Running m(o)TOR with the brakes on leads to catastrophe at mitosis

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Zbigniew Darzynkiewicz; Brander Cancer Research Institute and Department of Pathology; New York Medical College; Valhalla, NY USA;
Email: darzynk@nymc.edu; http://dx.doi.org/10.4161/cc.22932

Two parallel and concordant activities characterize cell cycle progression. One is associated with preparation for and execution of DNA replication followed by mitosis (“cell cycle”). Cyclins are the motors of the cell cycle, as they activate the respective CDKs and thereby drive the cell through the sequential phases of the cycle. Their antagonists are CKIs, which inhibit the CDKs stalling the cycle progression. The second type of activities involves anabolic processes that contribute to growth in cell size and mass (“cellular growth”). Constitutive signaling along the mTOR/S6K pathways is the key factor mediating these anabolic processes.1 During unperturbed and balanced growth these two activities are flawlessly coordinated. This synchronization ensures that the cell size, as well the ratio of protein or RNA content to DNA, remains invariant for cells in particular phases of the cycle or for particular cell type. However, during arrest in cell cycle progression, for example, when induced by inhibitors of DNA replication, these activities become uncoupled. The cell growth continues, resulting in an “unbalanced growth” phenotype when the ratio of cell protein/mass to DNA content is greatly augmented. While this phenomenon was initially observed nearly five decades ago,2 recent evidence underscores its importance and links it mechanistically with senescence and aging.3,5 Specifically, it has been postulated that cell cycle arrest when is concurrent with the ongoing or intensified mTOR/S6K signaling (growth cycle) results in induction of the unbalanced growth phenotype (cell hypertrophy), which is a characteristic feature of cell senescence as well as considered to be the primary cause of organismal aging.3,5

In the currently published article, Leontieva et al.6 describe that constitutive mTOR signaling during the cell cycle arrest, induced by upregulation of p21, contributed to cell senescence (geroconversion); these cells were characterized by greatly increased levels of cyclin D1 and cyclin E as well as being under replication stress, manifested by markers of DNA damage signaling. When the cycle progression was restored by downregulation of p21, the cells were able to pass through S and G2, and decrease the level of cyclins D1 and E, but then they underwent either mitotic catastrophe or entered higher DNA ploidy by endoreduplication. Suppression of mTOR signaling, either by rapamycin or by nutlin 3a in the cells arrested by p21, prevented geroconversion, lowered the level of cyclin D1 expression and, after removal of p21, restored ability to proliferate. In another model cell system, in which nutlin 3a was unable to suppress mTOR signaling but was inducing arrest in G1 and the senescent phenotype, removal of nutlin 3a led to initiation of DNA replication but could not restore capability to proliferate.

The findings presented by Leontieva et al.6 underscore the role of mTOR signaling during cell cycle arrest in the induction of either cell senescence or quiescence and restoration of replicative potential. Of particular interest is the observation of massive upregulation of cyclins D1 and E, which, upon restoration of the cycle progression, appeared to initially enhance DNA replication rate (providing the “hyper-mitogenic drive”), but then likely to contribute to the mitotic catastrophe. Clearly, progression of hypertrophic cells additionally accelerated during S appears to be catastrophic when passing later through mitosis. Highly unbalanced ("unscheduled") expression of not only cyclins D and E but also cyclins A and B1 was previously observed in cells arrested and synchronized at the G1/S boundary by the inhibitors of DNA replication aphidicolin, mimosine or excess of thymidine.7 These cells, in which the chromosome cycle and growth cycle were also dissociated, resulting in their hypertrophy, when released from the arrest and progressing through S phase, had several-fold higher levels of all the cyclins (D, E, A and B1) compared with S-phase cells in untreated cultures. The elevated level of cyclin A was likely reporting the replication stress, while the elevated level of cyclin B1 was considered to be due to the increased half-life of this protein stabilized by overexpression of cyclin E.7 Interestingly, following successful mitosis and cytokinesis, the immediate G0 progeny of these synchronized cells, while they had normal levels of the respective cyclins, still showed some degree of imbalance, characterized by > 30% higher protein to DNA ratio compared with G1 cells from exponentially growing cultures, and had proliferative capacity.7 These findings collectively with observations of Leontieva et al.6 suggest that there is a threshold level of the growth imbalance (cell hypertrophy) defining the “point of no return.” The cells that pass the threshold are losing the reproductive potential either through mitotic catastrophe or endoreduplication. The cells below this threshold attempt to return to balanced growth either through accelerated rate of cell cycle progression vis-à-vis growth cycle,8 by autophagy9 or by both mechanisms and preserve their replicative potential. The intensity of mTOR signaling combined with duration of the cell cycle arrest plays a critical role as to whether the cells do pass this point or not.

The possibility of decoupling “cell cycle” from the “growth cycle” to induce cell hypertrophy and irreversible senescence suggests an interesting anticancer strategy. The activation of many oncogenes and/or dysfunction of tumor suppressor genes, either one leading downstream to an increased mTOR signaling, is a hallmark of most cancers. Cells of such tumors, therefore, would be more predisposed to undergo senescence under conditions of persistent chromosome cycle arrest that induces DNA replication stress compared with normal cells. We have recently reported that prolonged treatment of the non-small cell lung carcinoma cells, characterized by strong mTOR signaling, with very low doses of DNA alkylating drug mitomycin C, induced cell cycle arrest, replication stress and, similarly, as in the case of p21-induced arrest,10 endoreduplication.
This led to their senescence and irreversible loss of reproductive capability.10 The induction of cell senescence by the low doses of DNA damaging drugs or other means of arresting the cell cycle rather than induction of apoptosis by high drug doses, typical of standard chemotherapy, may be therefore more effective in treatment of cancers characterized by high level of mTOR signaling, with less side effects for the patient. Screening tumors for the presence of activation of such oncogenes may select patients sensitive for this type of chemotherapy, providing a personalized cancer treatment approach.

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Faithful transmission and maintenance of the integrity of genetic information in the cell is controlled by a set of coordinated processes that involve chromatin remodeling, cell cycle checkpoint control, DNA replication, recombination and repair. A defect in any of these tightly regulated processes would result in gross chromosomal rearrangements, such as chromosome deletion, insertion, duplication, translocation and loss and lead to tumorigenesis or cell death.1 In eukaryotic cells, DNA is packaged into a highly compacted inaccessible structure called the chromatin by both histones and nonhistone proteins.2,3 Cells rely on post-translational histone modifications and ATP-dependent chromatin remodeling machines to gain access to, and perform various functions on, DNA. Histones can be reversibly modified in several ways, including methylation, ubiquitylation, acetylation and phosphorylation. Histone acetylation is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs are a diverse set of enzymes that are evolutionarily conserved from yeast to humans and are usually subunits of large co-activator complexes,2 namely the human TBP-free TAF complex (TFTC), SPT3/TAF9/GCN5 complex (STAGA) (human homolog of yeast SAGA complex) and the activation 2a (Ada2a)-containing (ATAC) complex. These co-activator complexes function in many cellular processes, including transcription, cell cycle progression and DNA replication and repair.2,3

Human Ada3 is an essential component of the ATAC complex, which is composed of ADA2, ADA3 and GCN5.2 Ada3 protein associates with and regulates transcriptional activity of estrogen receptor4 and retinoic acid receptor.5 Ada3 also binds and stabilizes the tumor suppressor p53 and is required for p53 acetylation by p300.6 Although several cellular functions of Ada3 as part of multiple HAT complexes have been identified, the in vivo physiological roles of mammalian Ada3 were unclear, until a recent study by the Band Laboratory that demonstrated an essential role of mammalian Ada3 in embryonic development as well as cell cycle progression.7 In a recent issue of Cell Cycle, Mirza et al.8 discovered another interesting function of Ada3: it participates in DNA repair and in the maintenance of genomic stability. Using adenovirus-Cre-mediated Ada3 deletion in Ada3fl/fl mouse embryonic fibroblasts, Mirza et al. showed that Ada3 depletion is accompanied by increased levels of pATM, pYH2AX, p53BP1 and PRADS1, even before ionizing radiation, indicating that Ada3 deficiency results in increased DNA damage in the cell.8 Ada3 does not appear to be required for DNA damage sensing or checkpoint activation; however, a delay in DNA repair was observed in Ada3−/− cells.8 Ada3 deficiency results in increased DNA damage and genome instability8 observed in Ada3−/− cells. Since CtIP is known to act in both cell cycle checkpoint and DNA repair,9 this delay in the disappearance of CtIP foci may explain both the checkpoint defects7 and DNA repair deficiency8 observed in Ada3−/− cells.

The findings of Mirza et al. not only establish a novel function of Ada3 in DNA repair and genome maintenance, but also open up a new direction for the studies of HAT complexes in DNA damage response. It would be interesting to further decipher the precise molecular mechanism underlying the functions of Ada3 and its associated HAT complexes in cell cycle checkpoint control, DNA repair and the maintenance of genome stability.

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Alteration/deficiency in activation 3 (ADA3): Regulator of DNA repair and genome stability

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Gargi Ghosal and Junjie Chen*; Department of Experimental Radiation Oncology; The University of Texas MD Anderson Cancer Center; Houston, TX USA; *Email: jchen8@mdanderson.org; http://dx.doi.org/10.4161/cc.22934
Micromanaging a large tumor suppressor

Comment on: Fang L, et al. Cell Cycle 2012; 11:4352–65; PMID:23111389; http://dx.doi.org/10.4161/cc.22670
Yanmei Li1 and David E. Spaner1,2,*; 1Division of Molecular and Cellular Biology; Sunnybrook Research Institute; Toronto, ON Canada; 2Department of Medical Biophysics; University of Toronto; Toronto, ON Canada; *Email: spanerd@sri.utoronto.ca; http://dx.doi.org/10.4161/cc.22935

Studies of microRNA (miRNA) in cancer are relatively recent but have exciting possibilities to improve our understanding and treatment of this disease.1 Over 1,000 miRNAs (1–4% of the human genome) exist in non-coding genes or gene clusters or in introns of coding genes. Primary miRNA transcripts are cleaved into stem-loop structures in the nucleus and trimmed into 19–24 nucleotide duplexes in the cytoplasm. One strand of a duplex is incorporated into an RNA-induced silencing complex (RISC), where it binds complementary sequences in 3'-untranslated regions (UTRs) and decreases protein expression by inhibiting translation or causing mRNA degradation. Each miRNA can potentially bind 200 mRNAs and regulate multiple genetic pathways.

Ablation of microRNAs (miRNAs) in cancer is overexpressed in lymphomas and lung cancers and may act like an oncogene.2 In contrast, let-7 family members, miR-15 and miR-16 are downregulated in a number of cancers and may act as tumor suppressors by decreasing the expression of oncogenes or anti-apoptotic proteins such as Ras or Bcl-2.1,3 While members of an miRNA cluster are often expressed coordinately, they may not have identical functions. For example, miR-17 acts like a brake on miR-92a, which appears to be the most potent oncogene of the miR-17-92 cluster.2

In this issue of Cell Cycle, Burton Yang’s laboratory identifies a new role for miR-93 in breast cancer.4 MiR-93 is part of the miR-106b-25 cluster on chromosome 7 and was found to be overexpressed in human primary breast cancer cells compared with normal breast tissue. Overexpression of miR-93 in MCF1 human breast cancer cells caused them to survive better in serum-free conditions and increased their invasive properties and interactions with endothelial cell lines in vitro. These properties were reflected in increased metastatic capability and angiogenesis in immunodeficient mice. To explain these observations, in silico studies implicated the tumor suppressor large tumor suppressor 2 (LATS2) as a miR-93 target. LATS2 is a serine-threonine protein kinase that is activated by mitotic stress, DNA damage and oncogenes and regulates a number of cell processes, including apoptosis and motility.5 LATS2 is downregulated in a number of cancers, including breast cancer, through epigenetic mechanisms involving promoter methylation. However, LATS2 has also been shown to be downregulated by miR-372, miR-373, miR-31 and miR-195 in a number of cancer cell lines.5,6 Fang et al. found a reciprocal relationship between LATS2 protein expression and miR-93 in breast cancer lines and primary cells. Expression of a luciferase construct harboring the target site in the LATS2 3'-UTR was decreased by miR-93, but not when this region was mutated to prevent binding of miR-93. Treatment of MT-1 cells with siRNA to LATS2 conferred the same phenotype as miR-93, decreasing by miR-93, but not when this region ing the target site in the LATS2 3'-UTR was decreased by miR-93, but not when this region was mutated to prevent binding of miR-93. Treatment of MT-1 cells with siRNA to LATS2 conferred the same phenotype as miR-93, while ectopic expression of a LATS2 gene lacking the 3'-UTR reversed the effects of miR-93. Taken together, these results establish miR-93 as an oncogene that downregulates LATS2 and promotes malignant behavior of breast cancer cells.

This study is an important contribution to our understanding of breast cancer. However, as with any good research, the findings raise additional questions. Why is miR-93 upregulated? Is there a cytogenetic basis for this finding? Interestingly, previous reports documented loss of heterozygosity at this locus rather than amplification.7 Alternatively, is there a constitutively activated signaling pathway that increases miR-93 expression? Is miR-93 associated with specific breast cancer types?8 It is interesting that so many different miRNAs appear to regulate LATS2. This observation probably attests to the biological importance of this molecule, but is LATS2 the only pathogenic protein that is regulated by miR-93 in breast cancer? Certainly, increased interactions with endothelial cells suggest that cytokines and adhesion molecules are likely also increased by miR-93 and may be independent of LATS2.

What is the role of the other members of the miR-106b-25 cluster? Are miR-106b and miR-25 also oncogenes, or do they function to balance the oncogenic properties of miR-93, as described for the paralogous miR-17–92 cluster?2 Intriguing therapeutic possibilities arise from the finding that miR-93 is overexpressed in breast cancer. In the future, the results of Fang and coworkers may lead to more effective breast cancer treatments that use agents such as anti-sense oligonucleotides1 to block miR-93.

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Mesenchymal traits and cancer stem cells in mammospheres: Chicken or egg?

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Mohammad Kohandel; Department of Applied Mathematics; University of Waterloo; Waterloo, ON Canada and Centre for Mathematical Medicine; Fields Institute; Toronto, ON Canada; Email: kohandel@uwaterloo.ca; http://dx.doi.org/10.4161/cc.22936

The cancer stem cell (CSC) hypothesis states that tumors are initiated and maintained by a subset of cancer cells with the ability to self-renew and to differentiate into cells of various lineages. Putative CSCs have been identified in a variety of human malignancies including breast cancer.1 Breast CSCs expressing the cell surface markers CD44high/CD24low were able to generate tumors when transplanted in small numbers in immunocompromised NOD/SCID mice.1 In addition to the cell sorting method, breast CSCs have been identified in vitro by their ability to grow as mammospheres (MS) in non-adherent, serum-free conditions.2 MS obtained in these pro-stem culture conditions have been shown to be enriched in cells expressing CD44high/CD24low markers.3 Beside stem-like features, the epithelial-mesenchymal transition (eMT) appears to play a critical role in breast cancer progression. eMT is a process wherein epithelial cells undergo multiple changes that enable them to adopt mesenchymal features, including enhanced capacity for migration, invasiveness and elevated resistance to apoptosis.4 eMT programs are orchestrated by a set of pleiotropic transcription factors, including Slug, Snail, Twist and Zeb1, which can directly repress levels of mRNA for many proteins and transcription factors, including Slug, Snail, Twist and Zeb1. Moreover, Borgna et al.4 have also observed a global decrement in the expression of EMT transcription factors Zeb1, Zeb2, Snail2 and Twist 1. Overall, their data provide evidence for a shift toward a mesenchymal phenotype, along with stemness features, under proficient culture conditions. This supports the use of MS as an in vitro model for the study of therapeutic approaches targeting mesenchymal phenotypes.

The enrichment in mesenchymal features under MS-proficient conditions may be due to an increase in the proportion of a pre-existing subpopulation of cells with mesenchymal phenotype, or by de novo induction of EMT. Interestingly, Borgna et al.4 have observed the gain of mesenchymal features in short-term MS in MCF7 cells (a luminal cell line) lacking enrichment in the CD44high/CD24low fraction, suggesting that the gain of mesenchymal phenotype may precede the acquisition of stem-like features. This is an important observation that can be further validated by a functional assessment of MS under different culture conditions, as cells acquire an EMT and then a stem cell profile. We have recently developed a quantitative approach5 to validate different possible scenarios that can lead to the enrichment of stem cell activity following induction of EMT.6 Additionally, we have suggested the utility of comparing mammosphere data to computational mammosphere simulations in elucidating the growth characteristics of mammary CSCs. I anticipate that this “modus operandi” of using quantitative modeling (grounded in experimental data) to gain new insights may well provide a rational means for predicting the timeline of the acquisition of mesenchymal and stem features. The process of EMT and its relationship to CSCs are novel and rapidly converging directions of research, and many related questions may be considered. For instance, it would be interesting to use the suggested MS culture conditions to develop and validate novel and effective therapeutic strategies, followed by in vivo validations. Moreover, in vitro MS studies under tumor microenvironmental conditions, such as hypoxia, may provide further information, which can also be validated by in vivo experiments.

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