Cell Cycle 12:7, 1018–1021; April 1, 2013; © 2013 Landes Bioscience

Cell Cycle News & Views

Vitamin D and cancer

Comment on: Ma Y, et al. Cell Cycle 2013; 12:743–52; PMID:23388458; http://dx.doi.org/10.4161/cc.23846

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Already in the eighties it was shown that the active form of vitamin D_3, 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$], can inhibit the proliferation of melanoma cells[1] and stimulate the differentiation of myeloid leukemia cells.[2] The almost universal presence of the vitamin D receptor (VDR) and the presence of 1α-hydroxylase (CYP27B1) activity in non-classical tissues together with the antiproliferative and prodifferentiating effects suggests a paracrine role for 1,25$(OH)_2D_3$. 1,25$(OH)_2D_3$ directly regulates the expression of a whole set of genes through binding to the VDR which heterodimerizes to the retinoid X receptor (RXR), and 1,25$(OH)_2D_3$-VDR/RXR complexes bind to vitamin D response elements in the promoter region of target genes. In most cancer cell types that express a functional VDR, exposure to 1,25$(OH)_2D_3$ results in the accumulation of cells in the G_0/G_1 phase of the cell cycle. This effect is not due to one single gene or a single pathway but depend on a multiple pathways among which epidermal growth factor, insulin like growth factor, transforming growth factor, prostaglandins and Wnt-β-catenin signaling cascades.[3] In $VDR^{-/-}$ mice there is no apparent increase in spontaneous cancer but these mice have an increased rate of proliferation of colonic, prostate and breast cells.[4] If $VDR^{-/-}$ mice are challenged with carcinogenic agents the incidence of skin tumors increase or more pre-neoplastic lesions in the mammary glands are present. Mouse mammary tumor virus (MMTV)-neu mice on VDR heterozygous background also show accelerated mammary tumorigenesis compared with MMTV-neu mice on VDR wild type background.[5] $VDR^{-/-}$ mice crossed with APC heterozygous mice ($Apc^{-/+}$), develop more colonic aberrant crypt foci.[6] Similarly $VDR^{-/-}$ mice develop more skin tumors when exposed to UV-B. These data suggest that vitamin D deficiency may be a predisposing environmental factor for cancer. Cross-sectional and especially prospective studies indicate that a low vitamin D status (25-hydroxyvitamin D_3 levels) is associated with a higher risk for several types of cancer, particularly colorectal cancer.[6] Whether vitamin D supplements may decrease the risk of cancer (prevention) awaits the results of ongoing randomized controlled trials as the limited number of available studies are inconsistent. A different question is whether 1,25$(OH)_2D_3$ can be used to treat cancer but supraphysiologic doses are needed and leads to calcemic side effects. To overcome this problem analogs of the parent compound have been synthesized with a clear dissociation between antiproliferative and calcemic activity. One such promising superagonistic analog is the 14-epi analog inecalcitol being 10-fold more potent to inhibit the proliferation of breast cancer cells and 400 fold less calcemic than 1,25$(OH)_2D_3$.[7] Inecalcitol is 100 times more potent than 1,25$(OH)_2D_3$ to protect keratinocytes of UV-B induced damage. Trump et al. show in the present issue that inecalcitol is 30× more active to inhibit squamous cell carcinoma (SCC) proliferation and the induction of apoptosis by inecalcitol is much higher compared with 1,25$(OH)_2D_3$. The superagonistic action of inecalcitol correlates with its ability to induce coactivator-VDR interactions[7] and co-crystallization studies show that inecalcitol forms closer contact points with the human VDR-LBD.[7]

The potential clinical use of 1,25$(OH)_2D_3$ analogs such as inecalcitol as an anti-cancer drug has been demonstrated in in vivo animals models of breast cancer,[7] prostate cancer[8] and by the current study of Trump in SCC. Moreover a phase II study in patients with hormone-refractory prostate cancer demonstrated that 27 of the 31 patients treated with inecalcitol (at doses up to 600 μg/day) and Taxotere during 18 weeks showed a decrease in prostate specific antigen levels of more than 30% within 3 m of initiation of treatment without any changes in calcium parameters.[7] Although these preliminary results look promising more clinical trials are needed to evaluate 1,25$(OH)_2D_3$ and its analogs as compounds that prevent and/or delay cancer progression. As can be expected from nearly all anticancer drugs combination therapies are more likely to generate long-term effects than single therapy.

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Aurora B: Hooking up with cyclin-dependent kinases

Comment on: Trakala M, et al. Cell Cycle 2013; 12; In this issue;
PMID:23428904; http://dx.doi.org/10.4161/cc.24004

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Aurora B (Aurkb) is one of the major protein kinases that ensures the proper execution and fidelity of mitosis.[1] A member of the chromosomal passenger complex, Aurkb has been implicated in various mitotic functions, including chromosome-microtubule interactions, sister chromatid cohesion, the spindle-assembly checkpoint and cytokinesis.[2] As it is upregulated in several human cancers and correlated with poor prognosis, Aurkb is believed to be an important anti-cancer drug target. In this connection, a number of small-molecule inhibitors have been developed and are currently at various stages of clinical trials.[3] Therefore the effects of Aurkb inactivation is of considerable interest.

In this issue of *Cell Cycle*, Malumbres and colleagues examined the effects of Aurkb inactivation in mouse cells.[4] As genetic ablation of Aurkb results in mitotic aberrations and lethality after implantation in mice,[5] the authors made use of conditional knockout mouse embryonic fibroblasts and chemical inhibition to tackle the issue. These tantalizing results establish a linkage between Aurkb and another major cell cycle regulator, cyclin-dependent kinase, through the CDK inhibitor p21*Cip1/Waf1*.

Trakala et al. discovered that although mitotic entry is unaffected in Aurkb-deficient MEFs, the majority of the cells are unable to form a metaphase plate and exit mitosis prematurely. Moreover, these Aurkb-deficient MEFs also exit Taxol-mediated mitotic block precociously. One of the characteristic features of premature mitotic exit is the formation of polyploid cells, which can lead to cell death or genome instability in the subsequent division cycles. This may in part contribute to the increase in tumor incidence in Aurkb heterozygous mice.[5] Premature mitotic exit in mammalian cells typically involves mitotic slippage, which is caused by the gradual destruction of cyclin B1 during the mitotic arrest.[6] Intriguingly, the premature mitotic exit in the absence of Aurkb is associated with high expression of cyclin B1, suggesting that the process may resemble adaptation instead of classic slippage.

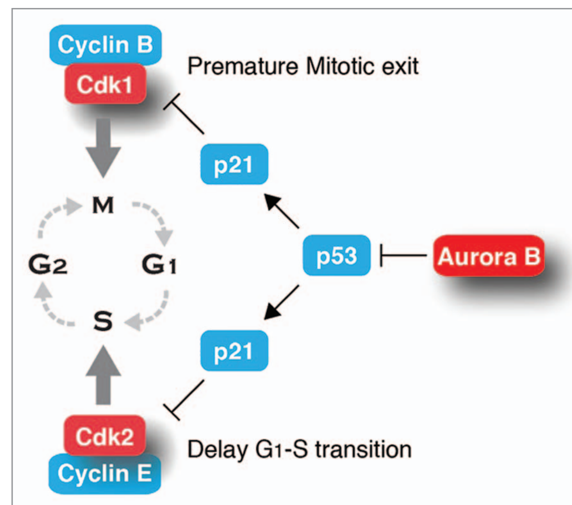

Figure 1. A model of links between Aurkb and cyclin-dependent kinases.

The observation that the CDK inhibitor p21*Cip1/Waf1* is induced after Aurkb inactivation provides a possible mechanistic basis of the premature mitotic exit.[4] Indeed, downregulation of p21*Cip1/Waf1* reverses the unscheduled mitotic exit. Yet a conceptual obstacle for p21*Cip1/Waf1* in causing premature mitotic exit is that the canonical p21*Cip1/Waf1* pathway is well established to be involved in interphase arrest (such as after DNA damage). To extricate from this problem, Trakala et al. proposes that the p21*Cip1/Waf1* induced after Aurkb inactivation is at a level that is insufficient to prevent mitotic entry but result in premature exit due to partial inhibition of Cdk1.

A critical question is why inhibition of Aurkb leads to an accumulation of p21*Cip1/Waf1*. Aurkb has been shown to phosphorylate p53 and downregulate its transactivation activity and protein stability.[7,8] Hence inhibition of Aurkb is expected to activates p53 and its downstream targets such as p21*Cip1/Waf1*. In addition, it is conceivable that the mitotic stress induced after Aurkb inhibition can also lead to p53 activation.

A somewhat unanticipated result of Trakala et al. is the pronounced effect of Aurkb-deficiency on interphase. Entry into S-phase from quiescence is delayed in Aurkb-deficient MEFs or after treatment with the Aurora kinase inhibitor ZM447439. A reduction of Aurkb in heterozygous mice also delays cell cycle entry after partial hepatectomy or skin wound healing. Although not tested directly, the accumulation of p21*Cip1/Waf1* after Aurkb inhibition may blunt the activity of Cdk2, thereby causing the observed delay in G_1-S transition. However, as Aurkb is degraded at the end of mitosis by APC/C-mediated ubiquitination, one has to speculate that the Aurkb present during interphase is adequate to suppress the accumulation of p53 and p21*Cip1/Waf1* during normal cell cycle.

A recurring theme in the regulation of mitosis is the inextricable links between the key players. Although the biological significance of the regulation of p53-p21*Cip1/Waf1* axis by Aurkb remains to be defined, these observations suggest the possibility of a novel mechanism that regulates CDKs in both mitosis and interphase. In addition to the effects on the cell cycle, other consequences of p53 activation after the Aurkb inactivation will also be interesting for further investigation. Finally, whether a similar mechanism is present in cancer cells, and whether the loss of p53 confers different sensitivity to Aurkb inhibitors may have important implications in cancer therapies.

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Potentially novel options for treatment of HPV-attributable head and neck cancer

Comment on: Li C, et al. Cell Cycle 2013; 12:923–34; PMID:23421999; http://dx.doi.org/10.4161/cc.23882

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Squamous cell carcinomas of the head and neck originate in the mucosal linings of the oral cavity, the oropharynx, the larynx and hypopharynx. Most patients present with advanced stages of disease and are treated by surgery with postoperative (chemo) radiotherapy or chemoradiation, the combination of systemic cisplatin with concomitant locoregional radiotherapy, sometimes followed by salvage surgery. Patients who are unfit to receive chemoradiation are treated with a combination of cetuximab, an antibody inhibiting the EGF receptor, combined with locoregional radiotherapy, also called bioradiation.[1]

Since last decades it has been shown that human papillomaviruses (HPV), known as the cause of cervical cancer, also cause a subgroup of head and neck cancers, most particularly those arising in the oropharynx. The proportion of HPV-attributable disease varies over the world, but appears to increase rapidly. In the USA and Canada the proportion of HPV+ve tumors is between 50 and 70%,[2,3] and in Europe usually somewhat less (30%). However, both the proportion and incidence rates of HPV+ve tumors are increasing.[4] In the Netherlands the proportion increased from 5% in 1990 to 30% in 2010.[5]

Tumors caused by HPV infection form a different disease entity. They are different at the molecular and clinical level. HPV+ve tumors have a very favorable prognosis when compared with HPV–ve tumors. In fact the predominant factor that predicts prognosis of oropharyngeal cancer is the presence of HPV.[2,3] The prognosis is so favorable that two studies have been initiated to de-intensify therapy: the RTOG1016 trial in the USA and the De-ESCALaTE HPV study in Europe. In both studies patients with HPV+ve tumors are randomized between a chemoradiation arm and a bioradiation arm, with the aim to reduce toxicity in the bioradiation arm while maintaining favorable prognosis. However, it could be questioned whether cetuximab is the optimal substitute of cisplatin for HPV+ve tumors. In this issue Li and Johnson[6] provide data that there might be other options at the horizon, also less toxic than cisplatin and more targeted to HPV.

The HPV genome encodes two viral oncoproteins named E6 and E7. The virus uses these proteins to create an S-phase environment in the host cell to allow viral replication using the host cell DNA replication machinery. The E6 protein binds and inactivates p53 and the E7 protein the pocket proteins pRb, p107 and p130, key proteins in the regulation of the G_1 and G_2 cell cycle checkpoints. These host proteins are subsequently ubiquinated and targeted for proteosomal degradation. Previously it has been shown that the knockdown of these E6/E7 genes inhibits proliferation of HPV-positive head and neck cancer cell lines.[7] In this issue Li and Johnson,[6] focusing on E6 and the p53 pathway, convincingly show that the cell cycle arrest and apoptosis caused by E6(/E7) knockdown is p53 and p21 mediated, as expected. Intriguingly, they also showed that bortezomib, a drug that inhibits proteosomal protein degradation and approved for treatment of multiple myeloma, was able to liberate and restore p53 and p21 expression specifically in HPV+ve cell lines, also causing cell death. This would suggest that bortezomib might be an interesting alternative for cisplatin in the combination with radiotherapy for HPV+ve tumors. Bortezomib is considerably less toxic than cisplatin.

Obviously a lot of additional work needs to be done before considering a clinical study. First, all data reported by Li and Johnson were collected by in vitro experiments. Therapy experiments in mouse models of xenografted cell lines or even better, human-in-mouse tumor models,[8] are required. In addition, other questions remain. Although the authors convincingly showed that p53 and p21 are upregulated by bortezomib in HPV+ve cell lines and cause apoptosis, the HPV+ve cell lines are not more sensitive to bortezomib than the HPV-ve cell lines. That is somewhat unexpected. Moreover, one of the HPV+ve cell lines showed a G_1 arrest and the other two a G_2 arrest after bortezomib treatment. Although p53 and p21 play a role in both checkpoints, the nature of this difference should be solved, and also whether it is relevant in the context of irradiation. Bortezomib-based treatment protocols would include radiotherapy. Notwithstanding, irrespective of these considerations that clearly deserve attention, the observation is very interesting and should be further evaluated.

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One among many: ODF2 isoform 9, a.k.a. Cenexin-1, is required for ciliogenesis

Comment on: Chang J, et al. Cell Cycle 2013; 12:655–62; PMID:23343771; http://dx.doi.org/10.4161/cc.23585

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An elegant study from the Kyung Lee laboratory[1] resolves the confusion over the role of the centrosome protein "human outer dense fiber protein 2" (hOdf2) vs. its splice variant, Cenexin-1 (Odf2 isoform 9), in the assembly of centriolar appendages and primary cilia. Previous studies suggested that these polypeptides had overlapping or distinct functions in ciliogenesis,[1] but the different isoforms led to uncertainty about this claim. The Lee group provides solid data to demonstrate that Cenexin-1 but not Odf2 is required for these functions.

Primary cilia are microtubule-based sensory organelles projecting from the surface of most cells. They assemble from the centrosome, more specifically from the mother centriole or basal body of the centrosome. This centriole contains specialized substructures called appendages that are lacking in the daughter centriole. Subdistal appendages appear to position the basal body at the cell cortex through contact with the microtubule cytoskeleton, whereas the distal appendages have been proposed to anchor the basal body to the plasma membrane.[2] Chang et al., 2013 used an invaluable tool established by Ishikawa et al.—an Odf2/cenexin-1 null cell line ($Odf2^{-/-}$)[3] that lacks both appendage types and cannot make primary cilia.[1,3]

The authors use this cell line for complementation experiments designed to test whether expression of either hOdf2 or hCenexin-1[1] rescues the $Odf2^{-/-}$ phenotypes. The outcome of this experiment was difficult to predict because hOdf2 was primarily characterized in testes, where it played a role in sperm outer dense fiber component required for sperm tail function.[4] On the other hand, hCenexin-1 was not examined in testes but was found to be the major *ODF2* isoform in somatic cells, where its unique C-terminal extension was required for recruiting Plk1 during mitosis.[5] A clue to their cilia functions was suggested by the localization of hCenexin-1 to the mother centriole, the site of cilia formation, and the localization of hOdf2 along the entire ciliary axoneme.[1]

Results from the $Odf2^{-/-}$ complementation experiments show that hCenexin1 expression rescues subdistal appendage formation, whereas expression of hOdf2 does not. The *ODF2* splice variant, hCenexin1, is able to rescue cilia formation. Other less direct data consistent with a role for cenexin1 in cilia formation is its interaction with Rab8 through its C-terminal extension, which is lacking in hOdf2. This may be an important interaction, as Rab8 is required for membrane trafficking during ciliogenesis. Another result suggests that hCenexin1 is required for localizing Chibby, an essential cilia component, to mother centrioles.

This study redefines what was previously thought to be cooperative or distinct roles of hOdf2 and hCenexin-1 in the formation of centriolar appendages and cilia. The work shows that hCenexin-1 alone performs these functions arguing for hOdf2 function to be revisited, possibly through the use of the robust tools exploited in this study.[1] It will also be of great interest to gain a better understanding of the molecular mechanism of hCenexin-1 control over centriolar appendage organization and how this, in turn, influences ciliogenesis. This will likely involve structural roles such as building appendages and anchoring microtubules, as well as molecular roles in binding to Rab8 and localization of Chibby to centrioles. In this regard, the C-terminal extension of hCenexin-1 is required for both mother-centriole-specific-localization of the protein and for binding the activated form of the small GTPase, Rab8.[1]

Other work on Rab8 as well as Rab11 suggests interesting GTPase control mechanisms for cilia formation. For example, a Rab11-Rab8 GTPase cascade has been proposed for primary ciliogenesis.[6] Moreover, Rab8 associated with recycling endosomes localizes to the basal bodies of the growing primary cilium where it is thought to participate in ciliary vesicle formation.[6] In addition, Rab11 (and possibly Rab8) associated with recycling endosomes localize specifically to the appendages of the mother centriole.[7] These intriguing observations lead us to speculate that the mother centriole appendages, and more specifically, the Rab8-binding C-terminal extension of cenexin-1 at these appendages, may facilitate organization of the Rab11-Rab8 GTPase cascade at these sites for initiating ciliogenesis and the formation of the ciliary vesicle.

On a related topic, the ODF2 gene is required to establish planar cell polarity and basal foot formation at cilia.[8] It is unclear if it is the hOdf2 isoform, the hCenexin1 isoform, or other Odf2 spice variants that are required for these cellular functions. At this juncture, the best candidate for initiating and regulating planar cell polarity is hCenexin-1 since exogenously expressed hCenexin1 localizes to mother centriole appendages and contributes to microtubule organization.

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