Cell Cycle 11:23, 4299–4304; December 1, 2012; © 2012 Landes Bioscience

Cell Cycle News & Views

More tasks for Dna2 in S-phase

Comment on: Karanja KK, et al. Cell Cycle 2012; 11:3983–96; PMID:22987153; http://dx.doi.org/10.4161/cc.22215

Tomás Aparicio[1] and Jean Gautier[1,2,*]; [1]Institute for Cancer Genetics; Columbia University Medical Center; New York, NY USA; [2]Department of Genetics and Development; Columbia University Medical Center; New York, NY USA; *Email: peter.zhou@uky.edu; http://dx.doi.org/10.4161/cc.22807

Dna2 was first characterized in yeast as an essential gene encoding a protein with both helicase and endonuclease activities involved in maturation of Okazaki fragments during DNA replication. Dna2 also plays a role in double-strand break (DSB) repair by homologous recombination. The respective contributions of its replication and/or repair functions toward cell viability and resistance to genotoxic stress is not entirely clear. Recent studies, including that of Karanja et al.[1] in a recent issue of *Cell Cycle*, are starting to clarify the multifaceted roles of DNA2.

Together with the endonuclease Rad27 (Fen1 in higher eukaryotes), Dna2 removes 5′ flaps generated by strand displacement during synthesis by Pol δ on the lagging strand. Most 5′ flap processing during replication is due to the activity of Rad27, yet Rad27Δ yeast cells are viable, whereas Dna2Δ cells are not. This suggests that the essential role of Dna2 in genome maintenance is distinct from Okazaki fragment maturation.[2] Indeed, Dna2 is a target of the intra-S-phase checkpoint in fission yeast and stabilizes replication forks.[3] Dna2 phosphorylation by Cds1 promotes the association of Dna2 to replication forks to counteract fork reversal. Reversed forks can be erroneously recognized as recombination intermediates leading genomic rearrangements.[4] Thus, Dna2 maintains genome stability by processing stalled forks before they collapse into aberrant structures. Similarly, Exo1 nuclease also participates in preventing the generation of "chicken-foot" structures from blocked forks, but, interestingly, Exo1 appears to be functional, even in the absence of an active checkpoint.[4]

Homology-dependent repair requires the generation of 3′ ssDNA, a process called resection that is regulated by CDKs. Resection provides the template that is used by Rad51 recombinase to search for homologous sequences.5 Resection is initiated by the MRN (Mre11-Rad50-Nbs1) complex and its co-factor CtIP. More processive, long-range resection is then performed by two partially overlapping pathways involving Dna2 and/or Exo1.[5]

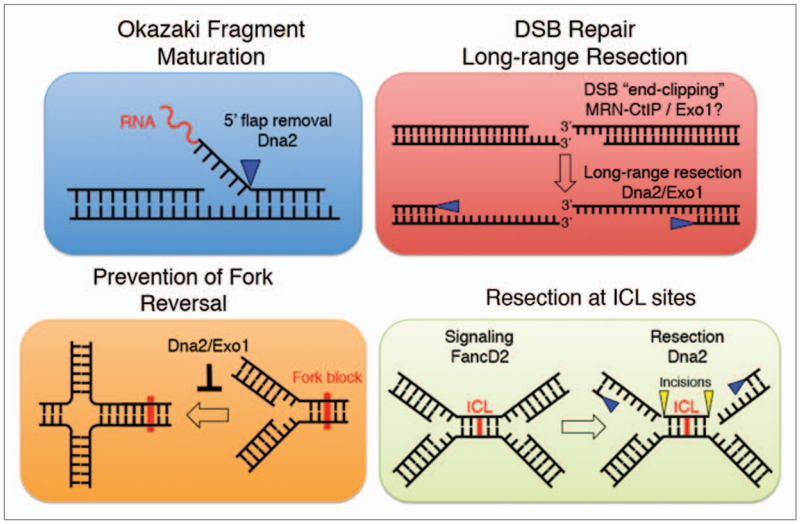

Figure 1. The many roles of Dna2 and Exo1 during S-phase.

In the November 2012 issue of *Cell Cycle*, Karanja et al.[1] provide additional evidence for the role of Dna2 and Exo1 in S-phase. Using siRNA-mediated Dna2 and Exo1 knockdown, they show that both nucleases contribute to cell viability following CPT, cisplatin or MMS treatments in a redundant manner. The data further support the conserved roles of Dna2 and Exo1 in the DNA damage response. Notably, the authors observe that Dna2-depleted cells have more profound defects in resection and Chk1 activation than Exo1-depleted cells when treated with cisplatin, a DNA-damaging agent that generates DNA interstrand cross-links (ICLs).

The Fanconi anemia/BRCA (FA/BRCA) pathway is critical for ICL repair in proliferating cells.[6] Recent work in the Xenopus system showed that the FA/BRCA pathway modulates the DNA damage response to ICLs and promotes ICL repair during S-phase.[7,8] Upon stalling at an ICL, the Fanconi anemia pathway promotes both stabilization of replication forks and recruitment of structure-specific nucleases to perform incision on both sides of the ICL. Rad51 loading takes place at the lesion before a DSB is generated,[9] suggesting that resection initiates from a partially processed ICL. Then translesion DNA synthesis is performed across the ICL site, the adduct is removed and the fork is most likely re-established by HDR. The mechanism of the resection step and the nature of the nucleases involved are still unknown, but given that Dna2 is present at replication forks, that it is involved in resection at DSBs and that it is regulated by the S-phase checkpoint, makes it an attractive candidate to perform this task. Notably, Karanja et al. detect a physical interaction between Dna2 and FancD2. Furthermore, experiments in FancD2-null cells show that Dna2 works downstream or parallel to FancD2, suggesting a function for Dna2 in ICL processing. FancD2 complex participates in signaling from ICL damage and in recruiting incision nucleases to the lesions, functions that could both involve Dna2. Nevertheless, it is also conceivable that Dna2 helps to prevent fork regression during ICL repair.

In summary, these findings further position Dna2 as a versatile checkpoint-regulated nuclease working during chromosomal replication and repair and essential for maintaining genome stability. Further studies are needed to understand the precise role of resection during ICL repair and the role of Dna2 and Exo1 nucleases, which appear to be partially redundant in this process. (**Fig. 1**)

References

1. Karanja KK, et al. Cell Cycle 2012; 11:3983-96; PMID:22987153; http://dx.doi.org/10.4161/cc.22215.
2. Budd ME, et al. Mutat Res 2000; 459:173-86; PMID:10812329; http://dx.doi.org/10.1016/S0921-8777(99)00072-5.
3. Hu J, et al. Cell 2012; 149:1221-32; PMID:22682245; http://dx.doi.org/10.1016/j.cell.2012.04.030.
4. Branzei D, et al. Nat Rev Mol Cell Biol 2010; 11:208-19; PMID:20177396; http://dx.doi.org/10.1038/nrm2852.
5. Symington LS, et al. Annu Rev Genet 2011; 45:247-71; PMID:21910633; http://dx.doi.org/10.1146/annurev-genet-110410-132435.
6. Deans AJ, et al. Nat Rev Cancer 2011; 11:467-80; PMID:21701511; http://dx.doi.org/10.1038/nrc3088.
7. Ben-Yehoyada M, et al. Mol Cell 2009; 35:704-15; PMID:19748363; http://dx.doi.org/10.1016/j.molcel.2009.08.014.
8. Knipscheer P, et al. Science 2009; 326:1698-701; PMID:19965384; http://dx.doi.org/10.1126/science.1182372.
9. Long DT, et al. Science 2011; 333:84-7; PMID:21719678; http://dx.doi.org/10.1126/science.1204258.

Unfolding tyrosine kinase inhibitor sensitivity in chronic myeloid leukemia

Comment on: Kusio-Kobialka M, et al. Cell Cycle 2012; 11:4069–78; PMID:23095523; http://dx.doi.org/10.4161/cc.22387

Danilo Perrotti* and Paolo Neviani; Comprehensive Cancer Center; The Ohio State University; Columbus, OH USA;
*Email: danilo.perrotti@osumc.edu; http://dx.doi.org/10.4161/cc.22808

The unfolded protein response (UPR) is a mechanism by which normal cells react to endoplasmic reticulum (ER) stress to maintain cell homeostasis. ER stress is triggered by a variety of stimuli, including nutrient deprivation, oxidative stress and higher metabolic demand. This often results in the accumulation of unfolded or misfolded proteins in the ER lumen, a phenomenon that triggers the switch-on of the UPR. Thus, a complex network of pathways will act together to protect, adapt and recover the "injured" cells from ER stress.[1] At molecular level, this translates into inhibition of protein translation and enhanced transcription of genes encoding molecular chaperones and other factors important for protein folding, degradation and quality control.[1] If the damage to the ER persists over a prolonged period of time, apoptosis is normally evoked to eliminate damaged cells.[2] Because cancer cells are generally exposed to a multitude of internal and external metabolic stressors, it is not surprising that molecular pathways regulating the cell response to ER stress have been found associated with autophagic and antiapoptotic signals and aberrantly activated in solid tumors and leukemias,[1,3] two characteristics that make this pathway suitable to be used for therapeutic intervention. For example, a suitable target for anticancer drug development is represented by the ER chaperone GRP78; in fact, its high level of expression in a variety of tumors, including hepatocellular carcinoma, breast cancer and chronic myeloid leukemia (CML), is a strong indicator of a deregulated and, likely, constitutively active UPR.[1,3-6]

CML is characterized by the presence of the Philadelphia chromosome carrying the fusion oncogene BCR-ABL1.[7] The presence of this constitutively active tyrosine kinase in myeloid progenitors is sufficient to induce and maintain their enhanced survival, a feature that is typical of the prolonged and indolent chronic phase (CP) of CML.[7] While in the mid-'90s allogeneic stem cell transplantation was the only curative, albeit risky, option for CML, from early 2000, first- and second- and, soon, third-generation TKIs (i.e., imatinib, nilotinib, dasatinib, bosutinib and ponatinib) are the elective therapeutic choice for chronic phase patients, the majority of which achieve and maintain major or complete molecular response.[7] However, in a small percentage of patients that are either refractory or become resistant to ABL1 tyrosine kinase inhibitors, CML undergoes blastic transformation, a still-fatal disease stage, historically termed blast crisis (BC) that is characterized by the increased expression and/or activity of BCR-ABL1 and the accumulation of secondary genetic and molecular abnormalities.[7] Thus, it is therefore imperative to explore alternative routes that may be helpful to prevent the arising of resistance to TKIs and, most importantly, offer patients in CML-BC new-targeted therapeutic options that may either eliminate the leukemic cell clone or make it responsive to TKIs and other available drugs.

In a recent issue of *Cell Cycle*, Kusio-Kobialka et al.[8] describe for the first time that in CML there is a correlation between ER stress, CML progression and response to imatinib treatment. In particular, they found that in human CML cell lines and primary cells, the PKR-like ER-resident kinase (PERK) is activated in a BCR-ABL1 expression-dependent manner.[8] PERK is one of the main initiators of the UPR and PERK-dependent phosphorylation of eIF2α impairs global cap-dependent mRNA translation, with the exception of ATF4 mRNA, whose product activates pathways controlling adaptation to stress and apoptosis.[1] Importantly, the activation of the PERK-eIF2α pathway seems to follow the natural progression of the disease and is enhanced in cells derived from patients in CML-BC as opposed to patients in the chronic phase or to cells derived from healthy individuals.[8] When BCR-ABL1-expressing cells were treated with imatinib, the authors saw a downregulation of PERK and eIF2α expression and phosphorylation levels in a dose-dependent manner, suggesting that the induction of the response to the ER stress may be mediated by BCR-ABL1 activity.[8] By using dominant-negative mutants of PERK or eIF2α, the authors have also been able to show that the PERK-eIF2α pathway serves a pro-survival role in CML; in fact, cells expressing their dominant-negative forms show a decreased ability to form colonies in clonogenic assays and also seem to be more sensitive to imatinib-mediated cell death.[8] In conclusion, this manuscript highlights the importance of exploring alternative pathways, like those involved in the UPR, as they might constitute the answer to overcoming the current therapeutic limitations we are facing in treating CML-BC and other acute leukemia patients.

References

1. Suh DH, et al. Ann NY Acad Sci 2012; 1271:20-32; PMID:23050960; http://dx.doi.org/10.1111/j.1749-6632.2012.06739.x.
2. Szegezdi E, et al. EMBO Rep 2006; 7:880-5; PMID:16953201; http://dx.doi.org/10.1038/sj.embor.7400779.
3. Li X, et al. J Hematol Oncol 2011; 4:8; PMID:21345215; http://dx.doi.org/10.1186/1756-8722-4-8.
4. Shuda M, et al. J Hepatol 2003; 38:605-14; PMID:12713871; http://dx.doi.org/10.1016/S0168-8278(03)00029-1.
5. Fernandez PM, et al. Breast Cancer Res Treat 2000; 59:15-26; PMID:10752676; http://dx.doi.org/10.1023/A:1006332011207.
6. Piwocka K, et al. Blood 2006; 107:4003-10; PMID:16469868; http://dx.doi.org/10.1182/blood-2005-04-1523.
7. Perrotti D, et al. J Clin Invest 2010; 120:2254-64; PMID:20592475; http://dx.doi.org/10.1172/JCI41246.
8. Kusio-Kobialka M, et al. Cell Cycle 2012; 11:4069-78; PMID:23095523; http://dx.doi.org/10.4161/cc.22387.

On the connections between cancer stem cells and EMT

Comment on: Borgna S, et al. Cell Cycle 2012; 11:4242–51; PMID:23095640; http://dx.doi.org/10.4161/cc.22543

Mario Cioce[1] and Gennaro Ciliberto[2,*]; [1]Department of Cardiothoracic Surgery; NYU Langone Medical Center; New York, NY USA; [2]IRCCS Istituto Nazionale Tumori Pascale; Napoli, Italy; *Email: g.ciliberto@istitutotumori.na.it; http://dx.doi.org/10.4161/cc.22809

In the last years, two independent concepts have improved our understanding of cancer recurrence and spread: (1) the cancer stem cell (CSC) hypothesis and (2) the occurrence of epithelial-to-mesenchymal transition (EMT). Recent evidences, such as the one presented in a recent issue by Borgna et al.,[1] point to a line of convergence of the two concepts.

EMT is a physiological cell reprogramming event utilized in tissue remodeling during embryonic development and activated in normal adult tissues during regeneration.[2] The presence of EMT-like cells in tumors has been linked to increased invasive and metastatic properties. The CSC hypothesis postulates the existence of hierarchically high-positioned, chemoresistant cells, which are responsible for disease relapse after treatment with debulking agents. These cells are endowed with the ability to reconstitute the histological heterogeneity of the originating tumor upon transplantation in immunodeficient hosts.[3] These properties of CSCs have strict resemblance to tissue remodeling and repair, which are typical features of mesenchymal tissues. Indeed, it has been shown that cultured breast cells that have undergone EMT in vitro also possess cancer stem cell signatures and properties.[4]

Growth of breast cancer cells as non-adherent spheroids in relatively non-differentiating conditions is regarded as a useful tool to enrich cells endowed with CSC-like features, such as chemoresistance and tumor-repopulating ability.[5] In the November 2012 issue of *Cell Cycle*, Silvia Borgna and colleagues provide evidence, at a molecular level and by using a large panel of cell lines corresponding to different breast cancer subtypes, that mammosphere-inducing growth conditions enrich for EMT-like cell subpopulations as well.[1] This is especially true for Claudin-low breast cancer cell lines, which are highly enriched for CSC-like, $CD44^{high}/CD24^{low}$ cells. Their work once more suggests that acquisition of EMT and CSC features are highly interconnected processes, possibly relevant for the organization of mammospheres. Indeed, recent evidence has been provided that the interaction of cell subpopulations with distinct mesenchymal and epithelial traits is instrumental for the maintenance of CSC-like cells[6] and relies upon cytokine-mediated signaling[7] (**Fig. 1**, see page 4). In light of this, it may be worth noticing that mammospheres are heterogeneous in composition and represent an ideal place for paracrine signaling to occur between different cell subpopulations. One may thus predict that compounds interfering with this crosstalk can block mammosphere formation (**Fig. 1**, see page 4). Indeed Butein, a naturally occurring STAT3 and NFκB inhibitor, impairs mammosphere formation from multiple breast cancer cell lines,[5] possibly by blocking IL-6 signaling.[6]

Interestingly, Borgna et al. found at least one of the known EMT-promoting transcription factors to be dynamically modulated in most of their cultures in time (mainly SNAI2 and TWIST1) when shifting from adherent to mammosphere culture conditions.[1] This underscores the relevance of such a process and its activation by distinct, converging and interconnected pathways. Indeed, forced expression of individual EMT-inducing transcription factors in stabilized cell lines has led Weinberg and collaborators to postulate the existence of an EMT interactome of transcription factors which are capable of reciprocally influencing each other. It will be interesting to evaluate the levels of EMT-promoting factors upon chemotherapy treatment of spheroids in vitro and to establish whether the enrichment for EMT-like cells is relevant to chemoresistance of mammospheres.

Culturing cancer cells as 3D spheroids may represent, therefore, a simplified albeit very useful tool for reproducing in vitro transient dynamic states of the tumor growth. It may also stimulate a shift in the way we envision hunting for novel therapeutic tools. Finally, this methodology is of general value, as it can be applied to cancers from other histotypes. For example, cells derived from biopsies or from malignant pleural effusions of patients with NSCLC give rise efficiently to propagating tumor spheroids in culture,[8] which are, again, enriched in CSC markers.[8,9] In conclusion, in vitro cultures of tumor spheroids from stabilized cell lines and from fresh tumor specimens may therefore be considered a useful in vitro model to screen for new agents capable of co-targeting both CSCs or EMT malignant features of cancer cells.

References

1. Borgna S, et al. Cell Cycle 2012; 11:4242-51; http://dx.doi.org/10.4161/cc.22543.
2. Kalluri R, et al. J Clin Invest 2009; 119:1420-8; PMID:19487818; http://dx.doi.org/10.1172/JCI39104.
3. Alison MR, et al. Cancer Treat Rev 2012; 38:589-98; PMID:22469558; http://dx.doi.org/10.1016/j.ctrv.2012.03.003.
4. Mani SA, et al. Cell 2008; 133:704-15; PMID:18485877; http://dx.doi.org/10.1016/j.cell.2008.03.027.
5. Cioce M, et al. Cell Cycle 2010; 9:2878-87; PMID:20581442; http://dx.doi.org/10.4161/cc.9.14.12371.
6. Liu S, et al. Cancer Res 2011; 71:614-24; PMID:21224357; http://dx.doi.org/10.1158/0008-5472.CAN-10-0538.
7. Scheel C, et al. Semin Cancer Biol 2012; 22:396-403; PMID:22554795; http://dx.doi.org/10.1016/j.semcancer.2012.04.001.
8. Mancini R, et al. PLoS One 2011; 6:e21320; PMID:21789168; http://dx.doi.org/10.1371/journal.pone.0021320.
9. Bartucci M, et al. Cell Death Differ 2012; 19:768-78; PMID:22117197; http://dx.doi.org/10.1038/cdd.2011.170.

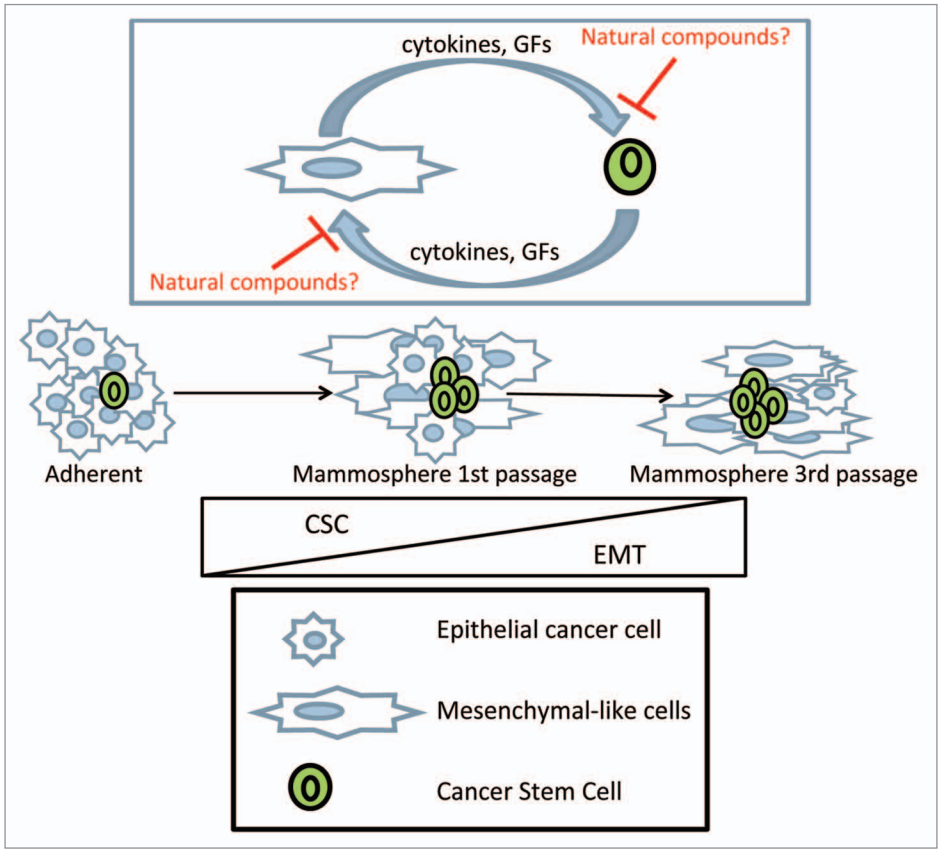

Figure 1. Schematic hypothetical working model. Mammospheres contain both CSC and EMT-like cells. When grown as mammospheres in non-adherent and no-serum conditions, breast cancer cell cultures are progressively enriched in EMT-like cells. Inset: A functional crosstalk is established between mesenchymal-like cells and epithelial-like cancer stem cells. This leads to enrichment for both CSC-like and EMT-like cell subpopulations within mammospheres. Please note that the number of CSC-like breast cells is almost constant in serially passaged mammospheres, while the proportion of EMT-like cells increases. Natural compounds, like butein, may interfere with paracrine signaling, sustaining the emergence of the mentioned cell subpopulations.

Transforming growth factor-β: Guardian of catabolic metabolism in carcinoma-associated fibroblasts

Comment on: Guido C, et al. Cell Cycle 2012; 11:3019–35;
PMID:22874531; http://dx.doi.org/10.4161/cc.21384

Husain Yar Khan[1] and Akira Orimo[2,3,*]; [1]Department of Biochemistry; Faculty of Life Sciences; Aligarh Muslim University; Aligarh, India; [2]Department of Pathology and Oncology; Juntendo University School of Medicine; Tokyo, Japan; [3]Atopy Research Centre; Juntendo University School of Medicine; Tokyo, Japan
*Email: aorimo@juntendo.ac.jp; http://dx.doi.org/10.4161/cc.22811

Transforming growth factor-β (TGF-β) never ceases to fascinate cancer researchers due to its pleiotropic effects and significant clinical relevance to human diseases. Over the past few decades, TGFβ has been the focus of considerable research efforts, but we still do not fully understand the complex mechanism(s) by which this cytokine influences tumorigenesis. It has become evident that TGFβ modulates carcinoma cell behavior in a cell context-dependent fashion during the early and late stages of tumorigenesis.[1] TGFβ is well-known to play tumor-suppressive roles that inhibit tumor cell proliferation and induce apoptosis in premalignant cells. In contrast, this cytokine often provides malignant cells harboring cancer-driving genetic mutations with the hallmarks of cancer-aggressive traits. The latter is exemplified by epithelial-mesenchymal transition and cancer stem cell phenotypes that promote tumor invasion and metastasis.[1,2] Cell-autonomous oncogenic signaling conferred upon carcinoma cells often abolishes their tumor-suppressive responsiveness to TGFβ during late stages of tumorigenesis. Interestingly, such paradoxical TGFβ-induced cellular responses may also depend on complex regulation by the tumor microenvironment.[3]

Carcinoma-associated fibroblasts (CAFs), which consist of fibroblasts and myofibroblasts, are a predominant cell type within the tumor-associated stroma. Carcinoma cell-secreted TGFβ appears to initiate, in a paracrine fashion, the conversion of resident fibroblasts to CAF myofibroblasts within the tumor stroma. During the course of tumor progression, such myofibroblasts markedly increase the level of TGF-β production, which,

in turn, enables these cells to activate TGFβ signaling in an autocrine fashion, thereby constitutively driving their myofibroblastic, tumor-promoting property.[4]

Caveolin-1 (Cav-1) is proposed to be essential for achieving the myofibroblastic state in CAFs and is a potential clinical biomarker for human breast cancers.[5] The Cav-1 expression level is inversely correlated with TGFβ signaling in stromal fibroblasts. Downregulation of Cav-1 expression also increases TGFβ signaling in these cells, whereas upregulation of TGFβ signaling suppresses Cav-1 expression.

In the August 15, 2012 issue of *Cell Cycle*, Guido et al. provided evidence supporting a critical role of TGFβ signaling in metabolic reprogramming via Cav-1 in CAFs.[6] Metabolism in cancer cells had long been considered to merely be an indirect secondary phenomenon that is simply associated with, i.e., does not cause, tumor progression. However, reprogrammed cancer metabolism now serves as one of the hallmarks of human cancers and not simply as a passive readout.[7] Guido and colleagues previously proposed the concept of "two-compartment tumor metabolism," wherein stromal Cav-1 loss induces a "Warburg effect" in tumor-associated stromal cells, thereby leading to energy-rich metabolites that fuel neighboring cancer cells.[5] In the 2012 study, they have indicated that activation of TGFβ signaling in fibroblasts leads to an attenuation of Cav-1 expression that increases oxidative stress, induces autophagy/mitophagy, elevates aerobic glycolysis and, thus, stimulates mammary tumor growth (**Fig. 1**).[6]

This work also shows that TGFβ released from either carcinoma cells or CAFs drives the canonical Smad2/3 signaling in CAFs via a paracrine or an autocrine mechanism, respectively (**Fig. 1**). The resulting decrease in Cav-1 expression is a prerequisite for the generation of energy-rich metabolites, thereby promoting apposed cancer cell growth. Collectively, activation of TGFβ signaling in CAFs is elucidated as being the force that drives catabolic metabolic reprogramming via Cav-1 downregulation in these cells, thereby stimulating tumorigenesis in human breast carcinoma cells.

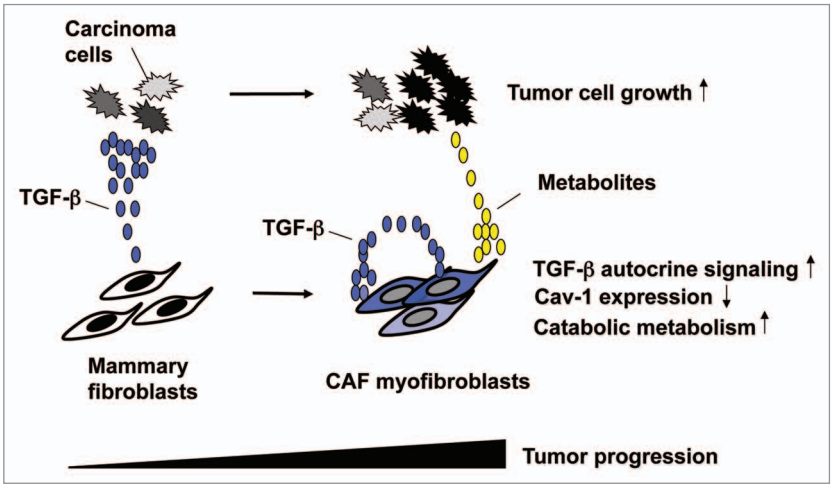

Figure 1. TGFβ-signaling causes metabolic reprogramming in CAFs to promote tumorigenesis. Cancer cells secrete TGFβ that initiates the conversion of mammary stromal fibroblasts to myofibroblasts in a paracrine fashion. During the series of tumor progression, myofibroblasts increase their TGFβ production and conversely decrease Cav-1 expression. The resulting myofibroblasts activate TGFβ signaling in an autocrine fashion, which leads to increased oxidative stress, induction of autophagy/mitophagy and subsequently aerobic glycolysis (Warburg effect), thereby generating metabolites (lactate, pyruvate, glutamine, ketone bodies, etc.). These metabolites, which are routed to the adjacent cancer cells, boost their anabolic metabolism and growth.

Notably, pharmacological inhibitors and neutralizing antibodies targeting TGFβ signaling, potentially in both tumor and stromal compartments, have indeed been reported to enhance the efficacy of conventional chemotherapies attenuating tumor growth in xenograft tumor models.[8,9] These effects presumably involved modulation of vascular permeability, ECM production and recruitment and activation of tumor-promoting stromal cells within tumors.

In summary, the recent study by Guido et al. has demonstrated the importance of TGFβ autocrine signaling and the concomitant Cav-1 downregulation in CAFs, which can promote catabolic metabolism in these cells and, consequently, lead to enhanced tumorigenesis in adjacent human breast carcinoma cells.[6] This work represents a step forward in our quest to understand the molecular mechanism(s) underlying CAF-promoted tumorigenesis and the development of novel therapeutic approaches.

References

1. Massagué J. Nat Rev Mol Cell Biol 2012; 13:616-30; PMID:22992590; http://dx.doi.org/10.1038/nrm3434.
2. Ikushima H, et al. Nat Rev Cancer 2010; 10:415-24; PMID:20495575; http://dx.doi.org/10.1038/nrc2853.
3. Bierie B, et al. Nat Rev Cancer 2006; 6:506-20; PMID:16794634; http://dx.doi.org/10.1038/nrc1926.
4. Kojima Y, et al. Proc Natl Acad Sci USA 2010; 107:20009-14; PMID:21041659; http://dx.doi.org/10.1073/pnas.1013805107.
5. Sotgia F, et al. Breast Cancer Res 2011; 13:213; PMID:21867571; http://dx.doi.org/10.1186/bcr2892.
6. Guido C, et al. Cell Cycle 2012; 11:3019-35; PMID:22874531; http://dx.doi.org/10.4161/cc.21384.
7. Ward PS, et al. Cancer Cell 2012; 21:297-308; PMID:22439925; http://dx.doi.org/10.1016/j.ccr.2012.02.014.
8. Liu J, et al. Proc Natl Acad Sci USA 2012; 109:16618-23; PMID:22996328; http://dx.doi.org/10.1073/pnas.1117610109.
9. Kano MR, et al. Proc Natl Acad Sci USA 2007; 104:3460-5; PMID:17307870; http://dx.doi.org/10.1073/pnas.0611660104.

Multiplying madly: Deacetylases take charge of centrosome duplication and amplification

Comment on: Ling H, et al. Cell Cycle 2012; 11:3779–91;
PMID:23022877; http://dx.doi.org/10.4161/cc.21985

Hui-Fang Hung, Heidi Hehnly and Stephen Doxsey*; Program in Molecular Medicine; University of Massachusetts Medical School at Worcester; Worcester, MA USA; *Email: Stephen.Doxsey@umassmed.edu; http://dx.doi.org/10.4161/cc.22812

In this volume of *Cell Cycle*, Ling et al. discovered acetylation-based control of centrosome duplication and amplification.[1] The centrosome is primarily recognized as a microtubule-organizing center (MTOC), capable of nucleating and anchoring microtubules. At the G_1/S transition of the cell cycle, centrosome duplication is initiated, and by G_2/M, the process is complete. Normally, vertebrate centrosomes duplicate once and only once during the cell cycle and contribute to the formation of the two spindle poles during mitosis.

Aberrant centrosome duplication can result in centrosome amplification, a condition found in many cancers. This can lead to multipolar spindles and, in turn, chromosome segregation errors, loss of tumor suppressor function and aggressive malignancies.[2,3] Centrosome duplication must be tightly controlled to prevent centrosome amplification and to couple it with DNA replication.

Several mechanisms can contribute to centrosome/MTOC amplification in tumor cells, including cytokinetic failure, centrosome overduplication, centriole pair splitting and acentriolar MTOC formation.[2,4] Certain tumor-derived cell lines undergo multiple rounds of centrosome duplication when DNA replication is blocked, delaying S phase.[2] Centrosome duplication is under cell cycle regulator control, which controls DNA replication and thereby coordinates the two events. Phosphorylation also contributes to centrosome duplication, but little is known about the role of other post-translational modifications in this process.[1] In this study by Ling et al., the authors addressed this question. They unexpectedly found that centrosome number is controlled by deacetylases in both normal and tumor cells.

Histone acetylation is a common form of acetylation, but non-histone acetylation is also significant and plays a major role in mRNA and protein stability, protein interactions and protein localization.[5] In this study, the authors unexpectedly found that several centrosome proteins are acetylated (centrin, Plk2 and SEPT7). They also made the surprising discovery that several deacetylases localize to centrosomes (8/18) and suppress centrosome amplification following expression above endogenous levels (7/8). In contrast, only 3/10 non-centrosomal deacetylases suppressed centrosome amplification, suggesting a role for acetylation/deacetylation in centrosome number control.

The authors next identified a subset of deacetylases with the highest centrosome amplification suppression activity (HDAC1, HDAC5, SIRT1). They showed that the deacetylase activity of HDAC1 and SIRT1 was required to suppress centrosome amplification, but not for HDAC5. In contrast, HDAC5 phosphorylation was required for suppression activity, suggesting that posttranslational events localize HDAC5 to centrosomes suppressing centrosome amplification. More work is required to understand this differential localization, as well as the mechanism of deacetylase action, possible links to the cell cycle and how deacetylases are regulated.

In a previous study, Fukasawa et al. found that cyclin A was required for centrosome reduplication in cells arrested in late S/G_2 phase.[6] Here, they found that HDAC1 overexpression suppressed cyclin A transcription.[1] Following completion of centrosome duplication, we speculate that the centrosomal localization of HDAC1 suppresses cyclin A expression, or that low cyclin A levels permit centrosome localization of HDAC1. Consistent with this model is a previous study showing that HDAC1 localizes to centrosomes in metaphase[7] when centrosomes are not replicating and cyclin A expression is low.

Does this work have significance for the etiology of cancer and in therapeutic strategies? Centrosome amplification has become a hallmark of carcinomas and other cancers. The finding that many deacetylases suppress centrosome amplification is inconsistent with the described increase in deacetylase expression in cancer cells.[8] Moreover, deacetylase inhibitors have anticancer effects.[8] However, it is unclear if the deacetylase inhibitors used in the cancer studies affect deacetylase localization to centrosomes. Additional studies will shed light on the roles of deacetylases/acetylases in centrosome duplication and amplification. For example, it is likely that these enzymes function in duplication control, but they could also participate in the many steps of centrosome assembly that have been uncovered over the last several years.[9]

This paper provides novel insights into regulation of centrosome duplication/amplification through the identification of new contributors to this process, acetylases/deacetylases. Moreover, the discovery of acetylated centrosome proteins establishes new frontiers to understanding how post-translational modifications regulate centrosome function. Based on the profound changes in centrosome numbers induced by the perturbation of deacetylases, it is clear that this new area of centrosome biology has high potential to yield important insights into centrosome duplication and, perhaps, into other aspects of centrosome biology for years to come.

References

1. Ling H, et al. Cell Cycle 2012; 11:3779-91; PMID:23022877; http://dx.doi.org/10.4161/cc.21985.
2. Fukasawa K. Cancer Lett 2005; 230:6-19; PMID:16253756; http://dx.doi.org/10.1016/j.canlet.2004.12.028.
3. Nigg EA. Nat Rev Cancer 2002; 2:815-25; PMID:12415252; http://dx.doi.org/10.1038/nrc924.
4. Marthiens V, et al. J Cell Sci 2012; 125:3281-92; PMID:22956721; http://dx.doi.org/10.1242/jcs.094797.
5. Spange S, et al. Int J Biochem Cell Biol 2009; 41:185-98; PMID:18804549; http://dx.doi.org/10.1016/j.biocel.2008.08.027.
6. Hanashiro K, et al. Oncogene 2008; 27:5288-302; PMID:18490919; http://dx.doi.org/10.1038/onc.2008.161.
7. Sakai H, et al. J Biol Chem 2002; 277:48714-23; PMID:12354758; http://dx.doi.org/10.1074/jbc.M208461200.
8. Bolden JE, et al. Nat Rev Drug Discov 2006; 5:769-84; PMID:16955068; http://dx.doi.org/10.1038/nrd2133.
9. Gönczy P. Nat Rev Mol Cell Biol 2012; 13:425-35; PMID:22691849; http://dx.doi.org/10.1038/nrm3373.