Primed to Perish: Heightened Mitochondrial Priming Explains hESC Apoptosis Sensitivity

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Human embryonic stem cells (hESCs) are hypersensitive to apoptotic stimuli, though the underlying mechanisms are poorly characterized. In this issue of *Cell Stem Cell*, Liu et al. (2013) report that mitochondria of human ESCs exist in an apoptosis-prone state, ready to act as cellular executioners upon detecting DNA damage.

Anti- and proapoptotic factors maintain the proper balance between protecting cells from premature elimination and protecting the organism when individual cells go haywire. Exogenous stresses, which can cause DNA double-strand breaks and loss of genome integrity, challenge this balance and force a life-or-death decision. Human embryonic stem cells (hESCs) are exceptionally sensitive to DNA damage, compared to differentiated cells, and quickly undergo apoptosis in response to stress rather than attempting repair of a compromised genome. The mechanisms behind this apoptosis-prone state, and the role of the tumor suppressor p53 in this process, are not well understood.

In this issue of *Cell Stem Cell*, Liu and colleagues (2013) demonstrate that this susceptibility of hESCs to undergo apoptosis is due to a high state of "mitochondrial priming": a lowered cell-intrinsic threshold for initiating apoptosis, based on the balance of pro- and antiapoptotic proteins. Antiapoptotic factors, such as BCL-X_L, protect the mitochondria from permeabilisation, allowing the cell to repair the DNA damage. In contrast, proapoptotic factors, such as Bak or Bax, generate pores in the mitochondrial membrane, which is an irreversible step in the termination of the runaway cell (Youle and Strasser, 2008). The balance between these two responses determines the intrinsic apoptotic threshold. The tumor suppressor protein p53 is an important mediator of the cell's response to DNA damage (Vogelstein et al., 2000; Vousden and Lane, 2007); DNA damage induces p53 and activates the transcription of proapoptotic genes. In the cytoplasm, p53 associates with mitochondrial proteins and triggers apoptosis, either by sequestering antiapoptotic proteins or activating the oligomerization of proapoptotic proteins, Bax and Bak (Green and Kroemer, 2009). The relationship between p53, mitochondrial priming, and hESC sensitivity was thus far not clear.

To investigate the mechanisms underlying the increased sensitivity of hESCs to DNA damage, Liu et al. (2013) first explored the role of p53 in the apoptotic response to neocarzinostatin (NCS)-induced DNA double-strand breaks (DSBs). They observed that siRNA knockdown of p53 eliminated the apoptotic response that, in line with findings of other laboratories (Grandela et al., 2007; Qin et al., 2007), confirms the essential role of p53 in DNA damage-induced apoptosis. Furthermore, inhibition of Mdm2, a negative regulator of p53, resulted in p53 stabilization and elicited an apoptotic response in hESCs. One potential mechanism explaining the heightened sensitivity of hESCs to DNA damage is differential expression of p53 downstream target genes in hESCs compared to differentiated cells. Unexpectedly, Liu et al. (2013) did not observe a difference in the p53 transcriptional response. A potent inhibitor of transcription, α-amanitin, did not affect the p53-dependent apoptotic response, suggesting that the induction of p53 downstream target genes is not the primary mechanism of DSB-induced apoptosis in hESCs. Instead, an exclusively cytoplasmic mutant of p53 could rescue the apoptosis defect in p53 knockdown hESCs, demonstrating that the cytoplasmic form of p53 is an important mediator of DNA damage-induced apoptosis in these cells. While these findings clearly define a role for p53 in controlling apoptosis in hESCs, they do not explain the heightened sensitivity of hESCs compared to differentiated cells.

Liu et al. (2013) therefore explored whether alternative, hESC-intrinsic properties could explain their heightened sensitivity to DNA damage. Liu et al. (2013) used a BH3 profiling assay to determine levels of mitochondrial priming. This assay measures mitochondrial membrane permeabilisation (MOMP) upon exposure to BH3 peptides from proapoptotic proteins and can therefore gauge the intrinsic balance of pro- and antiapoptotic proteins acting on the mitochondrial membrane. Liu et al. (2013) demonstrate that hESCs have a much stronger MOMP response following the addition of BH3 peptides of proapoptotic proteins than did differentiated cells. Remarkably, the heightened hESC mitochondrial priming was independent of p53, as it was also observed when p53 was silenced. Instead, Liu et al. (2013) observed that undifferentiated cells express lower levels of the antiapoptotic protein Bcl-2 and higher levels of the proapoptotic protein PUMA. Thus, it appears that the balance is shifted toward the proapoptotic end of the spectrum in hESCs, which may be responsible for their apoptosis-primed state.

To test this last hypothesis, Liu et al. (2013) asked whether inhibiting the

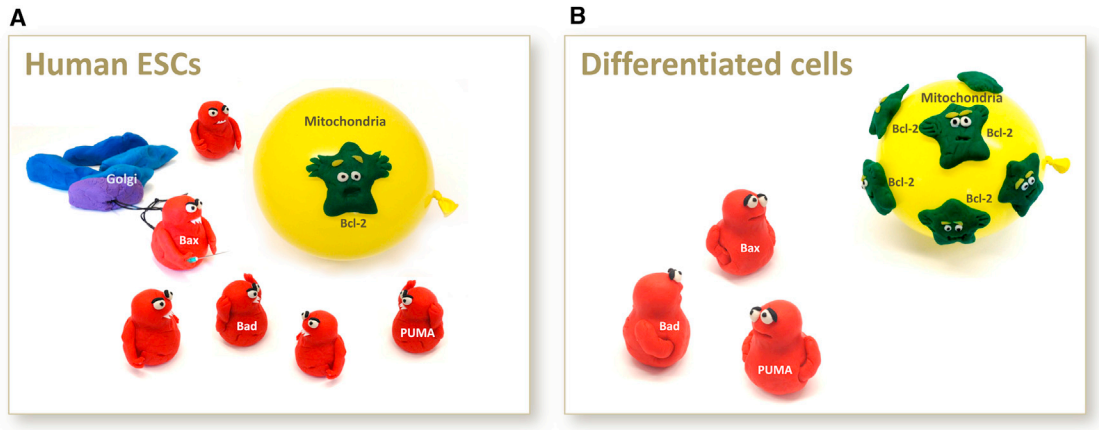

Figure 1. Lower Apoptotic Threshold in Human Embryonic Stem Cells Compared to Differentiated Cells

The apoptotic threshold of cells is determined by the balance between pro- and antiapoptotic factors. In this issue of *Cell Stem Cell*, Liu et al. (2013) demonstrate that human ESCs display high "mitochondrial priming," skewing this balance in favor of proapoptotic factors and lowering the apoptotic threshold. Their findings explain the high sensitivity of human ESCs to DNA damage.

(A) Human ESCs express higher levels of proapoptotic factors, such as Bax, Bad, and PUMA (red) and lower levels of antiapoptotic factors such as Bcl-2 (green). This is further supported by Dumitru et al. (2012), who demonstrated that the proapoptotic factor Bax exists in a preactivated state in human ESCs but is retained at the Golgi to prevent premature apoptosis.

(B) Differentiated cells express higher levels of antiapoptotic factors (green) and lower levels of proapoptotic factors (red), which are not in a preactivated state, making these cells less prone to apoptosis upon DNA damage.

antiapoptotic activity of Bcl-2 in differentiated cells would lower the apoptotic threshold to levels observed in hESCs. Indeed, upon inhibition of Bcl-2 with ABT-263, a BH3-mimetic drug (Tse et al., 2008), differentiated cells became more sensitive to DSB-induced apoptosis. Furthermore, while differentiated cells were insensitive to the ectopic expression of cytoplasmic p53, they rapidly underwent apoptosis in the presence of ABT-263.

The findings of Liu et al. (2013) raise new questions. How is the differential expression of pro-and antiapoptotic proteins regulated in hESCs, and how is it resolved upon differentiation? Are there additional players involved? How is cytoplasmic p53 activity coupled to mitochondrial priming in hESCs? A recent report from Dumitru and colleagues (Dumitru et al., 2012) provides further insight into this question, demonstrating that the proapoptotic protein Bax exists in a preactivated state in hESCs but not in differentiated cells. To prevent precocious apoptosis of hESCs, this preactivated Bax is sequestered at the Golgi. DNA damage results in rapid translocation of preactivated Bax to the mitochondria, and this translocation is p53 dependent.

Thus, hESCs appear to be continuously teetering on the brink of apoptotic cell death. By skewing the balance between pro-and antiapoptotic components toward proapoptotic proteins, which may exist in a preactivated state, hESCs lower their apoptotic threshold (Figure 1) and undergo apoptosis upon p53 induction. In contrast, differentiated cells have a higher threshold and, as a result, p53 does not induce apoptosis in these cells in response to the same stimuli. These unexpected findings provide new insight into how apoptotic networks participate in maintaining a high level of genomic integrity in hESCs, despite the high proliferative pressure that characterizes these cells. By lowering the tolerance for error, the emergence and propagation of mutations is strictly prevented. It will be interesting to explore whether similar protective mechanisms function in resident stem cell populations of rapidly proliferating tissues as well, such as the intestinal and skin epithelium and progenitors in the hematopoietic system. In addition, a recent study has shown that the level of mitochondrial priming in 85 tested tumors strongly correlated with clinical response to chemotherapy (Ni Chonghaile et al., 2011), suggesting that these protective mechanisms are activated even in genetically abnormal cells. Therefore, better understanding the regulatory mechanisms that maintain this lowered apoptotic threshold may provide novel insights in the pathogenesis of and/or therapeutic approaches for cancers as well.

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