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Cell Cycle News & Views

More isn't always better: Limiting centrosome size in interphase

Comment on: Jeffery JM, et al. Cell Cycle 2013; 12:899–906; PMID:23442802; http://dx.doi.org/10.4161/cc.23879

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The centrosome serves as the primary microtubule-organizing center in animal cells and, consequently, functions in many processes, such as migration and formation of the mitotic spindles. The centrosome consists of a pair of centrioles surrounded by pericentriolar material (PCM), the platform for microtubule nucleation. The pair of centrioles duplicate once per cell cycle to ensure the equal segregation of chromosomes in mitosis. The control of centrosome duplication and their capacity to nucleate microtubules is tightly coupled to cell cycle progression. Centriole duplication initiates at the beginning of the S phase and the duplicated centrioles elongate until the G_2 phase. At late G_2 phase, the centrosomes mature by recruiting PCM components, resulting in the increase in the microtubule-nucleating capacity that helps the formation of spindle microtubules later in mitosis. PCM recruitment in the centrosome maturation process has been intensively investigated and revealed to be regulated by mitotic kinases. However, the mechanism regulating interphase PCM recruitment remains largely unknown, especially in mammalian cells. In other organisms, centriole duplication factors, *C. elegans* ZYG-1[1] (Plk4 orthlog) and Drosophila Sas-4[2] (CPAP ortholog) were demonstrated to be involved in the interphase PCM recruitment.

In a recent issue of *Cell Cycle*, Jeffery et al.[3] proposed that centrosomal protein Centrobin regulates microtubule nucleation and organization by controlling the amount of PCM in interphase. Centrobin was initially identified as a daughter centriole-associated protein required for centriole duplication.[4] Centrobin has been shown to have microtubule-bundling activity[5] and plays a role in the stabilization of mitotic spindles by anchoring them to the centrosome,[6] while the role of Centrobin in interphase cells has not been well-defined. First, Jeffery et al. showed that Centrobin is exclusively localized at centrosomes in interphase cells[3] in contrast to its association with spindle microtubules during mitosis.[5,6] They next showed that when Centrobin is depleted in interphase cells, the microtubules become more focused around the centrosome and sparse in the cell cortex area. Furthermore, microtubules are less stable than those in control cells, as detected by sensitivity to microtubule depolymerizing conditions and by the acetylation state of the microtubules. They further demonstrated that altered microtubule organization is caused by increase in the number of short microtubules emanating from the centrosome without changes in the microtubule dynamics. Microtubule nucleation depends on the amount and integrity of PCM proteins, and Jeffery et al. observed an increase in the intensity of PCM proteins, including γ-tubulin, AKAP450, kendrin and PCM-1 at the centrosome, while total amount of them was not affected.

In summary, their data reveal a novel role for Centrobin in limiting PCM recruitment and microtubule nucleation. One interesting explanation for this function is that the presence of Centrobin at the daughter centriole is necessary to make it functionally different from the mother centriole, and in the absence of Centrobin, the daughter centriole may become more like the mother centriole, resulting in increased PCM recruitment and microtubule nucleation. Recently, it was reported that Drosophila Centrobin plays an important role in the asymmetric cell division of neuroblast in the generation of the central nervous system.[7] In this case, Centrobin functions in an opposite way, although this seems to be a neuroblast-specific phenomenon; the daughter centriole harboring Centrobin can organize PCM and microtubules in interphase to anchor at the apical cortex of the neuroblast, resulting in the formation of a specific axis in the following asymmetric cell division. Further investigation of Centrobin's function in various cellular events, including asymmetric cell division in mammalian systems, may provide valuable insights into the regulation of PCM recruitment as well as the functional difference between mother and daughter centrioles.

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Discovering smoking-related pathway alterations in urothelial cell carcinoma pathogenesis

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Urothelial cell carcinoma (UCC) represents the most common malignancy of the urinary tract. It is estimated that there were 73,500 new cases of UCC and 15,000 deaths for both sexes in the United States in 2012.[1] As the final recipient and reservoir of urine, the urothelium is inevitably exposed to carcinogens present in tobacco, which can create stepwise molecular alterations that eventually lead to transformation of urothelial cells. This concept is supported by epidemiologic studies that state that tobacco consumption is the most important factor for the development of this disease, contributing to approximately 50% of all cases.[2]

UCC is a heterogeneous disease; 70% of newly diagnosed bladder tumors are non-muscle invasive (NMIBC) and show a much better prognosis compared with those that invade the detrusor muscle (MIBC).[3] From the molecular point of view, evidence in the literature supports the existence of two distinct pathogenetic pathways involved in UCC development, corresponding to these two distinct (NMIBC and MIBC) biological and clinical phenotypes. In fact, while disruption to the PI3K-AKT-mTOR pathway and alterations in the tyrosine kinase receptor gene *FGFR3* and the oncogene *HRAS* are associated with NMIBC, the main genetic alterations underlying MIBC involve tumor suppressor genes encoding proteins that regulate cell cycle and apoptosis pathways, including *TP53*, *CDKN2A*, *CCND1*, *CDKN1B* and *RB1*.[4] Recent works have also suggested epigenetic mechanisms like promoter methylation in the pathogenesis of this disease.[5,6]

Understanding the multistep accumulation of genetic and epigenetic alterations related to environmental factors in the development and progression of this disease is crucial for the discovery of biomarkers that might be useful in predicting the behavior and prognosis of UCC in individual patients. In an intent to understand the genetic/epigenetic alterations that accumulates in the process of UCC development, the group led by Hoque has developed a very interesting cellular model for smoking-induced UCC.[7] In this study, SV-40 immortalized normal HUC1 human bladder epithelial cells were continuously exposed to 0.1% cigarette smoke extract (CSE) until transformation occurred. The authors observed morphological alterations and increased cell proliferation after 4 mo of exposure to CSE. After 6 mo the treated cells showed anchorage-independent growth and an increase in the migratory and invasive potential. The observed properties after 6 mo of CSE treatment were not noticeable at 4 mo of treatment, suggesting that some driver gene/genes might alter due to prolonged exposure to tobacco.

In order to assess key molecular alterations occurring in CSE-treated cells, the authors evaluated the expression level of specific genes involved in the PI3K-AKT pathway and found upregulation of *AKT1, AKT2, HRAS, RAC1* and downregulation of *PTEN, FOXO1, MAPK1* and *PDK1* among altered genes. Interestingly, immunohistochemistry for FOXO1 performed on UCC samples showed higher level and frequency of expression in the smokers group compared with non-smokers. In their view, this might reflect the fact that FOXO1 in smokers is subjected to an enhanced phosphorylation by AKT with consequent cytoplasmic translocation. Using genome-wide methylation analysis, the authors also found differentially methylated genes in CSE-treated and untreated HUC1 cell lines. They further confirmed methylation status of *MCAM, DCC* and *HIC1* in CSE-treated and untreated HUC1 cell lines by a complementary approach (QMSP). These findings support that epigenetic alterations are simultaneously related to smoke-associated UCC.

As stated above, *P53* represents the most frequently dysregulated gene in UCC, especially in the pathway related to muscle-invasive tumors. However, in this work, translational and transcriptional levels of *P53* were unchanged after 6 mo of CSE treatment. In this regard, the authors speculate that it could be possible that a prolonged period of exposure might be necessary to alter the *P53* pathway that is involved in the progression of NMIBC to MIBC. It would be therefore be useful to specifically investigate the mechanisms and the alterations necessary for this to happen, especially on a structural basis like LOH and copy number alterations. Detailed molecular studies using this cellular model will eventually help to identify related genes and pathways that are altered due to smoking in a stepwise fashion. Ultimately, accumulated knowledge will help to develop personalized management of UCC patients.[7]

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PP2A$^{Cdc55/B55}$, a possible therapeutic target in cyclin D1-dependent cancers

Comment on: McCourt P, et al. Cell Cycle 2013; 12:1201–10;
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Protein phosphatase 2A (PP2A) comprises a large family of heterotrimeric complexes required for a variety of cellular processes.[1] PP2A often functions to oppose the activity of oncogenic kinases and negatively regulates cell cycle progression in human cells. Therefore, PP2A is thought to contribute to tumor suppression, and PP2A-activating agents appear to reduce tumor burden.[2] However, conversely, PP2A inhibition may enhance cancer chemotherapy by DNA damaging agents. Survival of cancer cells in response to DNA damage depends on checkpoint-dependent cell cycle arrest. Therefore, PP2A inhibition, which promotes cell cycle progression and abrogates cell cycle arrest, may effectively induce mitotic catastrophes and subsequent cell death,[3] indicating that modulating PP2A activity may hold good promise as cancer therapy.[2] Thus, understanding how PP2A controls cell cycle progression is important.

In a recent report, McCourt et al. demonstrated an interesting link between PP2A and G_1 cyclins,[4] whose overexpression is frequently associated with human cancers.[5] In humans, cyclin D1, one of the G_1 cyclins that promote G_1/S transitions, is regulated by proteasomal-dependent proteolysis. Degradation of cyclin D1 is dependent on glycogen synthase kinase 3β (GSK3β)-dependent phosphorylation and subsequent recognition by the SCF$^{Fbx4/aB\text{-}crystallin}$ ubiquitin ligase.[6] In budding yeast, G_1 cyclins, Cln1 and Cln2, are known to undergo CDK1-dependent phosphorylation followed by ubiquitination via the SCF^{Grr1} ubiquitin ligase.[7] Thus, phosphorylation-dependent degradation of G_1 cyclins is conserved across evolution. However, phosphatases involved in dephosphorylation of G_1 cyclins were not well-documented. Considering that phosphorylation status of G_1 cyclins plays an important role in their stability, and that G_1 cyclins are often deregulated in human cancers,[5,6,8] identifying phosphatases involved in G_1 cyclin stability plays a significant role in the improvement of cancer therapy.

In the course of understanding how PP2A regulates cellular processes, McCourt et al.[4] identified an allele of *grr1* as a synthetic lethal mutation with the loss of Cdc55 (B55 in humans), one of two regulatory B subunits of budding yeast PP2A. This genetic interaction was specific to PP2A^{Cdc55}, because the *grr1* mutation was not synthetically lethal with the loss of Rts1, the second regulatory B subunit for PP2A. Grr1 is an F-box protein, which is a variable component of SCF ubiquitin ligases and responsible for substrate recognition.[7,8] Further mutational analyses of *grr1* revealed that mutations in domains required for substrate recognition are also synthetically lethal with *cdc55* deletion, suggesting that accumulation of SCF^{Grr1} substrates is toxic in the absence of Cdc55. Indeed, Cln2, one of the SCF^{Grr1} substrates, was highly accumulated in *grr1* mutant, and Cln2 overexpression was toxic in *cdc55*-deleted cells.

Interestingly, Cln2 was highly unstable in the absence of Cdc55. Cln2 degradation in *cdc55*-deleted cells was associated with the CDK1-dependent phosphorylation of Cln2, because the unphosphorylatable form of Cln2 was highly stable even in the absence of Cdc55. Furthermore, a temperature-sensitive mutation in Cdc53 (an SCF^{Grr1} component) stabilized Cln2 in *cdc55* cells, indicating that Cln2 is a better SCF^{Grr1} substrate in the absence of Cdc55. Considering that SCF^{Grr1} targets phosphorylated Cln2, these results suggest that PP2A^{Cdc55} regulates Cln2 stability through modulating its phosphorylation status. Consistent with this suggestion, the authors showed that PP2A physically associates with Cln2, indicating the role of PP2A in dephosphorylating Cln2. It would be interesting in the future to investigate whether PP2A^{Cdc55} indeed directly dephosphorylates Cln2.

The authors took a further step and genetically demonstrated that PP2A^{Cdc55} and SCF^{Grr1} act antagonistically to regulate G_1 cyclin-dependent cell cycle events.[4] Cellular amounts of human G_1 cyclins, such as cyclin D1, must be tightly regulated to prevent uncontrolled growth and genomic instability associated with a variety of cancers. Indeed, several F-box proteins or associated factors, which are known to regulate cyclin D1 levels, are mutated in cancers,[8] suggesting the importance of fine-tuning cyclin D1 levels in preventing cancer development. Therefore, targeting cyclin D1 is proposed to be an effective strategy in cancer therapy, and some compounds are reported to induce cyclin D1 degradation.[5] Therefore, the modulation of phosphorylation status by inhibiting PP2A may constitute a new way of regulating cyclin D1 levels in cancer. Further research into the role of PP2A in G_1 cyclin stability in human cancer cells would answer these questions. (**Fig. 1**)

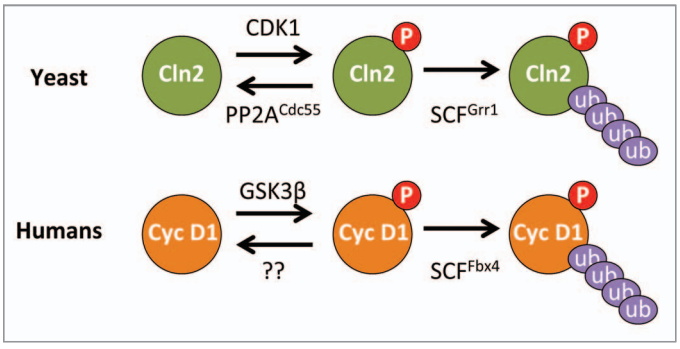

Figure 1. Phosphorylation-dependent degradation of G_1 cyclins in yeast and humans. In yeast, CDK1-dependent phosphorylation of Cln2 leads to Cln2 degradation via SCF^{Grr1}. This degradation can be inhibited by Cln2 dephosphorylation by the PP2A^{Cdc55} phosphatase. In humans, GSK3β phosphorylates Cyclin D1, resulting in Cyclin D1 ubiquitination by SCF$^{Fbx4/aB\text{-}crystallin}$.

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An expanded role for Caveolin-1 in brain tumors

Comment on: Quann K, et al. Cell Cycle 2013; 12:1510–20; PMID:23598719; http://dx.doi.org/10.4161/cc.24497

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Glioblastoma multiforme (GBM) arises from cells in the brain called astrocytes and can form in many different parts of the brain, including the cerebellum and spinal cord. GBM is both the most frequent and also the most deadly adult brain tumor, with an incidence rate of between two to three per 1,000 people. Post-surgical standard of care usually consists of radiation combined with temozolomide and dexamethasone.[1] However, even with aggressive intervention, GBM continues to be an aggressive, progressive disease with extremely high mortality rates. Because of the severity of the disease and the poor median and overall survival statistics for GBM patients, the need for identifying new and more effective targets and pathways to treat GBM is obvious and critical.

Caveolae are submicroscopic invaginations found in the cell membranes of a variety of tissue types and play an ever-expanding role in multiple cellular processes.[2] The predominant structural components of caveolae is the transmembrane-bound protein caveolin-1 (Cav-1). Cav-1 has been extensively studied and its activities characterized in a number of cancers, for which it has been shown to function as either a tumor suppressor or tumor promoter depending on tissue type and the underlying cellular proteome.

Cav-1 has only recently received increased attention in the brain cancer field, with approximately 25 published papers appearing in PubMed on Cav-1 and human brain cancers. The in vitro characterizations of the role of Cav-1 in GBM have largely been undertaken by Martin and colleagues, where Cav-1 was identified as a tumor suppressor, affecting proliferation in part through modulating TGFB/SMAD signaling.[3]

In a new study, Quann et al.[4] expanded upon this previous work by creating a stable Cav-1-overexpressing cell line based on the common GBM-derived cell line U-87MG. Microarray analyses comparing Cav-1-overexpressing cells to control cells established that critical cell cycle genes and cell survival proteins and pathways, such as cyclin D1 and AKT/mTOR, respectively, were downregulated. Perhaps more importantly, using a mouse xenograft model, they found that Cav-1-overexpressing tumors were significantly less proliferative and less invasive when compared with control cells, with explanted tumors displaying marked silencing of cell cycle and protein biosynthetic pathways. Finally, Cav-1-overexpressing cells were found to be sensitized to the antitumor effects of the most commonly used chemotherapy agent, temozolomide, and were significantly more likely to undergo apoptosis after treatment as compared with controls cells. These results extend the role of Cav-1 into the prognosis and possibly the treatment of GBM.

Interestingly, one of the most frequent point mutants in GBM occurs in the tumor suppressor protein, p53.[5] In certain p53-mutant tumors, glucose restriction, which induces oxidative stress, resulted in activation of autophagy and an autophagy-dependent degradation of mutant p53, leading to a feedforward acceleration of autophagy and tumor inhibition.[6] Furthermore, in the tumor stroma, Cav-1 expression has been found to be similarly downregulated by oxidative stress when autophagy was activated, which, in turn, resulted in a feedforward upregulation of stromal autophagy.[7] Termed the "reverse Warburg effect," this tumor microenvironment is defined by enhanced stromal aerobic glycolysis, oxidative stress and localized inflammation, which, in turn, promotes tumor cell survival through cancer cell parasitism of nutrients released from the autophagic stromal cells. Collectively, these results suggest that the expression levels of Cav-1, and certain mutant forms of p53, may be regulated in a similar fashion by autophagy, leading, however, to different phenotypic outcomes depending upon whether their expression occurs in the tumor or in the stromal component. Thus, it will be very important to determine whether and how Cav-1 and mutant forms of p53 cross-talk with the stroma and define their relationship with autophagy and the metabolism of tumor cells.

While the current study was limited to one cell line and an ectopic xenograft mouse model, the observations are extremely interesting, and further investigations are clearly warranted and encouraged. Given the great advances in mouse modeling of brain malignancies and the recent focus on perfecting non-invasive imaging of drug sensitivity/responses,[8] it is likely that more dynamic and comprehensive investigations into Cav-1 as an etiological mediator of GMB progression and treatment will be forthcoming. (**Fig. 1**)

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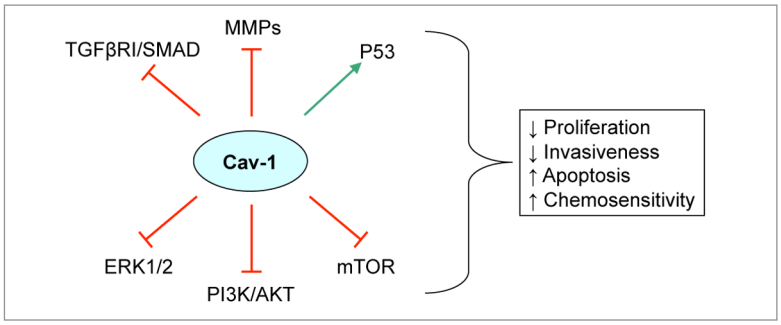

Figure 1. Caveolin-1 plays a central role in glioblastoma multiforme onset and progression and may be a biomarker for sensitivity to chemotherapy. Red lines denote genes or pathways inhibited by Cav-1, while green lines indicate those that are upregulated.