Focus on ART

Deadly decisions: the role of genes regulating programmed cell death in human preimplantation embryo development

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Abstract

Human preimplantation embryo development is prone to high rates of early embryo wastage, particularly under current in vitro culture conditions. There are many possible underlying causes for embryo demise, including DNA damage, poor embryo metabolism and the effect of suboptimal culture media, all of which could result in an imbalance in gene expression and the failed execution of basic embryonic decisions. In view of the complex interactions involved in embryo development, a thorough understanding of these parameters is essential to improving embryo quality. An increasing body of evidence indicates that cell fate (i.e. survival/differentiation or death) is determined by the outcome of specific intracellular interactions between pro- and anti-apoptotic proteins, many of which are expressed during oocyte and preimplantation embryo development. The recent availability of mutant mice lacking expression of various genes involved in the regulation of cell survival has enabled rapid progress towards identifying those molecules that are functionally important for normal oocyte and preimplantation embryo development. In this review we will discuss the current understanding of the regulation of cell death gene expression during preimplantation embryo development, with a focus on human embryology and a discussion of animal models where appropriate.

In most cases these anuclear cellular fragments will contain organelles and other cellular proteins (Jurisicova et al. 1996, Van Blerkom et al. 2002). The average blastomere size of fragmented embryos is significantly reduced by the degree of fragmentation (Hnida et al. 2004), leading to decreased cellular content and thus exerting a negative effect on the future mass of embryo. Embryo fragmentation, when accompanied by embryo cleavage and further development, is not by itself developmentally lethal, as in some instances fragments were resorbed or their presence did not impair embryo cleavage (Van Blerkom et al. 2001). Previously, it has been reported that fragment removal can improve the developmental potential of affected embryos (Alikani et al. 1999), as fragments may restrict and compromise the process of compaction. In our hands, fragment removal does not help embryos in which the fragments encompass more than 30% of the total embryo volume, as these embryos continue to fragment after treatment (A Jurisicova, unpublished observations). Thus it is possible that fragmentation is a normal coping mechanism of the embryo, and that it is the location or extent of embryo fragmentation that can negatively influence developmental potential (Antczak & Van Blerkom 1999).

One mechanism that has been proposed to be the cause of cytoplasmic fragmentation is the process of oncosis (Van Blerkom et al. 2001). This is a form of cell death characterized by the formation of organelle-deprived cytoplasmic blebs that can either be resorbed by the cell or detach and float away under conditions of oxygen deprivation (Majno & Joris 1995). As embryos are cultured in an oxygen-rich environment, it has been proposed that similar morphological changes could occur in blastomeres that failed to inherit a mitochondrial complement capable of supporting its energy demands (Van Blerkom et al. 2001). Mitochondrial membrane potential and energy...

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**Figure 1** The fate of human and murine embryos. The top panel represents successful development from the zygote stage to the blastocyst stage. The timing of these events is different between species, human embryos taking a longer time to cleave to the blastocyst stage than murine embryos, but all embryos follow the same sequence of events. The middle panel represents two-cell arrest, the most frequently observed developmental defect in murine embryos. After arrest, the two-cell embryo will degenerate, experience mild to moderate midline fragmentation, or fragment completely. The bottom panel represents human embryos, which often experience developmental difficulties between the four- and eight-cell stages. Embryos could then arrest, experience fragmentation coupled with the loss of blastomeres or fragmentation without the loss of blastomeres. Further discussion on the mechanisms and pathways involved in these modes of cell death can be found throughout the text.
production in preimplantation embryos have recently been the focus of much study and will be discussed by Van Blerkom in more detail elsewhere in this issue of Reproduction.

Another hypothesis credits embryo fragmentation to the activation of programmed cell death pathways, especially in instances in which fragmentation is accompanied by cleavage arrest (Jurisicova et al. 1996) or the complete destruction of all blastomeres (Yang et al. 1998). It is clear that there are several time points when in vitro produced embryos are particularly susceptible to cellular fragmentation, arrest and apoptosis. This is true not only for humans, but also for bovines, as recent data confirmed that the onset of apoptosis seems to be developmentally regulated in a stage-specific manner, but discrete features of the apoptotic process may be differentially regulated and independently modulated by the mode of embryo production (Gjorret et al. 2003). The underlying molecular mechanisms responsible for these embryonic defects remain to be determined.

A further phenomenon observed in embryos in almost all mammalian species is that of developmental arrest. The mechanisms leading to developmental arrest are unclear, yet, interestingly, the embryonic stage at which transcription-dependent proteins are first detected in the embryo coincides with the peak incidence of cleavage arrest in most species, suggesting that cleavage arrest could also be caused by a failure or delay in the onset of transcription (Telford et al. 1990). One cause of developmental arrest may be chromosomal abnormalities (Bongo et al. 1991, Munne et al. 1995), which could contribute to a disruption in mRNA transcripts and ensuing protein synthesis, leading to interference with normal embryo development (Devreker & Englert 2000). There is no simple explanation for developmental arrest, as there is a broad spectrum of cellular defects that occur during cleavage arrest, such as redistribution of mitochondria (Muggleton-Harris & Brown 1988, Acton et al. 2004) and abnormal subcellular localization of some proteins (Ohashi et al. 2001), including Bcl-2 family members (Jurisicova et al. 2003).

Maternal effects
Progression of fertilized mammalian oocytes through cleavage, blastocyst formation and implantation is dependent on the successful implementation of specific genetic and developmental programmes. It is not surprising that variability in oocyte quality has a profound impact on developmental competence and contributes to the high incidence of embryonic wastage observed during IVF procedures, as it was estimated that the factors predisposing an embryo to arrest are determined at or even before the zygote stage (Hardy et al. 2001). These factors are also regulated by maternal age, as increased frequencies of embryo fragmentation were observed during early cleavage stages in embryos from older IVF patients (Ziebe et al. 1997), and by an increased cell death index of blastocysts from older female mice (Jurisicova et al. 1998b). Maternal factors that could contribute to abnormal preimplantation development include cytoplasmic mRNAs, proteins, antioxidants, small peptides, lipids and organelles. Several recent studies have shown that oocyte survival is controlled by a number of cell death regulatory molecules responsible for either activation or suppression of cell death (reviewed by Tilly (2001)). It is also evident that deposition of maternal products in the form of transcripts, proteins and organelles dramatically influences the success of preimplantation embryo development (Muggleton-Harris & Brown 1988, Gandolfi & Gandolfi 2001). As many cell survival/death regulatory molecules are among these maternally stored transcripts in both mice and humans (Jurisicova et al. 1998b, Exley et al. 1999, Metcalfe et al. 2004), it is likely that they may contribute to developmental competence of a zygote. Cell survival molecules, when inadequately accumulated during oogenesis (either too few suppressors or too many activators; Fig. 3), or change during the ageing of oocytes in vitro (Metcalfe et al. 2003) or in vivo (Jurisicova et al. 2002), may lead to survival disequilibria, resulting in zygote or early embryo demise.

The ability to pass through the transition to zygotic control in vitro is believed to be a function of the cytoplasmic components of the oocyte and is not a reflection of the newly formed embryonic nucleus (Goddard & Pratt 1983). There must therefore be a cytoplasmic component(s) that supports development through the blocking stage and this component(s) must be lacking or non-functional in those embryos that do arrest. At the present time it is unclear which components of the ooplasm are responsible for this rescue effect, but in recent studies ooplasmic injection of isolated mitochondria was sufficient to rescue oocyte fragmentation in mice (Perez et al. 1999, Acton et al. 2001), making these cellular organelles likely candidates for mediating the positive action of transplanted ooplasm.

Genetic regulation of cell death in embryonic development
Programmed cell death is a precisely coordinated set of events dependent upon the actions and interactions of at least 100 gene products that either repress or activate the process of cellular self-destruction (for reviews, see Green & Reed (1998), Vaux & Korsmeyer (1999), Adams & Cory (2001)). The most widely studied of these genes are members of the Bcl-2 family, which can be subdivided into two groups: cell death suppressors (Bcl-2, Bcl-xL, Bcl-w, Mcl-1, A1) and cell death inducers (Bax, Bak, Mtd, BH3-only proteins) (Fig. 2). Cell death inducers can be further split into multidomain (containing BH1, BH2 and BH3 domains such as Bax, Bak, Mtd) and single domain (containing only BH3 domains, such as Bad, Bim, Hrk, Bid). Members of this gene family act through a complex
network of homo- and heterodimers (Adams & Cory 1998). The fate of the cell is determined by the ability of the cell death suppressors to sequester, and thus neutralize, the actions of the cell death inducers. Hence, the relative concentration of pro- and anti-apoptotic proteins determines whether a cell lives or dies (Antonsson & Martinou 2000). Many Bcl-2 family members localize permanently or transiently to mitochondrial membranes, where they are believed either to regulate, or to form, mitochondrial pores. Opening of these pores results in changes in mitochondrial membrane potential, accompanied by the release of several death-inducing factors from the mitochondrion into the cytoplasm.

On the basis of research on apoptosis in different cell types using various cell death triggers, it has become clear that there are at least two independent pathways leading to activation of apoptosis: intrinsic, mitochondrially driven, and extrinsic, without mitochondrial involvement. These pathways are not completely independent, as cross-talk and an augmentation loop exist between the intrinsic and extrinsic pathways (for reviews see Desagher & Martinou (2000), Hengartner (2000)). The finite control of cell death seems to be highly regulated by the interplay between Bcl-2 family members, and these interactions are purported to have an important role in embryo survival.

The relative rates of expression of the genes of several Bcl-2 family members in human MII oocytes were assessed through quantitative dot–blot RT-PCR, including pro-apoptotic members Bax and hara-kiri and anti-apoptotic members Bag-1, Bcl-x and Mcl-1. From the total amount of gene expression in each oocyte, the contribution from each family member was established and the results presented as pie graphs, in which each graph represents a single oocyte (Fig. 3). According to the pattern of expression profiles, the oocytes were grouped into three categories: those shifted towards cell death as a result of an imbalance shifted towards pro-apoptotic

**Figure 2** Interactions between Bcl-2 family members. Three main subfamilies of the Bcl-2 family are represented: pro-apoptotic BH3 proteins (range of orange squares), anti-apoptotic proteins (range of green circles) and pro-apoptotic channel forming proteins (range of blue ovals). The changes in shading and colour gradient indicate differences in binding partners and affinities between members in the pro-apoptotic subfamilies with members of the antiapoptotic subfamily. **Proapoptotic BH3 proteins**: Puma and Bim bind to all antiapoptotic members equally; Bik, Hrk and Bid bind to Bcl-xL, Bcl-w and Bfl1/A1; Bmf and Bad bind to Bcl-2, Bcl-xL and Bcl-w; Noxa binds to Bfl1/A1 and Mcl-1. **Proapoptotic channel forming proteins**: Bax and Bak bind to Bcl-2, Bcl-xL and Bcl-w, whereas Mtd/Bok binds to Bfl1/A1 and Mcl-1.

**Figure 3** Distribution of five pro- and anti-apoptotic genes in 15 individual MII stage human unfertilized oocytes obtained from several patients. The expression of Bag-1 (yellow), Bax (orange), Bcl-x (pale blue), Hrk (red) and Mcl-1 (dark blue) was determined using quantitative dot–blot RT-PCR. The total amount of gene expression was calculated for these pro-apoptotic (orange and red) and anti-apoptotic (yellow, pale blue and dark blue) genes, each pie piece representing the contribution for that particular gene. The oocytes were then grouped on the basis of their expression profiles. (A) Oocytes with a high level of pro-apoptotic gene expression, possessing an imbalance towards programmed cell death. (B) Oocytes with almost equal distribution of pro- and anti-apoptotic factors suggestive of a balance between survival and death molecule contributions. (C) Oocytes with an excess of anti-apoptotic factors indicative of a strong survival pathway.
genes, those predisposed to survival because of a strong level of anti-apoptotic gene expression, and those with a balance in expression of pro- and anti-apoptotic genes. These results suggest that there are inherent differences in the level of gene expression for these gene families, and that the balance between the expression of pro- and anti-apoptotic genes may be a central determinant of embryo survival.

**Anti-apoptotic Bcl-2 family members**

This group of proteins are the most widely studied genes during both oocyte and preimplantation embryo development and consists of several functionally overlapping homologues. Bcl-2, a founding member of this pro-survival gene family, had been reported to be expressed by many (Spanos et al. 2002), but not all, human embryos before the four-cell stage (Liu et al. 2000, Metcalfe et al. 2004). Immunolocalization confirmed expression of Bcl-2 in both good and poor quality embryos, but a subtle increase in accumulation of this protein was observed in the cellular fragments of some embryos (Spanos et al. 2002, Metcalfe et al. 2004).

In our hands, Mcl-1 is the most abundantly and consistently expressed Bcl-2 family member detected during the early cleavage stages (Jurisicova et al. 2003). These data are partially consistent with those of Metcalfe et al. (2004), who reported more variable expression between two- to four-cell stages, followed by equally variable Mcl-1 protein localization at the blastocyst stage. The lack of Mcl-1 expression observed in human blastocysts is intriguing, as Mcl-1 is the only pro-survival Bcl-2 family member of which the disruption results in preimplantation embryonic lethality.

Another important family member likely to regulate successful progression through preimplantation embryo development is Bcl-x. Although its RNA is less abundant than that of Mcl-1 (Jurisicova et al. 2003, Metcalfe et al. 2004), its protein is expressed in high levels during both early and late cleavage stages (Antczak & Van Blerkom 1999, Metcalfe et al. 2004). Using an antisense mRNA approach in murine embryos, we observed that the classical Bcl-xL isoform is not required for progression of eight-cell embryos through the blastocyst stage. In contrast, up-regulation of the alternative splicing form Bcl-xS renders blastomeres more susceptible to death and cell cycle arrest (Jurisicova et al. 2001), as previously observed in other cell types (Fridman et al. 1998, Lindenboim et al. 2000), suggesting that this gene may be an important component of the regulation of embryonic fate. Among other anti-apoptotic Bcl-2 family members, Bcl-w and Bfl-1/A1 are only weakly and inconsistently expressed (Jurisicova et al. 1998a, Metcalfe et al. 2004) and thus are unlikely to play a part in regulating early embryo demise. Moreover, their disruption in mouse does not interfere with progression of embryonic development (Print et al. 1998, Ross et al. 1998).

**Channel-forming pro-apoptotic family members**

Expression of the pro-apoptotic molecule Bax has been studied frequently and this gene appears to be consistently expressed throughout preimplantation development, with only a marginal increase in accumulation of the protein in poor quality embryos (Spanos et al. 2002, Metcalfe et al. 2004). Interestingly, at least one study described Bax immunoreactivity to be restricted to polarized domains of some cellular fragments (Antczak & Van Blerkom 1999), suggesting that this protein may participate in the restriction of subcellular compartments and possibly the removal of unwanted or harmful organelles or other cytoplasmic components. It is now known that both Bax and Bad are posttranslationally modified. Bad, having high binding affinity for Bcl-xL and Bcl-2, displaces Bax from Bcl-x/Bax complexes and allows Bax to homodimerize, change conformation, translocate to mitochondria and induce cell death (Capano & Crompton 2002). Bad itself is regulated by phosphorylation, which prevents its binding to Bcl-xL, and increases the affinity of Bad for the 14-3-3 sequestering protein, the binding of which inactivates the pro-apoptotic potential of Bad (Zha et al. 1996). It remains to be determined whether Bad and Bax are expressed during preimplantation development in their active or inactive conformation, and if these post-translational modifications, rather than transcriptional regulation, may explain the regulation of cellular fragmentation.

Two other channel-forming pro-apoptotic molecules, Bak and Bok/Mtd, have only recently been assessed (Metcalfe et al. 2004). Both are expressed, albeit more variably than Bax, throughout preimplantation development, Bok being more abundant during early and Bak during later embryonic stages. Interestingly, preliminary data suggest that fragmentation at the four-cell stage may be associated with increased expression of Bak (Metcalfe et al. 2004); however, this needs to be confirmed by immunolocalization.

**BH3 family members**

Very little is known about the expression of BH3-only molecules during preimplantation embryo development. This is clearly an unexplored field for new research, as these are the molecules that are the initiators of cell survival decisions and are responsive to cellular needs and requirements. Also, many of these genes are transcriptionally regulated (Puthalakath & Strasser 2002), making them the most suitable and meaningful candidates for detection via RT-PCR expression studies, a frequently used method for studying preimplantation embryo expression profiles.

From the few genes that have been studied, it is apparent that BH3 family members have a developmentally regulated pattern of expression with Bid being the least frequently expressed (Metcalfe et al. 2004). Both Bik and Bad are abundant during both early and later preimplantation stages, with some subtle differences between fragmented and non-fragmented embryos (Metcalfe et al. 2004).
2004). These results contrast with the lack of Bad expression by early cleavage stage embryos previously reported by Spanos et al. (2002) who detected Bad expression by RT-PCR in very few two- to four-cell embryos (two out of nine), but frequently around the time of compaction and at the blastocyst stage.

We have recently obtained descriptive correlative data suggesting that Hrk may be involved in the regulation of survival of preimplantation human embryos, as both its transcript and its protein concentrations were increased in a subset of arrested fragmented four- and eight-cell embryos (Jurisicova et al. 2003). It remains to be established what regulates the expression of this gene in preimplantation embryos and how this relates to embryo demise.

Extrinsic pathway

Involvement of the extrinsic pathway in the regulation of human preimplantation embryo development has been investigated by two groups. Kawamura et al. (2001) reported that human embryos at the two- and four-cell stage express Fas ligand, but this was not observed by Liu et al. (2000), as Fas ligand transcript was not detected in any of the viable or fragmented embryos they studied. Interestingly, the report by Kawamura et al. (2001) suggested a bimodal switch in death receptor expression between the two- and four-cell stages. Tumour necrosis factor receptor was detected at the two-cell stage, but Fas was detected only at the four-cell stage, and cellular fragmentation did not alter its expression profile. These two reports describe contradictory results and further investigation into the role of extrinsic signals in preimplantation embryo cell death is required.

Caspase requirement for cellular arrest and fragmentation

Caspases are a family of cysteine-dependent aspartate-directed proteases that are present in an inactivezymogen form before being cleaved to an active form. Upon activation, caspases cleave a variety of intracellular polypeptides, including, but not limited to, structural elements of the cytoplasm and nucleus. This leads to disruption of survival pathways and the disassembly of important architectural components of the cell, contributing to the well-documented morphological and biochemical changes that characterize apoptotic cell death (Earnshaw et al. 1999).

Caspases had been extensively studied, and yet their role in early embryo development remains unclear. Current studies have used fluorescent caspase substrates, which become trapped upon activation within a cell. Although a broad range of caspase inhibitors are commercially available, no systematic studies beyond the use of caspase 3/7 substrates have been reported. Martinez et al. (2002), who used the broad range caspase substrate, FITC-VAD, detected active caspases frequently in cellular fragments, but activity was only occasionally found in blastomeres of poor quality embryos. The percentage of caspase-positive fragments was increased in embryos with multinucleated blastomeres and this was unrelated to embryo morphology (Martinez et al. 2002).

These data also agree with reports by Spanos et al. (2002) and Van Blerkom et al. (2001), as in those studies caspase activity was observed only in arrested embryos or after compaction at the morula and blastocyst stages, frequently associated with apoptotic nuclei (Spanos et al. 2002). The group of arrested and fragmented embryos studied by our laboratory showed that active caspase 3/7 like activity was associated with cellular fragments containing nuclear material or in blastomeres with condensed chromatin, while the caspase 3 transcript was detected at all embryonic stages, and even increased in some fragmented embryos (Jurisicova et al. 2003). Other studies, however, have failed to demonstrate a strong correlation between caspase activation and embryo fragmentation. Attempts to reduce murine embryo fragmentation with caspase inhibitors (Xu et al. 2001) and a failure consistently to detect apoptotic markers in cytoplasmic embryo fragments from human embryos (Antczak & Van Blerkom 1999, Spanos et al. 2002) suggests that caspase independent mechanisms may play a part in this process, as has been observed in some cell types (Donovan & Cotter 2004).

Mouse models of early embryo loss

Animal models have a key role in investigations of the process of embryogenesis, in which murine embryos have been extensively studied in an effort to elucidate pathways and mechanisms common across mammalian species. Comparative gene expression studies of mouse and human preimplantation embryo development have identified striking similarities in the conservation of the molecular and cellular framework and in the execution of developmental decisions, making the mouse an excellent model for the study of human development. The mouse model is further attractive as it allows for easy genetic manipulation and induction of various pathological conditions.

Zygote arrest gene

Zygotic arrest gene (ZAR) is a putative transcription activator with a prototypical PHD domain. Female mice carrying two disrupted ZAR copies are infertile as a result of the inability of the embryo to proceed through the first cleavage division. Cleavage arrest is followed by cytoplasmic fragmentation and complete disintegration of the embryo (Wu et al. 2003). The functional importance of ZAR in the human is currently unclear, but may be clinically inconsequential, as zygote arrest is rare in the human and accounts for less than 1% of embryonic loss.

Mater

Mater is a protein that was originally identified in a screen for genes responsible for autoimmune oophoritis (Tong...
et al. 2000b). On the basis of a sequence homology, this oocyte-specific product belongs to a newly discovered group of NALP proteins (Tschopp et al. 2003). The majority of NALP proteins are adaptors involved in the regulation of inflammation and have a marginal effect on the regulation of apoptosis, but the precise function of Mater remains unclear. Oocytes ovulated by females carrying a Mater mutation are capable of fertilization and proceed through the first cleavage; however, this is followed by arrest and cellular fragmentation. Activation of the embryonic genome is impaired, accompanied by a decrease in protein synthesis (Tong et al. 2000a). A recent report on the localization of Mater protein revealed that its primary site is in the mitochondrion and nuclear envel-opes (Tong et al. 2004). The specific function of Mater protein and its relevance to the quality of human embryos remains to be established.

**Heat shock factor-1**

Heat shock factor (HSF)-1 is a protein responsible for the induction of expression of the heat shock protein (HSP) gene under both physiological and cell stress conditions. HSPs have numerous roles in cell function, including modulation of protein activity, regulation of protein degradation and transport across organelles, in addition to ensuring correct protein folding (Takayama et al. 2003). More recently, HSPs were implicated in the regulation of cell death, as Hsp 70 had been shown to prevent both caspase dependent and caspase independent cell death (Ravagnan et al. 2001). In addition, Hsp 27 is capable of interfering with cytochrome-c mediated apoptosome activation, leading to inhibition of the mitochondrial death pathway (Bruey et al. 2000). Both of these HSPs are regulated by HSF-1 (Sistonen et al. 1994, Cooper et al. 2000).

HSF-1 is expressed in high levels during preimplantation embryo development (Christians et al. 1997). Interestingly, embryos lacking a functional maternal copy of this gene during oocyte development arrest most frequently between the one- and three-cell stages, followed by partial cellular fragmentation. Zygote development is unimpaired and is followed by a delay in the onset of embryonic genome transcription (Christians et al. 2000). The connection of HSF-1 with other established cell death pathways in these embryos has not been explored.

**Myeloid cell leukaemia-1**

Myeloid cell leukaemia-1 (Mcl-1) is the only pro-survival gene the disruption of which in the mouse leads to early embryonic lethality. Blastocysts lacking Mcl-1 have been detected at day 4 in the reproductive tract, but these embryos failed to implant and were also unable to produce viable blastocyst outgrowths in vitro (Rinkenberger et al. 2000). No increase in apoptosis was observed in these blastocysts and the embryo loss could not be rescued by disruption of Bax or p53, suggesting that Mcl-1 may not act through the classical apoptotic pathway. The second, more subtle, phenotype relates to embryonic arrest, as embryos flushed at earlier stages arrest shortly after being placed in culture (Rinkenberger et al. 2000). This response is probably a result of embryonic stress encountered during in vitro culture. These findings clearly indicate an involvement of Mcl-1 in the regulation of cell cycle progression and place this molecule among a handful of proteins functionally important in the developmental decisions made by the early embryo.

**Defender against apoptosis-1**

Defender against apoptosis-1 (Dad-1) is an evolutionary highly conserved enzyme, which catalyses the transfer of preassembled high mannose oligosaccharides onto specific asparagine residues of nascent polypeptides (Sanjay et al. 1998). This enzyme is capable of interacting with and modifying Mcl-1 (Makishima et al. 2000). Thus it is not surprising that disruption of this gene leads to embryonic lethality at the blastocyst stage (Nishii et al. 1999, Brewster et al. 2000), phenocopying the Mcl-1 knockout mouse. Dad-1 mutants, however, display an increased rate of cell death in the inner cell mass both in vitro and in vivo, suggesting that this enzyme may have modification targets other than Mcl-1.

**Bax and p53**

Disruption of Bax and p53 does not result in any known developmental abnormalities observed during the preimplantation period under physiological conditions, but their embryonic phenotypes appear more obvious upon challenge. Lack of either of these genes provides a rescue of blastocysts from cell death induced by hyperglycaemia (Moley et al. 1998, Keim et al. 2001). Suppression of hyperglycaemia-induced cell death is dependent on both Bax and p53, and is accompanied by a decrease in the number of embryo resorptions, directly implicating cell death in postimplantation embryonic loss (Moley 2001).

**Survivin**

Survivin, an IAP family member (Vaux & Silke 2003a, b), has a pattern of expression restricted to a subset of tissues including gametes, embryos and some cancers (Kobayashi et al. 1999). Depending on the cell type and conditions used, this gene has been implicated both in the regulation of cell cycle progression and in the inhibition of apoptosis (Altieri 2003). Disruption of survivin leads to embryonic lethality between the morula and blastocyst stages, leading to formation of giant nuclei, decreased cell number, impaired mitotic figures (Uren et al. 2000) and increased apoptosis (Kawamura et al. 2003). Thus the mode of death in these embryos is more consistent with mitotic catastrophe, which can not be rescued by inactivation of caspases or other cell death genes (Okada et al. 2004). At the present time it is unknown whether
alterations in the expression of survivin can contribute to the broad range of nuclear defects described in human embryos.

**Endonuclease G**

Endonuclease G is a recently identified nuclear-encoded mitochondrial protein, reported to be important for both nuclear DNA fragmentation during apoptosis and mitochondrial DNA replication (Li et al. 2001). On induction of apoptosis, it is released from the mitochondria and translocates to the nucleus, where it is involved in the oligonucleosomal degradation of DNA through both caspase-dependent and caspase-independent mechanisms ( Lorenzo & Susin 2004). Disruption of the EndoG gene results in embryonic lethality during the morula to blastocyst transition (Zhang et al. 2003). Heterozygote animals show distinct phenotypes that could be attributed to a cell death imbalance, but oocytes from these females contained a similar number of mitochondria compared with wild-type controls, suggesting that a single copy of EndoG is sufficient to maintain mitochondrial DNA replication. These findings indicate that EndoG is essential during early embryogenesis, but its relationship to human embryo wastage has not yet been investigated.

**Conclusions and perspectives**

From all the references cited, it is clear that genes governing cell death pathways are involved in the regulation of preimplantation embryo survival. This is evident, not only from descriptive observational studies, but also from functional interventional animal experiments. Although some types of cell death in preimplantation embryos, especially during the postcompaction stages, are characteristic of the classical apoptotic mode of death, others display morphologies that are not fully consistent with classical apoptosis. To complicate the issue further, added to this is the difficulty in obtaining high quality, developmentally competent human embryos for study. It is clear that the majority of human embryos donated for research are suffering from various cellular defects, including abnormal nuclear ploidy, altered genomic imprinting and variable responses to culture conditions, and thus are likely to be a poor reflection of the true molecular pathways found in healthy embryos. Further studies with appropriate controls comparing ‘good’ with ‘poor’ quality embryos are needed to provide an accurate indication of preimplantation embryo development.

Current studies support the hypothesis that cellular fragmentation in a subset of human preimplantation embryos could be regulated by certain components of a genetic programme of cell death. As blastomeres are among the biggest cells in the human body, we speculate that they utilize some of the cell death machinery to remove the areas of cytoplasm, also called ‘highly polarized domains’, that may prove detrimental for future development, as proposed previously (Antczak & Van Blerkom 1999). Of particular interest to apoptosis is the polarized distribution of Bax, Bcl-x and Hrk, indicating that temporal changes in gene expression alone cannot be used to predict apoptosis, as spatial expression may also play a significant part. It is therefore feasible to propose that spatial localization could be an adaptive mechanism of the oocyte or embryo to sequester certain gene products. The formation of embryo fragments could be a survival mechanism in which pro-apoptotic gene products are removed from the embryo in an attempt to rescue the embryo from the damaging effects of an imbalance in pro- and anti-apoptotic gene products. Alternatively, as a result of fragmentation and selective removal of cytoplasm, an imbalance in protein expression in the affected blastomeres could result in the induction of programmed cell death pathways (Antczak & Van Blerkom 1999). Moreover, this process could be mediated by caspase, as caspase-positive fragments have been observed to detach from healthy blastomeres that subsequently underwent mitotic division in culture (Martinez et al. 2002). If attempted rescue is successful and the remaining blastomeres are not further challenged, embryo cleavage and development will proceed. If this rescue attempt fails and the remaining blastomeres experience other setbacks, the embryo will undergo arrest, followed by the execution of a caspase-dependent apoptotic programme in the remaining blastomeres.

Secondarily, we would like to propose that embryonic arrest in vitro is accompanied by cellular events that are partially consistent with programmed cell death type II or an autophagic mode of death (Levine & Klionsky 2004). It appears that, under certain conditions, when the classical apoptotic programme is heavily suppressed, cells can prolong their survival by using this catabolic pathway to produce energy, and only upon complete exhaustion of their intracellular energy supply do they succumb to autophagic death (Gozuacık & Kimchi 2004). The morphological hallmark of arrested embryos observed in vitro – the partial shrinking of blastomeres and formation of cytoplasmic vesicles – may be the result of an attempt of the cells to save themselves by catabolizing their own cytoplasm. Thus, if the embryo possesses a battery of transcripts that would strongly promote survival but finds itself in inadequate culture conditions, it may trigger the autophagic mode of death. The partial destruction of mitochondria, a process regulated by the Bcl family members via an autophagic mode of cell death (Tolkovsky et al. 2002), could also contribute to embryo demise. In contrast, an embryo originating from an oocyte with imbalanced cell death machinery or a zygote that fails to execute basic embryonic decisions would opt for the rapid apoptotic mode of death, accompanied by cellular fragmentation. These two modes of cell death may be intertwined and only further explorations of the genetic pathways involved in the regulation of death decisions will establish if these modes of death are truly genetically regulated and relevant during mammalian preimplantation embryo development.
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