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Review

DNA Damage in Stem Cells

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Both embryonic and adult stem cells are endowed with a superior capacity to prevent the accumulation of genetic lesions, repair them, or avoid their propagation to daughter cells, which would be particularly detrimental to the whole organism. Inducible pluripotent stem cells also display a robust DNA damage response, but the stability of their genome is often conditioned by the mutational history of the cell population of origin, which constitutes an obstacle to clinical applications. Cancer stem cells are particularly tolerant to DNA damage and fail to undergo senescence or regulated cell death upon accumulation of genetic lesions. Such a resistance contributes to the genetic drift of evolving tumors as well as to their limited sensitivity to chemo- and radiotherapy. Here, we discuss the pathophysiological and therapeutic implications of the molecular pathways through which stem cells cope with DNA damage.

Stem cells (SCs) are crucial for the generation and maintenance of intercellular heterogeneity—which is tightly linked to physiological tissue homeostasis—in higher eukaryotes (Blanpain and Simons, 2013). Accordingly, numerical or functional perturbations in various SC compartments have been linked to pathophysiological conditions including embryonic lethality, developmental defects, aging-related degenerative disorders, and oncogenesis (Goodell and Rando, 2015). Moreover, most neoplasms contain a population of cancer stem cells (CSCs) that drives oncogenesis and supports tumor progression (Kreso and Dick, 2014). Normal and malignant SCs share the ability to self-renew while generating differentiated cells (Blanpain and Simons, 2013). Until a decade ago, it was thought that differentiated cells would lose their potency, i.e., their ability to give rise to other cell types. Rather, even fully differentiated cells display some plasticity, enabling them to de-differentiate into SCs following specific stimuli. Besides supporting physiological tissue repair, such a plasticity allows for the generation of inducible pluripotent stem cells (iPSCs), which have considerable experimental applications and clinical potential (Takahashi and Yamanaka, 2016).

Genetic lesions of endogenous or exogenous origin are major threats to the survival and function of SCs (Blanpain et al., 2011). SCs are particularly exposed to sources of DNA damage, and this can have catastrophic consequences for tissue and organismal homeostasis (Behrens et al., 2014). Thus, both normal and malignant SCs rely on a very robust DNA damage response (DDR), which—on the one hand—is beneficial as it preserves optimal SC function in healthy tissues, and—on the other hand—is detrimental as it favors the survival and resistance to treatment of CSCs (Mandal et al., 2011). Of note, the DDR is also involved in the genetic reprogramming associated with the generation of iPSCs (González et al., 2013).

Here, we summarize the DNA repair mechanisms that operate in SCs, and we discuss the pathophysiological relevance of DNA damage in the SC compartment.

DNA Repair Mechanisms

The term DDR refers to a complex network of molecular circuitries that detect and control specific forms of DNA damage (Box 1). Such a control can involve repair, tolerance, or the permanent inactivation (via regulated cell death [RCD], or cellular senescence) of cells with non-reparable or non-tolerable lesions.

Base excision repair (BER) handles DNA lesions that do not significantly distort the double helix. The damaged base is excised to generate an abasic site that is recognized by apurinic/apyrimidinic endodeoxyribonuclease 1 (APEX1, best known as APE1), which initiates repair by DNA polymerase beta (POLB) and DNA ligase 1 (LIG1) or LIG3 complexed with X-ray repair cross complementing 1 (XRCC1). APE1 also recognizes single-strand breaks (SSBs), which are generally repaired upon the poly(ADP-ribose) polymerase 1 (PARP1)-dependent recruitment of BER components (Krokan and Bjørås, 2013) (Figure 1).

Nucleotide excision repair (NER) resolves a wide spectrum of DNA lesions, including adducts and structures distorting the double helix. These lesions are detected by a supramolecular complex containing XPC complex subunit, DNA damage recognition and repair factor (XPC), RAD23 homolog B, NER protein (RAD23B) and centrin 2 (CETN2), followed by recruitment of (1) the oligomeric transcription factor IIH (TFIIH), which promotes DNA unwinding; (2) XPA; (3) the heteromeric complex replication protein A (RPA); (4) ERCC excision repair 5, endonuclease

Box 1. Types of DNA Damage

DNA lesions that do not significantly distort the double helix include (1) single base alterations such as alkylations and deaminations arising spontaneously (e.g., conversion of cytosine into uracil), oxidations caused by ROS (e.g., the conversion of guanine into 8-oxoguanine), as well as erroneous methylations imposed by S-adenosylmethionine (SAM); (2) abasic sites produced by spontaneous hydrolysis (e.g., depurinations) or ROS; and (3) SSBs formed as intermediates during DNA repair, by ROS or by inhibition of topoisomerase activity. These forms of DNA damage are resolved by BER. In addition, 6-O-methylguanine sites generated by SAM or other endogenous/exogenous agents (e.g., N-methylnitrosourea or temozolomide) are corrected by the O-6-methylguanine-DNA methyltransferase (MGMT) (Ciccia and Elledge, 2010). DNA lesions severely distorting the double helix include (1) UV-induced lesions (e.g., cyclobutane pyrimidine dimers and 6-4 pyrimidine-pyrimidone photoproducts); (2) bulky adducts generated by endogenous/exogenous causes (e.g., cisplatin intrastrand crosslinks, polycyclic aromatic hydrocarbon, and malendialdehyde-related pyrimidopurinone adducts); and (3) some oxidative lesions (e.g., cyclopurines such as deoxyadenosine and deoxyguanosine). These lesions are repaired by so-called "Global Genomic Repair" NER. Lesions accumulating at transcription, including bulky adducts, discontinuities, or non-canonical DNA structures (e.g., R-loops) are resolved by so-called "Transcription-Coupled Repair" NER (Ciccia and Elledge, 2010). DNA base-base mismatches and/or small insertion/deletion loops (IDLs) are generated: (1) erroneously or as intermediates during DNA replication or HR; (2) by chemicals such as temozolomide and nucleoside analogs; or (3) by damaged bases like 8-oxoguanine and 5-hydroxycytosine. These lesions are resolved by MMR. DSBs arise directly or indirectly from several endogenous/exogenous causes, including ROS, the collapse of stalled DNA replication forks, telomere shortening, ionizing radiation, and chemotherapeutics (e.g., topoisomerase inhibitors). DSBs are repaired by NHEJ or HR. Interstrand DNA crosslinks (ICLs) can be caused by endogenous metabolites (e.g., formaldehyde, acetaldehyde, or malondialdehyde) as well as exogenous genotoxins (e.g., nitrogen mustards, platinum derivatives, mitomycin C, and psoralen) and are repaired by a dedicated system known as ICL repair (Ciccia and Elledge, 2010).

(ERCC5, best known as XPG); and (5) an heterodimer consisting of ERCC1 and ERCC4 (best known as XPF). This leads to incision of the damaged strand on both sides of the lesion by XPG and XPF (Marteijn et al., 2014) (Figure 1).

DNA mismatch repair (MMR) corrects base mismatches and small insertions or deletions that are recognized by mutS homolog 2 (MSH2)-MSH6 heterodimers (known as MutSα) or MSH2-MSH3 heterodimers (known as MutSβ). MutSα (or MutSβ) recruits a heterodimer consisting of mutL homolog 1 (MLH1) and PMS1 homolog 2, MMR system component (PMS2). Proliferating cell nuclear antigen (PCNA) activates the endonuclease activity of this complex to specifically incise the nascent DNA strand, followed by mismatch removal, usually dependent on exonuclease 1 (EXO1), and DNA repair synthesis (Kunkel and Erie, 2015) (Figure 1).

Non-homologous end joining (NHEJ) is the main pathway for repair of DNA double-strand breaks (DSBs). NHEJ (which operates throughout the cell cycle and often generates small insertions or deletions) proceeds upon formation of a heterodimer consisting of XRCC6 (best known as Ku70) and XRCC5 (best known as Ku80) on broken DNA ends, followed by recruitment and activation of protein kinase, DNA-activated, catalytic polypeptide (PRKDC, best known as DNA-PKcs). Upon assembly, this complex allows for association of nucleases like DNA cross-link repair 1C (DCLRE1C, best known as Artemis) and processing factors such as DNA polymerase mu (POLM) and lambda (POLL), which render non-ligatable DNA ends compatible with ligation by LIG4, XRCC4, and NHEJ factor 1 (NHEJ1, best known as XLF) (Chiruvella et al., 2013) (Figure 1).

Homologous recombination (HR) is an error-free DSB repair system operating only in the S and G_2 phases of the cell cycle. HR involves the 5′ to 3′ resection of broken DNA ends by (1) a heterotrimer consisting of MRE11 homolog, double-strand break repair nuclease (MRE11), RAD50 double-strand break repair protein (RAD50) and nibrin (NBN, best known as NBS1), and (2) RB binding protein 8, endonuclease (RBBP8, best known as CTIP). The two short stretches of single-stranded DNA (ssDNA) at DSBs are processed by exonucleases including CTIP and EXO1 to form long ssDNA overhangs that are coated by RPA to prevent degradation or annealing. RPA is replaced by RAD51 recombinase (RAD51) through a mechanism involving BRCA1, DNA repair associated (BRCA1), BRCA2, and partner and localizer of BRCA2 (PALB2). The resulting RAD51 filament invades the sister chromatid to perform homology search, and DNA repair synthesis is completed by (1) the DSB repair network, which generates double Holliday junctions and results in crossover or non-crossover products, or (2) the synthesis-dependent strand annealing pathway, in which the newly synthetized strand is released and anneals with 3′ ssDNA on the other side of the break (Prakash et al., 2015) (Figure 1).

Translesion synthesis (TLS) bypasses, without removing, DNA lesions that stall the replication machinery, which would otherwise lead to replication fork collapse and/or DSBs. TLS involves PCNA ubiquitination by a heterodimer composed of ubiquitin conjugating enzyme E2 A (UBE2A, best known as RAD6A) or UBE2B (best known as RAD6B) and RAD18, E3 ubiquitin protein ligase (RAD18), followed by recruitment of a DNA polymerase from the Y family, which catalyzes nucleotide (mis)incorporation opposed to the damaged site. Initial strand extension is usually mediated by polymerase ζ, after which a replicative DNA polymerase such as polymerase δ resumes normal DNA replication. TLS is an error-prone process generally linked to mutagenesis (Sale, 2013) (Figure 1).

The main DDR transducers are ATM serine/threonine kinase (ATM), which operates at DSBs, and ataxia-telangiectasia

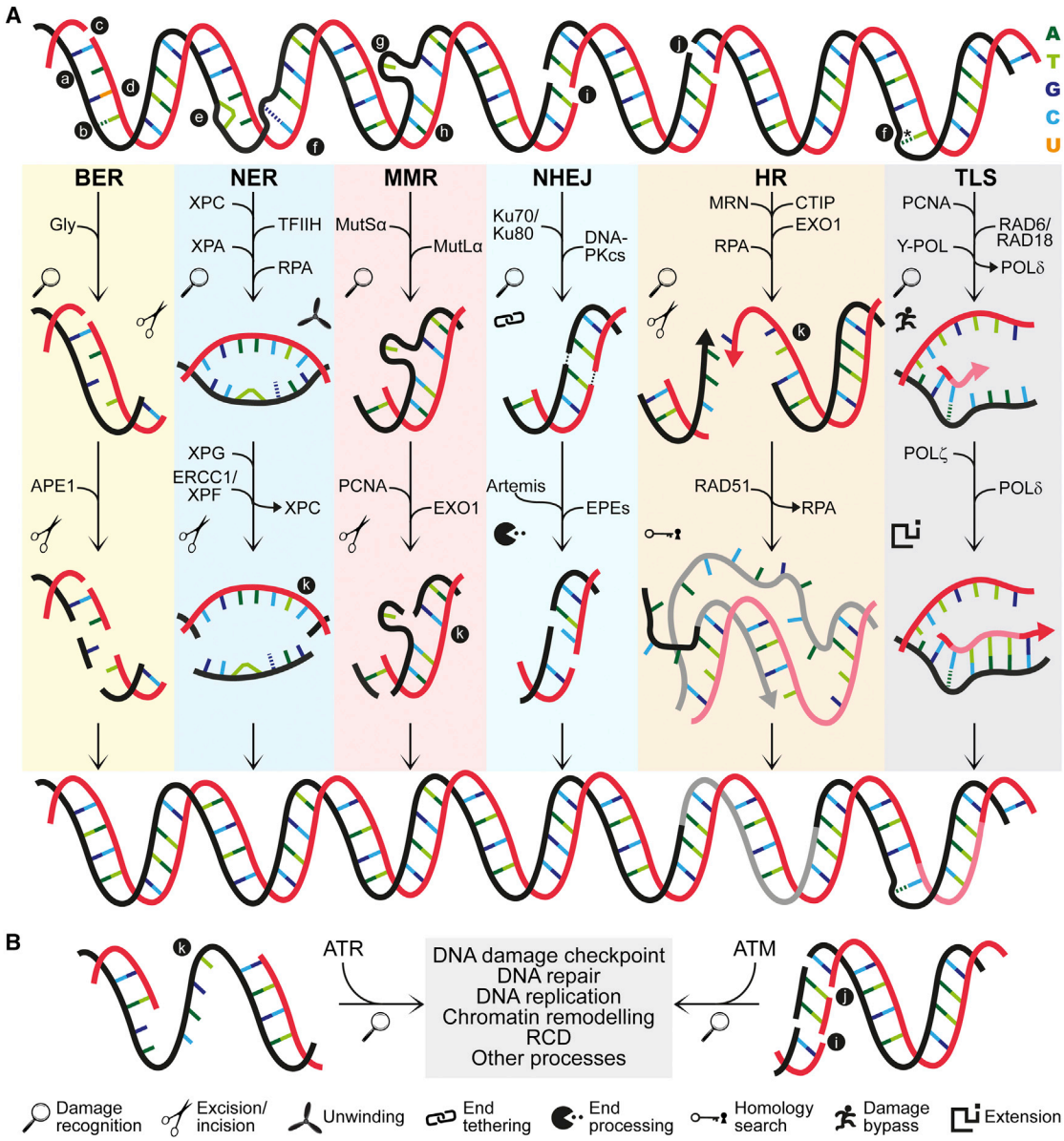

Figure 1. Main Molecular Pathways Underlying DNA Repair and the DDR

(A) Genetic defects as different as abasic sites (a), mildly distorting lesions (b), SSBs (c), deaminations (d), T-T dimers (e), distorting lesions (f), insertion/deletion loops (IDLs) (g), base-base mispairs (h), DSBs (i), end-resected DSBs (j), and ssDNA stretches adjacent to dsDNA (k) can be efficiently repaired by specialized mechanisms encompassing base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), non-homologous end joining (NHEJ), and homologous recombination (HR). Alternatively, multiple DNA lesions can be tolerated upon translesion synthesis (TLS). Please refer to the main text for the official and common names of the proteins and multiprotein complexes involved in these pathways.

(B) In response to ssDNA stretches adjacent to dsDNA or DSBs, ATR and ATM, respectively, activate signal transduction pathways that are involved in the maintenance of nuclear, cellular, or organismal homeostasis, including regulated cell death (RCD).

EPE, end-processing enzyme; Gly, glycosydase; MRN, MRE11-RAD50-NBS1; POLδ, polymerase δ; POLζ, polymerase ζ; Y-POL, Y-family polymerase.

and Rad3-related (ATR), which operates at long ssDNA stretches adjacent to dsDNA. Activated ATM and ATR phosphorylate various substrates involved in nuclear and extranuclear homeostasis. In particular, ATM and ATR phosphorylate H2A histone family member X (H2AFX, best known as H2AX), and activate checkpoint kinase 2 (CHEK2, best known as CHK2) and CHEK1 (best known as CHK1), respectively, hence enforcing a temporary cell-cycle arrest. The ATM-CHK2 and ATR-CHK1 axes also stimulate the transcriptional activity of tumor protein p53 (TP53, best known as p53), which promotes a temporary arrest in cell cycle (potentially allowing for DNA repair and restoration of homeostasis), or RCD (if damage is beyond recovery) (Maréchal and Zou, 2013) (Figure 1).

DNA Damage in Embryonic Stem Cells

Embryonic stem cells (ESCs) are pluripotent cells (they can generate cells of different lineages) with a key role in development and represent not only a unique experimental model, but also a potential tool for regenerative medicine (Kimbrel and Lanza, 2015). ESCs rely on a robust DDR machinery to compensate for their pronounced propensity to acquire DNA lesions, mostly reflecting a high proliferation rate and shortened G_1 phase of the cell cycle (Kapinas et al., 2013). A short G_1 phase may indirectly affect the fidelity of DNA synthesis and mitosis and also weaken the response to unreplicated and/or damaged DNA (Ahuja et al., 2016). Moreover, despite some debate, ESCs reportedly fail to activate the p53-dependent G_1-S and intra-S checkpoints in response to DNA lesions (van der Laan et al., 2013) but can temporarily arrest in the G_2 phase upon ATM activation (Stambrook and Tichy, 2010). In spite of these weaknesses, ESCs accumulate mutations at low rates, suggesting the existence of a particularly efficient and constitutively active DDR, possibly due to persistent replication stress (as per elevated levels of phospho-H2AX and chromatin-bound RPA or RAD51 at baseline, which decrease on differentiation) (Ahuja et al., 2016). Accordingly, ESCs are particularly sensitive to replication inhibitors (Desmarais et al., 2012). Moreover, genotoxic byproducts including reactive oxygen species (ROS) are kept to low levels by ESCs, mostly via a reversible uncoupling protein 2 (UCP2)-dependent and hypoxia inducible factor 1 alpha subunit (HIF1A)-dependent metabolic rewiring that supports anaerobic glycolysis and antioxidant defenses (Xu et al., 2013).

Human ESCs repair various DNA lesions much more efficiently than differentiated cells, possibly owing to increased expression of multiple components of the DNA repair machinery (Maynard et al., 2008). Moreover, ESCs preferentially employ HR over NHEJ (Tichy et al., 2010), hence repairing DSBs with increased fidelity. This may depend on high RAD51 expression levels (Tichy et al., 2010) or on transient expression of zinc finger and SCAN domain containing 4 (ZSCAN4), a transcription factor that promotes telomere lengthening in mice via a HR-dependent mechanism (Zalzman et al., 2010). Moreover, the superior efficacy of the DDR in ESCs may derive (at least in part) from the overexpression of (1) KH domain containing 3, subcortical maternal complex member (KHDC3, best known as FILIA), which promotes PARP1 activity (Zhao et al., 2015); (2) spalt like transcription factor 4 (SALL4), which favors ATM activation by interacting with RAD50 (Xiong et al., 2015); and (3) dicer 1, ribonuclease III (DICER1), which preserves genomic integrity by resolving replication stress (Swahari et al., 2016).

When DNA damage is unresolvable, ESCs avoid the propagation of genetic lesions by undergoing RCD or losing pluripotency. ESCs are particularly prone to DDR-associated RCD as (1) they maintain the cytotoxic protein BCL2 associated X, apoptosis regulator (BAX) in a constitutive active state at the Golgi apparatus, from where it can quickly translocate to mitochondria to promote RCD (Dumitru et al., 2012), and (2) they display constitutive mitochondrial priming (the balance between anti- and pro-apoptotic members of the BCL2 protein family is close to threshold for RCD induction) (Liu et al., 2013). p53 favors loss of potency by repressing the transcription factors Nanog homeobox (NANOG) and POU class 5 homeobox 1 (POU5F1, also known as OCT3/4) (Lin et al., 2005), and by transactivating differentiation-associated genes (Li et al., 2012). A similar activity is mediated by FILIA, at least in mice (Zhao et al., 2015). The ATM/ATR-p53 axis has also been involved in the preservation of pluripotency by ESCs exposed to replication-interfering agents (Gonzales et al., 2015). Possibly, this apparent discrepancy reflects the capacity of p53 to support DNA repair or initiate DDR-driven RCD, depending on the extent of damage (see above).

Thus, ESCs efficiently avoid the stabilization of mutations by buffering endogenous genotoxins, accurately repairing lesions, or preventing cell-cycle progression or survival when damage is beyond recovery.

DNA Damage in Adult Stem Cells

Adult stem cells (ASCs) are multipotent cells (they can generate cells of a single lineage) contributing to tissue homeostasis and repair upon injury. ASCs can persist for prolonged periods in a reversible non-proliferating state (quiescence or G_0 phase), which renders them prone to accumulate mutations upon microenvironmental insults (Cheung and Rando, 2013).

Hematopoietic Stem Cells

Hematopoietic stem cells (HSCs) establish and preserve hematopoiesis throughout post-natal life by dividing asymmetrically to generate myeloid or lymphoid progenitors (Orkin and Zon, 2008). HSCs co-exist in two dynamic pools of active (cycling) and dormant (quiescent) cells: the former actively supports hematopoiesis; the latter ensures HSC preservation (Li and Clevers, 2010). HSCs are exquisitely sensitive to acquire mutations upon exposure to endogenous and exogenous sources of DNA damage, including replication stress and microenvironmental genotoxins (Adams et al., 2015). Such pronounced sensitivity, at least in part reflecting the lack of an efficient G_1-S DNA damage checkpoint (Moehrle et al., 2015) and the preferential use of NHEJ over HR (Mohrin et al., 2010), constitutes the rationale for lymphoablating/myeloablating regimens administered prior to HSC transplantation (Gyurkocza and Sandmaier, 2014). Moreover, it explains the increased frequency of hematological malignancies caused by cancer therapy (Bhatia, 2013). Deep-sequencing studies confirm that the illicit expansion of rare HSC clones bearing advantageous mutations drives multiple hematological cancers (Adams et al., 2015).

The accrual of mutations by HSCs promotes aging-related disorders other than cancer, such as bone marrow failure and anemia (Behrens et al., 2014). Accordingly, *Prkdc*$^{-/-}$, *Lig4*$^{-/-}$, and *Xrcc5*$^{-/-}$ mice spontaneously develop HSC defects ranging from reduced self-renewal to functional exhaustion (Rocha et al., 2013). The aging HSC compartment displays defects in multiple DNA repair pathways, extensive cell-cycle deregulation, high degrees of replication stress, and ROS overgeneration (Kowalczyk et al., 2015). Oxidative stress is particularly prominent once quiescent HSCs are activated by perturbations of hematological homeostasis (Walter et al., 2015). However, quiescent HSCs are more prone than their active counterparts to accumulate mutations as they age, owing to reduced DNA repair proficiency (Beerman et al., 2014). In this pool of HSCs, endogenous

aldehydes and telomere shortening are major sources of DNA damage (Garaycoechea et al., 2012). Moreover, mesenchymal inflammation has recently been shown to mediate genotoxic effects in HSCs, de facto fostering leukemogenesis in mice (Zambetti et al., 2016).

HSCs harness multiple mechanisms to prevent the accrual of mutations or their transmission to the progeny. Baseline DNA repair is highly efficient in HCSs. Indeed, microenvironmental factors such as thrombopoietin (THPO) increase the accuracy of NHEJ in quiescent HSCs (de Laval et al., 2013). Moreover, minichromosome maintenance complex component 3 (MCM3) licenses a high number of dormant replication origins during G_1, as a backup mechanism to limit replication stress in S phase (which is particularly relevant for HSCs, see above) (Alvarez et al., 2015). Some components of the DNA repair machinery are upregulated during sensitive processes, including the transition from quiescence to active cycling (Beerman et al., 2014). ROS production is minimized as HSCs reside in a hypoxic environment and display a low metabolic activity based on glycolysis (Suda et al., 2011). The glycolytic state in HSCs depends on 3-phosphoinositide-dependent protein kinase 1 (PDPK1), which is required for both quiescence and stemness (Takubo et al., 2013). HSCs also limit oxidative stress by activating ATM and forkhead box O (FOXO) signaling (Ito et al., 2004; Tothova et al., 2007) and autophagy (Mortensen et al., 2011). p53 rapidly controls HSCs accumulating genetic damage beyond repair (via RCD or senescence) (Wang et al., 2014). In this context, protein phosphatase 1 regulatory subunit 13B (PPP1R13B, best known as ASPP1) plays a critical role by (1) limiting self-renewal and favoring quiescence in physiological conditions and (2) promoting the lethal activity of p53 in response to DNA damage (Yamashita et al., 2015). Runt-related transcription factor 1 (RUNX1), which is frequently mutated in (pre-)leukemic blasts, is also involved in the elimination of HSCs experiencing genotoxic stress (Cai et al., 2015). Absent in melanoma 2 (AIM2) has recently been shown to detect DSBs induced by radiation in HSCs, hence precipitating their demise upon caspase 1 (CASP1) activation (Hu et al., 2016). Finally, HSCs accruing genetic lesions differentiate (and hence lose stemness) in a basic leucine zipper ATF-like transcription factor (BATF)-dependent manner (Wang et al., 2012). Altogether, these observations suggest the existence of a poorly characterized checkpoint specifically activated in HSCs exiting G_0 to repair DNA lesions accumulated during quiescence or to limit their propagation via RCD or differentiation. The cytotoxic protein BH3 interacting domain death agonist (BID) appears to participate in this process by preserving quiescence (Maryanovich et al., 2012).

Additional signaling networks strengthen the ability of HSCs to cope with DNA damage, including (1) a pathway relying on cyclin-dependent kinase inhibitor 1A (CDKN1A, best known as p21), which acutely promotes DNA repair and HSC survival but supports exhaustion on chronic activation (Insinga et al., 2013); (2) period circadian clock 2 (PER2), which modulates the DDR to limit stemness (Wang et al., 2016b); (3) aldehyde dehydrogenase 2 family (ALDH2), which supports DNA repair and acetaldehyde detoxification (Garaycoechea et al., 2012); and (4) BMI1 proto-oncogene, polycomb ring finger (BMI1), which controls oxidative stress and promotes the DDR (Liu et al., 2009).

Non-hematopoietic SCs

The sensitivity of non-hematopoietic ASCs to genotoxic stress depends on proliferation rate and global regenerative capacity of the tissue of origin (Mandal et al., 2011). Highly proliferating tissues such as the intestinal epithelium are highly sensitive to DNA damage of endogenous (e.g., replication stress) and exogenous (e.g., irradiation) source. Conversely, slowly proliferating or quiescent ASCs present an increased resistance to DNA damaging agents, which is key for tissue homeostasis.

Trp53-proficient murine mammary stem cells (MaSCs) efficiency repair DSBs (and hence resist ionizing radiation) as they increase NHEJ activity (Chang et al., 2015). Similarly, murine hair follicle stem cells (HFSCs) quickly initiate NHEJ and upregulate the cytoprotective protein BCL2 in response to DSBs to prevent RCD (Sotiropoulou et al., 2010). Human mesenchymal stem cells (MSCs), but not their differentiated progeny, also display high resistance to DNA damage, which depends on ATM and NHEJ (Oliver et al., 2013). Of note, the radioresistance of murine MSCs is enhanced by hypoxia, as HIF1A (which responds to low O_2 levels) controls the expression of various NHEJ and HR components (Sugrue et al., 2014). Skeletal muscle stem cells (MuSCs, also known as satellite cells) accurately resolve DSBs induced by radiation via a DNA-PKcs-dependent mechanism (Vahidi Ferdousi et al., 2014).

ASC quiescence is often linked to increased resistance to genotoxins and recovery potential. Robust evidence indicates that two distinct pools of intestinal stem cells (ISCs) co-exist to ensure tissue homeostasis and regeneration following injury. One pool involves crypt base columnar (CBC) ISCs, which are cycling and express high levels of leucine-rich repeat containing G-protein-coupled receptor 5 (LGR5). The other pool consists of quiescent ISCs, which are located further away from the crypt base, are quiescent, and express BMI1 and HOP homeobox (HOPX) (Barker, 2014). Proliferating CBC ISCs are more sensitive to irradiation than quiescent ISCs (Barker, 2014). In this scenario, quiescent ISCs compensate for CBC ISCs succumbing to radiation by re-entering the cell cycle upon a metabolic rewiring orchestrated by musashi RNA binding protein 1 (MSI1) (Yousefi et al., 2016). WNT signaling via LGR5 seems to be involved in the response of ISCs to radiation as well as in the regeneration of damaged intestinal epithelium. Indeed, WNT hyperactivation sensitizes CBC ISCs to DNA damage-driven RCD (Tao et al., 2015). Conversely, boosting irradiation-elicited WNT signaling in quiescent cells promotes intestinal regeneration upon damage by upregulating LIG4 and enhancing NHEJ (Jun et al., 2016). Accordingly, activation of WNT signaling with recombinant agonists potentiates intestinal regeneration and increases the survival of mice exposed to chemoradiotherapy (Zhou et al., 2013). Moreover, the LRG5 effector SRY-box 9 (SOX9) contributes to ISC radioresistance and regeneration by preserving quiescent ISCs (at least in mice) (Roche et al., 2015). Transplanted MSCs have recently been shown to promote crypt regeneration in the small intestine upon irradiation by increasing the number of $LGR5^+$ ISCs and activating WNT signaling (Gong et al., 2016). Moreover, macrophage-derived WNT has been ascribed with a crucial role in protecting ISCs from radiation injury and promoting regeneration (Saha et al., 2016). Thus, WNT signaling is important for ISCs to cope with DNA damage.

Some observations suggest that CBC ISCs may be more (not less) resistant to DNA damage than their quiescent counterparts and drive regeneration after damage. Depletion of LGR5$^+$ CBC ISCs affected the recovery of crypts upon irradiation (Metcalfe et al., 2014). Moreover, CBC ISCs seemed to efficiently resolve DSBs by using HR and drive crypt repopulation after irradiation (Hua et al., 2012). Finally, yes-associated protein 1 (YAP1)—a transcriptional effector of HIPPO signaling—has been involved in intestinal recovery after irradiation by counterbalancing excessive WNT signaling (Barry et al., 2013) and reprogramming LGR5$^+$ ISCs toward regeneration (Gregorieff et al., 2015). Possibly, this apparent contrast reflects the existence of multiple sub-pools of ISCs and high degrees of functional plasticity (Barker, 2014). In support of this interpretation, the LGR5$^+$ ISC pool responsible for recovery of intestinal crypts post-irradiation has been proposed to derive from radioresistant keratin 19 (KRT19)$^+$ ISCs residing above the crypt base (Asfaha et al., 2015) or from dedifferentiating enterocyte precursors (Tetteh et al., 2016). Moreover, LGR5 and BMI1 have rather broad and overlapping expression profiles in crypts (Barker, 2014), which complicates precise lineage tracing. An improved characterization of proliferating and quiescent ISCs is required to elucidate the intestinal response to DNA damage.

Multiple mechanisms prevent the propagation of genetic lesions to the progeny of non-hematopoietic ASCs. Ionizing radiation rapidly triggers cellular senescence in neural stem cells (NSCs) as it promotes differentiation via a pathway depending on ATM, bone morphogenetic protein 2 (BMP2), and signal transducer and activator of transcription 3 (STAT3) (Schneider et al., 2013). Similarly, melanocyte stem cells (McSCs) lose stemness following genotoxic insults, which results in premature differentiation (Inomata et al., 2009). Thus, the DDR promotes the differentiation of ASCs in the presence of excessive DNA damage. In line with this notion, BRCA1 and Fanconi anemia complementation group D2 (FANCD2) have recently been shown to prevent the epithelial-to-mesenchymal transition (EMT, a form of dedifferentiation) in human mammary epithelial cells (Wang et al., 2016a). Further corroborating the importance of the DDR for SCs, mice lacking *Brca1* in the epidermis are hairless as they lack HFSCs (Sotiropoulou et al., 2013), while *Exo1* is required for the function of NSCs (but not HSCs) (Zhang et al., 2014a). Thus, different SC pools may preferentially rely on different modules of the DDR for the maintenance of genetic (and hence functional) homeostasis.

Mounting evidence indicates that p21 has a key role in protecting non-hematopoietic ASCs from genetic insults. Mammary stem cells (MaSCs) activate a p21-dependent pathway that promotes DNA repair and inhibits p53-dependent RCD, hence preserving stemness (Insinga et al., 2013). In the presence of excessive DNA damage, p21 limits the proliferation of potentially tumorigenic ISCs that escape from p53-dependent RCD (Sperka et al., 2011). Accordingly, inhibition of cyclin-dependent kinase 4 (CDK4) and CDK6 confers intestinal radioprotection to mice by preventing p53 activation while leaving the p21 network operational (Wei et al., 2016). p21 also controls the expansion of subependymal NSCs by limiting genetic defects upon the downregulation of the pluripotency-associated transcription factor SRY-box 2 (SOX2) (Marqués-Torrejón et al., 2013). NSCs rely on the antioxidant functions of autophagy for stemness preservation, as demonstrated by the conditional deletion of RB1-inducible coiled-coil 1 (*Rb1cc1*) (Wang et al., 2013a). Similarly, autophagy activation by caloric restriction increases the pool of ISCs, hence boosting radioresistance and intestinal regeneration (Yilmaz et al., 2012).

Despite such a multipronged control, non-hematopoietic ASCs tend to accumulate chromosomal abnormalities at similar frequency than ESCs, HSCs, and iPSCs (see below) (Ben-David et al., 2011). Accordingly, non-hematopoietic ASCs can acquire and propagate genetic mutations that contribute to age-related disorders including cancer, as demonstrated by elegantly targeting common oncogenes and oncosuppressor genes in cultured ISCs (Drost et al., 2015). Thus, in vivo oncogenesis may involve ASCs that acquire relevant mutations. Indeed, in a mouse model of sebaceous adenomas, HFSCs had mutations in lymphoid enhancer binding factor 1 (*Lef1*) and reduced p53 activity (Petersson et al., 2015). Highly proliferating tissues have an increased neoplastic incidence linked to mutagenesis, which results from intrinsic and extrinsic factors (Wu et al., 2016). However, the type of mutation may be more important than crude mutational load in the oncogenic process (Blokzijl et al., 2016). Thus, caution should be employed before drawing a direct link between stemness/proliferation rate and oncogenesis.

The accumulation of DSBs in aging MuSCs impairs skeletal functions, a detrimental phenotype that can be reverted (along with a reduction in genetic lesions) by growth differentiation factor 11 (GDF11) (Sinha et al., 2014). Along similar lines, administration of the NAD$^+$ precursor nicotinamide riboside to mice limits senescence in multiple non-hematopoietic ASCs pools, including MuSCs, NSCs, and McSCs, as it extends organismal lifespan (Zhang et al., 2016). Further studies are required to elucidate the effects of these interventions on DNA repair in ASCs.

DNA Damage in iPSCs

iPSCs are pluripotent cells established experimentally upon reprogramming of terminally differentiated cells (Box 2). iPSCs can be safely derived from patients and can be driven into multiple pathways of differentiation, hence constituting very powerful investigational tools and promising platforms for hitherto developing clinical interventions (Kimbrel and Lanza, 2015). One of the major concerns about the clinical implementation of iPSC-based therapeutics is their intrinsically high mutational load, partially reflecting the somatic mutations of differentiated cells from which they derive (Tapia and Schöler, 2016). iPSCs also display high epigenetic and transcriptional variability as a consequence of the genetic background of the donor (Choi et al., 2015; Rouhani et al., 2014). Moreover, iPSCs accumulate genomic aberrations that were absent or present at low frequencies in parental cells (Liang and Zhang, 2013). These defects may be ascribed to the preferential reprogramming of somatic subclones harboring advantageous genetic traits within a heterogeneous starting cell population, as well as to the genetic and epigenetic remodeling imposed by the reprogramming protocol (Box 2) (Gore et al., 2011). Reportedly, iPSC reprogramming is associated with the generation of DSBs independently of vector integration or use of oncogenic transcriptional factors (González et al., 2013). This explains why the successful

Box 2. Protocols for the Generation of iPSCs

The reversion of fully differentiated somatic cells to an ESC-like state was first achieved in 2006 through the retroviral/lentiviral vector-mediated overexpression of stemness-associated transcriptional factors in groups: (1) OCT3/4, SOX2, and Kruppel like factor 4 (KLF4) and MYC (known as OSKM or Yamanaka factors), or, alternatively, (2) OCT3/4, SOX2, NANOG, and lin-28 homolog A (LIN28A) (Takahashi and Yamanaka, 2016). During the last decade, the efficiency and safety of reprogramming have been significantly improved using multiple approaches (Takahashi and Yamanaka, 2016). These include (but are not limited to) the ectopic co-expression of OSKM with undifferentiated embryonic cell transcription factor 1 (UTF1), the inactivation of the p53 pathway, as well as the replacement of KLF4 with NANOG (within the Yamanaka cocktail). Additional improvements were obtained by replacing MYC with: (1) v-myc avian myelocytomatosis viral oncogene lung carcinoma derived homolog (MYCL1, best known as L-MYC), which displays reduced oncogenic activity; (2) the ESC-associated transcription factors SALL4, in the human system, or T-box 3 (Tbx3), in the murine system; (3) ESC-specific microRNAs including miR-291-3p, miR-294, and miR-295, or (4) antisense inhibitors of the microRNA let-7, which increases the level of the tripartite motif containing 71 (TRIM71). Lately, third-generation methods for somatic cells reprogramming have been developed based on non-integrative platforms including Sendai viral particles, adenoviruses, episomal vectors, recombinant proteins, miRNAs, synthetic mRNAs, minicircle vectors, and PiggyBac transposons, as well as on the administration of cocktails of small molecules (Takahashi and Yamanaka, 2016). Notwithstanding considerable technical improvements, which allow for the successful derivation of iPSCs from a wide panel of somatic cells, the efficiency of somatic cell reprogramming is still relatively low. This constitutes a prominent hurdle for the application of iPSCs as cellular therapeutics as well as for their use as a research tool in disease modeling, drug discovery, and developmental biology (Kimbrel and Lanza, 2015).

generation of iPSCs requires multiple DDR pathways including NHEJ and HR (González et al., 2013; Tilgner et al., 2013). The expression of reprogramming factors also promotes replication and oxidative stress, which can be counteracted by CHK1 upregulation as well as by nucleoside or antioxidant supplementation (Ji et al., 2014; Ruiz et al., 2015). Of note, replication stress in iPSCs is aggravated by the acquisition of aberrant karyotypes (Lamm et al., 2016). Defects in specific DDR pathways have also been linked to genetic instability in iPSCs, owing to inefficient DNA repair and/or the preferential use of error-prone mechanisms (Felgentreff et al., 2014). These observations highlight the importance of evaluating DDR status before any putative clinical application of iPSCs.

The activation of p53 acts as a barrier for cell reprogramming by inducing cell-cycle arrest, RCD, or senescence (Rivlin et al., 2015). Accordingly, strategies targeting p53 have been shown to improve the efficacy of iPSC generation, but at the cost of increasing genetic instability and tumorigenic potential (Sarig et al., 2010). Rather, expression of ZSCAN4 reportedly facilitates iPSC generation by limiting DNA damage and p53 activation induced by reprogramming factors while preserving genomic stability (Jiang et al., 2013). These findings indicate that the outcome of somatic cell reprogramming is dictated by a balance between the activity of DNA repair processes and p53 activation.

Established iPSCs resemble ESCs in their propensity to accumulate DNA lesions, at least in part owing to a shortened G_1 phase, and the lack of efficient G_1 and intra-S checkpoints (Desmarais et al., 2016; Momcilovic et al., 2010). Similar to ESCs, iPSCs harness various strategies to minimize or cope with DNA damage. First, iPSCs can count on robust MMR, NER, and BER systems (Luo et al., 2012) and efficiently resolve DSBs, most likely as they overexpress multiple DDR components (Momcilovic et al., 2010). Second, iPSCs limit ROS levels by (1) mounting robust antioxidant responses (Dannenmann et al., 2015) and (2) preferentially employing anaerobic glycolysis as energy source (Panopoulos et al., 2012). The glycolytic switch that accompanies somatic cell reprogramming reportedly relies on hexokinase 2 (HK2) overexpression, inactivation of the pyruvate dehydrogenase complex, and UCP2 upregulation (Xu et al., 2013). Of note, despite strong antioxidant defenses at baseline, antioxidant supplementation improves iPSC maintenance (Ji et al., 2014), meaning that these cells normally produce ROS in excess of their endogenous buffering capacity. Finally, iPSCs are highly sensitive to RCD triggered by DNA damage (Desmarais et al., 2016). This is mainly due to high p53 levels and constitutive mitochondrial priming and appears to involve ATM signaling (Dannenmann et al., 2015). In summary, iPSCs repair or control DNA damage similar to ESCs, although parental cell- and reprogramming-dependent factors render them exquisitely prone to bear genetic alterations that may limit their clinical potential.

DNA Damage in CSCs

According to a debated model, CSCs are multipotent SCs responsible for the long-term clonal maintenance and growth of most human neoplasms (Kreso and Dick, 2014). CSCs—whose relative abundance is highly variable (2.5%–40%)—are normally identified based on CD24, CD44, CD117, or CD133 but display high degrees of genotypic, phenotypic, and functional heterogeneity. CSCs resemble ASCs as they can (co)exist in a cycling and quiescent state and as they acquire mutations that further increase heterogeneity (Kreso and Dick, 2014).

Patient-derived as well as cultured CSCs exhibit a robust DDR as compared to relatively more differentiated malignant cells, which underlies their elevated resistance to antineoplastic agents with genotoxic activity. Patient-derived glioblastoma stem cells (GSCs) display increased HR efficiency as compared to neural progenitors (Lim et al., 2012), as well as superior NHEJ activity, which appears to rely on RNA binding motif protein 14 (RBM14) and confers radioresistance (Yuan et al., 2014). Similarly, CSCs isolated from p53-deficient mouse mammary tumors efficiently repair radiation-induced DSBs via NHEJ

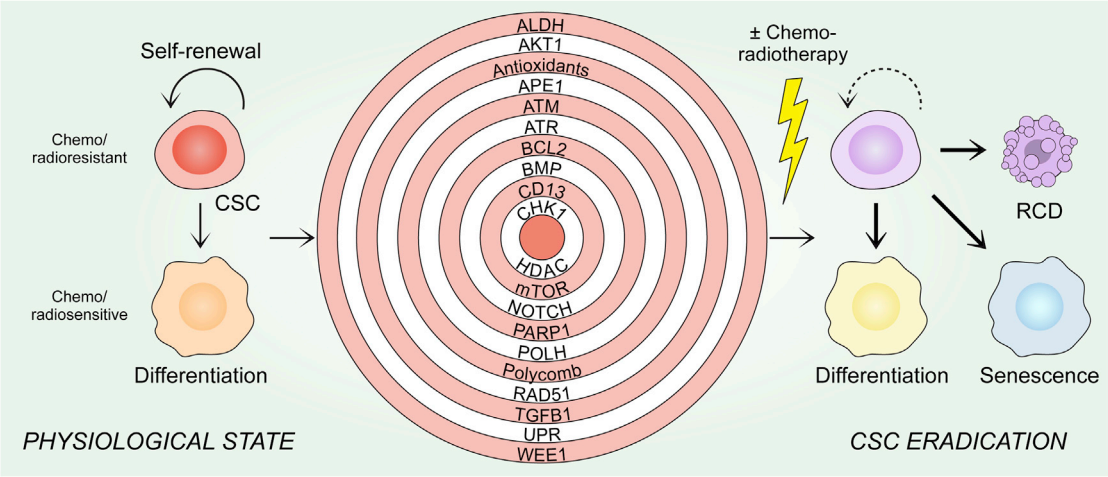

Figure 2. Targeting DNA Repair in Cancer Stem Cells
In physiological conditions, chemo- and radioresistant CSCs self-renew as they generate relatively more chemo- and radiosensitive malignant cells with limited stemness. Multiple components of the DDR can be directly or indirectly targeted as a standalone strategy for the preferential eradication of CSCs or as a means to boost their chemo- and radiosensitivity, via differentiation, regulated cell death (RCD), or cellular senescence.

(Banerjee et al., 2014). Along with increased repair activity, the $CD133^+$ fraction of cultured lung cancer cells exhibits increased levels of multiple HR and NHEJ components in basal conditions (Desai et al., 2014). Moreover, GSCs derived from patients or xenografts contain high levels of RAD51 and RAD17 (which is involved in ATR signaling) at baseline, together with signs of replication stress (Bao et al., 2006). Patient-derived GSCs also exhibit increased PARP1 activity, which ensures the accurate repair of SSBs induced by oxidative stress (Venere et al., 2014) and contributes to radioresistance (Ahmed et al., 2015). SSB repair is particularly active in $CD24^{-/low}CD44^+$ breast CSCs, linked to APE1 upregulation (Karimi-Busheri et al., 2010). Finally, $CD44^+CD117^+$ CSCs isolated from ovarian cancer cell lines or primary tumors show enhanced tolerance to DNA damage mediated by DNA polymerase eta (POLH). Accordingly, genetic inhibition of POLH by small interfering RNAs (siRNAs) or microRNAs (miRNAs) reportedly sensitizes ovarian CSCs to the genotoxic effects of cisplatin, in vitro and in vivo (Srivastava et al., 2015) (Figure 2).

The basal activation status of checkpoint kinases (rather than the hyperactivation of DNA repair) may constitute a key mechanism for CSCs to resist genotoxic agents. CSCs derived from glioblastoma or lung cancer patients preferentially activate CHK1 in response to the ATM- and ATR-dependent G_2-M checkpoint to survive radiotherapy (Bartucci et al., 2012). On the contrary, GSCs are unable to activate the G_1 and intra-S checkpoints (McCord et al., 2009). As compared to their relatively more differentiated counterparts, breast CSCs display reduced levels of oxidative DNA damage, both at baseline and after irradiation (Diehn et al., 2009). Consistently, high frequencies of leukemic stem cells (LSCs) are associated with limited ROS production and increased ROS scavenging dependent on glutathione peroxidase 3 (GPX3) upregulation (which is a bad prognostic indicator for acute myeloid leukemia patients) (Herault et al., 2012). In addition, head and neck cancer SCs with low levels of ROS are poorly sensitive to cisplatin, and this can be reversed by ROS scavenger inhibitors (Chang et al., 2014). Apparently at odds with these findings, ROS levels do not differ between CSCs and bulk malignant cells isolated from *Trp53*$^{-/-}$ mouse mammary tumors (Zhang et al., 2010), whereas GSCs display high ROS production and signs of DNA damage at baseline (Venere et al., 2014). The reasons underlying these apparent discrepancies remain to be elucidated (Figure 2).

CSCs activate robust cytoprotective mechanisms to preserve stemness in spite of DNA damage. Primary colorectal CSCs resist DNA-damaging agents as they display limited mitochondrial priming than their differentiated counterparts, representing a target for pharmacological interventions with BCL2 antagonists (Colak et al., 2014). Similarly, pancreatic $CD133^+$ CSCs exhibit increased expression levels of BCL2 and baculoviral IAP repeat containing 5 (BIRC5, a cytoprotective protein best known as survivin) than their $CD133^-$ counterparts (Banerjee et al., 2014). The impact of p21 in CSC self-renewal is a matter of debate. On one hand, p21 seems to be critical for LSCs to preserve stemness. Indeed, p21 activation limits the accumulation of DNA lesions in LSCs by decelerating cell-cycle progression and hence preventing exhaustion (Viale et al., 2009). Consistently, inhibitor of DNA binding 1, HLH protein (ID1), and ID3 have been shown to control stemness in colorectal CSCs by upregulating p21 (O'Brien et al., 2012). Moreover, colorectal cancer cells acquire a stemness signature and enter quiescence upon exposure to DNA-damaging agents, via a mechanism-dependent on YAP1 signaling (Touil et al., 2014). On the other hand, gremlin 1, DAN family BMP antagonist (GREM1) reportedly promotes stemness in GSCs by inhibiting p21 and accelerating cell-cycle progression (Yan et al., 2014). Possibly, this apparent discrepancy relates to the strength or duration of p21 signaling, but this hypothesis remains to be experimentally addressed (Figure 2).

Multiple common SC markers favor chemo- and radioresistance in CSCs. The radioresistance of stem-like nasopharyngeal carcinoma cells reportedly relies on the transactivation of *CHEK1* and *CHEK2* by MYC, resulting in a proficient DNA damage checkpoint (Wang et al., 2013b). Accordingly, L1 cell adhesion molecule (L1CAM) triggers ATM signaling and DNA repair in irradiated GSCs by MYC-dependent *NBN* transactivation (Cheng et al., 2011). Moreover, Polycomb proteins appear to regulate the DDR in CSCs. BMI1 is enriched at chromatin in irradiated $CD133^+$ glioblastoma cells, promoting the recruitment of ATM and DNA-PKcs for DSB repair (Facchino et al., 2010). On similar lines, oxidative stress limits the tumorigenicity of patient-derived GSCs by promoting BMI1 degradation (Sato et al., 2014). Enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2) contributes to GSC radioresistance by a pathway dependent on maternal embryonic leucine zipper kinase (MELK) (Kim et al., 2015). EZH2 is also involved in the expansion of $CD24^{-/low}CD44^+$ breast CSCs by epigenetically repressing DNA repair (Chang et al., 2011). The EMT-inducing transcription factor zinc finger E-box binding homeobox 1 (ZEB1) participates in an ATM-dependent mechanism that supports the DDR in CSCs by stabilizing CHK1 (Zhang et al., 2014b). The semiquiescence marker alanyl aminopeptidase, membrane (ANPEP, best known as CD13) protects human liver CSCs from genotoxic agents by limiting ROS-driven DNA damage and consequent RCD, in vitro and in vivo (Haraguchi et al., 2010). Finally, ALDH is overexpressed by chemoresistant gastric CSCs and protects them from DNA damage induced by oxidative stress (Raha et al., 2014) (Figure 2).

Such a robust armamentarium allows CSCs to tolerate high levels of replication stress and resist DNA-damaging agents but often increases their basal dependence on specific DDR components like ATR or RAD51 for survival and proliferation (Gallmeier et al., 2011). Effective strategies for sensitizing CSCs to therapy rely on the inhibition of ATM or ATR signaling. Specific pharmacological inhibitors of CHK1 and CHK2 have been shown to reverse radioresistance in $CD133^+$ GSCs (Bao et al., 2006) and stem-like neuronal progenitors (Wang et al., 2013b). CHK1 inhibition increases the sensitivity of lung CSCs to anticancer agents like gemcitabine or cisplatin (Bartucci et al., 2012), as well as that of pancreatic CSCs to a gemcitabine plus a radionuclide-antibody conjugate (Al-Ejeh et al., 2014). Inhibition of the CHK1 effector WEE1 G2 checkpoint kinase (WEE1) also overcomes the resistance of GSCs to radiation (Mir et al., 2010). Moreover, the combinatorial administration of histone deacetylase (HDAC) inhibitors with WEE1- or CHK1-targeting agents appears to be selective for LSC-like cells while sparing normal $CD34^+$ hematopoietic cells (Zhou et al., 2015) (Figure 2).

ATM and PARP1 also appear as promising targets for CSC eradication. Patient-derived GSCs are radiosensitized more efficiently by ATM inhibitors than by ATR or CHK1 inhibitors, possibly related to the pleiotropic functions of ATM (Ahmed et al., 2015). PARP1 inhibitors reportedly sensitize GSCs to DNA-damaging therapeutics (Venere et al., 2014). In addition, PARP or HDAC inhibitors are active against liver CSCs in vitro and in vivo, perhaps as they inhibit the DDR by affecting chromodomain helicase DNA binding protein 4 (CHD4) (Nio et al., 2015). Finally, combining PARP and ATR inhibitors seems a particularly efficient strategy for CSC radiosensitization, suggesting that radioresistance involves a complex rewiring of the DDR (Ahmed et al., 2015) (Figure 2).

Other processes have been targeted to force differentiation or reduce DDR signaling and hence increase CSC sensitivity to DNA damage. These include (1) BMP signaling, which induces colorectal CSC differentiation by inhibiting WNT signaling (Lombardo et al., 2011); (2) transforming growth factor beta 1 (TGFB1) signaling, whose blockade radiosensitizes CSCs by abrogating the DDR (Hardee et al., 2012); (3) Notch signaling, whose inactivation increases the sensitivity of ovarian SCs to platinum therapy by enhancing DDR-driven RCD (McAuliffe et al., 2012); (4) AKT1 signaling, whose abrogation increases the radiosensitivity of breast CSCs by inhibiting WNT signaling (Zhang et al., 2010); (5) mechanistic target of rapamycin (MTOR) signaling, whose inhibition augments the radiosensitivity of GSCs by preventing DNA repair (Kahn et al., 2014); and (6) unfolded protein response (UPR), whose activation reportedly promotes the differentiation of colorectal CSCs (Wielenga et al., 2015). Finally, CSCs may be selectively targeted with epigenetic drugs including DNA methyltransferase 1 (DNMT1) and HDAC inhibitors (Pathania et al., 2016), as well as with inhibitors of BMI1 (Kreso et al., 2014) (Figure 2).

In conclusion, the DDR stands out as a promising therapeutic target for CSC eradication.

Concluding Remarks

SCs are prone to acquire genetic lesions owing to intrinsic and environmental factors. Such lesions are especially detrimental to SC maintenance and function and may promote a variety of disorders ranging from aging-associated degenerative conditions—which are linked to dysfunctional SC pools and limited tissue regeneration/repair—to cancer—which is linked to the uncontrolled expansion of pre-malignant SCs, CSCs, or their progeny. Similarly, severe DNA damage is potentially detrimental to CSCs, providing the rationale for the development of specific therapeutic strategies. However, both normal and neoplastic SCs count upon robust antioxidant defenses (which limit genotoxicity) and a superior DDR (to repair unavoidable damage). These mechanisms are in place to protect cellular homeostasis. In addition, normal SCs undergo differentiation, senescence, or RCD in response to unrepairable DNA damage, which contributes to the maintenance of organismal (over cellular) homeostasis. The same does not hold true for CSCs, which rely on robust cytoprotective pathways to survive despite extensive genetic lesions. Thus, the mechanisms for preservation of cellular and organismal homeostasis are well interconnected in normal SCs but disconnected from each other in CSCs, which provides a therapeutic window for the selective eradication of the latter. It will be interesting to see whether strategies targeting CSCs will enter the clinical practice. Alongside, it will be important to determine whether and to what extent preserving genetic stability in normal SCs and iPSCs might have clinical benefits in the treatment of degenerative conditions. Future studies will have to elucidate these unknowns and characterize in more detail the molecular mechanisms that allow SCs to cope with DNA damage for extended periods.

AUTHOR CONTRIBUTIONS

I.V. and L.G. wrote the manuscript, centralized and integrated comments from co-authors, and revised the review upon editorial feedback. I.V. and G.M. designed figures under supervision by L.G. G.M., R.D.M., and G.K. corrected the article and provided valuable input to preparation.

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