in all these processes. (**a**) Schematic model of the process of cavitation based on the formation of the pro-amniotic cavity in mouse embryos.[11,12] These studies suggest that a death signal is released from the surrounding tissue (indicated by large shaded arrow) while a survival signal is present in the basement membrane (small arrow). The combination of these two signals allows cells adjacent to the basement membrane to survive, while those in the centre die by apoptosis and exfoliate into the lumen. (**b**) Sagittal section of an E6.5 mouse embryo, showing the presence of the pro-amniotic cavity, p. (**c**–**e**) *In vitro* culture methods which mimic epithelial remodelling processes observed *in vivo.* (**c**) Cavitating embryoid bodies produced by the PSA1 ES cell line. (**d**) Intestinal epithelial remodelling in catenary culture. (**e**) Confocal optical section of MCF-10A mammary acini grown in matrigel and stained with the DNA dye TOPRO3. Arrows indicate the presence of apoptotic cells in the lumens of these tissues. Panels b and c are reproduced from Coucouvanis and Martin[12] with permission from Professor Gail Martin (UCSF, CA, USA) and the Company of Biologists Ltd. The image in panel e was supplied by Mr. Luke Dow and Dr. Patrick Humbert, Peter MacCallum Cancer Institute, Melbourne

Conclusions

There are several striking similarities between the morphological events and signalling pathways involved in directing the selective apoptosis of cells in different developing tissues. This suggests that similar mechanisms and signals may be involved in different developmental processes. A similar pattern of apoptosis is observed during the formation of hollow tubes lined with epithelial monolayers in egg cylinder formation, intestinal development and mammary acinar development (Figure 1). The most central cells are susceptible to apoptosis, while those associated with the basement membrane develop apico-basal polarity and survive. BMP signalling has been implicated in several instances of programmed cell death, suggesting that this pathway is commonly activated in the initiation of apoptosis during development. Other factors must ultimately contribute to cell death, however, as there are many cellular contexts where exposure to BMP signals does not result in cell death. The development of the *in vitro* methods described here should allow the contribution of specific signalling pathways to be dissected in more detail by the experimental modulation of signalling. Coordination of cell proliferation, migration and cell death in highly specialised tissues such as the intestine and the mammary gland are not only important for the establishment of these tissues but also are essential for tissue homeostasis. Disruption of tissue architecture is associated with early stages of tumour formation. Filling of the luminal space is a hallmark of ductal carcinoma *in situ* in the breast,[24,25] and loss of cell polarity and crypt architecture is characteristic of early adenoma formation in the colon.[26] Elucidation of the regulators that control apoptotic pathways during development will not only contribute to our understanding of tissue and organ formation but also provide potential targets for tumour prevention and/or therapy.

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