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Seminars in Cell & Developmental Biology 16 (2005) 261–269

seminars in
CELL & DEVELOPMENTAL
BIOLOGY

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Review

Programmed cell death in the embryonic vertebrate limb

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Available online 16 January 2005

Abstract

The developing limb bud provides one of the best examples in which programmed cell death exerts major morphogenetic functions. In this work, we revise the distribution and the developmental significance of cell death in the embryonic vertebrate limb and its control by the BMP signalling pathway. In addition, paying special attention to the interdigital apoptotic zones, we review current data concerning the intracellular death machinery implicated in mesodermal limb apoptosis.

Keywords: Apoptosis; Caspases; BMPs; Morphogenesis

Contents

1. Introduction

The vertebrate limb has always been an important developmental context for the study of programmed cell death during embryogenesis as in the developing appendages cell death is a constant feature. The correlation between the areas of cell death and limb morphogenesis has been reported in amniote embryos including birds, reptiles and mammals (see Ref. [1]). In contrast, in the anamniote embryos limb development occurs in absence of cell death [2].

2. Cell death in the limb bud mesoderm

The most remarkable apoptotic areas of the limb bud are observed in the undifferentiated mesodermal cells. In the avian embryo, all these areas seem to have the role of regulating the amount of pre-skeletal cells accounting for skeletal morphogenesis. The elimination of interdigital cells (Interdigital Necrotic Zones; INZ) in species with free digits constitutes a paradigmatic example of morphogenetic cell death. In amniota embryos, digits develop as chondrogenic condensations separated by interdigital regions containing undifferentiated mesenchymal cells. The fate of these interdigital cells is dependent on the final morphology of the digits in each species (Fig. 1). In species with free digits such as chicken [3,4], lizard [5], and mouse or human [6,7], apop-

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doi:10.1016/j.semcdb.2004.12.004

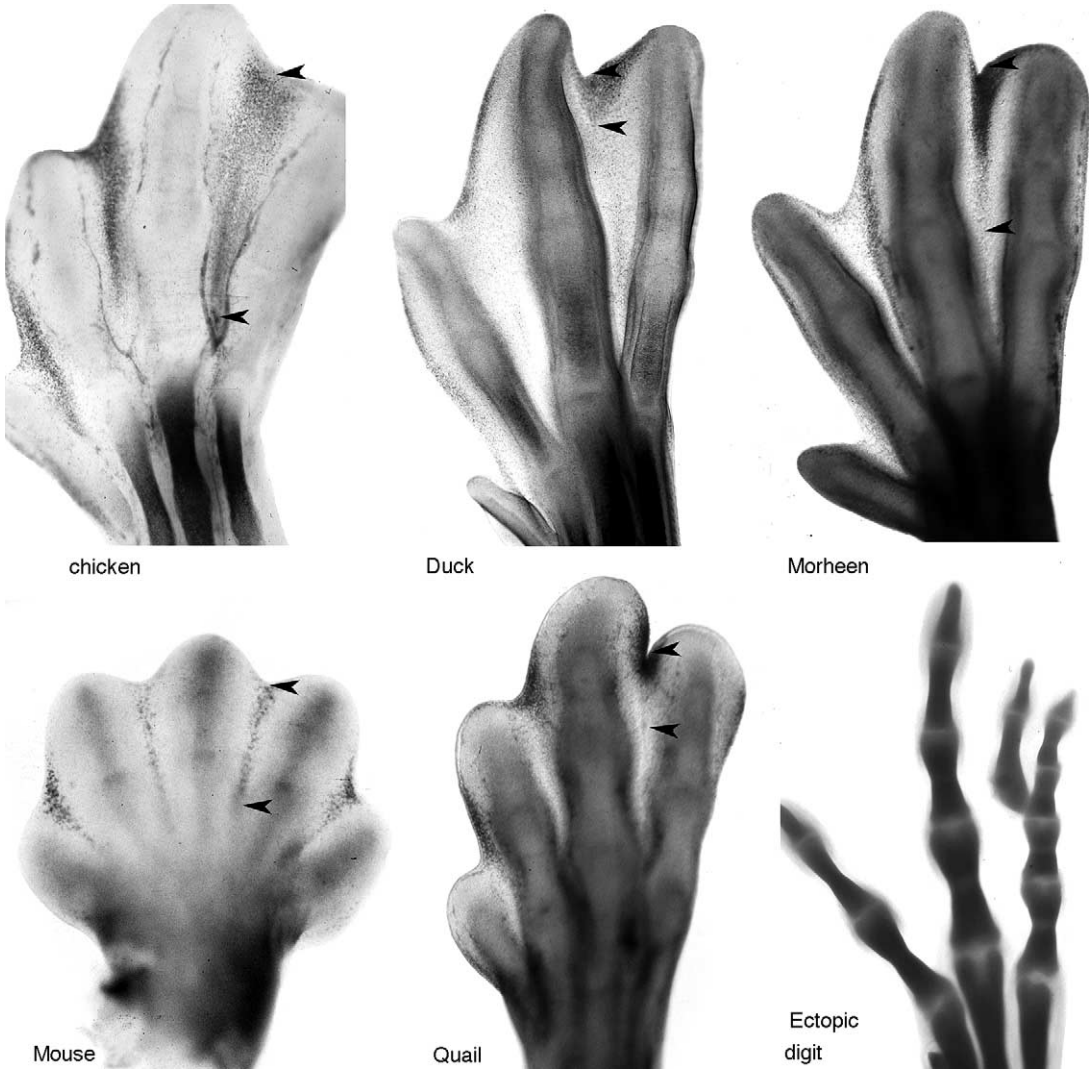

Fig. 1. Pattern of interdigital cell death in different species after neutral red vital staining and ectopic interdigital chondrogenesis resulting from inhibition of interdigital cell death in the chick (lower right panel).

tosis extends through all the interdigital space. In species with webbed digits such as duck [3,8], or tortoise [5] apoptosis is limited to the distal part of the interdigit. In species with autopods of singular morphology, such as the moorhen (*Gallinula chloropus*) or the coot (*Fulika atra*) [9], which have digits with lateral membranous lobulations or the split autopod of the chameleons [10], the pattern of interdigital cell death correlates closely with the specific phenotype of each species. Numerous experimental approaches have shown that the interdigital mesoderm contains cells with chondrogenic potential and when cell death is inhibited they are able to form an extra digit (Fig. 1; [11]).

In addition to the INZ there are other well characterized areas of apoptosis which are also correlated with the skeletal pattern of the limb. In the early avian limb two areas of cell death, the anterior necrotic zone (ANZ) and the posterior necrotic zone (PNZ) have been related with the reduced number of digits in birds, since they are absent in the polydactylous avian mutants [12]. In accordance with this interpretation in the mouse pentadactylous limb ANZ and PNZ are not present [13]. In contrast with that avian limb, the early limb bud of mouse and rat embryos exhibit a pattern of mesodermal cell death functionally associated with limb outgrowth and with the regression of the AER ([14,15] and see below).

A further area of cell death is also present in the central limb mesenchyme of avian embryos. This area which has been termed the opaque patch (OP) accompanies the formation of independent rudiments for the zeugopodial bones (tibia-fibula; ulna-radius). The *talpid3* chick mutant lacks this area of cell death and exhibits fusion of the zeugopodial skeletal pieces [16].

Although ANZ, PNZ, INZ and OP are the best characterized areas of cell death that occur during limb development, programmed cell death is observed in other limb domains with distinct and often unclear functions.