Article

P53 Regulates Rapid Apoptosis in Human Pluripotent Stem Cells

Kiyoko Setoguchi [1], Tara TeSlaa [2], Carla M. Koehler [2,3,4] and Michael A. Teitell [1,2,4,5]

*1 - **Department of Pathology and Laboratory Medicine,** University of California Los Angeles, 675 Charles Young Drive South, 4-762 MRL, Los Angeles, CA 90095, USA*
*2 - **Molecular Biology Institute,** University of California Los Angeles, 675 Charles Young Drive South, 4-762 MRL, Los Angeles, CA 90095, USA*
*3 - **Department of Chemistry and Biochemistry,** University of California Los Angeles, 607 Charles Young Drive East, 4041A Young Hall, Los Angeles, CA 90095, USA*
*4 - **Jonsson Comprehensive Cancer Center,** University of California Los Angeles, 675 Charles Young Drive South, 4-762 MRL, Los Angeles, CA 90095, USA*
*5 - **Department of Bioengineering,** Department of Pediatrics, California NanoSystems Institute, and Broad Center for Regenerative Medicine and Stem Cell Research, University of California Los Angeles, 607 Charles Young Drive East, 4041A Young Hall, Los Angeles, CA 90095, USA*

***Correspondence to Michael A. Teitell:** Department of Pathology and Laboratory Medicine, University of California Los Angeles, 675 Charles E. Young Drive South, Los Angeles, CA 90095, USA. mteitell@mednet.ucla.edu*
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Abstract

Human pluripotent stem cells (hPSCs) are sensitive to DNA damage and undergo rapid apoptosis compared to their differentiated progeny cells. Here, we explore the underlying mechanisms for the increased apoptotic sensitivity of hPSCs that helps to determine pluripotent stem cell fate. Apoptosis was induced by exposure to actinomycin D, etoposide, or tunicamycin, with each agent triggering a distinct apoptotic pathway. We show that hPSCs are more sensitive to all three types of apoptosis induction than are lineage-non-specific, retinoic-acid-differentiated hPSCs. Also, Bax activation and pro-apoptotic mitochondrial intermembrane space protein release, which are required to initiate the mitochondria-mediated apoptosis pathway, are more rapid in hPSCs than in retinoic-acid-differentiated hPSCs. Surprisingly, Bak and not Bax is essential for actinomycin-D-induced apoptosis in human embryonic stem cells. Finally, P53 is degraded rapidly in an ubiquitin-proteasome-dependent pathway in hPSCs at steady state but quickly accumulates and induces apoptosis when Mdm2 function is impaired. Rapid degradation of P53 ensures the survival of healthy hPSCs but avails these cells for immediate apoptosis upon cellular damage by P53 stabilization. Altogether, we provide an underlying, interconnected molecular mechanism that primes hPSCs for quick clearance by apoptosis to eliminate hPSCs with unrepaired genome alterations and preserves organismal genomic integrity during the early critical stages of human embryonic development.
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Introduction

Human pluripotent stem cells (hPSCs) have both the ability to self-renew and the ability to differentiate into all cell types in the human body [1,2]. Genomic integrity of hPSCs in utero and in tissue culture is essential for cell lineage fidelity during further development and differentiation, and therefore, a rapid response to cellular damage is required. Human embryonic stem cells (hESCs) have been shown to undergo rapid apoptosis in preference to repair in response to DNA damage when compared to differentiated cells [3–8]. The mechanisms, however, that potentiate the rapid response of DNA-damaged hESCs toward apoptosis instead of repair are still not fully understood. The tumor suppressor protein P53 is a known regulator of apoptosis in response to DNA damage in hESCs [3–5,7,8]. In somatic cells, following

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DNA damage, P53 contributes to apoptosis induction and execution through transcriptional activation of pro-apoptotic genes, through sequestration of anti-apoptotic proteins, or by interacting with and positively influencing pro-apoptotic proteins, Bax and Bak, in the cytosol [9–15]. Knockdown of *P53* in hESCs eliminates the apoptotic response to DNA damage. hESCs expressing P53 lacking a nuclear localization signal can activate apoptosis in response to DNA damage, indicating that cytosol-localized P53, in addition to nuclear P53, contributes to apoptosis in hESCs [5]. Inhibition of cyclin-dependent kinase CDK1 can selectively induce the DNA damage response and P53-dependent apoptosis in hESCs, in contrast to only causing transient cell cycle arrest during DNA repair in differentiated cells [16]. Expression of P53 target genes is rapidly induced in response to DNA damage in embryonic stem cells, but this rapid response is also seen in differentiated cells [5]. Whereas P53 plays a large and potentially distinct role in the DNA damage responses of hESCs and somatic cells, no actual differences in the apoptosis-inducing behavior of P53 or its regulation have yet been identified between hPSCs and differentiated cells. Instead, what has been reported is that the mitochondria in hPSCs are primed for apoptosis due to a difference in the balance between pro-apoptotic and anti-apoptotic proteins, leading to a higher sensitivity and lower apoptotic threshold for hESCs compared to differentiated cells [5,17].

Activation of apoptosis by cell intrinsic stimuli, such as DNA damage, occurs through mitochondrial outer membrane permeabilization (MOMP), which requires the activation of pro-apoptotic BCL-2 family member protein Bax or Bak [11,18,19]. Some hESC lines show constitutively activated pro-apoptotic Bax localized to the Golgi apparatus during S phase, where it is unable to activate apoptosis until DNA damage induces its translocation to the mitochondria to induce MOMP [20]. Knockdown of *Bax* in hESCs decreases apoptosis in response to DNA damage. Additionally, P53 is required for the translocation of Bax from the Golgi apparatus to the mitochondria with DNA damage in hESCs [20]. In other cell types and hESC lines, Bax is localized to the cytosol in an inactive state. Once activated by BH3-only proteins, Bax undergoes a conformational change and insertion into the mitochondrial outer membrane [18,21]. Nonetheless, since activated Bax is not detectable in the Golgi apparatus of most hESC lines, this potential sensitizing mechanism cannot be exclusively responsible for the rapid activation of apoptosis in response to DNA damage in these hESC lines [20].

Here, we further investigate apoptotic mechanisms in hPSCs and discover that differential regulation of P53 stability sensitizes hPSCs to apoptosis. Initially, we evaluated the similarities and differences in the apoptotic machinery between hPSCs and differentiated cells to elucidate the pathways underlying the rapid activation of apoptosis in hPSCs. We discovered that hPSCs activate apoptosis rapidly not only in response to DNA damage but also in response to transcriptional inhibition and the induction of endoplasmic reticulum (ER) stress. In addition, we identified important roles for the mitochondrial fission protein Drp1 and pro-apoptotic BCL-2 family member protein Bak in hESC apoptotic hypersensitivity. Finally, we report that P53 is rapidly degraded at steady state in hPSCs, but inhibition of ubiquitin-proteasome-dependent degradation by Mdm2 causes prompt stabilization of P53 and the induction of apoptosis in hESCs.

Results

hESCs are hypersensitive to diverse mitochondria-mediated apoptotic stimuli

In addition to rapid apoptosis in response to DNA damage, hPSCs also undergo mitochondria-dependent apoptosis upon dissociation into single cells, which can be suppressed by Rho-dependent protein kinase inhibitors [22,23]. Therefore, we considered whether hPSCs are more generally hypersensitive to mitochondria-mediated apoptosis by evaluating whether hPSCs are more sensitive to multiple intrinsic apoptotic stimuli than their differentiated counterparts. We used retinoic acid (RA)-induced differentiation of hPSCs as a differentiated comparative cell derivative of hPSCs [24–26]. RA is an important morphogen during development and is commonly used for neuronal differentiation [27–29]. RA treatment of monolayer culture of hESCs causes preferential differentiation into ectodermal and mesodermal lineages [30–32]. Undifferentiated hPSCs and RA-differentiated cells were evaluated for apoptosis by incubation with various apoptosis-stimulating agents. Exposure of H9 hESCs to RA reduced the expression of Nanog and Oct4, key transcription factors for maintaining hPSC self-renewal, to undetectable levels by days 3 and 5, respectively (Fig. 1a). Actinomycin D, a potent inducer of apoptosis through transcriptional repression, was used to induce apoptosis in H9 and H1 hESCs and in a human induced pluripotent stem cell (hiPSC) line, HIPS2, on days 0, 1, 3, and 5 of RA-induced differentiation (Fig. 1b and Fig. S1b and c). The fragmentation of cells into apoptotic bodies is carried out by a family of intracellular cysteine-dependent, aspartate-directed proteases (caspases). Upon activation of mitochondria-mediated apoptosis and MOMP, procaspase-3 is cleaved and in turn cleaved caspase-3 executes apoptosis through digestion of other intracellular proteins including the DNA repair protein poly(ADP-ribose) polymerase (PARP) [33–35]. Therefore, apoptosis was evaluated

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