

# Cell Cycle News & Views

## MRE11 and ATM AKTivate pro-survival signaling

Comment on: Fraser M, et al. *Cell Cycle* 2011; 10:2218–32

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Whether during normal cell growth or after genotoxic stress, there is a continuous battle between survival and death that determines cell fate. Tumor cells have acquired enhanced anti-apoptotic and pro-survival properties that make them resistant to cancer therapy. It is well-established that AKT is hyperactivated in many cancers, and that this protects against cell killing in response to DNA damaging agents, including ionizing radiation (IR). A plethora of events are triggered in response to IR, including the DNA damage response (DDR), apoptosis/death as well as pro-survival signaling via ERK and PI3K/AKT.<sup>1</sup> Many laboratories have reported that IR activates AKT (seen as increased levels of AKT phosphorylation at S473 and T308). ATM (ataxia telangiectasia mutated), the master regulator of the DDR, in part controls insulin and IR-induced phosphorylation of AKT,<sup>2,3</sup> and AKT has been shown to regulate DSB repair in a number of different cell systems.<sup>4-8</sup> However, it is unclear how the DDR signal originating from DSBs is transduced to AKT.

The article by Fraser et al. published in the July 1st issue of *Cell Cycle* reports that MRE11 promotes the accumulation of phosphorylated AKT (S473) at DSBs via ATM.<sup>9</sup> They demonstrated that AKT is important for conferring radioresistance and for promoting the ligation of linearized plasmids in vitro, suggesting that AKT might facilitate the resealing of DSBs, thereby enhancing cell survival. Using IR, nuclear UV laser micro-irradiation or an inducible restriction endonuclease to induce DSBs, they showed that pAKT (S473), but not pAKT (T308) or total AKT, accumulated in the vicinity

of IR-induced DSB and colocalized with  $\gamma$ H2AX and pATM (S1981).

Knowing that MRE11-RAD50-NBS1 (MRN) is positioned upstream of ATM, they knocked down MRE11 expression and showed that pAKT (S473) foci formation was dependent on MRE11. However, MRE11 did not require the MRE11 endonuclease domain, suggesting that DNA resection was not necessary to attract pAKT to repair centers. They went on to show that the histone ubiquitin ligase RNF168 was also required for DSB-induced pAKT localization. However, DNA-PKcs, PI3K and ATR were not. These results demonstrate that DSBs activate a signaling cascade that directly promotes a PI3K-independent pathway of AKT phosphorylation that is dependent on MRE11-ATM-RNF168 signaling. Altogether, the authors suggest that ubiquitin-dependent and DNA-PKcs-independent non-homologous end joining (NHEJ) repair is the target for pAKT.

What remains unclear is whether the global effects resulting from AKT knockdown on radiosurvival can be fully accounted for by the relatively small fraction of total AKT that localized to DSBs as pAKT (S473). Furthermore, is NHEJ specifically influenced by AKT, or is homologous recombination repair (HRR) also affected? Since resection was not necessary for attracting pAKT to repair foci, this might argue against a role for HRR. As the kinetics of foci removal was not closely examined in the study (except for a single 24 h time point), it is difficult to say whether the presence of pAKT at DSBs influences repair in vivo. Another question is the role of other AKT isoforms. Only AKT1 was manipulated by RNAi. It would

be interesting to know whether AKT2 and AKT3, two other very similar AKT isoforms that provide overlapping and back-up roles to AKT1, influence pAKT foci localization and repair. Most commercially available antibodies against pAKT (S473) also recognize phosphorylated AKT2 (S474) and AKT3 (S472). Thus, the roles of AKT2 and AKT3 in this response, if any, should be explored further.

The results by Fraser et al. are complementary to recent findings by Khalil et al.,<sup>10</sup> who demonstrated that DSBs resulting from BrdU photolysis or endonuclease electroporation triggered an ATM-dependent, pro-survival signaling cascade. This cascade required functional AKT in order to transmit the signal to MEK/ERK and promote cell proliferation. Collectively, conclusions from these two studies add weight to the notion that after low levels of DNA damage pro-survival signaling might be yet another arm of the DDR regulated by ATM. Thus, AKT could play the role of gatekeeper, somehow weighing cues from the DDR and growth factor signaling, to make balanced decisions together with ATM as to whether a cell will repair its DNA and live or be unable to and die.

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# The HIF-1 $\alpha$ -c-Myc pathway and tumorigenesis: Evading the apoptotic gatekeeper

Comment on: Hayashi M, et al. *Cell Cycle* 2011; 10:2364–72

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The imbalance between oxygen delivery and consumption results in hypoxia (low oxygen concentration), a hallmark of human cancers that contributes to resistance to radiation therapy and chemotherapy and ultimately to poor patient prognosis. The master regulator of the adaptive response to oxygen deprivation is hypoxia-inducible factor-1 (HIF-1), a transcription factor that operates by activating the expression of genes related to angiogenesis, glycolytic metabolism, oxygen consumption, migration and invasion.<sup>1</sup> HIF-1 protein complex consists of a  $\beta$  subunit, which is constitutively expressed, and a HIF- $\alpha$  subunit that is oxygen responsive and regulated by ubiquitin-proteasomal degradation. HIF-1 $\alpha$  is frequently overexpressed in human cancers and is an attractive target for therapy.<sup>2</sup>

HIF-1 $\alpha$  has also been implicated in a non-canonical pathway that does not require DNA binding activity and counteracts the effects of c-Myc on gene expression. Indeed, work from Huang's laboratory has provided evidence that the PAS (Per-ARNT-Sim)-B domain of HIF-1 $\alpha$  is responsible for inhibiting the c-Myc-dependent expression of *MSH2* and *NBS1*, enzymes involved in DNA mismatch and double-strand break repair, respectively.<sup>3</sup> Thus, the HIF- $\alpha$ -c-Myc pathway seems to act as an essential component in mediating genetic instability in a hypoxic environment that promotes tumor progression.<sup>4</sup>

These results, however, raised the important question as to whether normal tissues would respond differently to the selection pressure toward genetic instability exerted by the hypoxic environment. In the July 15th issue of *Cell Cycle*, Hayashi and her colleagues pursued this question by looking at one of the

major features of cancer, evasion of apoptosis and whether non-cancerous cells would experience similar accumulation of genetic alterations induced by the HIF-1 $\alpha$ -c-Myc pathway, or whether these normal cells would somehow be protected.<sup>5</sup> Indeed, they found that hypoxia or HIF-1 $\alpha$  expression inhibited DNA repair and induced DNA damage in all malignant and benign mouse cells tested. However, only apoptosis-deficient malignant cells accumulated DNA damage and acquired anchorage-independent growth and features consistent with epithelial-mesenchymal transition (EMT), implicating a protective function of apoptosis against HIF-1 $\alpha$ -induced malignant development. Furthermore, the growth advantage observed was further associated with resistance to etoposide treatment, at least in part attributed to increased Akt activity and inhibition of autophagy, thus, once again, implicating the HIF-1 $\alpha$ -c-Myc pathway in promoting the survival of apoptosis-deficient malignant cells. Taken together, Hayashi et al. provide evidence that normal cells proficient in apoptosis are, for the most part, safe under hypoxic stress, and genetic alteration produced by HIF-1 $\alpha$  is largely cell-context dependent.

The contribution of HIF to tumor progression is largely attributed to its ability to induce the expression of genes whose products contribute to essential features of the malignant phenotype, including metabolic reprogramming, angiogenesis and metastasis. The elegant work of Hayashi and his colleagues extends the involvement of HIF-1 $\alpha$  in tumorigenesis by implicating a non-canonical mechanism of action that counteracts c-Myc effects on gene expression. However, to what extent this pathway is active in the presence of

deregulated or oncogenically activated c-Myc remains to be established. Indeed, under these circumstances, the interaction between HIF and c-Myc appears to be much more complex.<sup>6</sup> For instance, HIF-1 and c-Myc can cooperate for the induction of glycolytic enzymes or angiogenic factors, and HIF-2 enhances c-Myc transcriptional activity in renal cell carcinogenesis.<sup>7</sup>

The work published by Hayashi et al. in the July 15th issue of *Cell Cycle* is consistent with a model in which apoptosis proficiency is a gatekeeper to limit the potential damaging genetic changes effected by a hypoxic microenvironment. Indeed, evidence has been provided that HIF-1 $\alpha$  may induce apoptosis, although the context in which this occurs and the pathways implicated are still poorly understood. Evasion of apoptosis is a hallmark of human cancers and selection of genetically altered, apoptosis-resistant clones by hypoxia has been previously suggested.<sup>8</sup> The interplay between cell-autonomous genetic changes and the selection pressure exerted by the tumor microenvironment is crucial to fully execute the tumorigenic program. Hayashi and colleagues provide further experimental evidence to corroborate a critical role played by the HIF-1 $\alpha$ -c-Myc pathway in tumorigenesis.

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# Cyclooxygenase (COX)-1 takes control of adult hippocampal neurogenesis

Comment on: Russo I, et al. *Cell Cycle* 2011; 10:2568–73

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The subventricular zone (SVZ) of the lateral ventricles and the subgranular zone of the dentate gyrus (DG) of the hippocampus are well-characterized germinal niches of the central nervous system (CNS), in which stem cells support neurogenesis and gliogenesis throughout adult life. The maintenance and differentiation of brain stem cells is orchestrated by cellular contacts to the basal lamina, which acts as a scaffold, sequestering and/or modulating soluble factors derived from local cells.<sup>1</sup>

While investigating the molecular basis of the cognitive decline that follows cranial radiation as adjuvant treatment of primary brain tumors in humans, Monje et al. first observed that experimental cranial irradiation significantly altered neurogenesis in the rat DG. The irradiation-disrupted stem cell niche had a remarkable decrease of blood vessel-associated clusters of proliferative neural progenitors as well as a significant increase of activated microglia. This microglial phenotype led to the hypothesis that inflammation may perturb the endogenous stem cell compartment and, ultimately, neurogenesis.<sup>2</sup> The same authors developed a model of lipopolysaccharide (LPS)-induced inflammation characterized by a significant impairment of hippocampal neurogenesis mediated by activated microglia releasing interleukin-6 in the DG.<sup>3</sup> Striking restoration of DG neurogenesis was achieved by either decreasing microglial activation with the non-selective inhibitor of cyclooxygenase (COX)-1 and COX-2 indomethacin<sup>3</sup> or with metabolites/chaperones that protect mitochondrial function.<sup>4</sup>

Following these first reports, we showed that in chronic experimental autoimmune encephalomyelitis (EAE), persistent CNS inflammation impaired the proliferative and migratory properties of SVZ-resident stem

cells, leading to significant accumulation of non-migratory neuroblasts within the SVZ.<sup>5</sup> However, when challenged within a relapsing-remitting EAE model, the SVZ stem cell compartment underwent significant acute increase of proliferation, migration and oligodendrogenic potential. This was lost along with progression towards more chronic disease stages. Interestingly, activated microglial cells were found closely associated with CNS stem and progenitor cells in the dysfunctional EAE SVZ, and delayed (i.e., started 20 days after immunization) treatment with the microglial modulator minocycline reduced the number of microglia while increasing the proliferation in the SVZ.<sup>6</sup>

These data indicate that adult neural stem cell physiology is greatly influenced by the cross-talk between the immune system and the CNS, and suggest that both states of persistent hyper- or underactivation (e.g., under immune deficiencies) of the immune system may lead to dysfunction of the CNS stem cell compartments.

Beyond the holistic (but true) view that soluble factors released by immune cells greatly affect stem cells, there is also parallel evidence that CNS stem cells express functional immune-like molecules, such as cell adhesion molecules, chemokine receptors and Toll-like receptors (TLRs), that enable them to interact with the inflamed CNS microenvironment. Interestingly, TLR2 and 4 also orchestrate proliferation and differentiation of CNS stem cells, with TLR2 being a positive regulator of neurogenesis only and TLR4 acting as a negative regulator of both proliferation and neurogenesis.<sup>7</sup>

COX-1 is another very interesting candidate to study when looking at neuro-immune interactions at prototypical CNS stem cell niches. A previous study from Bosetti and colleagues

investigated the critical role of COX-1 in the neuroinflammatory response to intracerebroventricular LPS and established that either gene ablation or pharmacological inhibition of COX-1 significantly reduced microglial activation, release of pro-inflammatory and oxidative stress mediators as well as blood-brain barrier disruption and recruitment of peripheral leukocytes.<sup>8</sup>

In the August 1st issue of *Cell Cycle*, Russo and colleagues revealed a role of COX-1 in the impairment of hippocampal neurogenesis and proliferation following LPS-induced inflammation. The authors showed that LPS reduces progenitor proliferation and neurogenesis in wild-type but not in COX-1<sup>-/-</sup> mice, pointing to an essential role for COX-1 in propagating the inflammatory response and modulating the neurogenic niche.<sup>9</sup> Hence, COX-1 emerges as a potential therapeutic target in inflammatory neurodegenerative diseases. Intriguingly, the epidemiological data indicating that non-steroidal anti-inflammatory drugs (NSAIDs) can affect the pathophysiology of major inflammation-driven neurodegenerative diseases, such as Alzheimer disease and multiple sclerosis, indirectly suggest that some of their protective effects may be related to COX-1 inhibition.<sup>8</sup> Further investigations will be required to elucidate the downstream effectors of this modulation of neurogenesis and how neuroinflammation relates to the pathophysiology of neurodegenerative diseases.

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# DNA double-strand breaks make bedfellows of ATM and AKT

Comment on: Fraser M, et al. *Cell Cycle* 2011; 10:2218–32

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Cells respond to myriad cues from neighboring cells or from their extracellular environment by various mechanisms, the best understood of which involves signaling at the cell membrane by receptor tyrosine kinases (RTKs). Interestingly, recent reports indicate that certain RTKs, such as EGFR, as well as their downstream components, PI3K and AKT, translocate into the nucleus in response to DNA double-strand breaks (DSBs), accumulate at the sites of DNA damage and directly influence DNA repair and other processes.<sup>1,2</sup> In response to DSBs, three central kinases, ATM, ATR and DNA-PKcs, trigger a DNA damage response (DDR) that culminates in chromatin remodeling, DNA repair, cell cycle arrest, programmed cell death and gene expression changes.<sup>3</sup> Importantly, all three kinases have been implicated in the activation of AKT in response to DNA damage.

AKT is a serine/threonine kinase and constitutes a common downstream node at which signals from multiple RTKs converge. The activation of AKT upon mitogenic stimulation requires its phosphorylation at Thr-308 by PDK1 and at Ser-473 by mTORC2. Once activated, AKT influences multiple biological responses, including cell survival, proliferation, translation and metabolism.<sup>4</sup> The activation of AKT upon DNA damage involves its phosphorylation at Ser-473 in a DNA-PKcs-dependent manner; this occurs in the nucleus and results in the triggering of pro-survival pathways.<sup>5</sup> Other reports have also implicated ATM in the activation of AKT in response to both IR and insulin, but the underlying mechanism(s) are not well worked out.<sup>6</sup>

A report in the July 1st issue of *Cell Cycle* by Bristow and colleagues solidifies the connection between ATM and AKT by demonstrating that “Mre11 promotes AKT phosphorylation in direct response to DSBs.”<sup>7</sup>

Mre11 is a component of the multifaceted Mre11-Rad50-Nbs1 (MRN) complex, which influences key responses to DSBs, including ATM activation and DSB repair.<sup>8</sup> The authors demonstrated that Mre11 is essential for the phosphorylation of AKT at Ser-473 upon DNA damage. This is presumably due to the role of the MRN complex in ATM activation, as AKT phosphorylation is similarly abrogated in ATM-null fibroblasts and in normal fibroblasts treated with an ATM inhibitor. Importantly, AKT phosphorylation requires histone ubiquitination, and AKT activation is abrogated in cells deficient in the ubiquitin ligase RNF168, whose recruitment to DSBs is dependent upon ATM. Histone ubiquitination is important for the assembly of DDR proteins at DNA damage sites<sup>9</sup> and could also serve to stabilize AKT and/or a putative AKT kinase at DSBs. These results reveal a Mre11-ATM-RNF168 axis that might work at multiple levels to recruit AKT to DSBs and to promote its activation.

On another significant note, while a number of studies have shown that AKT is activated by DSBs, the localization of AKT at damage sites was not convincingly demonstrated previously. However, Fraser et al. unequivocally showed the colocalization of AKT with  $\gamma$ H2AX (a surrogate marker for DSBs) and with phospho-ATM (the active form of this kinase) by using three complementary methods to generate DSBs.<sup>7</sup> Intriguingly, they found that only pAKT-S473 accumulates at breaks, while pAKT-T308 is pan-nuclear. Future studies should shed more light on the dynamics of AKT activation at DSBs.

Interestingly, this report also demonstrates that AKT contributes to the repair of DSBs by non-homologous end joining (NHEJ).<sup>7</sup> This is consistent with a number of reports indicating that AKT might act upstream of the NHEJ protein DNA-PKcs.<sup>2</sup> Moreover, a recent study

demonstrated that AKT also promotes DSB repair by upregulating Mre11 expression in response to IR.<sup>10</sup> These data raise the possibility of positive feedback loops between AKT and DNA repair proteins at damage sites, the net outcome of which would be efficient DSB repair as well as enhanced cell survival.

It is important to point out that results from different groups implicating either DNA-PKcs or ATM in AKT activation are not that discrepant, as it is becoming increasingly clear that ATM and DNA-PKcs have some overlapping roles *in vivo*.<sup>11,12</sup> It will be important to elucidate whether the effect of ATM on Ser-473 phosphorylation occurs via activation of a putative kinase (that is, perhaps, distinct from mTORC2) or inactivation of a phosphatase acting at this site or due to conformational changes in AKT triggered by protein-protein interactions at a DSB. This report and preceding studies suggest a great degree of complexity in the regulation of AKT and validate the existence of cross-talk between RTK-PI3K-AKT and DDR pathways. It is critical to understand the mechanisms underlying this cross-talk for the design of rational therapeutic strategies that could disrupt these connections for the radiosensitization of tumors with hyperactivated PI3K-AKT signaling.<sup>2,4</sup>

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# Autophagic tumor stroma: A biofuel for cancer growth

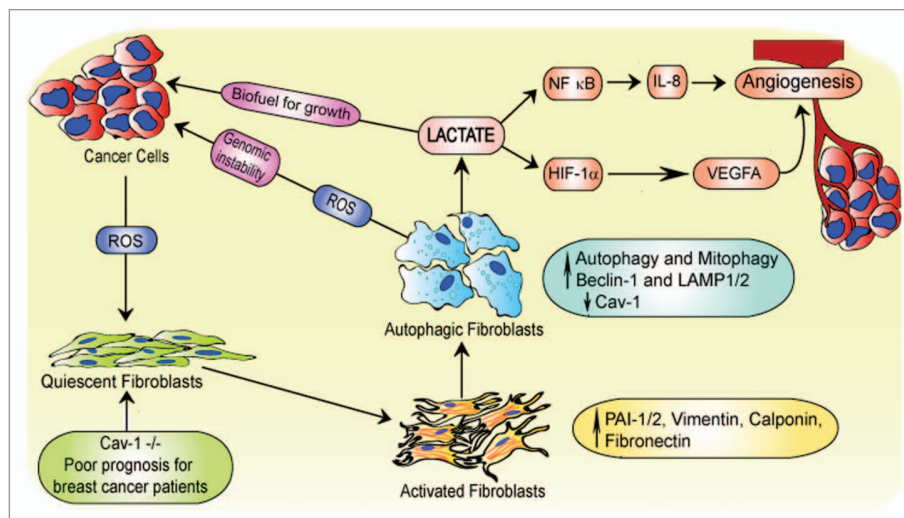
Comment on: Castello-Cros R, et al. *Cell Cycle* 2011; 10:2021–34

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To fulfill the energetic demands for growth, cancer cells support their metabolism with several nutrients available in circulation and interstitial fluid, including glucose, glutamine and lactate.<sup>1</sup> Notably, oncogenic mutations coupled to hypoxic conditions allow the tumor cells to metabolize glucose to pyruvate then pyruvate to lactate. This allows the regeneration of the NAD<sup>+</sup> pool required for the glycolytic flux to meet the demand for ATP production and biosynthetic precursors, a phenomenon called the “Warburg effect” when it occurs in the presence of oxygen.<sup>2</sup>

Recently, Lisanti and colleagues have put forward an iconoclastic hypothesis that focuses on the tumor stroma as the central fuel generator for cancer growth, the so-called “reverse Warburg effect.”<sup>3–5</sup> During starvation, cells undergo autophagy (self-eating). This process is mediated by the formation of large autophagosomes within which hydro-lases, acting at low pH, digest organelles, such as mitochondria and endoplasmic reticulum, thus providing amino acids, lipids and sugars for survival. The prevailing theory is that tumor cells modify their metabolism in order to fuel their own growth. The new hypothesis of Lisanti and colleagues argues that the Warburg effect is occurring in tumor stromal cells, such as fibroblasts, which lose their mitochondria by autophagy and mitophagy, thus generating the energy necessary to fuel cancer growth.

How is the reverse Warburg effect accomplished at the cellular and molecular level? First, cancer cells induce oxidative stress in quiescent fibroblasts by releasing reactive oxygen species (ROS) and inducing caveolin-1 (Cav-1) degradation (Fig. 1).<sup>6</sup> Cav-1-deficient fibroblasts dramatically promote tumor growth in breast carcinoma xenografts,<sup>7</sup> and the absence of stromal Cav-1 expression predicts poor clinical outcome in breast cancer patients.<sup>8</sup> In the June 15th issue of *Cell Cycle*, Castello-Cros and colleagues report that loss of Cav-1 in mammary stromal fibroblasts leads to upregulation of plasminogen activator inhibitor type 1 and 2 (PAI-1/2).<sup>9</sup> Fibroblasts overexpressing PAI-1/2, similar to Cav-1-deficient fibroblasts, promote tumor growth and metastasis when



**Figure 1.** Schematic representation of the autophagic tumor stroma. Cancer cells produce ROS and downregulate Cav-1. This process leads to activation of quiescent fibroblasts and overproduction of PAI-1/2, vimentin, calponin and fibronectin, all markers of activated fibroblasts. This leads to an autophagic phenotype characterized by increased autophagy markers (Beclin-1 and LAMP1/2), and loss of Cav-1 with subsequent production of lactate and ROS. Both lactate and ROS feed back onto the cancer cells providing “biofuel” for cancer growth and inducing additional mutations that would enhance survival. Notably, lactate can also induce Hif-1 $\alpha$  and thus VEGFA, as well as NF $\kappa$ B and thus IL-8, which both stimulate angiogenesis.

co-injected with breast cancer cells and induce oxidative mitochondrial metabolism in adjacent cancer cells. Thus, loss of Cav-1 and overexpression of PAI-1/2 lead to activation of fibroblasts with increased expression of vimentin, calponin and fibronectin, all markers of activated fibroblasts.<sup>9</sup> Subsequently, the activated fibroblasts undergo autophagy and mitophagy with increased expression of the autophagic markers Beclin-1 and LAMP-1/2 (Fig. 1). The autophagic fibroblasts then release ROS and lactate. ROS induces genomic instability in adjacent cancer cells, thereby favoring mutations that support survival and proliferation. Lactate, on the other hand, provides the major biofuel for cancer growth. The soluble lactate generated by autophagic fibroblasts together with tumor-derived lactate have two additional important bioactivities. First, lactate can be taken up by endothelial cells via the monocarboxylate transporter MCT1, thereby stimulating an autocrine NF $\kappa$ B/IL-8 pathway<sup>10</sup> to drive angiogenesis (Fig. 1). Lactate released by the tumor cells—both breast and colon cancer cells—via MCT4 is sufficient to

stimulate IL-8-dependent angiogenesis and tumor growth.<sup>10</sup> Second, it is known that lactate can also induce HIF-1 $\alpha$  and, thus, VEGFA expression. Both IL-8 and VEGFA would trigger pro-survival and pro-angiogenic activities, leading to additional “biofuel” advantageous to cancer growth.

The discovery that PAI-1/2 induces autophagy and indirectly stimulates cancer growth and angiogenesis adds another piece to the puzzle of this rapidly evolving story. However, much still needs to be done. First and foremost, we need to elucidate the precise signaling mechanisms involved in the complex cancer-stroma interactome. Although overwhelming evidence points to the necessity of direct cell-to-cell contact, soluble factors could prove to be important. In addition to cell-permeable constituents, such as ketones and lactate, larger receptor signaling complexes embedded in cancer-released exosomes could mediate this dialogue. The emerging evidence that DNA, mRNA and microRNAs could be transferred from one cell to another by piggy-backing in exosomes provides another clue

to this puzzle. Thus, cancer cells could transfer molecular “tool kits” that may, in turn, lead to a radical modification of the quiescent stroma into a self-destructing and energy-producing one that would promote angiogenesis and feed the tumor cells with extra biofuel.

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## miR-27a regulation of SCF<sup>Fbw7</sup> in cell division control and cancer

Comment on: Lerner M, et al. *Cell Cycle* 2011; 10:2172–83

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Protein degradation via the ubiquitin proteasome system (UPS) is an important aspect of cell division control. The ability of a cell to rapidly and temporally degrade proteins allows for the phases of the cell cycle to proceed in an orderly fashion and prevents potential interferences with downstream events. Protein degradation is essential for the initiation of DNA replication, chromosome segregation and exit from mitosis. However, studies over the past decade have shown that protein degradation is often perturbed in cancer cells, and this can lead to abnormal cell cycle progression, genetic instability and uncontrolled proliferation.

Proteins are targeted to the UPS by a class of enzymes termed ubiquitin ligases. An important family of these are the SCF-type ubiquitin ligases which are multiprotein complexes generally composed of a cullin (usually Cul1), the RING-finger protein Rbx1, Skp1 and an F-box protein. F-box proteins (named for their ~40 amino acid F-box motif, which allows for their association with the adaptor protein Skp1) function as substrate recognition components of the ubiquitin ligase. There are ~70 different F-box proteins in humans, each targeting a unique set of protein substrates for ubiquitylation.

The first evidence that suggested SCF ubiquitin ligases could play an important role in cell division control came from reports that showed a protein complex consisting of Skp1, Cdc53 (Cul1 homolog) and the F-box protein Cdc4 targeted the cyclin-dependent kinase (Cdk) inhibitor Sic1 for degradation in budding yeast.<sup>1-2</sup> Over the past decade, the homologous ubiquitin ligase in humans (termed SCF<sup>Hcdc4</sup>, SCF<sup>Fbw7</sup>

or SCF<sup>Fbw7</sup>) has been a subject of intense investigation.<sup>3,4</sup> We now know that SCF<sup>Fbw7</sup> mediates the degradation of several key regulators of cell division, including cyclins E1/2, c-Myc, c-Jun, c-Myb, Notch and mTor.<sup>3,4</sup> Recently, SCF<sup>Fbw7</sup> was also linked to apoptosis regulation through targeted degradation of the Bcl-2 family member and pro-survival protein Mcl1.<sup>5,6</sup>

In the July 1st issue of *Cell Cycle*, Lerner et al. reported that SCF<sup>Fbw7</sup> activity is regulated by the micro-RNA miR-27a.<sup>7</sup> In normal cell division cycles, Fbw7 expression was shown to be subject to miR-27a-dependent repression from G<sub>2</sub> through early G<sub>1</sub> phase, thus allowing SCF<sup>Fbw7</sup> substrates to accumulate and cyclin E to initiate an S-phase program. However, at G<sub>1</sub>/S boundary, the miR-27a-dependent repression of Fbw7 is lifted, which promotes the degradation of cyclin E and presumably other SCF<sup>Fbw7</sup> substrates. Therefore, miR-27a coordinates with transcriptional regulation (of CCNE) and temporal phosphorylations to ensure that cyclin E expression is restricted to late G<sub>1</sub> phase of the cell cycle. The importance of miR-27a in cell division control was demonstrated by experiments that showed enforced expression of miR-27a induced severe cell cycle defects, including premature entry into S phase, delayed S-phase progression and signs of replication stress. Interestingly, these phenotypes are also found in cells that express deregulated cyclin E, which is known to interfere with pre-replication complex assembly.

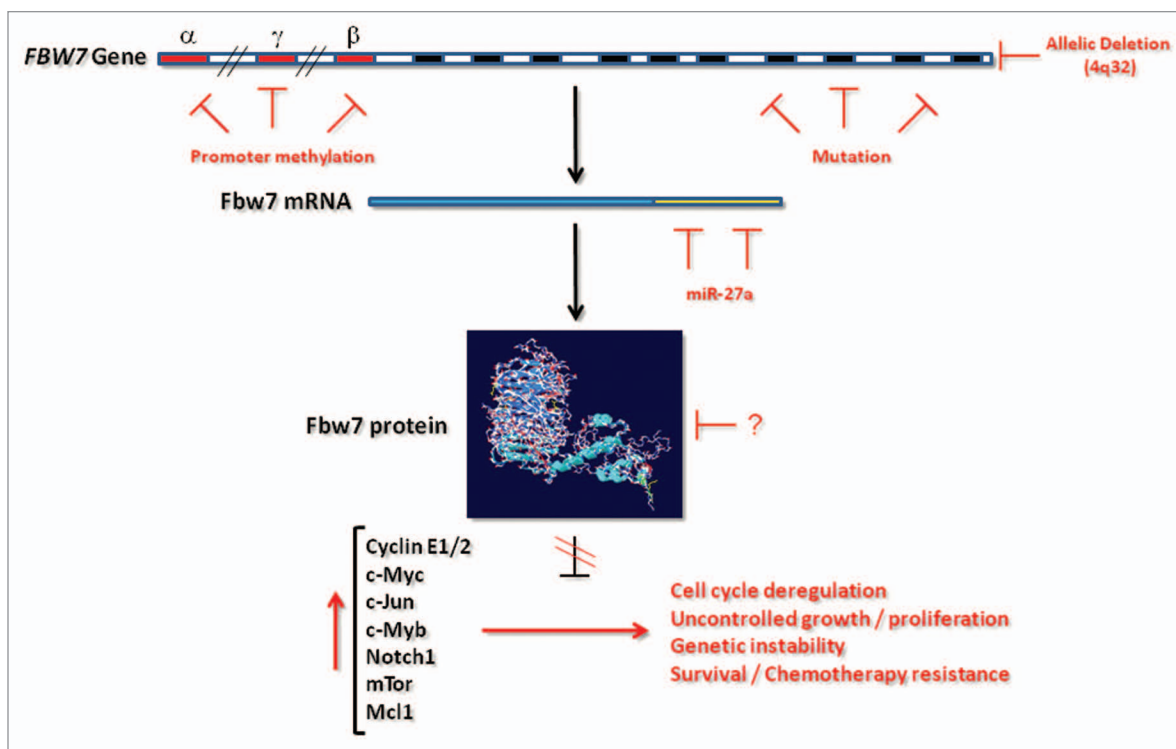
Since SCF<sup>Fbw7</sup> regulates the degradation of several key oncoproteins, it is subject to intense pressure for functional inactivation in cancer cells. Loss of SCF<sup>Fbw7</sup> function has been shown to promote cell cycle dysregulation,

uncontrolled proliferation, genetic instability and chemotherapy resistance.<sup>3-4</sup> Data from M. Lerner et al. suggests that overexpression of miR-27a may represent a novel mechanism of SCF<sup>Fbw7</sup> inactivation in cancer cells. miR-27a was found to be overexpressed in many pediatric B-ALLs, and its expression inversely correlated with Fbw7 levels in hyperdiploid pre-B-ALLs.<sup>7</sup> This data suggests that miR-27a overexpression could be oncogenic in these cancers by repressing Fbw7 expression, thus promoting the dysregulation of SCF<sup>Fbw7</sup>'s oncoprotein substrates (Fig. 1).

However, several questions of miR-27a's regulation of SCF<sup>Fbw7</sup> remain unanswered. How does miR-27a-dependent repression of Fbw7 become derepressed at the G<sub>1</sub>/S boundary to activate SCF<sup>Fbw7</sup> in normal cell cycles? Is miR-27a overexpression sufficient to functionally inactivate SCF<sup>Fbw7</sup> in cancer cells, and what is the importance of this mechanism relative to other known mechanisms of inactivation (Fig. 1)? Future studies aimed at understanding the molecular regulation of miR-27a-dependent repression of Fbw7 and functional and tumorigenicity assays will likely provide answers to these questions and, ultimately, help to define the importance of miR-27a in the regulation of normal cell division cycles and its dysregulation in cancers.

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**Figure 1.** Mechanisms of SCF<sup>Fbw7</sup> functional inactivation in cancer. Allelic deletions, promoter methylations and mutations of the *FBW7* gene have been detected in a variety of human cancers.<sup>3-4</sup> M. Lerner et al. recently found that Fbw7 expression is also negatively regulated by the micro-RNA miR-27a, which is overexpressed in pediatric B-ALLs and hyperdiploid pre-B-ALLs.<sup>7</sup> Functional inactivation of SCF<sup>Fbw7</sup> has been shown to promote cell cycle deregulation, uncontrolled proliferation, genetic instability and resistance to chemotherapy. Alterations found in cancer cells are indicated in red.

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## Taccalonolides: A microtubule stabilizer poses a new puzzle with old pieces

Comment on: Risinger AL, et al. *Cell Cycle* 2011; 10:2162-71

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The structural diversity of microtubule targeting agents (MTAs) is breathtaking, from simple bicyclics, like combretastatins, to complex compounds, like epothilones and halicondrins. The taccalonolides, plant-derived steroids described by Risinger and Mooberry in the July 1st issue of *Cell Cycle*,<sup>1</sup> fall somewhere in the middle of this structural complexity.

Interest in MTAs as chemotherapeutics began with the use of vincristine to treat acute lymphoblastic leukemia in the 1950s and shows no sign of abating. All MTAs inhibit mitosis in rapidly growing cells, though clinical efficacy against patient tumors is likely due to non-mitotic activities.<sup>2</sup> The success of MTAs as chemotherapeutics has fueled searches for new compounds, one result being the taccalonolides. Notably, unlike agents against other cellular targets, MTAs remain principally

natural products or semi-synthetic derivatives. That diverse organisms produce MTAs as toxins underscores the crucial roles of MTs in many cellular functions, continuing to make MTs attractive targets. The variability of MTAs likely explains their diverse antitumor profiles and varying neurotoxicity. This variability may yet lead to agents with different antitumor activity and hopefully less neurotoxicity, and this possibility continues to encourage pursuit of MTAs as cancer therapeutics.

In the July 1st issue, Risinger and Mooberry discussed several features of taccalonolides that distinguish them from paclitaxel and other MT-stabilizers: (1) a novel structure, (2) MT activity in cells not reproduced with purified tubulin or cell extracts, (3) cellular activity much less reversible than with other MTAs and (4) concentrations effective in interphase and mitotic cells that are very similar,

unlike the differential seen with paclitaxel. We discuss these below.

The MT activity of taccalonolides was demonstrated by mitotic arrest and accumulation of MT bundles in interphase cells, evidence that taccalonolides, like taxanes, epothilones, laulimalide and peloruside, stabilize MTs. Taccalonolides as steroids are quite different structurally from other MT stabilizers, but taccalonolides are not the only MT-stabilizing steroid. A synthetic derivative with paclitaxel-like activity was discovered years ago in a search of analogs of the natural estrogen metabolite 2-methoxyestradiol, itself a weak MT destabilizer. This steroid derivative demonstrated pronounced MT stabilization with purified tubulin but not with cells,<sup>3</sup> the opposite effect of that observed with taccalonolides.

Taccalonolides are unusual, since their cellular activity is not reproduced in vitro. Unlike

paclitaxel and other MT stabilizers, taccalonolides do not induce MT assembly using purified tubulin or cell extracts. It is assumed that taccalonolides act by binding tubulin, but direct binding data demonstrating this are lacking, and, in fact, one study found an absence of binding and an inability to displace other MT stabilizers from tubulin.<sup>4</sup>

Other MTAs exhibit discordance between activity in cells and with purified proteins. Dolastatin 15 and the 2-methoxyestradiol derivative mentioned above are examples. Dolastatin 15 is a MT-destabilizing peptide that is very potent in cells ( $GI_{50}$  1000-fold lower than taccalonolides), but it inhibits polymerization of purified tubulin very poorly and does not inhibit binding of other MTAs to tubulin. Dolastatin 15 was ultimately shown to bind tubulin,<sup>5</sup> but the discordance between its *in vivo* and *in vitro* activities

remains notable compared to other MTAs and is worth remembering in the context of taccalonolide activities described by Risinger and Mooberry.

The cellular effects of taccalonolides are less reversible on removal of drug than is observed with paclitaxel. This persistence suggests that the drug may be significantly retained once inside the cell compared to most MTAs. Again, MTA diversity provides another example. Dolastatin 10 binds tightly to tubulin and is highly retained in cells;<sup>6</sup> these properties likely contribute to its very low  $GI_{50}$ . In contrast, taccalonolides show  $GI_{50}$  values  $\sim 10^5$ -fold higher than dolastatin 10 and appear not to bind tightly to tubulin. It will be interesting to reevaluate this with a labeled taccalonolide. While durability of drug effect may be seen as desirable, it may be a mixed blessing for a chemo-

therapeutic, since irreversibility could increase neurotoxicity.

Finally, the suggestion that taccalonolides may target interphase MTs more efficiently than other MTAs is intriguing. If this is a new wrinkle on MTA activity, it may manifest as greater activity against slow-growing cells and human tumors compared to other MTAs. These various activities make the taccalonolides compounds to watch as the puzzles described in this paper are solved.

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## Checking before changing: Cell cycle checkpoints inhibit muscle differentiation

Comment on: Simonatto M, et al. *Cell Cycle* 2011; 10:2355–63

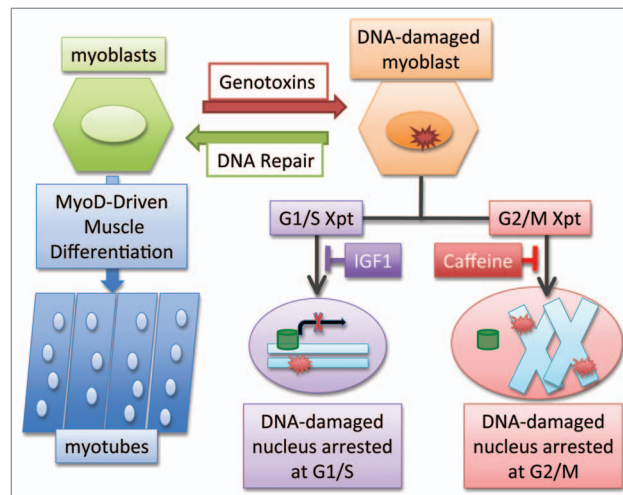
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In proliferating cells, genome integrity is maintained by checkpoint mechanisms that “recognize” lesions in DNA and/or chromatin and “orchestrate” a coordinated response of the cell cycle and DNA repair machines to prevent the replication or segregation of damaged genome.<sup>1</sup> Almost a decade ago, Puri et al.<sup>2</sup> provided the first evidence for a “differentiation checkpoint,” showing that muscle-specific gene expression is inhibited when DNA damaged myoblasts are induced to undergo terminal differentiation. That study identified tyrosine phosphorylation of MyoD by the nuclear ABL tyrosine kinase as a key event in inhibiting the transactivation function of MyoD following DNA damage.<sup>2</sup> However, it was unclear whether MyoD inhibition is part of the cell cycle checkpoint mechanism or a parallel pathway unique to myoblasts. Simonatto et al.<sup>3</sup> provided answers to these questions in the July 15th issue of *Cell Cycle*.

By treating myoblasts with a panel of genotoxins that induce  $G_1/S$  or  $G_2/M$  arrest, Simonatto et al. showed that MyoD is inhibited at both checkpoints. This inhibition of muscle-specific gene expression is reversed with time and closely correlated with the repair of DNA lesions. Thus, a myoblast with damaged DNA does not differentiate until the

genome is repaired (Fig. 1). It is shown that IGF1 and caffeine specifically and respectively abolish genotoxin-induced MyoD inhibition

and the  $G_1/S$  and the  $G_2/M$  cell cycle checkpoints in myoblasts (Fig. 1), demonstrating that the differentiation checkpoint is part



**Figure 1.** DNA damage-induced muscle differentiation checkpoints. Genotoxins activate two differentiation checkpoints at  $G_1/S$  and  $G_2/M$  to inhibit MyoD-dependent muscle gene expression in DNA damaged myoblasts. These checkpoints are reversible, as differentiation can resume after DNA is repaired. Inhibition of MyoD (depicted as the small green cylinder) is mediated by the cell cycle checkpoints, because inhibitors of the  $G_1/S$  or  $G_2/M$  cell cycle arrest, i.e., IGF1 or caffeine, also block the differentiation/arrest, respectively. In  $G_1/S$ -arrested myoblasts, MyoD remains associated with the promoters of muscle-specific genes but does not stimulate transcription. In  $G_2/M$ -arrested myoblasts, MyoD dissociates from DNA and is thus unable to stimulate muscle-specific gene expression.



of the cell cycle checkpoint mechanisms. It is also shown that MyoD association with muscle-specific promoters is maintained in G<sub>1</sub>/S-arrested cells; however, MyoD is unable to elaborate the appropriate histone modifications required for transcription (Fig. 1). However, MyoD is not able to associate with DNA in G<sub>2</sub>/M arrested cells (Fig. 1). These results demonstrate that the G<sub>1</sub>/S and the G<sub>2</sub>/M cell cycle checkpoints employ different means to prevent MyoD from driving muscle differentiation.

The differentiation-arrest activated in myoblasts appears to contradict the biological principle that cell cycle arrest and terminal differentiation are tightly linked. For example,

the RB family of pocket proteins, p130, p107 and pRB, inhibit G<sub>1</sub>/S transition but stimulate muscle differentiation.<sup>4</sup> A role for nuclear ABL in inhibiting MyoD,<sup>2</sup> also demonstrated in mice,<sup>5</sup> is initiating the differentiation checkpoint, but because activated RB inhibits ABL,<sup>6</sup> this only results in a transient modification of MyoD.<sup>2</sup> Thus, cell cycle checkpoints must inhibit MyoD through multiple mechanisms. Of note is the physiological factor Myostatin, which inhibits both the proliferation and the differentiation of myoblasts.<sup>7</sup> Myostatin inhibits the activity of the Cyclin E-Cdk2 complex, leading to the dephosphorylation of NPAT, a nuclear protein required for histone gene expression.<sup>8</sup> NPAT also plays a key role in DNA

damage-induced G<sub>1</sub>/S arrest.<sup>9</sup> Thus, Cyclin-E-Cdk2 inhibition and possibly NPAT dephosphorylation may also contribute to the G<sub>1</sub>/S differentiation checkpoint. Much remains to be learned about the differentiation checkpoints, and the work of Simonatto et al. has pointed the way forward.

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## Novel role of Wip1 in p53-mediated cell homeostasis under non-stress conditions

Comment on: Park HK, et al. *Cell Cycle* 2011; 10:2574–82

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The wild-type p53-induced phosphatase 1 (Wip1, also-called PPM1D) is a type 2C protein phosphatase (PP2C $\delta$ ) that requires Mg<sup>2+</sup> or Mn<sup>2+</sup> for catalytic efficacy and is insensitive to phosphatase inhibitor okadaic acid. Wip1 was originally identified as a p53-induced gene in response to ionizing radiation.<sup>1</sup> Subsequent studies indicated that Wip1 acts as a homeostatic regulator of the DNA damage response, facilitating the return of cells to a normal pre-stress state following repair of damaged DNA.<sup>2-3</sup> In addition, Wip1 inhibits several tumor suppressor pathways, including ATM-CHK2-p53 and p38MAPK-p53.<sup>2</sup> Furthermore, Wip1 is found to be aberrantly expressed in several types of human cancers, and Wip1-null mice are resistant to cancer.<sup>4-5</sup> These unique features make Wip1 an oncogenic phosphatase and a promising drug target for cancer therapy.

The p53 tumor suppressor plays a pivotal role in preserving the integrity of the genome and in preventing cancer development. The importance of p53 in this process is demonstrated by the fact that inactivation of p53 occurs in over 50% of human cancers, and loss of p53 function is known to be essential for carcinogenesis.<sup>6</sup> In response to various cellular stresses, p53 is activated and functions as a sequence-specific DNA binding transcriptional factor to induce its downstream targets, which mediate cell cycle arrest, DNA repair, and apoptosis.<sup>7</sup> Consistently, numerous

studies have shown that p53 exerts pivotal roles in guarding genomic stability under stress conditions.<sup>6</sup>

Most studies on Wip1 have been focused on how Wip1 facilitates the return of cells to homeostasis after DNA damage. The study by Park et al. convincingly showed that under non-stress conditions, Wip1 plays a critical role in p53-mediated cell homeostasis. Specifically, they showed that Wip1 is able to induce G<sub>2</sub>/M arrest in cells with wild-type p53 but not the ones with mutant p53. In addition, Wip1-mediated G<sub>2</sub>/M arrest is able to decrease cell death in mouse embryonic fibroblasts. To uncover the underlying mechanism, they showed that Wip1 is able to decrease Cdc2 kinase activity by increasing phosphorylation of the inhibitory tyrosine-15 of Cdc2. Together, the current studies suggest that, under non-stress conditions, Wip1 collaborates with p53 to induce G<sub>2</sub>/M arrest, which enables normal cells to be ready for mitosis and avoid mitotic catastrophe. Similarly, this regulation favors cancer cells with wild-type p53 to escape from the mitotic checkpoint and, thus, promotes tumor formation. Therefore, targeting Wip1 in cancer cells with wild-type p53 may lead to a shorter G<sub>2</sub> preparation and render cell death via mitotic catastrophe. In support of this notion, Wip1 inhibition has been shown to decrease cancer cell proliferation in a p53-dependent manner.<sup>8-9</sup>

At this point, it still remains unclear how Wip1 induces G<sub>2</sub>/M arrest in cells with wild-type p53. It will be interesting to address how Wip1 increases the phosphorylation of the inhibitory tyrosine-15 of Cdc2. Additionally, is there any other mechanism by which Wip1 inhibits Cdc2 kinase activity? Finally, it will also be interesting to address how p53 modulates Wip1-induced G<sub>2</sub>/M arrest under non-stress conditions, which will advance our understanding of how p53 makes cell fate decisions. Although much work remains to be explored, these findings clearly highlight a novel role of Wip1 in p53 guarding homeostasis under non-stress conditions. The work also shed a light on a novel mechanism by which Wip1, in addition to its ability to inhibit p53, promotes tumorigenesis by helping cancer cells escape from the mitotic checkpoint.

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# Lamins, guardians of the soma and the genome

Comment on: Redwood AB, et al. *Cell Cycle* 2011; 10:2549–60

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Lamins are components of the nuclear lamina, a protein network that provides a framework for the nuclear envelope and interacts with chromatin and the cytoskeleton. Lamins A and C are encoded by the same gene (*LMNA*) by alternative splicing. Lamin A undergoes serial post-translational modifications on the CaaX-box at its C terminus: (1) farnesylation of the cysteine, (2) cleavage of the -aaX tripeptide, (3) methylation of the farnesylated cysteine and (4) proteolytic removal of the 15-residue C-terminal peptide containing the farnesylated cysteine. Mutations in *LMNA* that preclude this proteolytic processing or, in *FACE1/Zmpste24*, the gene encoding its processing enzyme,<sup>1</sup> cause human premature aging diseases, such as Hutchinson-Gilford progeria syndrome (HGPS) and restrictive dermopathy (RD).<sup>2</sup> Other mutations in *LMNA* are responsible for a variety of human diseases, collectively known as laminopathies. However, the molecular mechanisms underlying the phenotypic heterogeneity of these genetic disorders remain obscure, although a genome-wide alteration of the epigenome might explain it partially.<sup>3</sup>

In a previous study, Susana Gonzalo's group shed some new light onto the functional role of lamin A.<sup>4</sup> It was already known that lamins participate in the maintenance of genomic stability through the stabilization of 53BP1, a component of the DNA damage response pathway (DDR).<sup>5,6</sup> Thus, embryonic fibroblasts lacking lamin A/C exhibited increased basal levels of  $\gamma$ H2AX, chromosome breaks, defects in telomere chromatin architecture, impaired maintenance of telomere length and, most importantly, a hindered ability to process dysfunctional telomeres by long-range NHEJ.<sup>5</sup> In their more recent work, the same group went beyond and provided experimental evidence showing that lamins also participate in the short-range DSB repair pathway induced by ionizing radiation, apparently in a 53BP1-dependent manner.

The competition between the NHEJ and the high-fidelity homologous recombination repair pathways (HR) is well-established. However, and contrary to this general rule, lamin A/C-deficient cells with decreased NHEJ

repair were not more proficient in the HR pathway. Intriguingly, Redwood et al. showed that the HR pathway is compromised in lamin A/C-deficient cells by transcriptional downregulation of *RAD51* and *BRCA1*, two key components of the HR machinery. Accordingly, lamins-deficient cells exhibit increased radiosensitivity.<sup>4</sup> By following an intelligent candidate approach, they were able to experimentally demonstrate that this transcriptional inhibition was carried out specifically by the repressor complex formed by the Rb family member p130 and E2F4. This is the first time that lamins were shown to be involved in the transcriptional regulation of the HR repair pathway. This observation is of special interest considering that HGPS and *Zmpste24*<sup>-/-</sup> fibroblasts have increased NHEJ activity at expense of a reduction of HR (Fig. 1).<sup>6</sup> Thus, subtle variations in lamin A function might affect the DDR pathway in very different manners, such as destabilizing 53BP1 and RB or downregulating *RAD51/BRCA1*. Unraveling the specific alterations associated to each *LMNA* mutation will facilitate understanding the role of DDR defects in the development of human laminopathies.

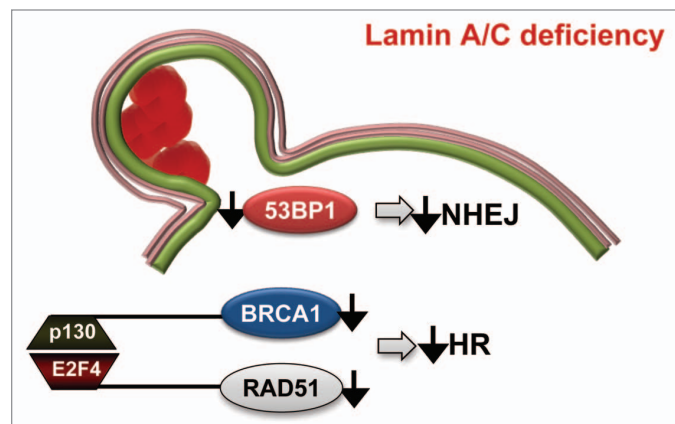
Much has been learned about the function of lamins in DNA repair over the last years. Lamin A/C-deficient mice do not show an increased rate of tumorigenesis<sup>7</sup> despite

the severe defects in the two main pathways of DNA repair (NHEJ and HR) reported by Gonzalo's group. This fact, together with the observed repair of DNA DSBs in the absence of canonical NHEJ and HR in lamin A/C-deficient MEFs, points towards the in vivo operation of alternative DDR repair pathways and opens up new avenues for research in the field.

Finally, it is interesting to note that *LMNA* expression is reduced/absent in undifferentiated or proliferative cells but is observed in differentiated or non-proliferative cells, such as quiescent adult stem cells. Taken together, and considering the link between age-related nuclear envelope defects, DNA damage accumulation and stem cell dysfunction in HGPS,<sup>8,9</sup> it becomes now more evident that the therapeutic response of a tumor (i.e., radioresistance) can be drastically affected by the status of *LMNA* expression in the cancer stem cells and their niches.

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**Figure 1.** Lamin A and genome stability. In the absence of lamin A/C, the structure of the nuclear envelope is compromised and the stability of 53BP1 is decreased, causing a reduction in the NHEJ repair pathway. However, lamin A/C deficiency also leads to increased formation of p130/E2F4 complexes, which in turn bind to the *RAD51* and *BRCA1* promoters, inhibiting their transcription and ultimately provoking a reduction in the HR repair pathway.