

# Regulation of cell differentiation by the DNA damage response

Mara H. Sherman<sup>1</sup>, Craig H. Bassing<sup>2,3</sup> and Michael A. Teitell<sup>1,4</sup>

<sup>1</sup> Molecular Biology Institute, University of California, Los Angeles, CA 90095, USA

<sup>2</sup> Division of Cancer Pathobiology, Department of Pathology and Laboratory Medicine, Center for Childhood Cancer Research, Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA

<sup>3</sup> Department of Pathology and Laboratory Medicine, Abramson Family Cancer Research Institute, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA

<sup>4</sup> Department of Pathology and Laboratory Medicine, Department of Pediatrics, Jonsson Comprehensive Cancer Center, Broad Stem Cell Research Center, California NanoSystems Institute, and Bioengineering Interdepartmental Program, University of California, Los Angeles, CA 90095, USA

**When faced with DNA double-strand breaks (DSBs), vertebrate cells activate DNA damage response (DDR) programs that preserve genome integrity and suppress malignant transformation. Three established outcomes of the DDR include transient cell cycle arrest coupled with DNA repair, apoptosis, or senescence. However, recent studies in normal and cancer precursor or stem cells suggest that a fourth potential outcome, cell differentiation, is under the influence of DDR programs. Here we review and discuss the emerging evidence that supports the linkage of signaling from DSBs to the regulation of differentiation, including some of the molecular mechanisms driving this under-appreciated DDR outcome. We also consider the physiologic and pathologic consequences of defects in DDR signaling on cell differentiation and malignant transformation.**

## The vertebrate DNA damage response

DSBs are common and unavoidable genetic lesions that are also essential for vertebrate biology. Although extrinsic factors such as ionizing radiation (IR) can induce DSBs, intrinsic factors are the major source of DSBs induced throughout the genome. Reactive oxygen species (ROS) that arise as byproducts of cell respiration continuously cause DNA DSBs during all phases of the cell cycle, whereas stalled DNA replication forks and DNA replication through single-strand breaks result in DSBs during each S phase in proliferating cells. In vertebrates, the induction of DSBs by tissue-specific nucleases is crucial for the generation of genetic diversity during meiosis in germ cells and is required for the assembly and diversification of antigen receptor genes in developing lymphocytes. The misrepair or aberrant repair of DSBs can lead to apoptosis, genome instability, genetic and epigenetic changes, and cell transformation. Thus, to survive, maintain cell identity and function, yield viable progeny, prevent transformation, and ensure viability and health of host organisms, vertebrate cells must sense and respond to DSBs arising from a variety of insults [1].

The vertebrate DDR is orchestrated by the DNA damage-sensing kinases ATM, ATR, or DNA-PKcs, which can phosphorylate hundreds of proteins [2] including the p53 tumor suppressor and other tumor-suppressing and cell cycle-regulating proteins [3,4]. ATM and DNA-PKcs are themselves tumor suppressors and at least one of these kinases is required for vertebrate development; ATR is required for DNA replication and cell proliferation [5–9]. Depending on the manner, extent, and cellular context of DSB formation, several long-recognized outcomes of DDR signaling are widely appreciated: transient cell cycle arrest coupled with DNA repair, apoptosis, or senescence [10]. Recent studies now also suggest a fourth potential outcome for developing cells with DNA DSBs. In these cells the DDR appears to take on unanticipated roles of regulating precursor or stem cell differentiation programs. This function of the vertebrate DDR is distinct from known DNA DSB responses that maintain genome integrity, suggesting that DDR programs could have broader functions in precursor and stem cell development than previously recognized. The sections that follow provide examples of DDR programs that promote differentiation in several vertebrate cell lineages. We also contrast this DDR activity in differentiation with its better-appreciated role in preventing malignancy.

## DDR programs that promote cell differentiation

We highlight two recent studies that define molecular links between ATM-dependent DDR signaling from antigen receptor locus DSBs and the differentiation of B lymphoid lineage cells. We also discuss the implications for DSBs induced by genotoxic agents in influencing B-cell differentiation programs. Finally, we summarize additional experiments that collectively suggest links between p53-mediated DDR signaling from DSBs induced by extrinsic or intrinsic factors and neuronal cell differentiation.

### *B lymphocytes*

The development and function of vertebrate adaptive immune systems requires the programmed induction and subsequent repair of DSBs during antigen receptor gene rearrangements. B-cell antigen receptors (BCRs), or

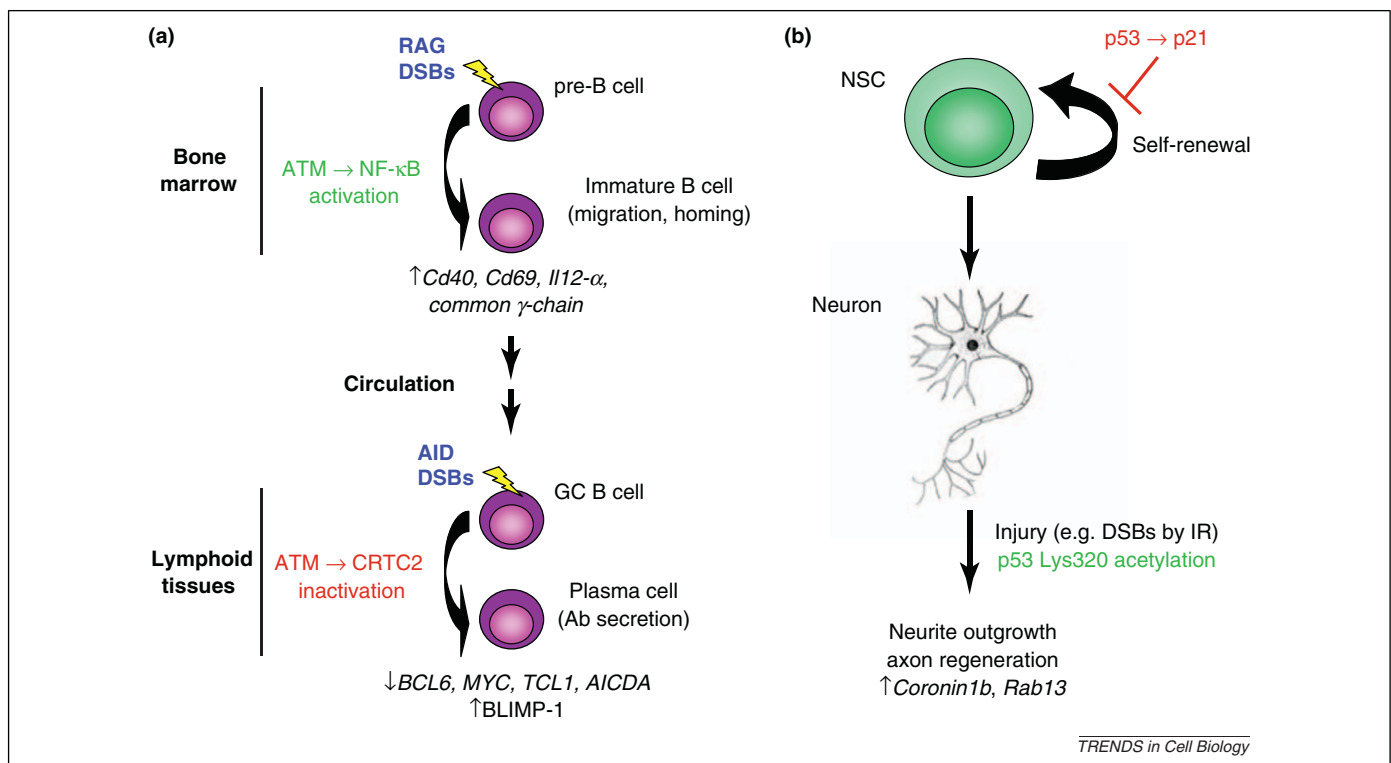
Corresponding authors: Bassing, C.H. (bassing@mail.med.upenn.edu); Teitell, M.A. (mteitell@ucla.edu).

antibodies, are composed of immunoglobulin (Ig), heavy (H), and light (L) chains encoded by different genes, which are assembled via the recombination of variable (V), diversity (D), and joining (J) gene segments. In developing B cells in the bone marrow the recombinase-activating gene (RAG) endonuclease cleaves DNA at the border of two *Ig* gene segments and their flanking recombination signal sequences [11]. RAG DSBs are then processed and joined by the non-homologous end-joining (NHEJ) DSB repair pathway [12] to assemble a complete *Ig* gene, enabling Ig chain expression at the surface of a developing B cell. V(D)J recombination of *Ig* genes occurs in a developmental stage-specific manner in which *IgH* and *IgL* genes are assembled in pro-B cells and pre-B cells, respectively. The expression of a functional *IgH* gene in pro-B cells is required for signaling developmental progression to the pre-B cell stage, whereas expression of a functional *IgL* gene in pre-B cells is required for signaling differentiation into immature B cells and emigration from the bone marrow.

RAG-induced DSBs during *IgL* gene rearrangements in pre-B cells regulate a multifunctional genetic program that involves approximately 300 genes [13]. RAG DSBs signal gene expression changes through ATM-dependent and ATM-independent mechanisms, with one of the ATM-dependent mechanisms involving the activation of intracellular signaling cascades that regulate NF- $\kappa$ B-dependent gene transcription. This RAG-induced DSB genetic program involves many genes that are expressed only in B and T lymphocytes encoding proteins that regulate processes

required for normal lymphocyte differentiation and mature lymphocyte function [13]. Notably, the ability of RAG DSBs to signal through ATM is important for normal migration and homing of pre-B cells, thereby linking the DDR with functional B-cell differentiation (Figure 1a). In this context, the ability of RAG DSBs to regulate changes in the levels of expression of lymphocyte-specific genes in pre-B cells could be important for integrating the selection of the primary IgL repertoire with secondary IgL rearrangements to 'edit' auto-reactive BCRs. Future studies also are required to evaluate whether RAG DSBs induced during *IgH* gene rearrangements in pro-B cells and during T-cell receptor gene rearrangements in pro-T and/or pre-T cells similarly regulate a multifunctional genetic program that links the DDR to lymphocyte differentiation and mature lymphocyte function.

Following successful V(D)J recombination and emigration from the bone marrow, immature B cells enter the circulation and home to lymphoid tissues throughout the body. These so-called transitional B cells continue developing into antigen-naïve mature B cells, with many residing in clusters called primary follicles within the white pulp of lymphoid tissues, and these can form secondary follicles under the influence of T-cell-dependent antigens. Secondary follicles comprise a mantle zone of non-responding B cells and a germinal center (GC) of clonally-selected antigen-responsive B cells undergoing rapid expansion coupled to *Ig* refinements and antigen-driven selection [14]. GCs are sites of class-switch recombination (CSR) and somatic hypermutation (SHM), DNA strand-breaking



**Figure 1.** DNA DSBs promote the differentiation of B-lymphocyte precursors and NSCs. **(a)** During B-cell development, DNA DSBs, introduced by RAG endonuclease in pre-B cells or by AID in GC B cells, activate gene expression programs that promote functional lineage differentiation, including cell migration, homing, and antibody secretion [13,17]. Chemical and physical agents that induce genotoxic DSBs outside antigen receptor loci also regulate the same multifunctional genetic programs as RAG and AID DSBs, respectively. **(b)** p53 → p21 signaling downstream of DSBs opposes NSC self-renewal [61,62]. Injury of neuronal precursor cells, such as by DSBs from IR, activates p53-dependent axon regeneration and neurite outgrowth via induced *Coronin1b* and *Rab13* expression [24]. Green text indicates an increase or activation, whereas red text indicates a decrease or inactivation.

processes that result in the expression of high-affinity antibodies of different isotypes that enable secretion from plasma cells. During CSR, deoxycytidine deamination mediated by activation-induced cytidine deaminase (AID) occurs in switch regions between *Ig* constant (C) region exons, giving rise to staggered single-strand breaks that resolve into DSBs [15]. Subsequent ligation of distal S regions by NHEJ [12] and/or an alternative end-joining pathway [16] enables the expression of downstream C exons, thereby modifying the effector function of an antigen-selected antibody.

In peripheral B cells stimulated to undergo a GC-like reaction *in vitro*, AID DSBs activate an ATM-dependent signaling pathway that ends by phosphorylating and inactivating the transcriptional coactivator CRTC2 [17]. CRTC2 ChIP-on-chip and global gene expression analyses in GC B cells revealed that CRTC2 regulates a network of genes that promotes proliferation, GC B-cell self-renewal, and inhibits plasma cell differentiation. During CSR, DSBs activate ATM to CRTC2 signaling, which causes CRTC2 phosphorylation and inactivation by exclusion from the nucleus. CRTC2 inactivation is required to end an ongoing GC reaction and differentiate B cells into antibody-secreting plasma cells (Figure 1a). AID-knockout or ATM-knockdown GC B cells continue to proliferate, are impaired for antibody secretion, and fail to execute the plasma cell differentiation program properly. Humans with AID deficiency are immunodeficient and suffer from hyper-IgM syndrome type 2 (HIGM2). HIGM2 B cells lack AID-induced DSBs, fail to class-switch, have reduced serum antibodies, and show lymphoid hyperplasia with massively enlarged GCs that result from a defect in plasma cell differentiation [18]. In human B-cell lymphoma patient samples, molecular alterations in the pathway from AID-induced DSBs to CRTC2 inactivation include repressed ATM and LKB1 expression and sequence alterations in the CRTC2 kinase target domain [17]. Future studies to evaluate the tumorigenic role of these alterations, and also the use of strategies to repair pathway defects and promote differentiation with DSBs, will be required to assess differentiation blockade as a driver in GC B-cell lymphomagenesis.

Exposure of pre-B and GC B cells to chemical and physical agents that induce genotoxic DSBs regulate the same multifunctional genetic programs as RAG and AID DSBs, respectively [13,17]. These results demonstrate that DSBs outside of antigen receptor loci also activate DDR programs that regulate the expression of B-lymphocyte-specific genes. These data further suggest that the induction of non-physiologic DSBs, such as during chemotherapy, in B-lineage and potentially other lineage cells could have adverse effects upon cell development and function. In this context, follow-up studies of children and young adults with Hodgkin and non-Hodgkin lymphomas treated with genotoxic therapies showed an increased incidence of secondary malignancies, most commonly leukemias and lymphomas [19,20]. Notably, adult survivors of childhood cancers treated with genotoxic drugs exhibited a higher incidence of both immunological and neurological disorders than the general population [21]. This type of emerging evidence suggests a possible connection between

neurologic disorders and the corruption of cell differentiation programs caused by increased frequencies of DSBs in the neural tissues of children.

### Neuronal cells

The results of several experiments collectively suggest links between p53-mediated DDR signaling from DSBs induced by extrinsic or intrinsic factors and neuronal precursor or stem cell (NSC) differentiation. IR-induced DSBs result in p53 Lys320 acetylation [22] in the CNS [23], and acetylated p53 Lys320 promotes neurite outgrowth *in vitro* and axon regeneration *in vivo* [24]. IR also induces p53 expression in subventricular zone (SVZ) NSCs, with loss of p53 resulting in increased NSC proliferation and impaired neuronal differentiation *in vitro* and *in vivo* [25]. Consistent with a role for p53 in promoting DSB-induced neuronal differentiation, p53-deficient SVZ NSCs show increased proliferation and self-renewal capacity and impaired expression of neuronal or glial lineage differentiation markers [26]. These studies altogether suggest the need for additional and more direct evidence that DSBs promote p53-dependent NSC or neuronal precursor differentiation. It is also notable that p21, a major target of p53 signaling, inhibits NSC self-renewal possibly through its action in blocking cell cycle progression (Figure 1b) [27]. Loss of p53 could relax p21-dependent cell cycle checkpoints, resulting in increased NSC self-renewal and inhibited differentiation. Combined, these data suggest a potential role for DSB-initiated p53-dependent DDR signaling in suppressing NSC and multipotent precursor cell self-renewal in favor of differentiation. In addition to direct tests of this idea, studies that identify a molecular pathway linking DSB-initiated signaling to the control of neuronal differentiation are required to establish a definitive role for the DDR in neuronal differentiation.

### The flip side: DDR programs that inhibit cell differentiation

In this section we discuss the opposing influence that the DDR response can have on cell differentiation, highlighting studies conducted with myoblasts, melanocyte stem cells, and hematopoietic stem cells.

### Myoblasts

Whereas many stem and progenitor cell types could be induced to proliferate, the terminal differentiation of specialized cells almost invariably coincides with withdrawal from the cell cycle [28]. In this regard, genotoxic stress-induced cell cycle arrest could potentially coactivate a cell differentiation program, which could result in differentiated cells with deleterious genetic alterations. To prevent this, it was proposed that there could be a DDR-regulated differentiation checkpoint akin to a cell cycle arrest checkpoint [29]. To test this idea, C2C12 myoblasts were examined for a DDR-regulated differentiation checkpoint during muscle differentiation. C2C12 cells actively divide as undifferentiated, mononucleated cells in the presence of serum and also express a transcriptionally inactive form of MyoD, a key muscle lineage-promoting transcriptional regulator [30]. Serum withdrawal results in MyoD activation, cell cycle exit, and terminal differentiation of C2C12

myoblasts into multinucleated myotubes. C2C12 cells underwent serum withdrawal following exposure to a panel of genotoxic agents, including etoposide and IR which cause DNA DSBs [29]. In addition to inducing cell cycle arrest, these agents blocked myotube formation via c-ABL-dependent inhibition of MyoD activation, supporting the presence of a DDR-regulated differentiation checkpoint. Importantly, the block on muscle differentiation by genotoxic stress is reversible, because washout of these agents enables efficient differentiation of C2C12 cells into myotubes. The reversibility of a DDR-regulated differentiation checkpoint suggests that myoblasts can overcome this block following the repair of damaged DNA.

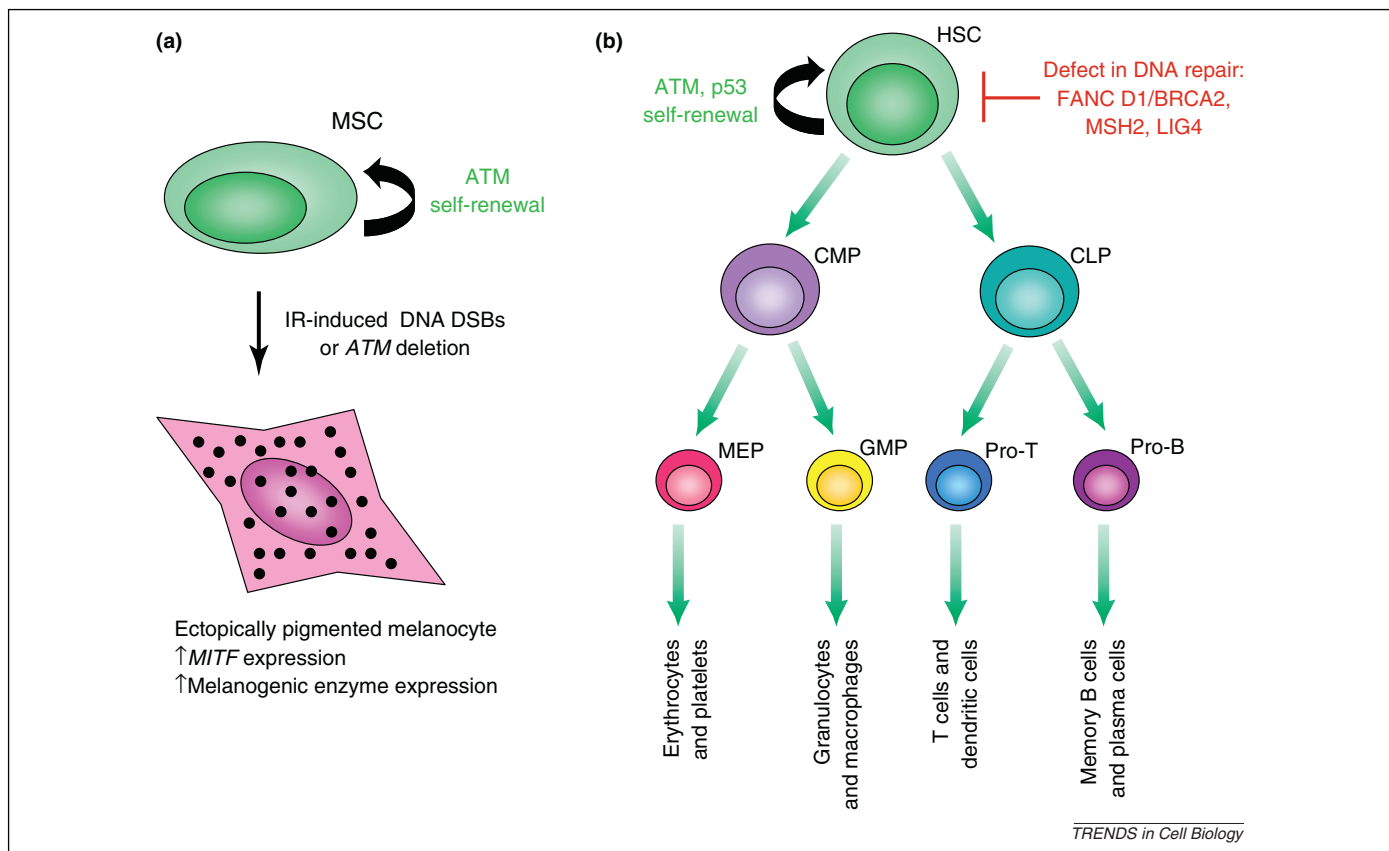
### Melanocyte stem cells

Somatic stem cell and progenitor cell functions decline with aging, and this is thought to contribute to the progressive dysfunction of tissues in organisms of advanced age [31–33]. The molecular and physiologic changes that arise in somatic stem and progenitor cells with aging remains incompletely understood, although increasing evidence suggests functional connections between DSB frequencies, DDR programs, and differentiation within these replacement cell pools [34,35].

Hair greying is one of the most obvious signs of aging in humans and results from the loss of melanocyte stem cells

(MSCs) from subcutaneous hair follicles over time [36]. DNA DSBs from IR oppose MSC self-renewal and trigger aberrant MSC differentiation into mature melanocytes within the stem cell niche, resulting in grey hair in the absence of apoptosis or cell senescence (Figure 2a) [37]. Ectopic differentiation of MSCs is further exacerbated by targeted deletion of *Atm*, resulting in defects in DNA repair and DDR signaling upon IR [37]. These results indicate that increased frequencies of DSBs, the absence of efficient DSB repair, and/or DDR signaling, promote the differentiation of MSCs by removing them from the repopulating pool within the replicative niche, and suggest that ATM functions as a ‘stemness checkpoint’ to maintain stem cell quality and amount. Therefore, it appears that cellular context matters a great deal, because DSB-initiated ATM signaling maintains MSCs and blocks differentiation, whereas DSB-initiated ATM signaling promotes the differentiation of B-lineage cells.

Deletion of the DDR gene *Atr* in adult mice leads to depletion of stem and progenitor pools in the intestine, bone marrow, and skin, resulting in tissue dysfunction and aging-related phenotypes [33]. Although it was proposed that the loss of stem and progenitor cells was from elevated apoptosis or senescence, an increase in apoptotic cells was not observed and  $\beta$ -galactosidase staining for senescent cells did not differ between *Atr*-deficient and control mouse



**Figure 2.** DNA DSBs inhibit melanocyte stem cell (MSC) differentiation and hematopoietic stem cell (HSC) reconstitution of blood-lineage cells. (a) ATM maintains the self-renewal of MSCs, which controls orderly hair-cell differentiation and hair color. Increased DSBs in MSCs from IR, or from ATM deletion, opposes MSC self-renewal and instead activates aberrant MSC differentiation via the induction of *MITF*, the master transcriptional regulator of melanocyte development. This gives rise to ectopically pigmented melanocytes in the stem cell niche and premature hair greying [37]. (b) DDR proteins p53 [40] and ATM [41] maintain HSC self-renewal, whereas defects in DNA repair proteins FANC D1/BRCA2, MSH2, or LIG4 impair the ability of HSCs to repopulate the hematopoietic system during cell stress or regeneration [38]. Key: CLP, common lymphoid progenitor; CMP, common myeloid progenitor; MEP, megakaryocyte-erythroid progenitor; GMP, granulocyte-macrophage progenitor; pro-T, pre-T-cell precursor; pro-B, pre-B-cell precursor

tissues. Thus, the possibility remains that mosaic adult mouse tissue deletion of *Atr* triggered aberrant, ectopic differentiation, leading to the loss of proliferating stem and progenitor cells and to the accelerated onset of aging-associated tissue dysfunction, although this notion requires further evaluation.

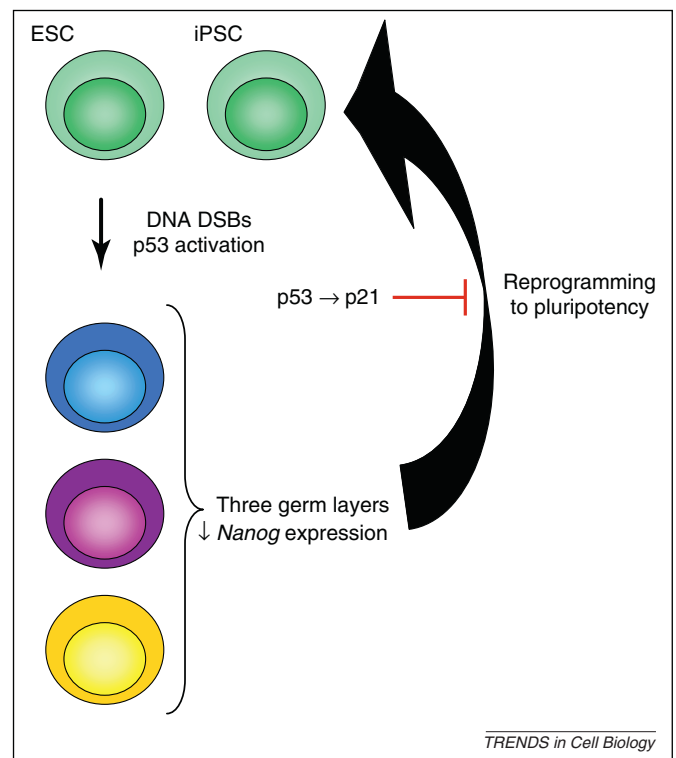
#### Hematopoietic stem cells

DNA damage from exogenous and endogenous sources, including DNA DSBs marked by  $\gamma$ H2AX foci, accumulate in hematopoietic stem cells (HSCs) with age; these could contribute to the functional decline of HSCs over time [32]. Suggesting a connection between DNA DSBs, DDR programs, and a functional decline in aging HSCs is the impairment of the ability of HSCs to reconstitute blood-lineage cells in mice deficient for the DNA-repair genes *FancD1/Brca2*, *Msh2*, or *Lig4* [38]. These mutant mouse strains do not show decreased numbers of biomarker-defined HSCs, but instead demonstrate severe defects in repopulating blood cell lineages in response to stress or regeneration cues. HSC expansion and maintenance is minimally affected in these DDR mutant mouse strains, probably as a consequence of the natural quiescent state of HSCs, which also protects them from accumulating DNA lesions. Aging HSCs exhibit a differentiation bias that favors the myeloid lineage with a concomitantly reduced potential for lymphoid lineage differentiation [39], although whether this aging bias relates to the accumulation of DNA damage over time has not been determined. When stressed to self-renew in bone-marrow transplantation assays, HSCs from DDR-deficient mice are defective [32]. Mutant HSCs show significantly increased apoptosis and decreased proliferation capacity, suggesting that the proper repair of DNA damage is required for efficient HSC self-renewal and reconstitution potential under duress. Both p53 [40] and ATM [41] positively regulate HSC quiescence and self-renewal (Figure 2b), whereas in the absence of ATM, ROS-induced DNA damage leads to the loss of self-renewal capacity and exhaustion of the HSC pool [42]. These findings suggest that increased DSBs from the lack of ATM, and/or impaired DDR signaling from absent ATM, exacerbate these HSC defects. As in MSCs, DDR signaling from normal physiologic frequencies of DSBs maintains HSCs, whereas the loss of a wide range of DDR genes leads to increased DSBs, impaired DNA repair, and depletion of the HSC pool, resulting in reduced reconstitution or differentiation capacity. Overall, these studies suggest a protective function for the DDR in stem and progenitor cell pools, potentially to promote normal development and prevent the propagation of genetic errors. Pluripotent embryonic stem cells, which can give rise to all vertebrate cell types of an adult, can also be subject to similar DDR-mediated regulation, as discussed in the next section.

#### DDR influences pluripotent stem cell differentiation

Pluripotent embryonic stem cells (ESCs) are unique in their capacity for self-renewal and differentiation into all three germ layers of the growing embryo. DNA lesions, including deletions, amplifications, or translocations resulting from aberrant DSB repair within ESCs, could

be propagated to daughter stem and differentiated cells, with the potential to affect all levels of the developmental hierarchy, including future generations of individuals within a species. Although apoptosis could clear severely damaged cells from the replicating stem cell pool, ESCs can activate/engage an alternative response to DSBs. In mouse ESCs (mESCs) with DSBs following doxorubicin exposure, p53 binds to the promoter of *Nanog*, a gene required for ESC self-renewal [43,44], resulting in repressed *Nanog* expression [45]. The repression of *Nanog* by p53 promotes the removal of damaged cells from the replicating stem cell pool by encouraging mESC differentiation, with subsequent establishment of efficient p53-dependent cell cycle arrest or apoptosis programs in differentiated progeny cells. Notably, additional studies are required to determine whether DDR programs directly induce the differentiation of the three vertebrate germ layers from ESCs. p53–p21 signaling also suppresses the generation of induced pluripotent stem cells by genetic reprogramming [46,47], in which quiescent differentiated cells re-enter the cell cycle and are returned to a stem cell-like state by the introduction of key pluripotency-regulating transcription factors [48]. These findings are consistent with a potential connection between DSB-initiated DDR signaling and cell differentiation or de-differentiation (Figure 3), and leave open the possibility that regulating pluripotent stem cell differentiation upon induction of DNA DSBs is an additional p53 function. Induced pluripotent stem cells represent an exciting therapeutic application in stem cell



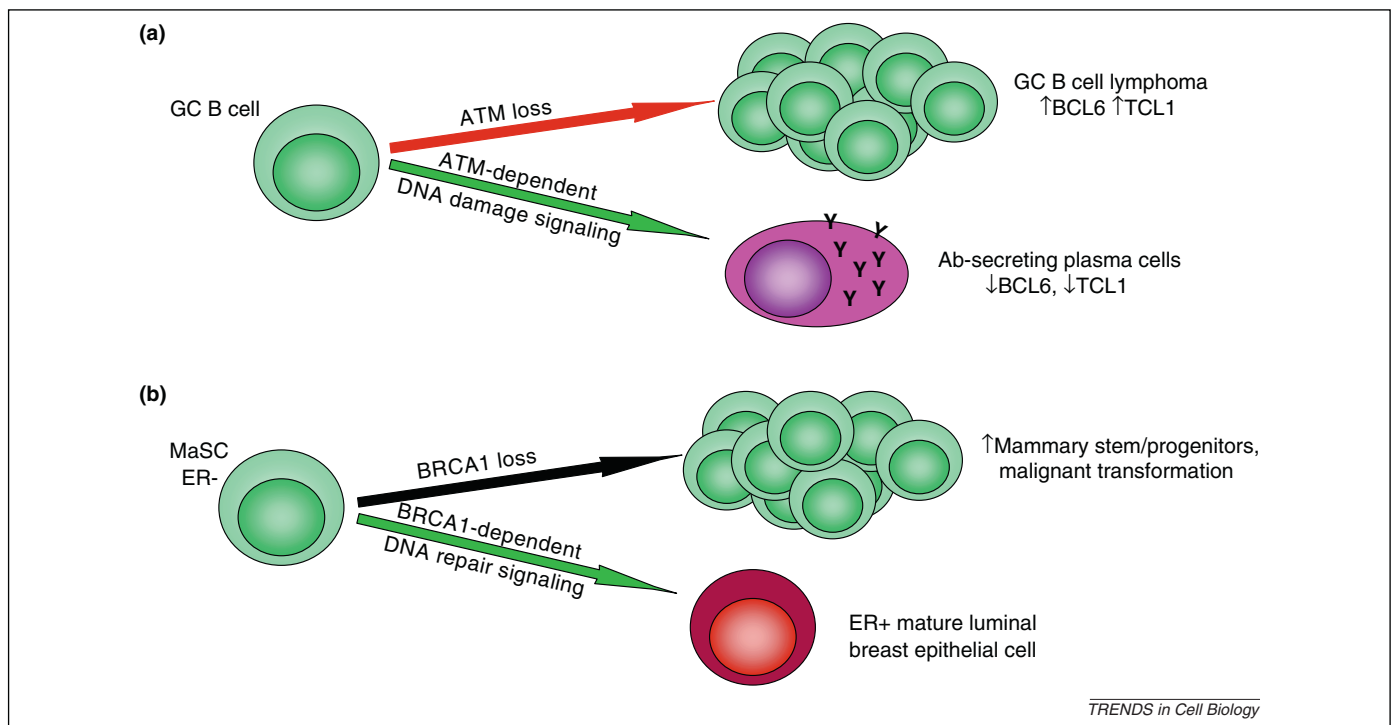
**Figure 3.** DDR molecules promote the differentiation ESCs and inhibit the reprogramming of differentiated cells to induced pluripotent stem cells (iPSCs). p53 activation by DNA DSBs in ESCs leads to p53 binding of the *Nanog* promoter and suppression of the pluripotency transcription factor, NANOG [45]. NANOG suppression supports the differentiation of ESCs into the three germ layers of a developing embryo. The reverse process – generation of iPSCs from differentiated cells by the introduction of key pluripotency-regulating transcription factors – is inhibited by p53 → p21 DDR signaling [46,47].

biology, and an improved understanding of the barriers to the reprogramming process is of great value. These recent studies suggest that inhibition of p53-dependent signaling cascades can improve the efficiency of somatic cell reprogramming. Although this is a desirable outcome, permanent inhibition of p53 would also increase the associated risk of malignant transformation. Future work could provide methods that achieve transient inhibition of the p53 pathway during reprogramming, possibly using chemical inhibitors or reversible genetic techniques, to generate a higher yield of clinically relevant induced pluripotent stem cells. This challenge highlights a practical role for understanding and manipulating DDR programs that promote differentiation while opposing malignant transformation.

### DSBs driving differentiation versus malignancy

Cell differentiation is regulated by networks of transcription factors and signaling pathways that can show cell type-restricted expression and/or temporally controlled activation or repression. Cells usually differentiate without undergoing malignant transformation, although genetic or epigenetic alterations that arrest differentiation are frequently observed in cancer. The role of the transcriptional repressor BCL6 in the B-cell compartment serves to illustrate the balance between DSB-initiated differentiation versus transformation during lineage development. The translation of BCL6 mRNA into protein is specifically induced in B cells during the GC reaction, and BCL6 directly or indirectly represses a network of DDR genes that regulate cell cycle arrest and apoptosis, including *ATR*, *p53*, and *p21* [14,49]. The repression of DDR genes

probably enables GC B cells to sustain the AID-induced DNA DSBs associated with *Ig* gene remodeling, while maintaining a rapid rate of expansion, because mice lacking *Bcl6* cannot form GCs [50]. BCL6 also inhibits the terminal differentiation of GC B cells into antibody-secreting plasma cells by repression of the master regulator of plasma cell development, *Prdm1*, which encodes BLIMP1. Not surprisingly, dysregulated *Bcl6* expression in mice causes increased GC formation, inhibition of plasma cell differentiation, and generation of diffuse large B-cell lymphomas [14,51]. Lymphomagenesis in this case could be due to aberrant continued repression of *DDR* genes and subsequent accumulation of transforming lesions, although this notion requires investigation. Mutations and chromosomal translocations activating *BCL6* expression are also frequently observed in GC-derived B cell lymphoma patient samples, replicating the findings seen in genetically engineered mice. Notably, a recent study demonstrated that IR- or etoposide-induced DSBs leads to ATM-dependent degradation of BCL6 protein [52], which could release GC B cells to differentiate into plasma cells. AID-induced ATM signaling during CSR also represses *TCL1* oncogene expression by inactivation of CRTC2 [17], and *TCL1* is a known promoter of mESC self-renewal [53] whose dysregulation also leads to GC-derived B cell lymphomas (Figure 4a) [54,55]. These data suggest that signaling components downstream of ATM activation could serve as novel therapeutic targets to promote BCL6 degradation or *TCL1* silencing and drive the differentiation or apoptotic response in B-cell lymphomas. Furthermore, the results also suggest that DDR-



**Figure 4.** DDR to DSBs influences outcomes between cell differentiation and malignant transformation. **(a)** In GC B cells, dysregulated expression of *BCL6* [14] or *TCL1* [54] oncoproteins promotes B cell lymphomagenesis. ATM-dependent DDR signaling promotes the degradation of BCL6 protein and the transcriptional repression of *TCL1* [17,52], favoring terminal B-cell differentiation to antibody-secreting plasma cells over malignant transformation. Not all GC B-cell lymphomas are ATM-deficient because there are additional lymphomagenic mechanisms. **(b)** Expression of the DNA repair enzyme BRCA1 is required for the differentiation of ER-negative MaSCs into ER-positive mature luminal cells, whereas the loss of BRCA1 causes an impaired DDR, robust expansion of MaSCs, and promotes malignant transformation [59]. Not all breast cancers are BRCA1-deficient because there are additional carcinogenic mechanisms.

initiated ATM signaling couples differentiation with the prevention of lymphomagenesis. A relationship between faulty differentiation and malignant degeneration has also been described for so-called cancer stem cells, as discussed further below.

### DDR and dysregulated cancer stem cell self-renewal

Studies of hematopoietic, breast, brain, colon, pancreas, and other cancers has provided evidence for a model of malignant transformation known as the cancer stem cell (CSC) hypothesis [56]. This model is based on the presence of a subset of cancer cells that exhibit certain stem cell properties within a tumor, such as the ability for indefinite self-renewal that can sustain or re-establish a malignancy [57]. Pioneering work in CSC biology has resulted in the identification of cell surface markers useful for the isolation of CSC populations within certain types of malignancies that maintain tumorigenic potential. However, the molecular mechanisms underlying dysregulation of self-renewal and differentiation in CSCs are not clear. BRCA1 is part of the DDR and is known to regulate DNA repair, cell cycle checkpoints, and overall genome stability [58]. BRCA1-positive breast cancers are usually of the basal-cell type and could resemble adult mammary stem cells (MaSCs) in appearance and biomarker expression; therefore, these tumors are thought to originate from MaSCs with defective differentiation. A recent study showed that *BRCA1* expression was required for the differentiation of estrogen receptor (ER)-negative MaSCs into ER-positive mature luminal cells, with the loss of BRCA1 causing a robust expansion of the mammary stem/progenitor cell pool and a concomitant decrease in cells expressing ER and other luminal epithelial markers (Figure 4b) [59]. These data suggest that BRCA1 has a key role in the differentiation of MaSCs, further suggesting a connection between the DDR and cell differentiation. The absence of BRCA1 could inhibit differentiation via the accumulation of genetically unstable MaSCs and consequent secondary oncogenic mutations. However, it also remains possible and untested that the BRCA1-dependent DNA repair machinery activates differentiation-inducing signaling pathways in MaSCs.

### Concluding remarks

Evidence accumulated over many years has connected deficiencies in DDR pathways with tumorigenesis. Defects in established components of the DDR, including DNA repair, cell cycle arrest, apoptosis, or senescence, have been shown to foster genomic instability and enable secondary genetic alterations. DDR-deficient chromosomal instability leads to genome alterations that are recurrently observed in multiple mouse and human tumor types, suggesting that defects in key DDR pathways promote tumorigenesis in part by allowing the onset and/or selection of driver alterations within the genome [60]. A distinct model that connects deficiencies in DDR signaling to the development of cancer involves the notion that differentiation and cell transformation possibly are related and alternative outcomes, and this is supported by several recent studies demonstrating molecular mechanisms that couple central DDR pathways to

tissue-specific differentiation programs. This model posits cell differentiation as a viable precursor cell outcome of the DDR. From an evolutionary standpoint, the DDR-enforced differentiation of stem or precursor cells would help to preserve the genome integrity of a cell type, tissue, organism, or species.

How does a cell decide between the four possible outcomes of the DDR? We suggest at least two candidate processes that control the decision-making process. The first is related to the extent of damage to the genome. When faced with extensive genome-wide damage, apoptosis or senescence of the cell is to the benefit of the organism, because it is unlikely that DNA repair processes would eradicate all deleterious genome aberrations. However, in the case of low levels of exogenous or physiologic DNA damage, as incurred by developing B cells subjected to DSBs by RAG and AID during *Ig* gene modifications, the needs of the organism are served by inducing differentiation. A second decision-regulating process could involve the differentiation state of a DNA-damaged cell within a specific lineage. For example, DDR signaling via ATM promotes the quiescence and self-renewal of HSCs [41], whereas in more advanced lineage progenitors, such as pre-B [13] and GC [17] B cells, ATM-dependent DDR signaling promotes cell differentiation.

Additional studies are required to uncover the mechanisms that block differentiation and potentially act as primary drivers for many tumor types, as *BCL6* dysregulation appears to do for some diffuse large B-cell lymphomas. Because differentiation therapy can be an effective treatment modality for specific cancers, an improved understanding of the role of the DDR in regulating cell differentiation within developing tissues could provide novel treatment opportunities. The studies discussed here suggest that specific components of DDR signaling pathways might be candidate therapeutic targets – not only for their effects on genome maintenance but also for their ability to direct the differentiation fates of stem and precursor cells. Future studies that determine the interactions between DDR and differentiation signaling networks will increase our understanding of the close interplay between cell differentiation and malignant transformation.

### Acknowledgments

Work in the Bassing and Teitell labs is supported by grants from the National Institutes of Health (GM07185 to M.H.S., CA136470 and CA125195 to C.H.B., and CA90571, CA156674, GM073981, P01GM081621, and PNEY018228 to M.A.T.) and by the California Institute for Regenerative Medicine (CIRM grant RB1-01397 to M.A.T.). C.H.B. is a Leukemia and Lymphoma Society Scholar.

### References

- 1 Harrison, J.C. and Haber, J.E. (2006) Surviving the breakup: the DNA damage checkpoint. *Annu. Rev. Genet.* 40, 209–235
- 2 Matsuoka, S. *et al.* (2007) ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* 316, 1160–1166
- 3 Banin, S. *et al.* (1998) Enhanced phosphorylation of p53 by ATM in response to DNA damage. *Science* 281, 1674–1677
- 4 Canman, C.E. *et al.* (1998) Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. *Science* 281, 1677–1679
- 5 Barlow, C. *et al.* (1996) Atm-deficient mice: a paradigm of ataxia telangiectasia. *Cell* 86, 159–171

- 6 Brown, E.J. and Baltimore, D. (2003) Essential and dispensable roles of ATR in cell cycle arrest and genome maintenance. *Genes Dev.* 17, 615–628
- 7 Costanzo, V. *et al.* (2003) An ATR- and Cdc7-dependent DNA damage checkpoint that inhibits initiation of DNA replication. *Mol. Cell* 11, 203–213
- 8 Gurley, K.E. and Kemp, C.J. (2001) Synthetic lethality between mutation in *Atm* and DNA-PK(cs) during murine embryogenesis. *Curr. Biol.* 11, 191–194
- 9 Jhappan, C. *et al.* (1997) DNA-PKcs: a T-cell tumour suppressor encoded at the mouse *scid* locus. *Nat. Genet.* 17, 483–486
- 10 Kruse, J.P. and Gu, W. (2009) Modes of p53 regulation. *Cell* 137, 609–622
- 11 Jung, D. and Alt, F.W. (2004) Unraveling V(D)J recombination; insights into gene regulation. *Cell* 116, 299–311
- 12 Rooney, S. *et al.* (2004) The role of the non-homologous end-joining pathway in lymphocyte development. *Immunol. Rev.* 200, 115–131
- 13 Bredemeyer, A.L. *et al.* (2008) DNA double-strand breaks activate a multi-functional genetic program in developing lymphocytes. *Nature* 456, 819–823
- 14 Klein, U. and Dalla-Favera, R. (2008) Germinal centres: role in B-cell physiology and malignancy. *Nat. Rev. Immunol.* 8, 22–33
- 15 Chaudhuri, J. *et al.* (2007) Evolution of the immunoglobulin heavy chain class switch recombination mechanism. *Adv. Immunol.* 94, 157–214
- 16 Yan, C.T. *et al.* (2007) IgH class switching and translocations use a robust non-classical end-joining pathway. *Nature* 449, 478–482
- 17 Sherman, M.H. *et al.* (2010) AID-induced genotoxic stress promotes B cell differentiation in the germinal center via ATM and LKB1 signaling. *Mol. Cell* 39, 873–885
- 18 Revy, P. *et al.* (2000) Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the hyper-IgM syndrome (HIGM2). *Cell* 102, 565–575
- 19 Lin, H.M. and Teitell, M.A. (2005) Second malignancy after treatment of pediatric Hodgkin disease. *J. Pediatr. Hematol. Oncol.* 27, 28–36
- 20 Sacchi, S. *et al.* (2008) Secondary malignancies after treatment for indolent non-Hodgkin's lymphoma: a 16-year follow-up study. *Haematologica* 93, 398–404
- 21 Oeffinger, K.C. *et al.* (2006) Chronic health conditions in adult survivors of childhood cancer. *N. Engl. J. Med.* 355, 1572–1582
- 22 Liu, L. *et al.* (1999) p53 sites acetylated *in vitro* by PCAF and p300 are acetylated *in vivo* in response to DNA damage. *Mol. Cell. Biol.* 19, 1202–1209
- 23 Chao, C. *et al.* (2006) Acetylation of mouse p53 at lysine 317 negatively regulates p53 apoptotic activities after DNA damage. *Mol. Cell. Biol.* 26, 6859–6869
- 24 Di Giovanni, S. *et al.* (2006) The tumor suppressor protein p53 is required for neurite outgrowth and axon regeneration. *EMBO J.* 25, 4084–4096
- 25 Gil-Perotin, S. *et al.* (2006) Loss of p53 induces changes in the behavior of subventricular zone cells: implication for the genesis of glial tumors. *J. Neurosci.* 26, 1107–1116
- 26 Zheng, H. *et al.* (2008) p53 and Pten control neural and glioma stem/progenitor cell renewal and differentiation. *Nature* 455, 1129–1133
- 27 Mori, S. *et al.* (2001) Supraspinal sites that induce locomotion in the vertebrate central nervous system. *Adv. Neurol.* 87, 25–40
- 28 Walsh, K. and Perlman, H. (1997) Cell cycle exit upon myogenic differentiation. *Curr. Opin. Genet. Dev.* 7, 597–602
- 29 Puri, P.L. *et al.* (2007) A myogenic differentiation checkpoint activated by genotoxic stress. *Nat. Genet.* 32, 585–593
- 30 Wei, Q. and Paterson, B.M. (2001) Regulation of MyoD function in the dividing myoblast. *FEBS Lett.* 490, 171–178
- 31 Nijnik, A. *et al.* (2007) DNA repair is limiting for haematopoietic stem cells during ageing. *Nature* 447, 686–690
- 32 Rossi, D.J. *et al.* (2007) Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. *Nature* 447, 725–729
- 33 Ruzankina, Y. *et al.* (2007) Deletion of the developmentally essential gene ATR in adult mice leads to age-related phenotypes and stem cell loss. *Cell Stem Cell* 1, 113–126
- 34 Hasty, P. *et al.* (2003) Aging and genome maintenance: lessons from the mouse? *Science* 299, 1355–1359
- 35 Lombard, D.B. *et al.* (2005) DNA repair, genome stability, and aging. *Cell* 120, 497–512
- 36 Nishimura, E.K. *et al.* (2005) Mechanisms of hair graying: incomplete melanocyte stem cell maintenance in the niche. *Science* 307, 720–724
- 37 Inomata, K. *et al.* (2009) Genotoxic stress abrogates renewal of melanocyte stem cells by triggering their differentiation. *Cell* 137, 1088–1099
- 38 Rossi, D.J. *et al.* (2008) Stems cells and the pathways to aging and cancer. *Cell* 132, 681–696
- 39 Rossi, D.J. *et al.* (2005) Cell intrinsic alterations underlie hematopoietic stem cell aging. *Proc. Natl. Acad. Sci. U.S.A.* 102, 9194–9199
- 40 Liu, Y. *et al.* (2009) p53 regulates hematopoietic stem cell quiescence. *Cell Stem Cell* 4, 37–48
- 41 Ito, K. *et al.* (2004) Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. *Nature* 431, 997–1002
- 42 Ito, K. *et al.* (2006) Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. *Nat. Med.* 12, 446–451
- 43 Chambers, I. *et al.* (2003) Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell* 113, 643–655
- 44 Mitsui, K. *et al.* (2003) The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. *Cell* 113, 631–642
- 45 Lin, T. *et al.* (2005) p53 induces differentiation of mouse embryonic stem cells by suppressing Nanog expression. *Nat. Cell Biol.* 7, 165–171
- 46 Hong, H. *et al.* (2009) Suppression of induced pluripotent stem cell generation by the p53-p21 pathway. *Nature* 460, 1132–1135
- 47 Kawamura, T. *et al.* (2009) Linking the p53 tumour suppressor pathway to somatic cell reprogramming. *Nature* 460, 1140–1144
- 48 Takahashi, K. and Yamanaka, S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126, 663–676
- 49 Ranuncolo, S.M. *et al.* (2007) Bcl-6 mediates the germinal center B cell phenotype and lymphomagenesis through transcriptional repression of the DNA-damage sensor ATR. *Nat. Immunol.* 8, 705–714
- 50 Fukuda, T. *et al.* (1997) Disruption of the Bcl6 gene results in an impaired germinal center formation. *J. Exp. Med.* 186, 439–448
- 51 Cattoretti, G. *et al.* (2005) Deregulated BCL6 expression recapitulates the pathogenesis of human diffuse large B cell lymphomas in mice. *Cancer Cell* 7, 445–455
- 52 Phan, R.T. *et al.* (2007) Genotoxic stress regulates expression of the proto-oncogene Bcl6 in germinal center B cells. *Nat. Immunol.* 8, 1132–1139
- 53 Ivanova, N. *et al.* (2006) Dissecting self-renewal in stem cells with RNA interference. *Nature* 442, 533–538
- 54 Hoyer, K.K. *et al.* (2002) Dysregulated TCL1 promotes multiple classes of mature B cell lymphoma. *Proc. Natl. Acad. Sci. U.S.A.* 99, 14392–14397
- 55 Teitell, M.A. (2005) The TCL1 family of oncoproteins: co-activators of transformation. *Nat. Rev. Cancer* 5, 640–648
- 56 Cho, R.W. and Clarke, M.F. (2008) Recent advances in cancer stem cells. *Curr. Opin. Genet. Dev.* 18, 48–53
- 57 Reya, T. *et al.* (2001) Stem cells, cancer, and cancer stem cells. *Nature* 414, 105–111
- 58 Venkitaraman, A.R. (2002) Cancer susceptibility and the functions of BRCA1 and BRCA2. *Cell* 108, 171–182
- 59 Liu, S. *et al.* (2008) BRCA1 regulates human mammary stem/progenitor cell fate. *Proc. Natl. Acad. Sci. U.S.A.* 105, 1680–1685
- 60 Maser, R.S. *et al.* (2007) Chromosomally unstable mouse tumours have genomic alterations similar to diverse human cancers. *Nature* 447, 966–971
- 61 Armesilla-Diaz, A. *et al.* (2009) p53 regulates the self-renewal and differentiation of neural precursors. *Neuroscience* 158, 1378–1389
- 62 Tedeschi, A. and Di Giovanni, S. (2009) The non-apoptotic role of p53 in neuronal biology: enlightening the dark side of the moon. *EMBO Rep.* 10, 576–583