

# Cell Cycle News & Views

## The RP-p53-Mdm2 pathway: A new link to genetic integrity?

Comment on: Llanos S, et al. *Cell Cycle* 2010; 9:4005–12.

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Deregulation of the cell cycle (cell division) has long been known to contribute to the induction of cancer. Similarly, disruption of protein synthesis (cell growth) has also been shown to lead to several pathological conditions including cancer.<sup>1</sup> The tumor suppressor p53 is pivotal in inducing cell cycle arrest in response to DNA damage, and it has recently been recognized that p53 also plays a role in linking cell division with cell growth by sensing nucleolar stress.<sup>2</sup> When there is stress to ribosome biogenesis, and thus protein synthesis, several ribosomal proteins (RPs) such as RPL11, RPL23 and RPL5 have been shown to interact with Mdm2 and inhibit its E3 ubiquitin ligase activity towards p53. This leads to p53 stabilization and activation and enables cells to stop cell cycle progression in the absence of functional ribosomal biogenesis conditions.<sup>3</sup> This places p53 as an important regulator of both the DNA damage and protein synthesis pathways and suggests the coupling of these two processes may be important to preventing oncogenesis.

In a previous issue of *Cell Cycle*, Llanos and Serrano link the ribosomal stress and p53 pathways with the DNA damage response. These authors show that depletion of endogenous L37 led to an increase in p53 protein levels as well as its downstream targets p21 and Mdm2. Silencing of L37 also induced a decrease in S-phase cells, suggesting activation of p53-mediated cell cycle arrest. The authors also showed that knockdown of L37 increased the level of Mdm2 and Mdm2/L11 complexes, suggesting that in L37-depleted cells, Mdm2

remains inactivated by L11 and that the ribosomal stress pathway mediates activation of p53 by L37 knockdown. The mechanism linking L37 depletion and increased L11-Mdm2 binding, however, is unclear and will need to be investigated in future studies. A variety of DNA damage agents have previously been shown to impair ribosomal biogenesis<sup>4</sup> and induce p53 stabilization dependent on L11 and S7.<sup>5</sup> However, the impact of DNA damage on the levels of ribosomal proteins has not been widely studied. In this report, Llanos and Serrano showed that cisplatin, UV light and doxorubicin decreased the level of ectopically expressed L37. These authors then investigated whether L37 could contribute to p53 activation in response to genotoxic stress. L11 normally helps to activate the p53 response by binding and inactivating Mdm2, and previous studies have shown that down-regulation of L11 abrogates the activation of the p53 response after DNA damage.<sup>5</sup> Conversely, stably expressing GFP-L37 in U2OS cells in the current study showed a reduced sensitivity to UVC-induced apoptosis, suggesting that a reduction in L37 protein levels can activate p53 response to DNA damage. The use of an ectopic overexpression system may have limitations though, and future studies will be needed to address the effect of DNA damage on endogenous L37. In addition, the mechanism for DNA damage causing L37 to decrease remains to be determined. Although it has previously been shown that damage to DNA can activate the p53 response signal, the work of Llanos and Serrano demonstrates

that oncogenic challenges signaling to p53 leading to cell cycle arrest can also be sensed by perturbations to a factor involved in protein synthesis, linking cell growth and cell division to genotoxic stress via p53. Whether this effect is L37-specific or is general for other RP depletion has not yet been determined.

If defects in DNA and problems with protein synthesis can both activate p53 to arrest the cell cycle, why would cells want to link cell duplication with cell growth through the same mechanism? It is notable that both elevated and reduced levels of ribosomal biogenesis are associated with cancer development.<sup>1,2</sup> Interestingly, p53 induces cell cycle arrest at G<sub>1</sub> and G<sub>2</sub>/M, two phases of the cell cycle where the cell engages in a high rate of protein synthesis. It may be that by being responsive to the DNA damage p53 checkpoint response, the nucleolus may be enabled to be a sensor to the cell to stop dividing at critical points if there is a problem in providing proteins to the cell that are required for the integrity of cell duplication. This study suggests that regulation of protein synthesis through the RP-p53-Mdm2 pathway may have a significant contribution to protecting cells against DNA damage initiated oncogenesis.

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## Hedgehog signaling in T-cell development: A non-redundant role for Gli1

Comment on: Drakopoulou E, et al. *Cell Cycle* 2010; 9:4144–52.

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The hedgehog (Hh) signaling pathway plays an important role in embryonic patterning and controlling cell growth and cell fate during animal development.<sup>1</sup> In the adult, Hh signals are involved in tissue maintenance and repair and stem cell function. The secreted Hh ligands (Sonic, Indian and Desert) initiate signalling by binding to and inactivation of the transmembrane receptor patched (Ptc1), thereby relieving inhibition of the transmembrane protein smoothed (Smo). Active Smo triggers a signalling cascade which culminates in the inactivation of repressors and activation of zinc finger transcription factors of the Gli family, Gli1, Gli2 and Gli3. Gli1, the first vertebrate Gli gene to be discovered in humans, was found to be highly amplified in malignant glioma. The combinatorial and cooperative function of Gli factors is likely cell context dependent and to integrate numerous signalling inputs. In the absence of Hh signalling Gli1 is transcriptionally repressed and Gli3 and Gli2 are proteolytically processed to truncated repressor forms, resulting in silencing of Hh-Gli targets. Upon Hh binding Gli1 is transcriptionally activated (which involves Gli3) and together with non-processed full length Gli2/Gli3 proteins regulates the activation of specific Hh-Gli target genes. These include cell cycle regulators like Cyclin D, proteins of the Wnt and TGFbeta family or several stem-cell marker genes. An important feature of Hh signalling is that the transcriptional executors have both unique and overlapping functions as evident from the phenotypes of single and double mutant mice.

Hh signalling and Gli proteins not only have multiple functions in mouse development

but also function during differentiation of double-negative (DN) and double positive (DP) thymocytes, thus being central for the proper maturation and selection of T cells.<sup>2,4</sup> Expression of Gli3 occurs in foetal but not adult thymocytes and is highest in the DN1 population. Gli1 and Gli2 are expressed in both foetal and adult thymocytes. While Gli2 dominates in DN1 and DN2 populations Gli1 is most highly expressed in DN2 and DN3 cells, downregulated in DN4 and DP cells and again expressed more highly in SP cells. Mouse mutants defective for various players of the Hh signalling cascade showed differentiation of thymocyte precursors at the DN1 to DN2 transition to be dependent on Gli2 and Gli3 and Gli2 was identified as a negative regulator of pre-TCR induced differentiation of DN3 to DP cells. Hh signals also influence positive and negative selection of DP cells but the relative contributions of Gli2 and Gli1 in TCR repertoire selection are still unknown.<sup>5-7</sup>

In a previous issue of *Cell Cycle* Drakopoulou et al. analyzed thymocyte differentiation in foetal and adult Gli1<sup>-/-</sup> thymus. Gli1 was found to be required for normal differentiation of DN3 cells prior to pre-TCR signalling and to negatively regulate DN to DP transition as the proportion and cell number of DP cells was increased in foetal Gli1<sup>-/-</sup> thymus. In the adult thymus, loss of Gli1 had no significant impact on the proportion of thymocyte subpopulations. However, on a TCR transgenic (tg) background, using the HY-TCR tg system which allows to monitor positive selection of DP cells into the CD8 lineage in female mice and negative selection in male mice, positive selection

of HY-TCR+ thymocytes was increased in Gli1<sup>-/-</sup> thymus. Loss of Gli1 had no effect on negative selection. Thus, although Gli1<sup>-/-</sup> mice show normal development the authors have uncovered a non-redundant function of Gli1 during several steps of thymocyte differentiation with Gli1 acting as positive regulator at the DN3 stage and as negative regulator at DN3 to DP transition and during positive selection of CD8 SP cells. Since positive selection in Gli2<sup>-/-</sup> HY-TCR tg thymus was also enhanced subtle changes in either Gli1 or Gli2 activity might have strong impact on TCR repertoire selection. Indeed, based on these stimulating results further studies on single and double mutant thymocytes in different selection systems and a closer look at the T-cell repertoire might reveal whether Gli1 and Gli2 act cooperatively or have independent targets. In view that Hh-Gli signalling also regulates peripheral T-cell function<sup>8</sup> and is involved in tumorigenesis<sup>9</sup> it will now be a formidable challenge to unravel the regulation of upstream regulators of Gli proteins and the Gli1/Gli2 transcriptome involved in control of proper T-cell generation.

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## Dissecting the functions of Gli transcription factors in T-cell development

Comment on: Drakopoulou E, et al. *Cell Cycle* 2010; 9:4144–52.

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The members of the Gli family of zinc finger transcription factors, Gli1, Gli2 and Gli3, are the primary mediators of the signaling pathway initiated by the secreted Hedgehog (Hh) proteins.

The Hh pathway is crucial for normal embryonic development and homeostasis of adult tissues, since can regulate cell survival and differentiation as well as cell cycle progression.<sup>1</sup>

In the murine and human thymus, Hh signaling is involved in multiple stages of T-cell development, and Gli proteins are differentially expressed in thymocyte subpopulations.<sup>2,3</sup>

Postnatal human CD34<sup>+</sup> intrathymic precursors express the three Gli transcription factors, and CD4-CD8<sup>+</sup> single positive (SP) thymocytes express mainly Gli1 and Gli3. In contrast, minimal levels of Gli transcription factors are detected in human CD4<sup>+</sup>CD8<sup>+</sup> double positive (DP) and CD4<sup>+</sup>CD8<sup>-</sup> SP thymocytes.<sup>4</sup> In mice, Gli3 is detected in fetal, but not adult, CD4-CD8<sup>-</sup> double negative (DN) thymocytes, with expression in the CD44<sup>+</sup>CD25<sup>-</sup> DN1 subpopulation, down-regulation in the subsequent CD44<sup>+</sup>CD25<sup>+</sup> DN2 and CD44<sup>+</sup>CD25<sup>+</sup> DN3 stages, and highest expression in the CD44<sup>+</sup>CD25<sup>-</sup> DN4 subpopulation.<sup>5</sup> The other two Gli proteins, Gli1 and Gli2, are expressed both in fetal and adult thymocyte subpopulations. Gli2 is most highly expressed in DN1 and DN2 cells, downregulated at the DN3 stage, and upregulated again in the DN4 subpopulation. Gli1 expression is highest in the DN2 and DN3 subpopulations and down-regulated in DN4 cells. The expression levels of the three Gli transcription factors in DP thymocytes are very low, and Gli1 levels are further up-regulated in SP thymocytes.<sup>6-8</sup> These expression data correlate well with the analysis of mutant mice which show that Gli2 and Gli3 act as transcriptional activators in response to Hh

signaling at the DN1-DN2 transition.<sup>5,6</sup> Gli2 and Gli3 would play redundant roles in the control of differentiation, proliferation, and probably also survival of thymocyte precursors in those developmental stages. In contrast, Gli2 and Gli3 function as a transcriptional activator and repressor, respectively, at the transition from DN4 to DP thymocytes, where Hh signaling seems to exert a negative control.<sup>5,6</sup> At later stages of thymocyte development, Gli2 partially mediates the effects of Hh signaling in TCR repertoire selection and the transition from DP to SP cells.<sup>8</sup>

The contribution of Gli1, which can only act as a transcriptional activator of Hh signaling, to thymocyte development has remained unexplored. The fact that Gli1, unlike Gli2 and Gli3, is not essential for mouse development and that adult Gli1 knockout mice do not exhibit any specific phenotype caused by Gli1 deficiency seem to indicate that the transcriptional activator function of Gli2 often replaces Gli1, and has led to the assumption that Gli1 and Gli2 have extensive overlapping functions.<sup>9</sup> However, in a previous of *Cell Cycle*, Drakopoulou et al. analyze Gli1<sup>-/-</sup> fetal and adult thymus and show that Gli1 is non-redundantly required at multiple stages of T-cell

development: Gli1 is necessary for normal differentiation of DN3 thymocyte precursors, independently of pre-TCR signaling; Gli1 cooperates with Gli2 to carry out the physiological negative regulation of differentiation from DN to DP cells; and Gli1 also participates, along with Gli2, in TCR repertoire selection at the transition from DP to CD8<sup>+</sup> SP thymocytes.<sup>10</sup> Therefore, these data demonstrate that the thymus is one of very few tissues in which Gli1 has non-redundant functions.

In the future, it will be interesting to study the cross-talk that take place in the thymus between Hh signaling and other morphogens, as has already been pointed out in human intrathymic precursors.<sup>11</sup>

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## Small RFC subunits make a big difference

Comment on: Maradeo ME, et al. *Cell Cycle* 2010; 9:4370-8.

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Sliding clamps are important conserved proteins with roles in DNA replication and repair (reviewed in refs. 1 and 2). These ring-shaped proteins encircle the DNA and can slide along the duplex. Sliding clamps appear to be a major landing pad for DNA replication and repair proteins and function to tether proteins to the DNA, thereby increasing the efficiency of or coordinating the recruitment of enzymes in these processes. Studies in eukaryotes have identified two clamps, the homotrimer PCNA and the heterotrimeric 9-1-1 complex (Rad1, Hus1, Rad9 in humans; Rad17, Mec3 and Ddc1 in budding yeast). Subsequent bioinformatic approaches have not revealed any additional clamp-like molecules in yeast.<sup>3</sup> Structurally, these ring-shaped clamps are formed by non-covalent, head-to-tail interactions of three subunits, which form a closed ring around duplex DNA.<sup>2</sup>

Clamps must be actively loaded onto and removed from duplex DNA. This is accomplished by the action of conserved AAA+ clamp loaders.<sup>1,2</sup> The binding of these loading complexes to clamps opens the clamp ring and the hydrolysis of ATP releases the clamp, closing the ring around the DNA. The reversal of this process allows clamp removal. Eukaryotic heteropentamer clamp loaders consist of four small core subunits (Rfc2-5) and one large subunit: Rfc1, Ctf18, Rad24 (hRad17), or Elg1.<sup>1,2</sup> Each of these four pentameric clamp loaders has unique function in DNA metabolism, but notably all function in some aspect of DNA repair. The Rfc1-complex forms the canonical clamp loader that loads PCNA on primed DNA during DNA replication and repair. The Rad24-complex plays a part in DNA damage checkpoint response, loading the Rad17/Mec3/Ddc1 clamp.<sup>4</sup> The

Ctf18-complex also assembles with Ctf8 and Dcc1 (through an interaction with Ctf18) and functions in sister chromatid cohesion to load or unload PCNA.<sup>5</sup> Recent evidence suggests that the Elg1-complex seemingly helps maintain genome stability by functioning in sister chromatid pairing<sup>6,7</sup> and unloading ubiquitinated PCNA following bypass repair.<sup>8</sup>

Since the structure of each clamp loader differs only in the identity of the fifth subunit, it has long been assumed that the specificity and function of each clamp loader was determined solely by the large subunit. Work presented in this issue by Maradeo et al. challenges this idea by illustrating that mutations in the core Rfc2-5 subunit can also alter specificity. Specifically, they show that mutations within Rfc5 (rfc5-1) mimic some, but not all sister chromatid defect phenotypes in elg1. This work implies that a more sophisticated

signaling pathway likely helps in the recruitment of specific clamp loaders for specific DNA repair and replication functions. Although these results are specific for Rfc5 and sister chromatid cohesion, it is likely that the specificity imparted by Rfc5 extends to other DNA metabolism functions and the other small RFC subunits. It is interesting to note that Rfc5 is the only complex member without a functional ATPase activity.<sup>9</sup> This may suggest a more structural or regulatory role for Rfc5 compared to the other small subunits and thus it is possible that Rfc5 uniquely affects complex specificity.

Despite this caveat, several models can be envisioned to account for the specificity imparted by the small RFC core subunits. First, the small subunits may provide docking sites for the cooperative binding of other cofactors to the large subunit. These cofactors could in

turn facilitate the function and specificity of the holocomplex. A second scenario suggests the small subunits directly act as signal sensors or transducers in alternative contexts. Finally, the small RFC subunits may also respond to alternative environmental conditions and differentially regulate the assembly of the large subunit.

Collectively, the results presented by Maradero et al. reveal a complexity of clamp loader regulation that was not previously appreciated. Moving forward, it will be interesting to determine if rfc5-1 is functionally impaired as a signal transducer of the Elg1-RFC pathway due to the production of an alternatively modified protein (e.g. non-phosphorylatable) or if it is defective in a specific protein-protein interaction. Additionally, further studies should focus on the identification and characterization of other small subunit alleles with specific clamp loader defects.

This information will be crucial for a clearer understanding of the factors facilitating global genomic stability.

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## MicroRNAs are invading the tumor microenvironment: Fibroblast microRNAs regulate tumor cell motility and invasiveness

Comment on: Aprelikova O, et al. *Cell Cycle* 2010; 9:4387-98.

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Malignant tumor cells exist in a constantly evolving tissue microenvironment comprising fibroblasts, immune cells, smooth muscle cells, pericytes, adipocytes, the blood and lymphatic vascular networks, and the extracellular matrix.<sup>1</sup> The bidirectional and dynamic interactions between tumor cells and stromal elements, by either cell-cell contacts or soluble mediators, control tumor formation, progression, spreading and response to therapy.

Fibroblasts associated with reactive tumor stroma (so-called cancer-associated fibroblasts, CAFs) or myofibroblasts, often represent the majority of the stromal cells within human carcinomas and participate at all stages of tumor growth and progression. Animal and human studies demonstrated that CAFs, but not normal fibroblasts, promote tumor proliferation, growth and invasiveness by secreting MMPs, cytokines, and growth factors. Although the origin of CAFs, their activation, function, and interaction with tumor cells have been characterized,<sup>2</sup> the molecular mechanisms contributing to CAF-mediated tumor spreading and invasiveness are currently not well understood. Genome analysis of tumor stroma indicates that the hot spots

for mutations in the stroma are not the same as those identified in the epithelium,<sup>3</sup> suggesting that an independent pathway of mutation or gene expression might work in stromal cells. However, genetic alterations are rare in CAFs,<sup>4</sup> signifying that alterations in gene expression in CAFs may result from epigenetic mechanisms pre-existing in precursors or induced in fibroblasts by tumor cells, inflammation or specific tumor tissue conditions.

As pleiotropic modulators of gene expression, microRNAs (miRNAs) are essential for normal development as well as development of many diseases, including cancer.<sup>5</sup> MiRNAs repress expression of targeting genes by inhibiting their translation or inducing mRNA degradation. Over the last several years, roles for miRNAs during all stages of cancer progression have been established, including cancer associated polymorphisms in miRNA target sites or miRNAs themselves, miRNAs as tumor suppressors or oncogenes, and miRNA-regulated metastatic spread.<sup>6</sup> However, the role of miRNAs in tumor stromal elements and their association with tumor progression have not yet been investigated. In a previous issue, Aprelikova et al. explored the miRNA signature

of human CAFs and revealed the unique role of CAF miRNAs in regulating tumor cell motility.<sup>7</sup>

Analyzing miRNA and mRNA in CAFs derived from endometrial cancer versus paired normal endometrial fibroblasts, Aprelikova et al. identified differential expression of 11 miRNAs, with miR-31 being the most suppressed in CAFs.<sup>7</sup> Overexpression of miR-31 impaired the ability of CAFs to stimulate tumor cell migration and invasion. The authors proved that miR-31 directly targeted the homeobox gene SATB2, which encodes a nuclear matrix-attachment protein responsible for chromatin remodeling and regulation of gene expression. SATB2 was significantly elevated in CAFs and increased tumor cell migration and invasion, while knock-down of endogenous SATB2 in CAFs reversed this phenotype.<sup>7</sup> Although the mechanism of miR-31 down-regulation in CAFs has not been addressed, presented findings provide new insights into tumor-stroma interaction and document that miR-31 and its target gene SATB2 are involved in the regulation of tumor cell motility.

These new results also suggest that miRNAs have now become important therapeutic



targets not only in tumor cells, but surrounding stromal elements. In addition to potential practical utilization, the results of Aprelikova et al. publication open new opportunities for future analysis of CAF functioning in the tumor milieu. It will be important to determine which factors are responsible for altered miRNA levels in CAFs and if they are originated from tumor cells or host inflammatory cells. It will be crucial to know whether miRNA misbalance in CAFs is tumor specific, similar in primary and metastatic tumors, and could be

regulated by chemotherapy, anti-inflammatory drugs or immunomodulators. Do miRNA alterations in CAFs stable and long-living or evolve with tumor progression? Do they have any prognostic potential or can be used for diagnostic purposes? The finding that SATB2 is responsible for CAF-mediated tumor cell invasiveness is of special interest since SATB2 can be considered as a new stromal target for therapeutic interventions. However, genes that are regulated by SATB2 in fibroblasts and corroborate the increase in tumor cell motility

should be identified before the anti-SATB2 therapeutic modalities can be considered for experimental testing.

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## Finding markers for cancer stem cells in renal cell carcinoma: Looking beyond CD133

Comment on: D'Alterio C, et al. *Cell Cycle* 2010; 9:4492–4500.

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Since the end of the 1960s, the identification of cancer cells with stem cells characteristics (cancer stem cells, CSCs) in hematologic malignancies has prompted intensive research in oncology in order to identify and characterize them in many different solid tumors.<sup>1,2</sup> CSCs represent a small proportion of tumor cells and possess morphologic markers (i.e. CD133) and functional properties (i.e. "self renewal" and migration) associated with normal stem cells. Many in vitro and in vivo studies demonstrated that chemotherapy is ineffective against CSCs which are responsible for cancer relapses.<sup>3,4</sup> Thus, this intriguing theory could have pragmatic and dramatic applications in oncology.

D'Alterio et al. (in this issue) described the expression of CXCR4 (CXC-chemokine Receptor 4) and CD133 in cancer cell lines and surgical biopsies and their prognostic role in a large series of paraffin-embedded renal cell carcinomas (RCC). CXCR4 and its ligand, SDF1 chemokine (Stromal Derived Factor 1), stimulate chemotaxis/invasion, survival and cell proliferation of different human tumors, including RCC. The concomitant expression of CXCR4 and CD133 has already been reported in normal and cancer stem cells and it could confer an aggressive phenotype resistant to conventional therapies.<sup>5</sup> Interestingly, CXCR4

was significantly related with biologic features (stage, furhman grade, clinical presentation) and with disease-free survival ( $p=0.0199$ , HR: 3.40, CI: 1.11-10.38). In addition CXCR4 was functional in RCC cell lines as demonstrated in migration assays toward SDF1 and the migration was specifically inhibited by AMD3100, a CXCR4 inhibitor. The study adds new data on the role of CXCR4 in RCC suggesting that CXCR4 antagonism might be a promising therapeutic option. However, the authors did not find any correlation between CD133 or CXC4/CD133 concomitant expression and clinico-pathologic features or prognosis in RCC. Thus they make an important contribution to the study of CSCs in RCC concluding that CXCR4-CD133 does not fully characterize renal cancer initiating cells. This is consistent with recent data by Bruno et al. showing that renal tumor-derived progenitor cells were not tumorigenic in vivo but rather supported angiogenesis and tumor growth in the presence of tumor cells.<sup>6</sup>

The molecular and cellular heterogeneity of cancer cells do not fit completely with a static model of CSCs. The parallelism with recent research in melanoma can help the comprehension of this concept. In fact, at the molecular level, four subtypes of functional melanoma

stem cells have been described: CD20-positive cells, CD133-positive cells, side population cells excluding dye using ABC transporters, and slow-cycling cells.<sup>7</sup> Thus, traditional stem cell markers (i.e. CD133) may not necessarily identify CSCs. Furthermore, recent data have shown that some CSCs may actually not be static and well defined entities, but rather tumor cells that transiently acquire stemness properties depending on the tumor context.<sup>8</sup> This supports a model of dynamic stemness. Another study by Mani et al. suggested epithelial-mesenchymal transition (EMT) as an alternative stemness-associated mechanism.<sup>9</sup>

The identification of CSC markers in RCC remains a high priority to develop new therapeutic strategies and to advance the comprehension of RCC biology.

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## p53, transcriptional repression and drug sensitivity: Fresh perspectives on an old activity

Comment on: McKenzie L, et al. *Cell Cycle* 2010; 9:4200–12.

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Upon exposure to multiple different types of cellular stress, including DNA damage, hypoxia, and oncogene activation, the p53 tumor suppressor protein becomes activated as a transcription factor. It then functions as either a transcriptional activator or a transcriptional repressor of gene expression in order to induce apoptosis, growth arrest, or senescence. To transactivate gene expression p53 binds in the upstream regulatory sequences of a gene to a consensus DNA binding motif of two repeats of the 10 base pair element 5'-PuPuPuC(A/T)(T/A)GpyPyPy-3', where Pu is a purine residue and Py is pyrimidine; these repeats can be separated by 0-13 nucleotides.<sup>1</sup>

That p53 also functions as a transcriptional repressor has been known for close to 20 years.<sup>2,3</sup> However, because many powerful transcription factors can commandeer components of the basal transcriptional machinery (so-called transcriptional 'squenching'), they also possess potent non-specific transcriptional repression activity. This makes it notoriously difficult to map necessary and sufficient p53 binding sites on repressed promoters. An added complication is the fact that p53 induces cell cycle arrest and apoptosis, making it difficult to separate these outcomes from transcriptional repression.

In the present study by Meek and colleagues, the authors perform a meticulous study documenting the sequence-specific transcriptional repression by p53 of the gene encoding Polo-like kinase 1 (PLK1).<sup>4</sup> PLK1 is a member of a family of serine-threonine kinases that control mitotic progression and the DNA damage-induced G<sub>2</sub>/M checkpoint. Because PLK1 belongs to a class of genes that are

repressed in the G<sub>1</sub> phase of the cell cycle by virtue of an element in their promoters termed a CDE/CHR (cell cycle dependent element/cell cycle genes homology region), the authors use several means to eliminate p53's ability to cell cycle arrest from its ability to repress PLK1. Notable among these is their finding that p53 induction can still repress PLK1 in cells in which the p21 gene, a critical mediator of p53-mediated cell cycle arrest, is silenced. The authors then use chromatin immunoprecipitation to identify the sites for p53 binding, and they map these to two canonical p53 consensus elements located approximately 800, and 200, nucleotides upstream of the transcription start site.

The study by Meek and colleagues echoes some findings reported previously for p53-mediated repression of the Cdc25c gene.<sup>5</sup> Like PLK1, Cdc25c is a critical mitotic checkpoint gene that possesses a CDE/CHR element in its promoter. Like Cdc25c, p53 binds to a consensus element in the PLK1 promoter that resembles a canonical p53 response element—two copies of the 10 base pair element 5'-PuPuPuC(A/T)(T/A)GpyPyPy-3'. How p53 represses transcription from this element remains a burgeoning question in the field. One answer may come from the studies of Meek and Manfredi; in both cases the authors discovered that the p53 consensus element overlapped with a binding site for another transcription factor: one that played a major role in the activated expression of these genes.<sup>4,5</sup> Therefore, p53 may repress the transcription of these genes by interfering with the activity of another factor.

The future of p53, repression and the G<sub>2</sub>/M checkpoint seems clear. Which other genes

that function in the G<sub>2</sub>/M checkpoint and contain p53 consensus binding sites belong to this 'class' of genes? One intriguing possibility is Aurora Kinase, which like Cdc25c and PLK1 contains a CDE/CHR element, and is expressed at abnormally high levels in cells with inactive p53.<sup>6</sup> Another question relates to the differences between p53 activating elements and p53 repressing elements; are they identical or are there subtle differences that we are unaware of? Computational analyses suggest that p53 activating elements typically have spacers of 0-1 nucleotides between the dimer binding sites, while repressed genes more often have spacers of five nucleotides or greater;<sup>1</sup> this might be predicted to place p53 dimers on different faces of the DNA helix, and thereby alter their protein-protein interaction partners. Finally, the clinical relevance of this line of research is important. Inhibitors of Aurora kinase, as well as PLK1, are actively in use in clinical trials for cancer. These genes are typically repressed in non-cycling (normal) cells, and overexpressed in tumor cells with mutant p53; therefore, identifying other G<sub>2</sub>/M kinases that might be repressed by p53, and using cocktails of such inhibitors of such on tumors with mutant p53, is likely to represent a productive area for cancer research.

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