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Interdigital tissue regression in the developing limb of vertebrates

CARLOS I. LORDA-DIEZ, JUAN A. MONTERO, JUAN A. GARCIA-PORRERO and JUAN M. HURLE*

Departamento de Anatomía y Biología Celular and IDIVAL, Universidad de Cantabria. Santander, Spain

ABSTRACT Here we have chosen the regression of the interdigital tissue which sculpts the digits from the hand/foot plate in tetrapod embryos to review the most relevant aspects concerning the regulation and biological significance of programmed cell death. We gather abundant information showing that the initiation of the degenerative process is the result of a complex interplay between the different signaling pathways which are also responsible for limb outgrowth and skeletal tissue differentiation, rather than being regulated by a specific signaling pathway. The model further shows that once the death response is triggered, several different routes of cell disruption, including caspase-dependent apoptosis, lysosomal-mediated cell death, and even a cell senescence process, are activated in the interdigits to ensure their elimination. Transcriptional and structural changes accompanying the degenerative process, and their posible contribution to the control of the death process, are also revised in detail. Finally we survey a number of issues still awaiting clarification, such as the functional implication of interdigital cell death as a source of signals acting on the surrounding tissues, as occurs in the so called "regenerative cell death".

KEY WORDS: *cell death, apoptosis, limb development, developmental senescence, lysosomal cell death*

Introduction

The basic cellular processes accounting for growth, differentiation or degeneration of tissues and organs are conserved in embryonic, tumoral and adult tissues. This mechanistic uniformity allows researchers to choose, among many options, the most appropriate and informative models and assays to analyze basic biological problems of relevance in physiology and pathology. From this point of view, the regression of the interdigital tissue which sculpts the digits in vertebrate embryos is a paradigm of tissue remodeling, which can provide information for a better understanding of degenerative processes, not only in the embryo but in adult organs or tumours also.

Digits differentiate as radial condensations whithin the distal paddle-shaped region of the limb bud termed the autopod. The autopod contains skeletal progenitors of mesodermal origin covered by the ectoderm and its initial morphogenesis involves the following coordinate events: 1) the autopod as a whole undergoes outgrowth directed by the marginal ectoderm (termed AER) which delivers growth factors to stimulate proliferation of the subjacent mesodermal progenitors; 2) in the deepness of the autopod the progenitors condensate to form radial cartilage blastemas which become segmented into phalanges, and mantain outgrowth by incorporation of further progenitors at their distal tip, located in the zone subjacent to the AER; 3) in the interdigital regions progenitors are maintaned undifferentiated, but retain potential to form extra digits. When digits have attained almost their final number of phalanges, the AER cesses functioning first in the interdigits, and next in the digit tips (Gañan *et al.,* 1988). Concomitantly with the regression of the AER, the interdigital tissue arrests proliferation and undergoes massive cell death followed by degeneration of all the remaining tissue components, including blood vessels, and extracellular matrix (Fig. 1). In addition, local healthy cells and invading macrophages remove by phagocytosis the degenerating tissue (see Hurle *et al.*, 1977; Francisco-Morcillo *et al.*, 2014). In the latest stages of the process of chick embryos, the degenerated tissue, including macrophages and epithelial tissue is detached into the amniotic sac (Hurle and Fernandez-Teran 1983).

Variations in the extent of interdigit degeneration account for differences in the morphology of digits related with the functional specialization of vertebrate species to swim (webbed digits in

Abbreviations used in this paper: AER, apical ectodermal ridge; AIF, apoptotic inducing factor; BMP, bone morphogenetic protein; ECM, extracellular matrix; FGF, fibroblast growth factor; IGF, insulin growth factor; RA, retinoic acid; ROS, reactive oxygen species.

***Address correspondence to:** Juan M. Hurlé. Departamento de Anatomía y Biología Celular. Facultad de Medicina, Santander 39011, Spain. Fax: 34-942-201903. E-mail: hurlej@unican.es

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duck or tortoises), to fly (bat wings), or to walk on land (chicken, human, lizards; reviewed by Hurle *et al*., 1996). Conversely, abnormal inhibition of interdigital cell death in species with free digits results in syndactyly. However, the morphology and estructure of the syndactylous tissue is quite variable (Malik, 2012). For example, there are *soft tissue syndactylies* caused by absence of interdigital tissue remodeling, and, *osseous syndactylies*, when two adjacent digits fuse each other, or when an ectopic skeletal pieces are formed in the interdigits. The later not always are due to inhibition of cell death.

Regulation of interdigital tissue regression

Inhibition of interdigital cell death followed by syndactlyly is observed after disruption of almost each of the growth-regulatory pathways active in the autopod at the stages of digit formation, including: BMP-signaling (Zou and Niswander, 1996; Wang *et al*., 2004; Maatouk *et al*., 2009); FGF-signaling (Wilkie *et al*., 2002; Pajni-Underwood *et al*., 2007); Wnt-signaling (Mukhopadhyay *et al*., 2001; Grotewold and Rüther, 2002; Ikegawa *et al*., 2008; Morello *et al*., 2008; Villacorte *et al*., 2010); all-trans-retinoic acid signaling (Ahuja *et al*., 1997b; Rodriguez-Leon *et al*., 1999; Zhao *et al*., 2010; Cunningham *et al*., 2011); and Notch-signaling (Jiang *et al*., 1998; Sidow *et al*., 1997; Pan *et al*., 2005). In other cases the function of secreted factors in the regressing interdigits is less understood. Diferent Slit ligands and their Robo receptors are expressed in the interdigits without a known functional significance (Vargesson *et al*., 2001).The participation of IGF-signaling in the control of interdigital cell death was also proposed on the basis of the interdigital expression of different members of the pathway during the stages of interdigital cell death (van Kleffens *et al*., 1998; Allan *et al*., 2001). However, gain- and loss-of-function mutations of this signalling pathway cause phenotypes related with growth rather than altered morphogenesis (see, Tripathi *et al*., 2009). Together these findings indicate that interdigital cell death and limb outgrowth are divergent outcomes resulting from imbalance in the intensity of activation of comun regulatory processes.

Treatments with growth factors and their antagonists, together with studies using conditional compound genetics have provided considerable advance to unravel the crostalk between those signaling pathways in the control of interdigital cell death. It has been shown that BMPs are pro-apoptotic signals because negatively regulate the expression of FGF in the AER, which in turn are survival signals for the undifferentiate mesoderm (Maatouk *et al*., 2009; Wong *et al*., 2012). It has been also shown that Wnt/β-catenin signaling in the ectoderm positively regulate expression of *Fgf8* in the AER by antagonizing epithelial BMP signaling (Villacorte *et al*., 2010). Notch signaling also functions by regulating the expression of FGFs in the AER (Pan *et al*., 2005). The role of Retinoic acid signaling in interdigital cell death has been associated with an antagonistic interplay with FGF (Hernandez-Martinez *et al*., 2009) and a positive transcriptional influence on interdigital BMP gene expression (Rodriguez-Leon *et al*., 1999). In summary, all these studies point to FGFs produced by the AER as key signals required for survival of the subjacent mesenchymal tissue. However, additional direct effects of each signaling on the dying machinery cannot be discarded (see Hernandez-Martınez and Covarrubias, 2011). For example, it has been shown that retinoic acid down-regulates anti-apoptotic factors (Crocoll *et al*., 2002) and activates matrix proteinases associated with tissue remodeling (Dupé *et al*., 1999).

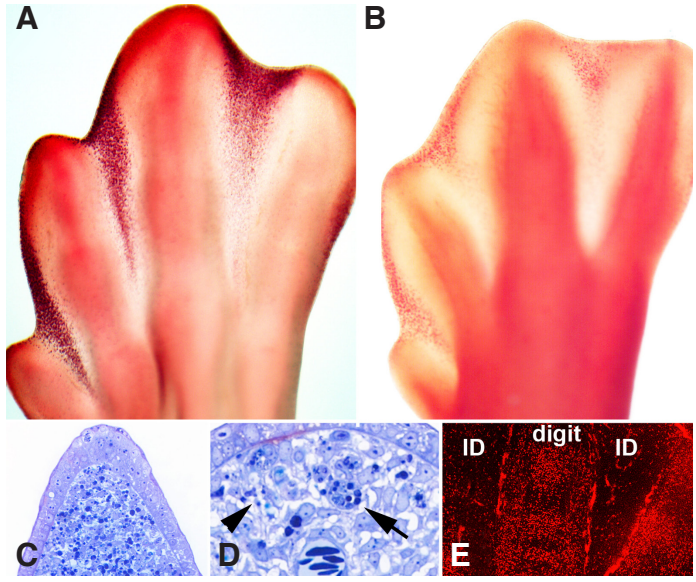

Fig. 1. Cell death and cell proliferation during the formation of the chick and duck toes. (A,B) *The leg autopods of chick* **(A)** *and duck* **(B)** *embryos vital stained with neutral red to show differences in the intensity of interdigital cell death between species with free and webbed digits.* **(C,D)** *A low and high magnification view of the regressing chick interdigit after toluidine blue staining. Note that the interdigital tissue contains only mesodermal progenitors and blood vessels covered by the ectoderm (Ect).* **(D)** *A high magnification view to show the presence of isolated dying cells (arrow head) and large macrophages (arrow) in the regressing interdigit.* **(E)** *A section of the chick autopode showing BrdU incorporation in the digit rays (digit) and interdigital tissue (ID). Note the low labeling of the interdigital tissue in comparison with the digit rays. The autopod was incubated for 30 min in BrdU and immulabeled with anti-BrdU antibody.*

Molecular machinery of cell death

Most evidence show that interdigital cells employ redundant molecular machinery for self-destruction. The predominant death mechanism in the interdigits is apoptosis (Garcia-Martinez *et al*., 1993), but syndactyly is never observed by blocking a single molecular component of the apoptotic machinery.Consistent, with the apoptotic nature of cell death, numerous caspases (cysteine-aspartic proteases), including initiator (caspase2, caspase 8, and caspase 9) and executioner caspases (caspase 3, caspase 6 and caspase 7; Fig. 2) are upregulated in the regressing interdigits (Nakanishi *et al*., 2001; Zuzarte-Luis *et al*., 2006), but interdigital cell death is not blocked in mice bearing single or compound caspase mutations, or deficient in caspase-activating adaptor protein, APAF-1 (Lakhani *et al*., 2006; Chautan *et al*., 1999; Kuida *et al*., 1998; Wang and Lenardo 2000; Nagasaka *et al*., 2009). It has been proposed that this apparent discrepancy is because interdigital mesenchyme die by necrosis if the apoptotic pathway is disrupted (Chautan *et al*., 1999).

Two distinct apoptotic pathways have been characterized in metazoa, the “extrinsic”, which is mediated by death receptors present on the cell surface, and the “intrinsic”, which results from the permeabilization of the mitochondria. Cytoplasmic release

of cytochrome C and nuclear translocation of the mitochondrial proapototic factor AIF are central events in the intrinsic pathway which have been observed in the interdigital dying cells (Zuzarte-Luis *et al*., 2006). Various pro- and anti-apoptotic factors involved in the permeabilization of the outer mitochondrial membrane, such as bcl-2, A1, bcl-x, Bag-1 (Carrio *et al*., 1996; Novack and Korsmeyer, 1994; Crocoll *et al*., 2002) show expression patterns consistent with their participation in interdigital cell death, but syndactyly is only observed in compound mutants afecting more than one of those pro-apoptotic factor. Syndactyly has been reported in triple-knockout, but not in single- or double-knockouts, of Bid, Bin and Puma (Ren *et al*., 2010); in double-knockout, but not in sigle-knockouts, of Bax and Bak (Lindsten *et al*., 2006; Ren *et al*., 2010); in double-knockout Bin and Bax, but not Bin and Bak (Hutcheson *et al*., 2005) and in double-knockout of Bin and Bmf (Hübner *et al*., 2010).

Lysosomes appear to be active partners of caspases during interdigital cell death. Lysosomal enzymes including cathepsins B, D, and L are up-regulated at the onset of interdigital cell death (Fig. 2B) and down-regulated in syndactyly (Zuzarte-Luis *et al*., 2007). Up-regulation is observed in TUNEL positive apoptotic cells and is accompanied by tissue acidification and up-regulation of acidic cytoplasmic DNAses (Montero *et al*., 2010). The involvement of lysosomes is not contradictory with the above described sydactylysm observed in mice deficient for regulators of mitochondrial permeability (Jäättelä *et al*., 2004). Various regulators of the mitochondrial membrane are also able to permeabilize lysosomes, and dying cells induced by a mild activation of lysosomes take an apoptotic, rather than necrotic, morphology (Boya *et al*., 2003, Boya and Kroemer, 2008). Furthermore, lysosomal enzymes are also able to activate caspases and induce mitochondrial permeabilization (Bidere *et al*., 2003). As observed for caspases, knockout of cathepsin genes lack syndactyly phenotype (Deussing *et al*., 1998) but interdigital cell death is inhibited *in vitro* by combined treatments with pan-caspase and lysosomal inhibitors (Zuzarte-Luis *et al*., 2007).

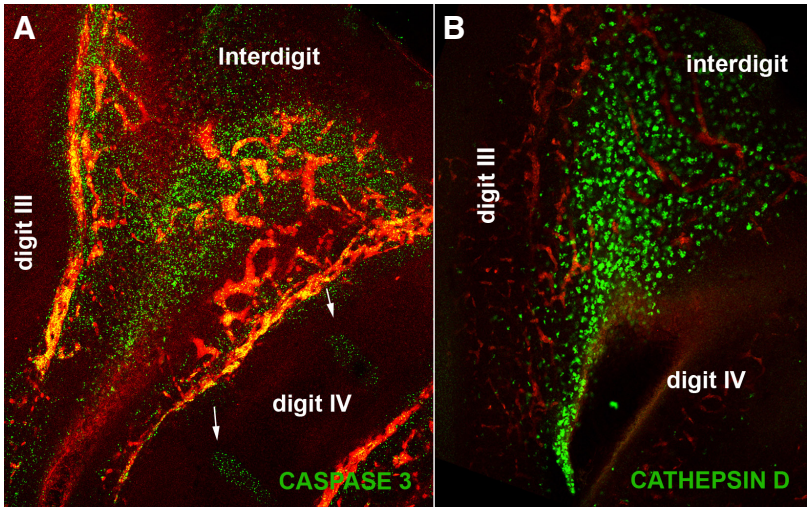

Fig. 2. Low magnification view of longitudinal sections of the third interdigit showning intense positivity for active caspase 3 (A) and cathepsin D (B) *consistent with the involvement of caspase-dependent apoptosis and lysosomal cell death in the regression of the interdigital tissue. Note transverse domains of caspase 3 in digit IV (arrows) which mark the developing interphalangeal joints.*

Morphological and immunohistochemical evidence indicates that, at advanced stages of interdigit tissue regression, autophagy also contributes to interdigital tissue regression (Montero *et al*., 2010), but this autophagic process has been related with a concomitant cell senescence process (see below; Storer *et al*., 2013).

In addition to the activation of apoptosis and lysosomes, oxidative stress might exert an important role in the establishment of the areas of interdigital cell death (Schnabel *et al*., 2006). The interdigital tissue, at difference of the digital regions, express low levels of genes encoding antioxidant enzymes such as superoxide dismutases, catalase and peroxidases (Schnabel *et al*., 2006; Shan *et al*., 2005), and reactive oxygen species increase in the interdigital tissue at the onset of cell death (Salas-Vidal *et al*., 1998). Furthermore, cell death is inhibited *in vitro* by antioxidant treatments (Salas-Vidal *et al*., 1998; Schnabel *et al*., 2006). Oxidative stress can damage most cell components, including mitochondrial and lysosomal membranes. Together these findings suggest a crosstalk between all the molecular players of interdigit regression. However, as observed for lysosomal enzymes and caspases, no syndactylous phenotype is appreciated in mice deficient in antioxidant enzymes (see Schnabel *et al*., 2006).

Interdigital tissue regression as a potential model of developmental senescence

Recent studies have shown that the molecular mechanisms characteristic of senescence also occur in a programmed manner during embryonic development (Nacher *et al*., 2006; Storer *et al*., 2013; Muñoz-Espin *et al*., 2013). There are not specific markers to define embryonic senescence processes but it is accepted that increased autophagy as detected by B-galactosidase activity, cell-cycle arrest, and matrix remodeling are indicators of physiological senescence. As described above, different degenerative cascades, including autophagy, are activated in the interdigital tissue (Montero *et al*., 2010; Storer *et al*., 2013). In addition, as will be analyzed below, interdigit remodeling is accopanied by growth arrest and matrix degradation.

Cytoskeletal disruption and growth arrest

Interdigit tissue regression is accompanied by inhibition of proliferation of interdigital cells (Fig. 1E; Toné and Tanaka, 1997). Some authors have emphasized that differential growth between the digits and interdigital regions exert a central influence in the process of digit individualization in the mouse (Salas-Vidal *et al*., 2001).

It has been shown that cycline M, the cyclin-dependent kinase inhibitor 1A (p21), and cyclin-dependent kinases 5 and 10 are regulated in the course of tissue regression. Cyclin-dependent kinase 5 is intensely expressed at protein level in the interdigital apoptotic cells (Ahuja *et al*., 1997a; Zhang *et al*., 1997) but its upregulation appears to be secondary rather than a cause of apoptosis (Ye *et al*., 2012). In humans, loss of function mutation of cyclin M causes STAR syndrome characterized by syndactylysm and other malformations due to inhibition of physiological cell death (Guen *et al*., 2013). Cyclin M activates cyclin-dependent kinase 10 and its loss of function up-regulates the activity of c-Raf.

The cyclin-dependent kinase inhibitor 1A, p21 is

expressed in the interdigital tissue (Vasey *et al*., 2011). p21 is a negative regulator of cell-cycle progression which binds and inhibits the complexes formed between cyclins and cyclin-dependent kinases. It is important to remark, that this factor is considered a key effector of cell senescence in developing systems (Muñoz-Espin, *et al*., 2013).

Members of the Rho family GTP-binding proteins including Rac1 and Cdc42, with have biological functions in the organization of the cytoskeleton have been also related with interdigital cell death (Suzuki *et al*., 2009; Aizawa *et al*., 2012). Silencing either Rac1 or Cdc42 in the limb mesoderm using a Cre-recombinase transgenic approach is followed by inhibition of interdigital cell death and subsequent syndactily. However, these syndactylous phenotype have been related with a down-regulation of BMP signaling rather than having a direct effect on the apoptotic process (Suzuki *et al*., 2009; Aizawa *et al*., 2012).

The role of other genes with a direct influence on cell proliferation have received relative little attention. Members of the Myc and the Growth arrest specific (Gas) gene families have been proposed to participate in the control of interdigit regression (Lee *et al*., 1999; 2001; Ota *et al*., 2007). According to those studies, Gas1 inhibits the function of c-Myc and arrest interdigital cells at G_0 (Lee *et al*., 2001) in turn, Gas2 is a cytoskeletal component which is cleaved by caspase 3 inducing changes in cell morphology such as cell fragmentation (Lee *et al*., 1999). The role of N-Myc in interdigit tissue regression is supported by the occurrence of syndactyly in humans haploinsuficient for N-Myc (van Bokhoven *et al*., 2005) and in mice with conditional deletion of N-Myc in the limb mesoderm (Ota *et al*., 2007). However, the role of N-Myc deficiency in the genesis of syndactyly has been related with a defect in the formation of interdigital tissue rather than inhibition of cell death.

Besides the role in cell proliferation, cytoskeletal alterations may also account for nuclear and cytoplasmic fragmentation which are central events of apoptotic cell death. It has been proposed, that the process of cell fragmentation might be modulated by protein crosslinking and autophagy through the activation of tissue transglutaminase in the dying cells via retinoic acid signaling (Moallem and Hales, 1996; Piacentini *et al*., 2000; D'Eletto *et al*., 2012).

Blood vessels and interdigital tissue regression

The regression of the interdigits involves a massive process of mesodermal cell death and also the physical elimination of the interdigital tissue. This fact implies that blood vessels and the extracellular matrix need to be eliminated during individualization of the digits. However, the regression of interdigital blood vessels occurs at considerable late stages of degeneration (Hurle *et al*., 1985), discarding that cell death was secondary to hypoxia subsequent to the loss of blood vessels. This interpretation is supported by the distribution of markers of hypoxia in the developing digits instead than the interdigital regions (Huang and Hales, 2012). It is well known that the differentiating cartilages lack blood vessels and hypoxia promotes chondrogenic differentiation of the embryonic limb skeletal progenitors (Amarilio *et al*., 2007). Consistent with those findings it has been recently proposed that interdigital vasculature exerts a positive influence on cell death promoting the local production of ractive oxygen species (Eshkar-Oren *et al*., 2015).

Extracellular matrix, cell adhesion, and interdigital cell death

Syndactyly is a comun phenotype in humans and mice deficient in extracellular matrix components. In the stages previous to cell death, the interdigital tisue contains a very complex extracellular matrix which requires to be removed in the course of interdigit regression (Hurle *et al*., 1994; Díaz-Mendoza *et al*., 2013). The interdigits also show regulated expression domains of various extracellular matrix proteases (Carrol *et al*., 1994; Zhao *et al*., 2010). Alterations in the extracellular matrix may influence cell death by disrupting cell adhesion, by modifiying availability of growth factors, or, by losing its scaffolding function. Syndactyly is observed in humans and/or mice deficient in laminin (Miner *et al*., 1998); nidogens (Böse *et al*., 2006); Fras1-related extracellular matrix gene 1 (Frem1; Smyth *et al*., 2004); SPARC(secreted protein acidic and rich in cysteine)-related modular calcium binding 1 (SMOC1; Okada *et al*., 2011); fibulin-1 (Debeer *et al*., 2002); fibrillin-2 (Arteaga-Solis *et al*., 2001; Chaudhry *et al*., 2001); versican degrading proteases ADAMTS (McCulloch *et al*., 2009; Nandadasa *et al*., 2014; Dubail *et al*., 2014).

In a complementary fashion, alterations of receptors responsible for anchoring cells to their surrounding matrix may be followed by disregulation of interdigit regression. It has been proposed the term "anoikis" to name cell death processes due to loss of cell anchoring to the extracellular matrix scaffold. Integrins are cell surface receptors that connect the ECM with the actin cytoskeleton. In the course of interdigit regression the extracellular matrix is broken down accompanied by alterations of downstream effectors of integrins such as decreased phosphorylation of the focal adhesion kinase FAK, and disintegration of paxillin which, via integrins, connect the cytoskeleton with the surrounding matrix (Zuzarte-Luis *et al*., 2006; Diaz-Mendoza *et al*., 2013). Together these findings point to an important role of cell-matrix adhesion in the onset of interdigital cell death, but syndactylous phenotypes caused by alteration of integrins are not very common and may reflect other roles of these receptors. Hence, althouh digit fusion is observed in mice with compound mutations of integrins α3 and α6 (De Arcangelis *et al*., 1999), syndactyly, in this case, appears to be due to abnormal development of the apical ectodermal ridge responsible for limb outgrowth. Other syndactylies accompaniying mutations of cell-cell adhesion molecules appear also due to disorganization of the ectodermal tissue (Brancati *et al*., 2010).

Transcription factors and miRNAs associated with interdigital cell death and senescence

The direct implication of transcription factors in the control of interdigital tissue regression is not fully understood. There are mouse mutants and human syndactylous syndromes, in which syndactyly appears to be secondary to defective growth rather than caused by a primary modification of the cell death process (Malik, 2012; Al-Qattan *et al*., 2013; Talamillo *et al*., 2010; Schatz and Ben-Arie, 2014). In other cases syndactyly is consequence of disruption of a signaling pathway regulated by the transcription factor. An example is the syndactly present in mice deficient in HoxA13 due to impairment in the synthesis of retinoic acid in the developing limb (Shou *et al*., 2013). In a similar fashion, the homeobox containing genes *Msx1* and *Msx2* are highly expressed in the undiferentiated limb mesoderm and interdigital tissue regression

is inhibited in mouse double knockout for these genes, but not in single *Msx1* or *Msx2* mutant mice (Lallemand *et al*., 2005). It has been proposed that Msx2 have a direct effect on mesodermal cell death via regulation of BMP signaling (Ferrari *et al*., 1998). In addition, the morphological exam of the double mutants indicates that syndactyly is due to the persistence of an active AER in the interdigits (Lallemand *et al*., 2005).

Members of the Iroquois (*Irx*) homeobox gene family are expressed in a regulated and differential fashion with predominant digit (*Irx1*; *Irx2*) and interdigit (*Irx3*; *Irx5*; *Irx6*) domains (Zülch *et al*., 2001; McDonald *et al*., 2010; Díaz-Hernández *et al*., 2013). Consistent with a function of this gene family in interdigital tissue regression, syndactyly is a characteristic phenotype of the mouse mutant Fused toes (Ft) caused by a deletion of 1.6 Mb of genomic sequences which include *Irx3*, *Irx5*, and *Irx6* (Peters *et al*., 2002). Remarkably, Ft mice show abnormal patterns of expression of *Bmp4*, *Fgf8* and the transcription factor *Id3* in the developing autopod (Heymer and Rüther, 1999).

Of particular importance for the consolidation of the new hypothesis proposing the occurrence of an embryonic senescence process (Nacher *et al*., 2006; Storer *et al*., 2013; Muñoz-Espin *et al*., 2013) is the high expression of the transcription factor c-Rel in the regressing interdigits (Abbadie *et al*., 1993) and the presence of syndactyly in mice deficient in IKKα (Hu *et al*., 1999). The Rel/NF-kB transcription factor family control cell-cycle, apoptosis, oxidative stress, immunity and inflammation and are considered central factors in aging and senescence (Gosselin and Abbadie, 2003).

It has been recently proposed that members of the AP-1 family of transcrition factors are major players in the control of limb programmed cell death. Several members of the family, including *c-maf*, *Nfe211*, *Nfe212*, *Xbp1* and *MafB*, are expressed in the areas of programmed cell death (Lecoin *et al*., 2004; Suda *et al*., 2014). Functional studies of various members of the family have identified MafB as an important factor in the control of limb programmed cell death in the route of ROS-dependent apoptosis (Suda *et al*., 2014). According to this study, in the embryonic chick, but not in mouse, heterodimers of MafB/cFos are inhibitors of cell death whereas heterodimers MafB/cJun promote apoptosis.

MicroRNAs (miRNAs) are small (20-25 nucleotide-long) non-coding RNAs implicated in most biological processes by negatively regulating target specific genes through translational repression and/or by inducing mRNA degradation. Several miRNAs has been implicated in the control of embryonic programmed cell death in *Drosophila* (Ge *et al*., 2012), but no specific miRNAs has been yet involved in the control of interdigit tissue regression in vertebrates. However, knockdown of *Dicer*, a RNAase required for the formation of miRNAs, directed to the entire limb mesoderm caused intensification of mesodermal cell death suggesting the existence of miRNAs involved in cell survival (Harfe *et al*., 2005). It can be expected important advances in this topic in the near future.

Concluding remarks

Despite being a subject studied for many years through very different methodological approaches, we are still far from full understanding the molecular basis and the biological significance of interdigital cell death.

It is clear that various molecular mechanisms participate in this process of programmed cell death (caspases, lysosomes, autophagy) but we still need to clarify the precise contribution of each one, and to unravel how them interplay to ensure the separation of the digits when a dead route is blocked.

In a similar fashion we need to clarify the significance of developmental senescence in the regression of the interdigital tissue and its functional relation with cell death if they have it.

A emerging function of cell death in developmental systems and in tumours is to provide short and/or long-range signals to the neighbouring tissue able to modulate growth and differentiation (Boland *et al*., 2013; Vriz *et al*., 2014; Hurle 2014). Hence, we need to clarify if interdigital cell death is only a destructive process or if it is the source of signals for other tissues.

Although, a large body of information has been accumulated about the signaling regulators of the death machinery (FGFs; BMPs; RA; ROS), we still lack a comprehensive view of this important issue.

Advances in these and other topics related with interdigital cell death could provide insights of relevance to understand degenerative diseases in human pathology and to develop strategies to improve cancer treatment.

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