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# Cell Cycle News & Views

## Redundancy in response to DNA damage: The key to protection of genome integrity

Comment on: Ciznadija D, et al. Cell Cycle 2011; 10:2714–23

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Progression through the cell cycle is mediated by cyclin-dependent kinases (Cdks) that are activated by their respective cyclins. The cyclin D-Cdk4/6 and, subsequently, cyclin E-Cdk2 phosphorylate retinoblastoma tumor suppressor protein (pRb), leading to a release E2F transcription factors from the complex with pRB. The release turns on transcription of genes coding for proteins required to initiate cell cycle progression and enter the S phase.[1] Availability of cyclins, whose content is regulated transcriptionally and post-transcriptionally, is the opening mechanism of modulation of Cdks' activity.[2] Subsequent regulation is provided by the two families of cyclin-Cdk inhibitors (CKI), the INK4 and Kip/Cip proteins. The INK4 inhibitors (p15, p16, p19) target cyclin D-Cdk4/6 complexes preventing entry to cell cycle ($G_0$ to $G_1$ transition). The Kip/Cip proteins (p21, p27, p57) inactivate cyclin E-Cdk2, thereby blocking the $G_1$ to S transition.

Damage to DNA triggers several signaling pathways defined as the DNA damage response (DDR). Activation of these pathways has four critical goals: (1) halting cell cycle progression to prevent transfer of DNA damage to progeny cells; (2) increasing accessibility of the damage sites to repair machinery; (3) engagement of repair machinery and (4) preparing apoptotic response to eliminate cells whose DNA has not been successfully repaired.[3] For the fail-safe execution of these goals, individual steps of DDR are highly redundant and use diversity of mechanism. The capability to phosphorylate the same proteins by ATM, ATR, DNA-PKcs or Chk2 protein kinases is a classic example of such redundancy. Similarly, halting cell cycle progression in response to induction of p53 can be achieved by inactivation of cyclin E-Cdk2 by p21 as well as through Cdc25A and Cdc25C phosphatases that dephosphorylate cyclin E-Cdk2.

Numerous redundant pathways, ubiquitin-dependent as well -independent, are involved in regulation of abundance of p21 protein. Ciznadija et al.,[4] in a recent *Cell Cycle* article, address this subject and describe mechanisms modulating turnover of p21 upon induction of p53 in response to DNA damage by ionizing radiation. In elegant experiments utilizing multiparameter flow cytometry, cell sorting, elutriation and gel blot analysis of proteins from the sorted cells, the authors correlated expression of p53, p21, p27, Chk2 and hdm2 vis-à-vis cell cycle phase in the irradiated cells.[4] Similar to the case of DNA damage induced by DNA topoisomerase I inhibitor camptothecin, the damage by radiation led to induction of p53 in cells in all phases across the cell cycle.[5] However, the accumulation of p21 was limited to cells in $G_1$ and $G_2$ phases. The apparent reduction of p21 seen in S-phase cells was mediated by the proteasome-dependent turnover pathway but independent of $SCF^{SKP2}$ E3 ubiquitin ligase activity. Using hdm2 siRNA and hdm2 knockout cells, the authors also explored the role of hdm2 in turnover of p21 in the S phase in irradiated cells.

Interestingly, similar to the case of ionizing radiation, the induction of DNA damage by UV was shown in another study to lead to proteosomal degradation of p21 also in S-phase cells.[6] The degradation was also independent of $SCF^{SKP2}$ but was mediated by the $CRL4^{CDT2}$ ligase, whose activity, in this case, required the interaction of p21 with PCNA.[6] However, in the S phase of non-irradiated cells, a redundancy between $CRL4^{CDT2}$and $SCF^{SKP2}$ in their ability to ubiquitilate p21 has been observed.[6] Other ubiquitin ligases, such as $APC/C^{CDC20}$, $CRK^{LRR1}$, $DBD1^{CUL4}$, target nuclear p21 as well and play a role in its turnover,[7] while $CLR2^{LRR1}$ targets cytoplasmic p21.[8] The possible involvement of these ligases in stability of p21 after DNA damage has not been investigated.

Regulation of p21 turnover is also mediated by phosphorylation by the nuclear-Dbf2-related (NDR) kinases, the enzymes that are highly conserved from yeast to human.[9] Little is known about their possible involvement in p21 turnover after induction of DNA damage, specifically whether their activity contributes to the reduction of p21 expression in S-phase cells.

The multiplicity of mechanisms regulating abundance of p21 after induction of DNA damage, their redundancies and evolutionary stability all suggest that, highly controlled and likely associated with multiple signaling pathways related to the cell cycle phase, regulation of p21 turnover is essential for effective DNA repair and maintenance of genome integrity.



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# Lamins reach out to novel functions in DNA damage repair

## Comment on: Redwood AB, et al. Cell Cycle 2011; 10:2550–61

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The faithful maintenance of genomic DNA is essential for cell function and organismal life and is achieved by an intricate DNA damage recognition and repair machinery. External factors such as UV or ionizing irradiation and genotoxic chemicals as well as endogenous hazards (e.g. reactive oxygen species) and defects during DNA replication constantly damage DNA. The most severe lesions are DNA double-strand breaks (DSBs), which are repaired by three pathways, depending on the cell cycle phase: homologous recombination repair (HR) is predominantly used during late S and $G_2$ phase, utilizing sister chromatids as templates for high-fidelity repair. In $G_1/G_0$, DSBs are repaired by the more error-prone but faster nonhomologous end joining (NHEJ) pathway, during which repair factors synapse and join the broken DNA ends. NHEJ also mediates long-range end fusions of de-protected telomeres and chromosome regions.[1] In addition, a less-understood slow alternative NHEJ pathway (a-NHEJ) repairs DSBs via DNA end resection and microhomology-mediated ligation.

In the past years defects in lamin A, a major scaffold protein in metazoan cell nuclei, were linked to the premature aging syndromes, Hutchinson-Gilford progeria and Werner-like syndrome.[2,3] Lamin-linked accelerated ageing is associated with impaired DSB repair and genomic instability.[4-6] At the molecular level, disease-linked lamin A mutants were found to impair recruitment of essential components of the DNA damage response pathways, including ATM (ataxia-telangiectasia mutated), ATR (ATM- and Rad3-related) and p53 binding protein (53BP1) to DNA damage sites.[4-6] The specific role of lamin A in DNA damage repair, however, remained unclear. A recent report by Susana Gonzalo showed that lamin A also stabilizes 53BP1 protein. Lamin A loss caused 53BP1 downregulation and impaired fusion of de-protected telomeres,[7] consistent with reports on 53BP1's role in long-range NHEJ.[8] In their paper in the August 1st issue of *Cell Cycle*, the authors identified novel unexpected roles of lamin A in the repair of irradiation-induced DNA damage by short-range NHEJ and HR.[9] They found that lamin A-deficient cells have significantly reduced levels of 53BP1 and show a delayed DNA repair compared to lamin A-expressing cells. In particular, the initial, fast phase of DNA damage response, likely mediated by NHEJ, was impaired in lamin-deficient cells. While 53BP1 overexpression rescued this defect, direct 53BP1 downregulation by RNA interference did not affect short-range NHEJ, as seen after 53BP1 downregulation mediated by lamin A loss. These unexpected findings are consistent with a model in which 53BP1 indirectly promotes fast NHEJ by inhibiting slow repair mechanisms like HR and alternative-NHEJ. Since lower 53BP1 levels in lamin A-deficient cells did not promote HR, the authors concluded that lamin A may have additional, 53BP1-independent roles in HR. One clue on this novel role came from analyses of components of the HR pathway: both RAD51 and BRCA1 were reduced on the protein and, more surprisingly, also on the transcript level. Based on previous reports that RAD51 and BRCA1 transcript levels are regulated by the p130/E2F4 pathway, and that lamin A is involved in the regulation of the p130-related pocket proteins pRb and p107, the authors investigated the levels of p130/E2F4 upon lamin A depletion. Loss of lamin A was found to promote formation of p130/E2F4 complexes, which, in turn, may repress RAD51 and BRCA1 transcription.

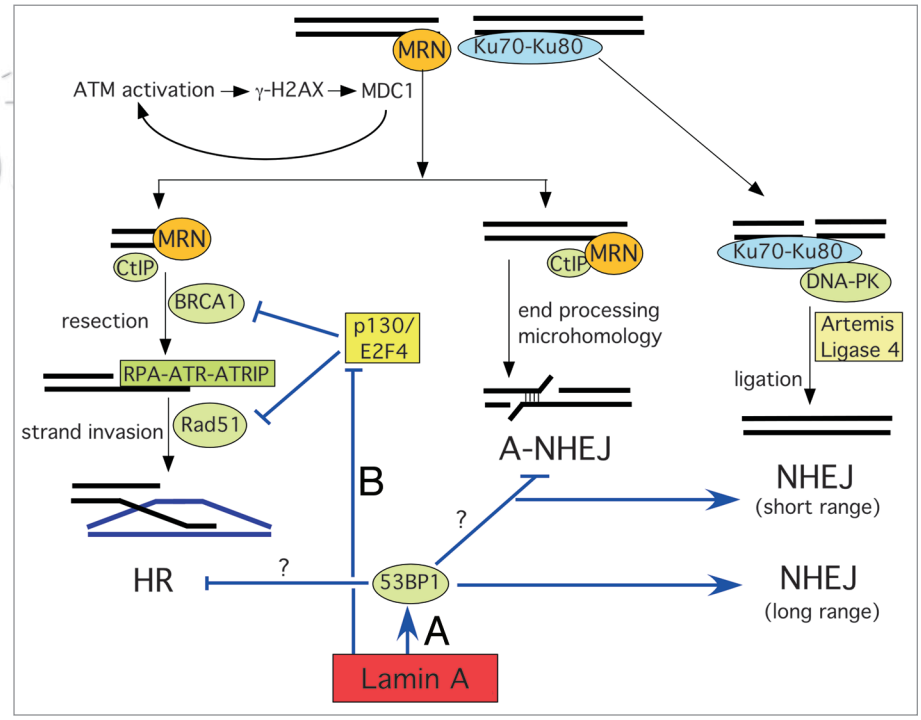


**Figure 1.** DSBs are sensed by the MRN (MRE11-RAD50-NBS1) complex, which activates ATM. ATM phosphorylates histone H2AX that recruits mediator proteins like MDC1 and ATM itself. In HR, MRN induces strand resection together with several proteins, including BRCA1 (breast cancer 1) and C-terminal binding protein (CtBP)-interacting protein (CtIP). Single-strand DNA overhangs are bound by replication protein A (RPA), ATR and ATR-interacting protein (ATRIP). RAD51 then promotes homology search and strand invasion. DNA ends bound by Ku70/Ku80 are repaired by NHEJ, involving also DNA-dependent protein kinases (DNA-PK), Artemis and Ligase 4. Alternative NHEJ (A-NHEJ) can be induced by MRN-CtIP, which facilitates DNA end fusion by strand resection and usage of sequence microhomologies. Lamin A has a dual role in these pathways: (A) It stabilizes 53BP1, which inhibits HR and A-NHEJ thereby favoring short range classical NHEJ. (B) Lamin A impairs formation of the p130/E2F4 complex, which represses BRCA1 and RAD51 transcription.

The molecular details of how lamin A influences 53BP1 stability and inhibits formation of p130/E2F4 repressor complexes remain to be identified. Lamins may directly bind to and affect these proteins, or these effects may be indirect. Altogether, the study by Redwood et al. provides a glimpse of novel functions of lamin A in both the transcription and stabilization of DNA damage repair components. Lamin A thus seems to also be involved in regulating the intricate cross talk between the different DSB repair pathways.


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# New role for Spinophilin in tumor suppression

## Comment on: Ferrer I, et al. Cell Cycle 2011; 10:2751–62


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Spinophilin was initially identified as a regulatory subunit of the PP1 protein phosphatase, responsible for its targeting to specific substrates in dendritic spines.[1,2] In the years after this discovery, the spectrum of Spinophilin partners and functions has expanded but has remained mostly in the field of neurobiology. The general picture emerging from these studies identifies Spinophilin as a scaffold protein that connects signal transduction to cell architecture, regulating processes critical for neural function, like spine morphology and density, synaptic plasticity and neuronal migration.[2]

In an article in the August 15th issue of *Cell Cycle*, Ferrer et al.[3] revealed that, in addition to its well-established role in neurophysiology, Spinophilin has a previously unappreciated role in cancer biology, acting upon the two major tumor suppressor circuits in mammals, the p53 and Rb pathways. Prompted by the location of the Spinophilin locus in a region of frequent LOH, Ferrer et al. set out to elucidate the potential role of this protein in tumorigenesis. Among the long list of PP1 substrates, they focused on the Retinoblastoma protein (Rb), an attractive candidate to mediate the tumor suppressive action of Spinophilin. Consistent with a role for Spinophilin and PP1 in phosphorylation of Rb and control of the $G_1$/S transition, Spn-knockout fibroblasts failed to fully dephosphorylate Rb at quiescence and showed premature entry into S phase after serum re-stimulation. Interestingly, this phenotype was paralleled by reduced PP1 activity.

Remarkably, Ferrer et al. showed that Spinophilin is also functionally linked to p53 in a variety of p53-mediated responses. MEF immortalization typically involves one of two equally frequent events: p53 mutation or inactivation of p19Arf or the entire Ink4a/Arf locus, reflecting the essential role of the ARF-p53 axis in senescence of this cell type. Spn-deficient fibroblasts underwent senescence and immortalization at normal rates. However, immortalization occurred in all cases through p53 mutation in a clear deviation from the pattern of wild-type MEFs. In additional experiments, loss of Spn accentuated p53-mediated cell-cycle arrest or the response to genotoxic agents, while silencing of Spinophilin enhanced the transformed phenotype of p53-deficient cells. Taken together, these observations clearly support a functional link between Spinophilin and p53, but they also suggest that the specific outcome can be context-dependent. Spinophilin loss may be beneficial by potentiating p53 in response to acute stress, but it can be deleterious under sustained mitogenic stress (as in serial passage or tumor formation), presumably because it poses a selective pressure that ultimately leads to p53 inactivation and increased tumorigenesis.

An interesting question is whether the links of Spinophilin to Rb and p53 are connected. It is conceivable that Rb deregulation due to Spn loss can provoke mitogenic stress that, in turn, leads to ARF-mediated p53 activation. In support of this model, ARF seems to mediate the enhanced p53 activation by oncogenic stress in Spn-deficient MEFs.[3] Also, the interaction between ARF and Spinophilin has been reported,[4] although its functional relevance is unclear. However, this has to be reconciled with unusually infrequent ARF loss in immortalized Spn-deficient MEFs or effects of Spinophilin on ARF-independent p53 responses. The involvement of PP1 in DNA damage signaling[5] could account for p53 regulation by Spinophilin in some cases. Even p53 or Rb-independent mechanisms could be considered, because Spinophilin can inhibit the growth of cells defective in either tumor suppressor.[3]

Can we extend these observations to the context of tumor formation? Spinophilin can restrain self-renewal of brain tumor initiating cells[6] and anchorage-independent growth of glioma cell lines.[7] Furthermore, the combined inactivation of Spinophilin and p53 correlates with increased tumorigenicity in vivo, as shown in two recent reports. First, Spinophilin-knockout mice display spontaneous mammary benign lesions, and this phenotype is exacerbated in mice expressing mutant p53 in mammary glands, leading to increased incidence of carcinomas.[8] Also, a subset of human lung tumors show reduced Spinophilin levels, which correlate with p53 inactivation and poor prognosis.[9] It would be interesting to extend these studies to other tumor types to establish the generality of these findings. In summary, although several interesting questions remain open, this report clearly identifies Spinophilin as a new player in tumorigenesis, in connection with PP1, Rb, and p53, and sets the basis for future work on the role of this protein in tumor biology.

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# Prostate cancer: JunD, Gadd45a and Gadd45g as therapeutic targets

## Comment on: Zerbini L, et al. Cell Cycle 2011; 10:2584–92

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Prostate cancer is among the most prevalent malignancies in older men and a frequent cause of death.[1-3] In addition to prostate-specific antigen (Psa) and Gleason grading, several molecular biomarkers have been proposed to predict outcome in patients with prostate cancer.[4] However, few of these biomarkers are used to guide clinical prognostic/diagnostic decision-making, since prostate cancer molecular pathology remains largely unknown. Nevertheless, plasma IL-6 and soluble IL-6 receptor (IL-6sR) levels are known to be significantly elevated in patients with metastatic, hormone refractory prostate cancer, and their levels in blood are predictive of prostate cancer progression and poor outcome.[5]

Zerbini and colleagues have previously shown that increased expression of the IL-6 gene in prostate cancer is primarily due to activation of NFκB p50 and p65 and the activating protein-1(AP-1) transcription factor heterodimer of JunD and Fra-1.[6] These authors now show that inhibition of JunD in prostate cancer cells results in induction of the stress sensors Gadd45a and Gadd45g but not Gadd45b, which, in turn, leads to activation of c-Jun N-terminal kinase (JNK), ultimately resulting in prostate tumor cell death and inhibition of tumor development (**Fig. 1**).[7]

Gadd45 proteins, including Gadd45a, Gadd45b and Gadd45g, have been implicated in stress signaling in response to physiological and environmental stress, including oncogenic stress, which can result in cell cycle arrest, DNA repair, cell survival, senescence and apoptosis (reviewed in ref. 8). The function of Gadd45 as a stress sensor is mediated via a complex interplay of physical interactions with other cellular proteins implicated in cell cycle regulation and the response of cells to stress, notably PCNA, p21, cdc2/cyclinB1 and the p38 and JNK stress response kinases. Altered expression of Gadd45 has been observed in multiple types of solid tumors as well as in hematopoietic malignancies (reviewed in ref. 9). Using genetically engineered mouse models and bone marrow transplantation, evidence has been obtained indicating that Gadd45 proteins can function to either promote or suppress tumor development and leukemia; this is dependent on the molecular nature of the activated oncogene and the cell type via engagement of different signaling pathways.

The findings by Zerbini et al., thus, are important. They extend the role of Gadd45 proteins as sensors of oncogenic stress and suggest that JunD as well as Gadd45a and Gadd45g proteins and their signaling targets represent a novel class of molecules for therapeutic intervention in prostate cancer.



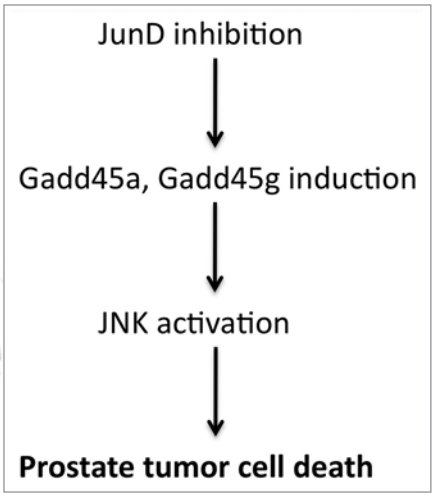


**Figure 1.** JunD, Gadd45a and Gadd45g therapeutic targets in prostate cancer. Inhibition of JunD in prostate cancer cells results in induction of the stress sensors Gadd45a and Gadd45g, which, in turn, leads to activation of c-Jun N-terminal kinase (JNK), ultimately resulting in prostate tumor cell death and inhibition of tumor development.

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# The sweet secrets of p27$^{kip1}$ regulation and function in cell migration

## Comment on: Nagano Y, et al. Cell Cycle 2011; 10:2593–603

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A paper in the August 1st issue of *Cell Cycle* describing regulation of p27$^{kip1}$ by the Siah1/SIP ubiquitin E3 ligase complex in the setting of glucose depletion reinforces the notion that a protein like p27$^{kip1}$, with its multitude of roles, has even more diverse and complicated mechanisms regulating its function.[1] p27$^{kip1}$ was initially identified as a kinase inhibitor protein (KIP) regulating cyclin E-Cdk2 (cyclin-dependent kinase 2) (**Fig. 1**),[2] and while the *CDKN1B* gene is rarely mutated in cancer, p27$^{kip1}$ protein levels are often low in human cancers.[2] Definitive evidence that p27$^{kip1}$ can function as a tumor suppressor protein includes the observation that mice with germline inactivating *Cdkn1b* mutations are predisposed to cancer,[2] and that germline inactivating *Cdkn1b/CDKN1B* mutations can underlie endocrine cancer predisposition in the rat and in man.[3] Besides its role in inhibiting the cell cycle, p27$^{kip1}$ has a positive role in cell cycle progression via effects on the assembly and/or stability of cyclin D-Cdk4 complexes.[2] A potential oncogenic role for p27$^{kip1}$ might be attributable to p27$^{kip1}$'s ability to enhance cell migration via p27$^{kip1}$ inhibition of RhoA,[4] a protein that functions with ROCKs (Rho-associated coiled-coiled kinases) to promote stress fiber formation and inhibit cell migration.[5]

The levels and function of p27$^{kip1}$ are regulated in part via the receptor tyrosine kinase/RAS/RAF/mitogen-activated protein kinase and phosphoinositol-3′ kinase (PI3K)/AKT/mTOR (mammalian target of rapamycin) pathways (**Fig. 1**).[2] The phosphorylation of p27$^{kip1}$ at specific sites by kinases in these pathways can increase p27$^{kip1}$ stability, enhance its cytoplasmic retention and/or increase its binding affinity to RhoA,[2] resulting in increased cell migration and, perhaps, enhanced invasion and metastatic capabilities of cancer cells.

The findings of Nagano and colleagues of a novel pathway regulating cytoplasmic p27$^{kip1}$ arose from the initial observation that mouse embryonic fibroblasts (MEFs) lacking the Siah1-interacting protein (SIP) had elevated p27$^{kip1}$ levels without apparent effects on other cell cycle factors.[1] Further work demonstrated that p27$^{kip1}$ levels were markedly decreased in MEFs in response to glucose depletion. Nagano and coworkers then found that glucose depletion led to induction of the Siah1 E3 ubiquitin ligase, followed by Siah1/SIP-dependent ubiquitination and degradation of the cytoplasmic p27$^{kip1}$ pool, with resultant decreased migration of MEFs in glucose-depleted conditions.[1]

The studies and data in the manuscript nicely support the authors' claims. Nonetheless, the results highlight unresolved issues and potential new research directions. The functional studies were pursued in fibroblasts, and it is not yet clear whether regulation of p27$^{kip1}$ by Siah/SIP1 in response to glucose stress can be generalized to other cell types. Also, while Siah1 induction may be key in mediating p27$^{kip1}$ degradation in response to glucose depletion, specific factors and mechanisms regulating Siah1 expression in response to glucose depletion are

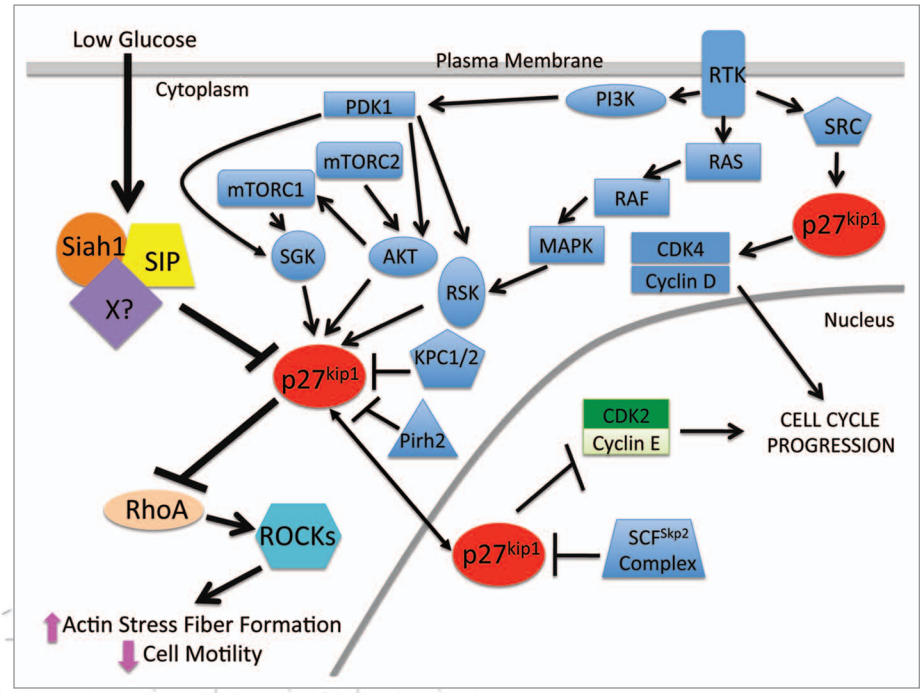




**Figure 1.** Siah1/SIP1 inhibition of p27$^{kip1}$ levels and decreased cell motility in fibroblasts in response to glucose depletion, as highlighted by Nagano and colleagues.[1] Other selected signaling pathways and factors impacting on p27$^{kip1}$ levels, localization and function in cell cycle progression and cell motility previously highlighted by Wander and colleagues[2] are also shown. Glucose depletion activates Siah1 expression. A Siah1/SIP E3 ligase complex, perhaps containing other proteins such as a yet-to-be-defined adaptor protein "X," promote ubiquitination and degradation of the cytoplasmic p27$^{kip1}$ pool. When present in the cytoplasm, p27$^{kip1}$ can antagonize RhoA, which, when not complexed with p27$^{kip1}$, can activate ROCKs (Rho-associated coiled-coiled kinases) to promote stress fiber formation and decreased cell motility. Net positive/enhancing signaling effects are indicated schematically by arrows, whereas net negative/inhibitory interactions are indicated by closed bars. Phosphorylation events play a major role in regulating p27$^{kip1}$'s subcellular localization, binding to various proteins, stability and function. Major ubiquitination factors for p27$^{kip1}$ include SCFSkp2, KPC1/2, Pirh2 and now also Siah1/SIP. SIP, Siah interacting protein; RTK, receptor tyrosine kinase; MAPK, mitogen activated protein kinase; PI3K, phosphoinositol-3′ kinase; CDK4, cyclin-dependent kinase 4; CDK2, cyclin-dependent kinase 2; PDK1, 3- phosphoinositide-dependent protein kinase 1; SGK, serum- and glucocorticoid-induced protein kinase; RSK, p90 ribosomal S6 kinase; mTORC1/2, mammalian target of rapamycin complex 1 and 2, respectively; KPC1/2, Kip1 ubiquitin promoting complex 1 and 2; Pirh2, p53-induced RING H2; Skp2, S-phase-associated kinase 2; SCF, Skp2, cullin, F-box protein complex.

uncertain. SIAH1 is a p53-regulated gene in some settings,[6] but it is unclear if p53 regulates Siah1 in response to glucose-depletion. Siah1/SIP degradation of $p27^{kip1}$ may require unknown adaptor proteins,[1] and whether phosphorylation or other post-translational modifications of $p27^{kip1}$ affect Siah1/SIP-mediated degradation is not yet clear, though phosphorylation events mediated by other pathways affect $p27^{kip1}$ function (**Fig. 1**). The findings of Nagano et al. indicate that Siah1/SIP-mediated degradation of $p27^{kip1}$ seems to be responsible for much of the inhibition of cell migration in fibroblasts in the setting of glucose depletion.[1] Given Siah1's function in regulating many other proteins in concert with SIP and/or other binding partners,[7] it remains to be seen if and how levels of other Siah1-regulated proteins are changed in response to metabolic stress. Yet another question is whether reduced $p27^{kip1}$ cytoplasmic levels uniformly inhibit cell migration in all cell types and settings. In contrast to the situation in fibroblasts and cancer cells where elevated $p27^{kip1}$ often stimulates cell migration in two dimensions,[2,4] increased $p27^{kip1}$ inhibits cell migration in endothelial cells and vascular smooth muscle cells.[8] Moreover, when fibroblast movement is studied in three-dimensional assays, deficiency of $p27^{kip1}$ leads to enhanced motility, perhaps in part due to $p27^{kip1}$'s ability to stabilize microtubules via binding to the microtubule-destabilizing protein stathmin.[9] Future studies will undoubtedly tackle these and other outstanding questions about the roles for Siah1/SIP in regulating $p27^{kip1}$ and cell migration in a variety of physiological and pathophysiological settings.


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# p21 regulates the cell cycle…or the other way around?

## Comment on: Ciznadija D, et al. Cell Cycle 2011; 10:2714–23


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For many years, the upregulation of the p53-p21 pathway was supposed to represent the universal response to genotoxic stress. Intriguingly, many genotoxic agents (hydroxyurea, aphidicolin, hypoxia, cisplatinun and UV irradiation) do not upregulate p21 despite p53 activation.[1] Remarkably, in the August 15th issue of *Cell Cycle*, Ciznadija and colleagues[2] showed that γIR (gamma ionizing radiation), a DNA damaging agent which was previously characterized as a bona fide inducer of p21, fails to promote such accumulation in S phase. Remarkably, this happens in the context of high p53 levels demonstrating that also in the context of γIR, as well as signals downstream of p53 specifically prevent p21 accumulation during S phase.

What is the connection between all these signals that negatively regulate p21? Despite the diversity in their nature, all the treatments that prevent p21 upregulation (see above) allow transition through or promote accumulation in S phase.[1] γIR seems to be the exception to this pattern, since it causes $G_1$/$G_2$ accumulation. However, a thorough look at the experiments performed by Ciznadija and colleagues[2] reveals that the S-phase population analyzed in this study was mostly the one that was already transiting the S phase when the insult was delivered. This suggests that, while a strong p53-dependent p21 induction stops S-phase initiation after genotoxic signals, S-phase entrance might be sufficient to revert and/or counteract those signals, preventing p21 accumulation until the finalization of DNA replication.

How does S phase prevent p53-dependent p21 accumulation when the DNA is damaged? Multiple evidences indicate that p21 levels could be controlled both transcriptionally and posttranscriptionally after genotoxic stress.[3] In particular, it is clear that basal levels of p21 are kept in check because of a SCF(Skp2)-dependent degradation of CDK-bound p21.[4] Genotoxic stimuli promote the degradation of the p21 pool that is bound to the proliferating cell nuclear antigen, PCNA. In this context, the action of the Cul4 (CDT2) E3 ligase degrades p21 molecules that are associated with chromatin-bound PCNA (revised in ref. 5). A third ubiquitin-independent but proteasome-dependent pathway of p21 degradation depends on Hdm2.[6,7] p21 proteolysis is facilitated by the interaction of free p21, with Hdm2 being the ring domain of Hdm2 dispensable for this process.[8] In agreement with previous reports, Ciznadija and colleagues[2] showed that the level of p21 mRNA increases steeply after γIR. Thus, p21 proteolysis during S phase must be sufficiently solid to counteract this challenge. In fact, the authors demonstrate that Hdm2 increases p21 turnover in cells transiting S phase after γIR. Interestingly, another report links p21 degradation after γIR with Cul4 (CDT2).[9] Thus, convergent degradation pathways could collaborate to counteract

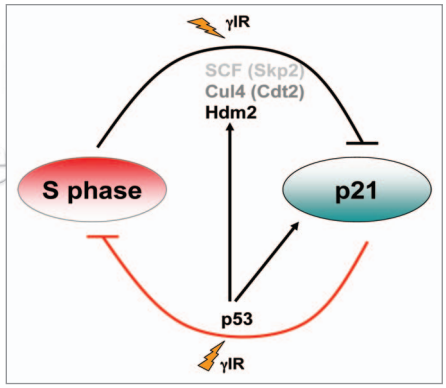


**Figure 1.** A novel signalling loop after γIR? While high p21 levels prevent S-phase entrance, p21 does not accumulate in cells transiting S phase. p53 transcriptionally activates p21 and promotes its accumulation in $G_1$, but Hdm2- and Cul4 (Cdt2)-mediated p21 degradation might prevent its upregulation in S phase.

γIR -induced p53-dependent p21 mRNA accumulation during S phase (**Fig 1**).

Why is it so important to avoid p21 upregulation in S phase, when cells are challenged with γIR? Our first simple speculation is that cells in S phase need to finish DNA replication. While checkpoint activation slows the progression of S phase, at least a part of the active replication forks will eventually encounter DNA lesions. p21 degradation might be required to maintain the processivity of DNA

replication at sites where damaged DNA must be used as templates.[1] Also, the resolution of double-strand breaks caused by γIR might also benefit from the duplication of the DNA around lesions that might promote error-free repair by homologous recombination. Thus, once started, S phase-driven p21 downregulation might facilitate the cell survival associated with the complete finalization of DNA duplication.


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# Caveolin-1: Would-be Achilles' heel of tumor microenvironment?

## Comment on: Agnieszka K, et al. Cell Cycle 2011; 10:1794–809


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The phenotype of solid tumors is largely determined by the surrounding tissue microenvironment composed of untransformed epithelium, stromal cells, soluble factors and extracellular matrix (ECM) produced by the interactions between these constituents.[1] Tumor cells modulate their microenvironment as they grow, which, in turn, synergistically supports and augments the tumor growth potential.[1] For example, proteases secreted by non-tumor cells not only degrade ECM to promote the motility and invasiveness of malignant cells, but also cause genomic instability through a Rac1b- and ROS-dependent mechanism to actually promote tumorigenesis and epithelial-to-mesenchymal transition.[1,2] Hypoxia within the growing tumor further exacerbates genomic instability, promoting growth and survival of malignant cells.[3] Additionally, cancer-associated fibroblasts (CAFs) in tumor stroma fuel malignant cells by transferring nutrients (a.k.a., reverse Warburg effect)[4] produced by oxidative stress-induced catabolic autophagy (a.k.a., autophagic glycolysis);[4] aerobic glycolysis then leads to acidosis within the tumor microenvironment and impairs the efficacy of alkaline chemotherapeutic drugs, such as bicarbonates.[3,5] These tumor-stroma interactions continue to evolve with time, helping malignant cells survive and thrive.[1]

Lisanti and his coworkers reported previously that co-culturing breast cancer cells with normal mammary fibroblasts causes loss of stromal caveolin-1 (Cav-1) through autophagic degradation, generating CAF-like cells.[4] They also showed that loss of stromal Cav-1 promotes mitochondrial oxidative stress and hypoxia, aggravating stromal autophagy and aerobic glycolysis that stimulate tumor growth by establishing a 'lethal tumor microenvironment."[4] These observations also carry significant clinical relevance, as loss of Cav-1 in breast cancer patient stroma correlates with poor prognosis and reduced therapeutic efficacy.[6,7]

Despite these studies, the means by which stromal Cav-1 suppresses mammary tumor growth remain poorly understood. Accordingly, in the June 1st issue of *Cell Cycle*, Lisanti et al. analyzed global gene expression profiles of cancer-associated stroma isolated by laser capture microdissection of breast cancer biopsies to explore how signaling pathways pertinent to loss of stromal Cav-1 are altered during breast carcinogenesis in vivo.[4] They divided the stroma into Cav-1 non-expressing and expressing groups and found that the former exhibits an upregulation of genes involved in oxidative stress, hypoxia, glycolysis, DNA damage repair, apoptosis, autophagy and myofibroblast differentiation when compared to the latter. This result supports their contention that loss of stromal Cav-1 contributes to the formation of a lethal tumor microenvironment through a process they term "the autophagic tumor stroma model of cancer," where tumor stromal cells destroy themselves to generate energy-rich nutrients that feed anabolic cancer cells and ultimately promote the severity of tumor grade and metastatic potential of the cancer.[4]

The body of work by Lisanti et al.[4] clearly raises the possibility that stromal Cav-1 status can be utilized as a prognostic/diagnostic marker for breast cancer with the worse clinical outcomes. The detailed mechanism by which Cav-1 is lost in tumor stroma, however, is yet to be explored. The same group previously showed that Cav-1 is downmodulated through lysosomal degradation in fibroblasts when co-cultured with breast cancer cells.[8] Although lysosomal degradation of Cav-1 is known to be mediated by ubiquitination following caveolar endocytosis,[9] it remains enigmatic how Cav-1 degradation in stromal fibroblasts is extrinsically induced by adjacent cancer cells. Additionally, the mechanistic basis for how the loss of Cav-1 in tumor stroma upregulates such gene sets as those involved in the formation of the "lethal tumor microenvironment" has yet to be determined. Lastly, it is important to know whether this particular mechanism is applicable to other solid tumors as well. A plausible explanation could be that the loss of Cav-1, a membrane scaffolding protein, alters critical intracellular/transmembrane signaling (e.g., G protein α subunit, eNOS, Src family kinases, EGF-R, PKCα, and Ras-related GTPases),[9] thus impairing the homeostatic balance of cells and upregulating stress-response machineries. Examination of this and other possibilities could help further evaluate the clinical use of stromal Cav-1 levels as an index of cancer progression and prognosis. What may, of course, be even more exciting is to see whether these findings could be manipulated for prevention and/or therapy.




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# Small cell lung cancer: New insights into origins

## Comment on: Park K, et al. Cell Cycle 2011; 10:2806–15

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Small cell lung carcinoma (SCLC) is one of the most aggressive tumor types. Typically, SCLC has already metastasized by the time of diagnosis, and, while initially responsive to chemotherapy, relapse usually occurs within a year.[1] Despite the aggressive nature and high prevalence of this tumor type, our basic understanding of the cells that give rise to SCLC is poor.

A mouse model for SCLC was previously generated by Anton Berns's lab.[2] In this model, adenoviral Cre (Ad-CMV-Cre) is administered to the lungs of adult animals harboring floxed *Rb* and *p53* alleles. The model recapitulates genetic characteristics of human SCLC, as *RB* and *p53* are both mutated in the vast majority of human SCLCs. Murine SCLC emerges over approximately a year with cell type characteristics and a metastatic pattern that mirrors human SCLC. Notably, the original mouse model is not reliant on knowledge of the SCLC cell of origin, as all cells in the lung that contact the Ad-CMV-Cre virus will delete *Rb* and *p53*.

Despite strong neuroendocrine characteristics in typical human SCLCs, the cell of origin is not clear. The presence of mixed tumors in which both neuroendocrine and alveolar or bronchiolar characteristics are present complicates our understanding of the SCLC-originating cells. It is possible that a non-neuroendocrine cell can adopt neuroendocrine characteristics (and vice versa), for example, through gene mutation. Indeed, a subset of non-small cell lung carcinoma (NSCLC) patients that developed resistance to EGFR inhibitors exhibited a change in tumor appearance, with initially non-neuroendocrine tumor cells acquiring neuroendocrine features.[3] Also, expression of *H-Ras* caused murine neuroendocrine tumor cells to lose neuroendocrine features.[4] Thus, given the ability of tumor cells to drastically alter their cell type characteristics, the SCLC-originating cells cannot be determined by the properties of late tumors.

Other than neuroendocrine cells, candidate adult lung stem cells and progenitor cells represent potential SCLC cells of origin. In the original Ad-CMV-Cre studies, pulmonary neuroendocrine cells, bronchioalveolar stem cell (BASCS), AT2, Clara cells and others are all infected. Two recent studies,[5,6] including one published in the August 15th issue of *Cell Cycle*,[6] for the first time have now assessed SCLC tumorigenesis upon *Rb/p53* mutation targeted to specific lung cell types.

These studies use *Cre* transgenics or Ad-Cre vectors with cell type-specific promoters to assess the response of different cell populations to Rb/p53 deletion.[5,6] Clara cells (*Scgb1a1*+) are a self-renewing lung population[7] located where SCLC is typically found, in the bronchi, bronchioles and terminal bronchioles. Through the use of Scgb1a1-Ad-Cre vectors[5] and *Scgb1a1-Cre* expressing mice,[6] both groups ruled out Clara cells as SCLC-initiating cells. They also both exclude BASCs (Scgb1a1+/SPC+), as initiating cells for SCLC following *Rb/p53* deletion. AT2 cells are another self-renewing population, which are located in the distal lung and express surfactant protein C (SPC). Sutherland et al. targeted SPC-positive cells with SPC-Ad-Cre and found partially penetrant SCLC with very long latency.[5] This differed from the Park et al. study,[6] where SPC-Ad-Cre-ER did not result in SCLC. This difference may be explained by lower efficiency of the SPC-Ad-Cre-ER infections vs. SPC-Ad-Cre. Park et al. also used the *SpC-rtTA/tetO-Cre* system to target *Rb/p53* mutation to AT2 cells, but still did not find neuroendocrine lesions, arguing that the SPC-positive AT2 cells are not the major cells of origin for SCLC.[6] It will be interesting to assess the extent of heterogeneity in the SPC-positive population and the factors that determine whether an SPC-positive cell can form SCLC. Critically, Sutherland et al. used neuroendocrine-specific CGRP-Ad-Cre vectors to positively show that neuroendocrine cells are by far the most efficient at initiating SCLC.[5]

The findings are likely to focus future analyses of the early events in SCLC on the neuroendocrine cell population. Pulmonary neuroendocrine cells are a very rare population in the adult lung and are poorly understood. With new tools now available, including neuroendocrine-specific adenoviral vectors, the biology of these rare cells may now be probed. Interrogating the roles for neuroendocrine cells in normal lung development/physiology and in tumorigenesis will undoubtedly lead to new inroads into combatting the most aggressive form of lung cancer.



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# Targeting JunD: A potential strategy to counteract hormone-refractory prostate cancer

## Comment on: Zerbini LF, et al. Cell Cycle 2011; 10:2583–91

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JunD is an intriguing member of the AP-1 transcription factor complex capable of activating or repressing a diverse array of target genes. Depending upon the cellular context and heterodimerization partner, JunD functions as a positive or negative regulator of cell proliferation, differentiation and apoptosis.[1] In human myeloblastic leukemia cells, JunD mediates UV-induced apoptosis while JunD knockout mice display augmented apoptosis, hypertrophic growth and angiogenesis in the heart upon pressure overload (reviewed in

ref. 1). Androgens play an important role in the initial development of prostate cancer. However, in its advanced stage, prostate cancer becomes androgen-independent and acquires a more virulent and aggressive phenotype. JunD plays important regulatory roles in both androgen-dependent and androgen-independent prostate cancer cells by functioning as a co-activator for androgen receptor to mediate androgen-induced oxidative stress or by interacting with the NFκB pathway to induce generation of interleukin-6, an important mediator of metastatic, hormone-refractory disease.[2,3] The versatile role of JunD as a potential proto-oncogene in prostate carcinogenesis makes it an important target to neutralize for potentially eliciting therapeutic benefits.

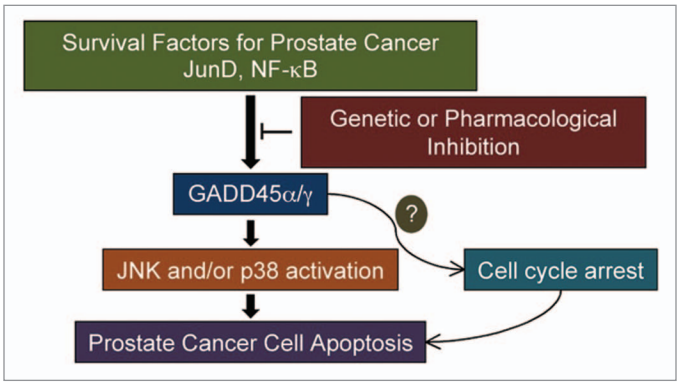


**Figure 1.** In hormone-refractory prostate cancer cells, inhibition of JunD by an adenovirus-mediated delivery of dominant-negative construct induces GADD45α and GADD45γ but not GADD45β, resulting in induction of apoptosis that is predominantly mediated though activation of JNK and p38

Growth arrest and DNA damage-inducible (GADD) genes GADD45α, GADD45β, GADD45γ, GADD34 and GADD153, are induced by a variety of cellular stresses, including nutrient deprivation and DNA damage.[4] The GADD proteins function primarily to protect cells and safeguard survival by inducing cell cycle arrest and DNA repair. However, sustained stresses causing accumulation of GADD proteins ultimately induce apoptosis. Overexpression of each GADD gene induces apoptosis, while combined overexpression of multiple different GADD genes synergistically augments this effect. The GADD45 gene family plays a prominent role in activation of stress-responsive kinases, such as p38 MAPK and JNK, by directly interacting with their upstream kinase MTK1 or MEKK4.[5] GADD45α, but not GADD45β or GADD45γ, induces $G_2$/M cell cycle arrest by inhibiting interaction of cdc2 with cyclin B1.[6] GADD45α is also a downstream gene of the p53 family and is required to maintain genomic stability.[7] Activation of several oncogenic signaling molecules, such as c-Myc, NFκB or Akt, leads to downregulation of GADD45 expression (reviewed in ref. 8). Mutational inactivation of GADD45α and silencing of GADD45γ by promoter hypermethylation has been detected in a number of cancers, suggesting its potential role as a tumor suppressor (reviewed in ref. 8). Several clinically relevant therapeutics, such as histone deacetylase inhibitors (HDACI), thiazolidinedione (TZD), non-steroidal anti-inflammatory drugs (NSAID) and melanoma differentiation associated gene-7/interleukin-24 (*mda*-7/IL-24) function by upregulating GADD family genes, and this induction plays a major role in the induction of apoptosis.[8]

The study by Zerbini et al. demonstrates that in hormone-refractory prostate cancer cells, inhibition of JunD by an adenovirus-mediated delivery of dominant-negative construct induces GADD45α and GADD45γ but not GADD45β, resulting in induction of apoptosis that is predominantly mediated though activation of JNK and p38 (**Fig. 1**).[9] In addition, JunD inhibition-induced cell death is diminished when GADD45α and GADD45γ are silenced. These studies highlight the importance of JunD expression and GADD45 repression in maintaining survival of prostate cancer cells. Moreover, these findings provide a rationale for targeting hormone-refractory prostate cancer via interference with JunD-mediated repression of GADD45α and GADD45γ. However, several intriguing issues require further clarification. Given that stresses often collectively induce all three GADD45 members, why does inhibition of JunD or NFκB specifically induce GADD45α and GADD45γ but not GADD45β? Does AP-1 bind to GADD45α and GADD45γ promoter and repress transcription? Is there no functional AP-1 binding site in the GADD45β promoter? Is there crosstalk between AP-1 and NFκB in active repression of GADD45α and GADD45γ? Another important angle is the possibility of translating the current observation into the clinic for possible therapy of prostate cancer. Proof-of-principle experiments in the paper show that an adenovirus expressing dominant-negative JunD inhibits prostate cancer xenografts in nude mice.[9] However, further experimentation is needed to establish the therapeutic potential of this approach. It is of interest to develop therapeutic small molecule inhibitors for JunD that interfere with its DNA binding or heterodimerization. Since JunD is required for normal development and differentiation, the potential side effects of a JunD inhibition in normal cells must also be investigated. Additionally, selective targeting of GADD45α and GADD45γ rather than JunD might achieve similar antitumor efficacy with less toxicity. The paper by Zerbini et al. carries important significance in raising these questions that will facilitate our in-depth understanding of prostate carcinogenesis and may lead to the development of targeted and more effective therapeutic strategies for advanced prostate cancer.



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# Multiple roles of ELG1 with different interactions determine various cellular processes

## Comment on: Parnas O, et al. Cell Cycle 2011; 10:2894–903

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The integrity of DNA replication and the repair of damaged bases are crucial to organismal survival. Proliferating cell nuclear antigen (PCNA) encircles double-stranded DNA to ensure proper accuracy and processivity of replication as well as functions in the DNA damage response by recruiting translesion synthesis (TLS) polymerases or promoting homologous recombination (HR). Post-translational modification of PCNA, including ubiquitylation, sumoylation and phosphorylation, regulates the DNA repair pathway of choice.[1] For example, PCNA is monoubiquitylated on K164 by the Rad6-Rad18 E3 complex in response to stalled replication forks, which recruits error-prone TLS polymerases to bypass damaged or misincorporated DNA bases. Further polyubiquitylation of K164 by Ubc13/Mms2/Rad5 directs error-free HR repair of the damaged DNA instead. Added complexity comes in the form SUMOylation, which suppresses unwanted HR at stalled replication forks by recruiting the helicase Srs2.[2] Because PCNA is so important in maintaining genome stability, the specific regulation of PCNA modifications that determine the DNA repair pathway of choice warrants further investigation.

The SUMO-targeted ubiquitin ligase (STUbL) complex of Slx5-Slx8 has been suggested to function to maintain genome stability by targeting SUMOylated proteins for destruction by the proteasome.[3] In this issue, Parnas and colleagues identified Elg1 as a protein that interacts genetically and physically with Slx5-Slx8.[4] Elg1 is a conserved protein that is required to maintain genome stability in organisms ranging from yeast to humans.[5-7] The multiple phenotypes of Elg1-deficient yeast and mammalian cells, including elevated rates of chromosome breaks and homologous recombination, telomere dysfunction and chromosome loss suggest that Elg1 may regulate multiple cellular processes.

The authors identified Slx5-Slx8 as an Elg1-interacting protein through a yeast-two-hybrid screen using Elg1's domain containing SUMO interacting motifs (SIM). Notably, all hits were known SUMOylated proteins or involved in the SUMO pathway, including Slx5, SUMO (encoded by *SMT3*), an E3 ligase Siz2, and SUMO-interacting proteins Sap1, Nis1, Fir1, Uls1 and Ufd1. Deletion of *SLX5* and *UFD1*, but not the other interacting genes, resulted in sensitivity to a DNA damaging agent. Based on the authors' previous report that yeast Elg1 also interacts with PCNA with a preferential affinity for SUMOylated PCNA,[8] Elg1 appears to interact with multiple SUMO-related proteins to regulate complex uncharacterized pathways.

Despite the physical interaction between Elg1 and Slx5-Slx8 and a similar affinity for SUMOylated proteins, epistatic relationships revealed that Elg1 and Slx5-Slx8 perform different functions to maintain genome stability.[4] Double *elg1 slx5* and *elg1 slx8* mutants possess a synthetic growth defect and a synergistic increase in genome instability, indicating that Elg1 and Slx5-Slx8 most likely function in related but separate pathways. Interestingly, while a Elg1-deficient yeast strain accumulates SUMOylated PCNA, the Slx5-Slx8 complex does not greatly affect levels of PCNA SUMOylation.[4,8] Furthermore, the interaction of Elg1 with Slx5 is independent of PCNA modification. Thus, while both Slx5-Slx8 and Elg1 interact with SUMOylated substrates, there is added complexity in different targets of their regulated pathways.

The interactions of Elg1 with SUMOylated proteins and various SIM-carrying proteins underscore the complex regulatory mechanisms by which Elg1 regulates different cellular processes. To support this complexity, mammalian ELG1 has also been shown to regulate PCNA ubiquitylation through its interaction with the USP1-UAF1 complex, which down-regulates the level of ubiquitylated PCNA.[9] This function of the mammalian ELG1 appears to contribute to elevated rates of chromosome breaks as well as hypersensitivity to DNA damaging agents observed in cells deficient in ELG1 expression.[5] It is intriguing that UAF1 contains putative SUMO-like sequences, underscoring the role of ELG1-SUMO interactions in regulating multiple cellular pathways in mammals. It will be important to further define the mechanisms by which Elg1-dependent regulation of PCNA and other SUMO-related proteins affects genome instability.



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