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Cell Cycle News & Views

The fox and the fat: An unexpected new treatment for brain tumors

Comment on: Wilk A, et al. Cell Cycle 2012; 11:2660–71;
PMID:22732497; http://dx.doi.org/10.4161/cc.21015

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When one thinks of the popular lipid-lowering drug fenofibrate, it is usually in connection with trying to prevent heart attack or stroke. One of the most unlikely discoveries of recent weeks is that fenofibrate may represent a new way to treat the deadly brain tumors, glioblastomas.[4] Glioblastoma multiforme is an astrocytoma form of glioma, or glial cell tumor, that accounts for more than 50% of all brain tumors. Glioblastoma is one of the deadliest tumors known, with the worst prognosis of any CNS neoplasia. Despite some hopeful advances, the median survival time after diagnosis is a dismal 14 mo.[3] Although glioblastoma strikes only about three out of 100,000 Americans, it does so without any clear genetic predisposition or environmental suspect. Glioblastoma was recently highlighted as the cause of death of Sen. Edward M. Kennedy, a strong proponent of cancer research.[2]

Krzysztof Reiss and colleagues, presently at the Neurological Cancer Research, Stanley S. Scott Cancer Center, LSU have been working on means of stimulating apoptosis, or programmed cell death, in gliomas. Their focus has been on the Bim pathway. Although other recent work focuses on Bim,[1] the signals triggering this pathway have been ill-defined. Working on the hypothesis that switching cancer cells from glycolysis to fat-burning could inhibit them, the LSU group tested fenofibrate, a drug used frequently along with statins to lower lipids and cholesterol by stimulating fat metabolism. They found that low doses of fenofibrate arrest growth of glioblastoma cells in culture, while higher doses induce massive apoptosis. Their results revealed that fenofibrate induced phosphorylation and nuclear translocation of the transcription factor, FoxO3A. FoxO3A, in turn, induced expression of Bim, resulting in glioblastoma cell death. The connection with FoxO3A is a promising new avenue of investigation for these deadly brain tumors. This is far from a cancer cure, but fenofibrate is a drug already on the market, and with few side effects. It can presumably be used immediately as an adjunct with existing anticancer agents (**Fig. 1**).

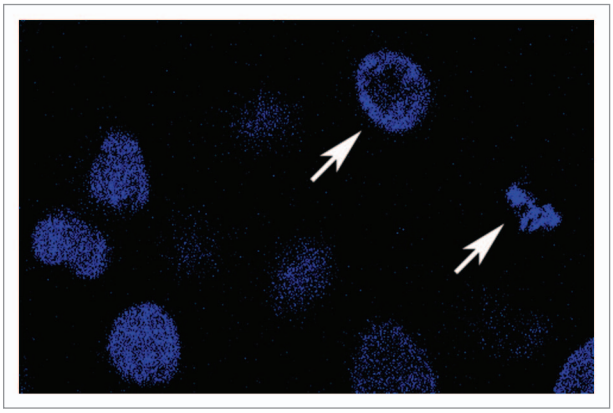

Figure 1. Glioblastoma cells in culture showing cells undergoing apoptosis (arrows). Blue, DAPI, is DNA stained to show cell nuclei.

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Directing p53 to induce autophagy

Comment on: Naidu SR, et al. Cell Cycle 2012; 11:2717–28;
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Macroautophagy is an ancient, evolutionarily conserved catabolic process involving the degradation of organelles and long-lived proteins, an important process for maintaining cellular homeostasis. Many cellular stresses, including hypoxia/anoxia and the lack of growth factors and nutrients, trigger autophagy to either mediate survival or cell death. Numerous intracellular factors have been implicated in promoting autophagy, including the tumor suppressor p53. Interestingly, p53 can promote cell fate decisions such as autophagy through transcriptional and post-transcriptional pathways. The complexity of how p53 can regulate cellular fate may be driven by specific pathways that are activated in response to cellular cues, while the understanding of intra- and extracellular signaling

that promotes post-translational modifications to p53 still remains incomplete. For example, various enzymes lead to phosphorylation, acetylation, glycosylation, ubiqutination, neddylation, sumoylation and methylation of p53, which are implicated in regulating the activity of p53. These specific posttranslational modifications would most likely change the recruitment of specific proteins, DNA binding or changes in compartmentalization of p53. The combination of specific modifications that are necessary to fine-tune p53 activity are still not well-defined. The signaling pathways that would activate specific enzymes to direct p53 to mediate cellular processes such as autophagy is important to understand, since manipulating these enzymes pharmacologically would be of therapeutic value.

A recent paper by Naidu et al. examines how post-translational modifications to p53 direct its activity to promote autophagy.[1] Since post-translational modifications to p53 in the C terminus (ubiquitin and sumoylation) have been implicated in redistribution of p53 to the cytoplasm,[2] the authors rationalized that post-transcriptional activity of p53 in the cytoplasm would be a key event in regulating autophagy. They show that in order for p53 to mediate autophagy sumoylation at K386 and acetylation of lysine 120 are necessary for facilitating the conversion of LC3, a key protein involved in autophagy. TIP60 can acetylate p53 at lysine 120, which is necessary for induction of the *p21* gene and the pro-apoptotic gene, *puma*.[3] Recent work by Lin et al. showed that TIP60 was necessary for the induction of autophagy,[4] which is also reported by Naidu et al. herein.[1] Collectively, Naidu et al.'s studies show that PIASγ can modify and activate TIP60, resulting in TIP60 and PIASγ post-translational modifications to p53 that redirects its activity to induce autophagy.

In light of these new findings, some additional biochemical questions remain, including how the regulator of p53, Mdm2, is involved in this pathway. Interestingly, Mdm2 forms a complex with, and is acetylated and sumoylated, by TIP60 and PIASγ, respectively.[5,6] Both modifications independently inactivate the ubiquitin ligase activity of Mdm2. Since TIP60/PIASγ regulate p53 and TIP60/PIASγ can regulate Mdm2, this suggests that Mdm2 may be serving as a scaffold to mediate these modifications to p53. It would be interesting to determine if Mdm2 can facilitate these modifications, as Mdm2 has been reported to have cellular-suppressor activity.[7] Additionally, considering that the Mdm2 family member Mdmx is found with p53 in the cytoplasm,[8] Mdmx may also be playing a role in regulating p53-mediated autophagy. Further work is also needed to establish a biochemical understanding of the events necessary to direct p53 for induction of autophagy and if preventing the p53-Mdm2 or p53-Mdmx complex using small-molecule inhibitors would promote or halt the induction of autophagy.

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Doubling the deck: Tetraploidy induces chromosome shuffling and cancer

Comment on: Lv L, et al. Cell Cycle 2012; 11:2864–75; PMID:22801546; http://dx.doi.org/10.4161/cc.21196

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Aneuploidy is ubiquitous in cancer and has been causally linked to tumorigenesis.[1] Although many decades of intense research have provided invaluable information on the causes and consequences of aneuploidy (reviewed in ref. 1), many questions still remain. For example, it is still unclear whether the degree of aneuploidy observed in cancer is the result of multiple subsequent losses/gains of one to few chromosomes, or if it is the result of an initial tetraploidization event followed by chromosome loss/gain events.[2] Furthermore, although tetraploidy has been observed in certain pre-cancerous lesions, thus making it a potential tumor promoter, it is not clear how tetraploidy affects aneuploidization and tumorigenesis.[1] In a recent study, Lv et al. began to provide answers to these questions by characterizing the karyotypes and cell division defects of mouse ovarian surface epithelial (MOSE) cells, which spontaneously transform after in vitro passaging over time.[3] Lv, et, al. showed that the rate of cytokinesis failure in MOSE cells increases with passage number, yielding more tetraploid cells.[3] These tetraploid cells continue to proliferate, but they display higher rates of chromosome mis-segregation compared to their diploid progenitors, leading to the generation of numerous aneuploid daughter cells.[3] Recent studies showing that clustering of supernumerary centrosomes is a major cause of chromosome mis-segregation[4-6] support the findings of Lv et al. To investigate how the observed cellular events affect tumorigenesis, Lv et al. injected late-passage (p35) MOSE cells into syngenic mice and saw tumors in 100% of mice.[3] The rapid transformation of MOSE cells with the concurrent generation of aneuploidy underscores the importance of aneuploidy in tumorigenesis. Indeed, it may be more appropriate to describe the transformation of MOSE cells as aneuploidy-induced instead of spontaneous, since passaging of chromosomally stable immortalized cells for a similar amount of time does not result in tumorigenesis.[6] Similar to the study by Lv et al., previous work in mouse mammary epithelial cells showed the same sequence of events: cytokinesis failure-tetraploidy-aneuploidy-tumorigenesis.[7] However, unlike the work by Lv et al., this sequence of events in mouse mammary epithelial cells relied upon p53 mutation or loss.[7]

It is unclear whether or not this is true for the MOSE cells used by Lv et al. Preliminary microarray data indicate a decrease in p53 expression levels in MOSE cells during progression (Schmelz, personal communication), but loss of p53 in MOSE cells has not been reported to date. Thus, it is unlikely that p53 was spontaneously lost in the Lv et al. and other independent studies using MOSE cells,[8] adding to the controversy of a "tetraploidy checkpoint."[9] While more work may be needed to understand whether p53 plays any role in modulating a response to tetraploidy and in the MOSE cancer progression model, it is clear from the work by Lv et al. that tetraploidy can occur early in tumorigenesis, act as an intermediate for aneuploidization and, ultimately, cause cancer (**Fig. 1**).[3]

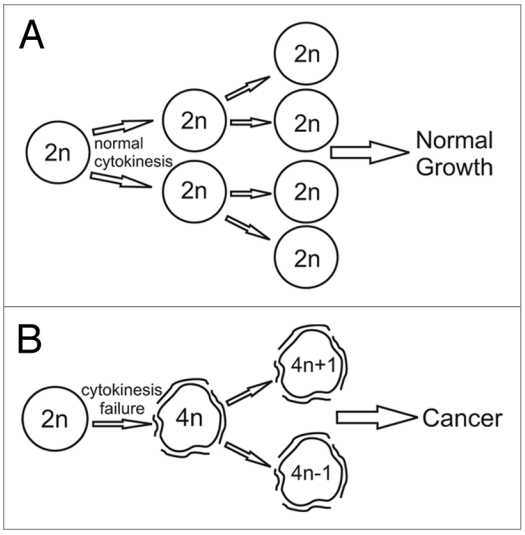

Figure 1. Tetraploidy can act as an intermediate for aneuploidization and cause cancer. Cells undergoing normal cytokinesis (A) maintain a stable karyotype and exhibit normal growth. Cytokinesis failure (B) generates tetraploid cells whose karyotype becomes unstable due to high rates of chromosome segregation errors. Such instability leads to high rates of aneuploidy and tumorigenesis.

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Switch of FANCL, a key FA-BRCA component, between tumor suppressor and promoter by alternative splicing

Comment on: Panneerselvam J, et al. Cell Cycle 2012; 11:2947–55; PMID:22828653; http://dx.doi.org/10.4161/cc.21400

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Fanconi anemia (FA) is a rare inherited disease. Besides anemia and other symptoms, the patients also show a high cancer penetrance, due to a defect in one of the genes that repair DNA crosslink, and, thus, constitute a potent tumor-suppressive pathway. These genes include *BRCA2*, also called FA-BRCA pathway, of which the *FANCL* is a key member, and the catalytic subunit of a protein complex of E3 ubiquitin ligase. Deficiency in crosslink repairs also makes cancer cells in FA patients supersensitive to crosslink-causing agents, such as the chemotherapeutic drug cisplatin.

A paper in the August 1, 2012 issue[1] from Fei's lab, together with a previous one,[2] shows that a shorter alternative splice variant of FANCL, dubbed FAVL, is highly expressed in sporadic bladder cancer as an oncoprotein capable of promoting cancer formation and rendering cells resistant to cisplatin. Restated, while the long form of FANCL, considered as the wild-type (wt), is a tumor suppressor, the short one (FAVL) resembles an oncogene, somewhat opposite to the case of *Bcl-x* wherein the long splice form (Bcl-xL) is oncogenic, but the short splice form (Bcl-xS) is tumor-suppressive. Hence, in bladder cancer, the FA-BRCA-suppressive pathway can be flipped to an oncogenic one simply by alternative splicing of a key component. This exciting finding raises an intriguing question as to whether a similar flip, which fortunately may be reversible and manageable, occurs also in other types of sporadic cancer. Relevant studies in sporadic malignancies have hitherto been focused on the *BRCA1* and *BRCA2* in some reproductive cancers. Fei's study incites us to interrogate *FANCL* and other FA members in other types of sporadic malignancy, not only for mechanistic insights, but also for therapeutic purposes, because tumors with a normal or abnormal FA-BRCA-suppressive pathway may respond differently to crosslink-causing drugs, as seen in bladder cancer cells.

Besides *Bcl-x* and *FANCL*, many other genes can also be switched between an oncogenic and a suppressive status to meet cells' needs in different situations. The switches can occur via many reversible mechanisms including alternative splicing, alternative transcription or translation initiation, etc., or via irreversible

mechanisms like mutation or partial deletion (**Fig. 1**). Some genes, like *c-myc*,[3] may not be switched often, because their wt is versatile, e.g., can cause proliferation or cell death, although some of their variants or mutants pro-and-con proliferation or apoptosis.

Nobody was born as a good or bad person; environment makes what we are, basically. Similarly, none of our genes initially favors or disfavors cancer. In other words, there probably is no such thing called tumor suppressor gene or oncogene, although such dichotomy of cancer-related genes has helped in delineating our research. For instance, although *p53* represents tumor suppressor genes and k-*ras* represents oncogenes, they are actually much alike: overexpression of their wt form often causes cell death,[4] but overexpression of their mutants promotes cancer formation. *Ras*-induced cell death is a paradigm of oncogene-induced senescence.[5] *p53* was initially classified as an oncogene for its high level in cancer, but later reclassified as a suppressor, because the alleles in cancer were found to be mutants. Probably the reclassification is unnecessary, since some of its 30,000 mutants[6] and some of its normal variants derived from alternative splicing or transcription initiation[7] are oncogenic. Another suppressor gene, *Rb1*, which sometimes also helps cell proliferation or survival, is overexpressed or amplified in some cancers and induces some tumors in animals,[8] although whether some of its 932 mutants[9] are oncogenic is understudied. Why do cancer cells not simply delete the *p53* or *Rb1* (which occurs only at low frequency), but instead undergo complicated mutations? Probably, switching these genes to a less suppressive or more oncogenic form helps their survival, since genes are actually more multi-faceted dice than two-sided coins, and cells holding more of those switches live better. This "switch" hypothesis, with the FANCL-FAVL flip as a paradigm, inspires us to conceptualize, and thus act, differently. For instance, those of us who consider *p53* also an oncogene may use knockout models more cautiously because of a greater concern of going too far from the reality where most cancers do not lack the whole gene, but instead, highly express (mutated) *p53*.

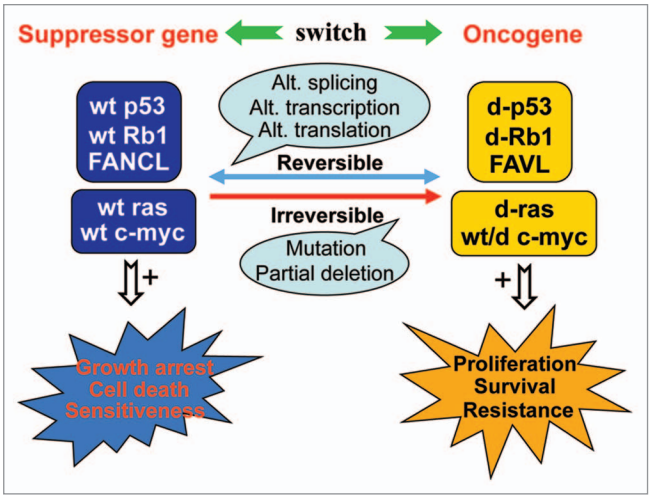

Figure 1. The wild-type (wt) of tumor suppressor genes exemplified by p53, Rb1 and FANCL can be switched to a different (d) form with oncogenic features, whereas the wt of canonical oncogenes, exemplified by k-*ras* and c-*myc*, are versatile or can be switched to a form with suppressive traits. The switch may occur via reversible mechanisms such as alternative (Alt.) transcription, splicing or translation, or via irreversible mechanisms such as mutation or partial deletion. Tumor-suppressive functions are typically manifested as enhanced (+) growth arrest, cell death or sensitivity to cancer treatments, whereas oncogenic traits include increased (+) proliferation, survival or resistance to therapies.

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New evidence that SAC can tolerate misaligned chromosomes in mouse oocytes

Comment on: Sebestova J, et al. Cell Cycle 2012; 11:3011–8; PMID:22871737; http://dx.doi.org/10.4161/cc.21398

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Spindle assembly checkpoint (SAC) is a biochemical pathway that monitors the attachment state of kinetochores and delays anaphase onset until all kinetochores are attached to spindle microtubules (**Fig. 1**).[1] Thus, SAC is crucial for proper segregation of chromosomes during both mitosis and meiosis. However, it is well established that chromosome segregation in female meiosis I is error-prone and is the major cause of miscarriages, birth defects, infertility and genetic disorders in humans.[2] These findings raised the possibility that the stringency of SAC may be reduced during female meiosis I.[3-5] Sebestova et al. now provide new evidence that SAC can tolerate misaligned chromosomes in mouse oocytes.[6]

Sebestova et al. used elegant confocal live-cell imaging experiments to monitor chromosome movements, spindle assembly, APC activity and polar body extrusion in mouse

oocytes with high rate of aneuploidy. The authors took advantage of the fact that oocytes isolated from aged mice and hybrid oocytes (*Mus musculus* x *Mus spretus*) exhibit high rates of chromosomal abnormalities. A series of careful analyses led to several important observations. First, they found that the presence of univalent chromosomes does not delay the onset of anaphase I in oocytes isolated from aged mice.[6] Importantly, in the presence of nocodazole, which depolymerizes microtubules and induces SAC-dependent arrest, oocytes arrested in meiosis I, indicating that the SAC was functional. This suggests that the stringency of SAC is reduced during female meiosis I or, alternatively, that univalents are able to satisfy the SAC by forming bi-polar (amphitelic or merotelic) attachments.[7-9]

Sebestova et al. further noticed that oocytes isolated from aged mice and hybrid oocytes often (up to 85%) contained unaligned chromosomes (these are chromosomes that presumably failed to establish proper microtubule-kinetochore attachments) at the time of anaphase I onset, as indicated by securin degradation. In fact, about 50% of DNA was positioned outside of the central quarter of the spindle shortly before the onset of anaphase I in hybrid oocytes.[6] These are significant observations, because the importance of the alignment of chromosomes at the equatorial plane for satisfying the SAC is a matter of debate. Although there are claims that alignment of all chromosomes on the equatorial plane of the spindle is required for satisfying the SAC, other results suggest that the SAC is not sensitive to the position of a chromosome on the spindle.[1]

Since the production of normal offspring requires accurate chromosome segregation, it is difficult to understand why female meiosis is unable to efficiently detect and correct aberrant chromosome behaviors. This topic will undoubtedly remain the focus of many future studies. It will also be important to establish to what extent the weakening of the SAC with age contributes to maternal age-related aneuploidy.[2]

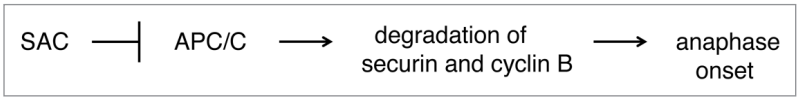

Figure 1. Schematic of the spindle assembly checkpoint pathway. The spindle assembly checkpoint (SAC) controls cell cycle progression during mitosis and meiosis. The anaphase-promoting complex/cyclosome (APC/C) promotes anaphase onset by targeting cyclin B and securin for degradation by the proteasome. If chromosomes are not attached to microtubules, then the SAC inhibits the APC/C and the cell delays anaphase onset to provide more time for proper attachment of kinetochores to microtubules.

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