Seminars in Cell & Developmental Biology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Mitochondria in human pluripotent stem cell apoptosis

Tara TeSlaa[a], Kiyoko Setoguchi[b], Michael A. Teitell[a,b,c,*]

[a] Molecular Biology Institute, University of California, Los Angeles, CA 90095, USA
[b] Department of Pathology and Laboratory Medicine, University of California, Los Angeles, CA 90095, USA
[c] Department of Bioengineering, Department of Pediatrics, California NanoSystems Institute, Jonsson Comprehensive Cancer Center, and Broad Center of Regenerative Medicine and Stem Cell Research, University of California, Los Angeles, CA 90095, USA

ARTICLE INFO

Article history:
Received 17 November 2015
Received in revised form 11 January 2016
Accepted 21 January 2016
Available online xxx

Keywords:
Apoptosis
Human pluripotent stem cells
p53
Tumorigenicity

ABSTRACT

Human pluripotent stem cells (hPSCs) have great potential in regenerative medicine because they can differentiate into any cell type in the body. Genome integrity is vital for human development and for high fidelity passage of genetic information across generations through the germ line. To ensure genome stability, hPSCs maintain a lower rate of mutation than somatic cells and undergo rapid apoptosis in response to DNA damage and additional cell stresses. Furthermore, cellular metabolism and the cell cycle are also differentially regulated between cells in pluripotent and differentiated states and can aid in protecting hPSCs against DNA damage and damaged cell propagation. Despite these safeguards, clinical use of hPSC derivatives could be compromised by tumorigenic potential and possible malignant transformation from failed to differentiate cells. Since hPSCs and mature cells differentially respond to cell stress, it may be possible to specifically target undifferentiated cells for rapid apoptosis in mixed cell populations to enable safer use of hPSC-differentiated cells in patients.

Contents

Abbreviations: BAK, BCL-2 antagonist killer; BAX, BCL-2 associated X protein; CDK1, cyclin dependent kinase 1; CLDN-6, claudin-6; CM, cardiomyocyte; CPE, *Clostridium perfringens* endotoxin; GPX2, glutathione peroxidase-2; GSH, glutathione; GSR, glutathione reductase; GSSG, glutathione disulfide; hESC, human embryonic stem cell; hiPSC, human induced pluripotent stem cell; hPSC, human pluripotent stem cell; IMS, intermembrane space; MDM2, mouse double minute 2 homolog; MMR, mismatch repair; MOMP, mitochondrial outer membrane permeabilization; NAMPT, nicotinamide phosphoribosyltransferase; OXPHOS, oxidative phosphorylation; PPP, pentose phosphate pathway; ROCK, Rho-associated kinase; ROS, reactive oxygen species; SCD1, stearoyl-coA desaturase; UCP2, uncoupling protein 2.

* Corresponding author at: Department of Pathology and Laboratory Medicine, University of California, Los Angeles, 675 Charles E. Young Drive South, Los Angeles, CA 90095, USA. Fax: +1 310 267 0382.
E-mail address: mteitell@mednet.ucla.edu (M.A. Teitell).

http://dx.doi.org/10.1016/j.semcdb.2016.01.027
1084-9521/

1. Introduction

New possibilities in regenerative medicine have been enabled by the production of human embryonic stem cells (hESCs) and the reprogramming of somatic cells into human induced pluripotent stem cells (hiPSCs) [1–4]. Both cell types classify as human pluripotent stem cells (hPSCs) and each can self-renew indefinitely in culture. hPSCs also have the potential to develop into all of the mature cells in our bodies, including the germ cells that pass genetic information on to our progeny. Therefore, protection against harmful DNA mutations and other heritable lesions is vital for propagation of our species. Consistent with this fidelity, hPSCs exhibit a low rate of DNA mutation due to enhanced mechanisms of DNA damage protection and repair [5–9]. In the event that irreparable DNA or other forms of heritable damage do occur, however, suboptimal hPSCs are prevented from replicating by sensitive and rapid apoptotic mechanisms [10–13]. hPSCs also uniquely regulate the cell cycle and cell cycle checkpoints to prevent damaged cells from further differentiation or cell division [14–16]. In this review we will examine and discuss the mechanisms employed by hPSCs to prevent the propagation of damaged cells, which centers on increased sensitivity to apoptosis triggers. Furthermore, teratoma formation in a rodent is the current gold standard test for hPSCs, but this occurrence also portends a potential tumorigenic threat for using hPSC derivative cells clinically [17]. An increased sensitivity to apoptotic stimuli for hPSCs could enable the selective removal of cells which fail to differentiate from a therapeutic cell population. Metabolic differences between hPSCs and their differentiated progeny are also substantial [18–23], potentially enabling metabolic selection against hPSCs in cell mixtures. Therefore, we also examine emerging methods for selective elimination of hPSCs that do not harm healthy populations of differentiated cells intended for cell therapies.

2. hPSCs show increased sensitivity to apoptosis

2.1. Mitochondria and BCL-2 family proteins in apoptosis

Apoptosis can be regulated by BCL-2 (B cell lymphoma-2) family proteins and their interactions with the mitochondrion. BCL-2 family proteins can be divided into three functional groups: pro-apoptotic effector proteins BAK (BCL-2 antagonist/killer) and BAX (BCL-2 associated X protein), a set of pro-apoptotic BH3 (BCL-2 homology domain 3)-only proteins, and a set of pro-survival proteins that include BCL-2 itself [24]. BAK and BAX can initiate the apoptosis cascade by induction of mitochondrial outer membrane permeabilization (MOMP) [25,26]. BAK and BAX are activated through protein interactions that induce structural changes and their homo-oligomerization. Activation of BAX causes exposure of its N-terminal domain and insertion of its C-terminal domain into the mitochondrial outer membrane, which contrasts with BAK, which is localized to the mitochondrial outer membrane in both inactive and active conformations [27–29]. Homo-oligomerized BAK or BAX forms a pore that causes MOMP [25,26,30]. MOMP subsequently leads to the release of pro-apoptotic proteins in the mitochondrial intermembrane space (IMS), including cytochrome *c*, which activates caspase-9 protease in the cytosol [28,31,32]. In turn, caspase-9 activates additional effector caspases, unleashing a cascade of proteases that culminates in apoptotic cell death [24,33,34]. BAK and BAX have overlapping roles in apoptosis, but differ in their mechanisms of activation [36–38]. *Bak* or *Bax* knockout mice have limited abnormalities, whereas a double knockout of both *Bak* and *Bax* is perinatal lethal due to defects in apoptosis induction [35]. In somatic cells, inactive BAX is located in the cytosol until an apoptotic stimulus causes BAX to interact with p53 or BH3-only proteins, activating BAX [27,39–41]. BH3-only proteins can directly bind and activate BAK or BAX, or they can bind and neutralize pro-survival BCL-2 family member proteins. When pro-survival BCL-2 proteins are not bound by BH3-only proteins, they can bind directly to activated BAK or BAX, inhibiting their pro-apoptotic activities [24]. Additional proteins can also interact with BCL-2 family member proteins to sensitize or deaden cellular responses to apoptosis induction [40,42–44]. Therefore, the cellular apoptotic threshold is a complex balance between pro-survival and pro-apoptotic proteins and their interactions.

2.2. Differential roles of BAK and BAX

Some hESC lines show a unique pattern of BAX regulation during S-phase of the cell cycle, poising these lines for rapid apoptosis induction upon DNA damage. In these lines, during S-phase BAX is sequestered in the Golgi apparatus in its activated conformation, held away from the mitochondrion. Cell stress from DNA damage causes a rapid p53-dependent translocation of active BAX from the Golgi to the mitochondria by an unknown mechanism, causing apoptosis (Fig. 1). By maintaining BAX in its active conformation in the Golgi, certain hESC lines bypass the BAX activation step and are 'primed' for a cell death response [11]. It is not yet known, however, how active BAX is localized to the Golgi and what holds it there prior to p53-dependent translocation to the mitochondrion. Active BAX forms homo-oligomers and pores activating MOMP [25] making it unclear whether active BAX also causes pore formation in the Golgi membrane stack and, if not, what prevents pore formation or oligomerization. Active BAX is detected by an antibody that recognizes its exposed N-terminal domain [11], but it is not known whether this antibody-detected conformational change is sufficient for BAX activity. The H1 hESC line lacks Golgi-localized active BAX but still exhibits hypersensitivity to apoptosis induction that is typical of other hPSC lines [10,11,45]. One interpretation of this data is that hPSC hypersensitivity to apoptosis may be independent of active BAX in the Golgi, or there may be additional mechanisms of hypersensitivity. In addition, whereas BAX has a key role in the apoptotic response of hPSCs to DNA damage, BAK is actually more important in response to other apoptotic insults and cell stresses. For example, the induction of rapid apoptosis in hPSCs by transcriptional inhibition using actinomycin D depends more on BAK than BAX [10]. Overall, some hPSC lines localize active BAX to the Golgi [11], but this unique localization is not required for apoptosis hypersensitivity in all hPSC lines, raising questions about its role in mitochondrial priming of hPSCs for apoptosis.

2.3. Enhanced mitochondrial priming in hPSCs

hPSCs also undergo rapid apoptosis with activation of the unfolded protein response and transcriptional inhibition [10,12,13]. These data strongly suggest that hPSCs are hypersensitive to multiple and perhaps all activators of mitochondrial-

Please cite this article in press as: T. TeSlaa, et al., Mitochondria in human pluripotent stem cell apoptosis, Semin Cell Dev Biol (2016), http://dx.doi.org/10.1016/j.semcdb.2016.01.027