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Cell Cycle News & Views

KAPping PML nuclear body number

Comment on: Kepkay R, et al. Cell Cycle 2011; 10:308–22

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The mammalian nucleus is organized into multiple subdomains. The Promyelocytic Leukemia Nuclear Body (PML-NB) is believed to occupy interchromatic space and to associate with the nuclear matrix.[1,2] There are usually 10–30 PML-NBs in the mammalian nucleus, but their number and size varies depending on the cell type and cell cycle distribution.[1,2] A large number of nuclear proteins, with functions spanning from tumor suppression to catabolism,[1,2] have been shown to associate with this structure, either stably or transiently. The PML-NB is named after PML, a growth and tumor suppressor originally identified at the t(15;17) translocation of acute promyelocytic leukemia.[1,2] PML is the essential constituent of PML-NB, as its loss results in delocalization of all its known components. PML functional inactivation in APL has been proposed to contribute to tumor development.[2] However, PML's role is not confined to hematopoietic cancers, as its loss promotes disease progression in solid tumors, such as lung and prostate carcinomas.[2] Several studies have proposed that some PML functions rely on the integrity of this nuclear structure, but a functional coincidence between these two entities is still a subject of intense discussion in the PML field. In this respect, a number of studies have proposed that PML may also operate outside the PML-NB.[2]

PML-NB dynamics and mechanisms underlying its formation have been under intense scrutiny in the last few years. Recent work from de The's group has demonstrated that this subnuclear structure is sensitive to intracellular redox conditions and that its formation relies on intermolecular disulfide bonds.[3] However, oxidation-dependent PML aggregation has its own downturn, as it promotes PML SUMOylation and subsequent degradation, thus highlighting the complex dynamics regulating this nuclear substructure. This represents a change in paradigm and may open a completely new field of research into the interplay between redox signaling, formation of intranuclear subdomains and tumor suppression.

A recent study by Dellaire and collaborators published in *Cell Cycle*[4] focuses on mechanisms controlling PML nuclear body number upon DNA damage and their relationship with chromatin regulators. This is an extremely interesting area of investigation. The authors show that DNA damage results in increased PML-NB number (approximately 1.4–1.5-fold). This effect occurs concomitantly with ATM activation and KAP1 phosphorylation. KAP1 is a co-repressor involved in the control of chromatin modification/dynamics, which is tightly regulated via ATM-dependent phosphorylation at serine 842.5 Interestingly, KAP1 knockdown in undamaged cells results in similar increase in PML-NB number, suggesting that ATM inhibits KAP1-dependent suppression of PML-NB number. Furthermore, KAP1 downregulation causes reduction in chromatin density and altered heterochromatin organization. Finally, loss of KAP1 led to overall increase in acetylation of histone H4, suggesting that this could be involved in regulation of PML-NB number. Subsequent experiments demonstrate that KAP1 knockdown-dependent increase in PML-NB number is only modestly augmented by irradiation. Introduction of RNAi-insensitive wt KAP1 into KAP1 knockdown cells restores basal PML-NB number and rescues the response to DNA damage. Instead, the non-phosphorylatable S842A mutant has a blunted effect. Thus, phosphorylation of KAP1 at S842 appears to inhibit its function in regulating PML-NB number. Interestingly, the phosphomimetic S842D mutant is partially impaired in restoring normal PML-NB number, suggesting a gain-of-function effect.

This work poses a number of questions, some of which are discussed by the authors. First, why does body number matter? The authors hypothesize that number and/or

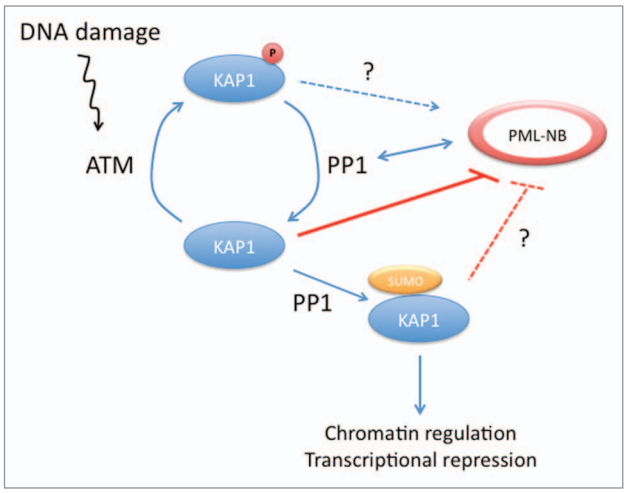

Figure 1. KAP1-dependent regulation of PML-NBs. KAP1 is phosphorylated by ATM at serine 842 upon DNA damage. The PP1 phosphatase dephosphorylates KAP1 and also associates with PML-NBs. Dephosphorylation of KAP1 results in its SUMOylation, thus promoting its co-repressive function. Unphosphorylated KAP1 inhibits PML-NB number. It is also possible that phosphorylated KAP1 could gain a PML-NB-inducing function. It is presently unclear (1) whether SUMOylated KAP1 affects PML-NB formation and (2) if PML-NB/PP1 interaction has any effect on KAP1 dephosphorylation and subsequent SUMOylation.

composition of PML-NBs can be used to modulate the "intensity" of DNA damage signaling. As the composition of PML-NBs is not altered upon irradiation (presented as data not shown), how would increased PML-NB number affect signaling? The authors propose that the "state-of-subdivision" of PML-NBs may be proportional to the total surface area available for interactions with signaling components and their subsequent post-translational modifications. As the authors also recognise, this is rather speculative model that require further testing/investigation. What remains to be established is how small changes in PML-NB number could affect DNA damage signaling. Furthermore, it is key to determine which signaling components are affected by this increase in PML-NB number.

Another important question is whether other posttranslational modifications of KAP1 regulate PML-NB number. In this respect, KAP1 regulates its own SUMOylation by acting as an E3 ligase through the PHD domain.[6] Interestingly, this activity is modulated by KAP1 interaction with PP1beta, which promotes SUMOylation of KAP1 upon dephosphorylation of S842.5 It would be worth investigating whether this modification could affect KAP1 role in regulation of PML-NBs. Interestingly, KAP1 forms subnuclear structures that are found adjacent to PML-NBs,[7] and PAROT, a KAP1-interacting protein, associates with PML-NBs,[8] thus suggesting a potential transient contact between KAP1 nuclear bodies and PML-NBs. Interestingly, PP1alpha, which dephosphorylates KAP1, has been proposed to functionally interact with PML,[9,10] thus suggesting that PML-dependent PP1 regulation may influence PML-NB formation via KAP1.

Finally, it would be worth investigating whether KAP1, as inhibitor of PML-NB formation, is able to modulate PML growth/tumor suppressive functions. A recent study by Ferbeyre and collaborators has further highlighted that PML-NB regulation could play an important role during the transition from preneoplastic lesions to malignant tumors.[10] Does KAP1 regulate PML-NB number during neoplastic transformation?

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Unleash the wild type: Restoration of p53 suppressive activity in skin cancer

Comment on: Bao MC, et al. Cell Cycle 2011; 10:301–07

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In a previous issue of *Cell Cycle* Bao et al. extended our perception of wild-type p53 restoration in tumor cells.[1] In what could be a ground breaking work, the authors showed that PRIMA-1^{met}, a drug thought to be exclusively efficient for mutant p53 bearing tumors, might also be valid for treatment of tumors which inactivate wild-type p53 in a non-genetic manner.

p53 is one of the most potent tumor-suppressors, which carries out cell fate decisions such as cell cycle arrest and apoptosis thus inhibiting cellular transformation.[2] Hence, it's the most frequently mutated gene in cancers.[3] Accordingly, a variety of drugs such as low molecular weight compounds and short peptides were developed, aimed at restoring p53 wild-type activity by shifting the wild-type and mutant equilibrium towards the wild-type conformation. Such are the compound MIRA-14 and the short peptides CDB3 and CP-31398.[5-6] Since p53 tumor suppression activity cannot be tolerated by the developing tumor, the fact that half of human cancers express the wild-type form surmises that p53 inactivation is achieved via additional, non-genetic manipulations. Indeed, almost 20 years ago a seminal work performed by

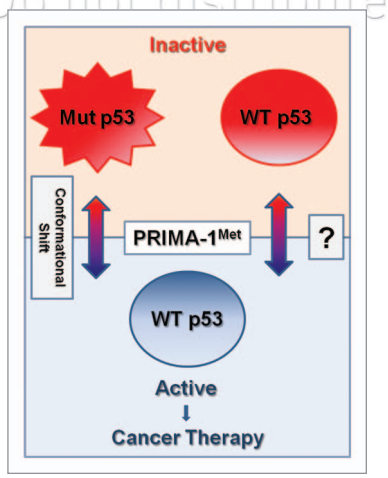

Figure 1. A schematic illustration representing the ability of PRIMA-1^{met} to restore p53 suppressive activity in tumors bearing p53 inactive forms.

Gannon and Lane predicted the presence of a short lived protein responsible for restricting p53 in the cytoplasm thus preventing its transcriptional activity.[7] Consequently, a second group of drugs has emerged, which employs a different strategy. Compounds such as RITA and Nutlin3A are designed to release wild-type p53 from the hold of its extinguisher, mdm2 thus stabilizing wild-type p53 and restoring its activity.[8-9]

In a previous study, Bao et al. demonstrated that wild-type p53 DNA binding activity is inactivated by integrin αVβ3 without any change in p53 level in a skin cancer model.[10] The authors decided to follow up on their initial observation and test PRIMA-1^{met}, a drug known to rescue mutant p53 function by restoring the wild-type p53 conformation,[11] on wild-type, p53-bearing tumor cells. For that purpose, a 3D-collagen gel model that mimics the pathophysiological microenvironment of malignant melanoma in the dermis was utilized. Indeed, three different cell lines which express wild-type, yet inactive, p53 became apoptotic upon PRIMA-1^{met} treatment. This outcome was reversed when p53 was depleted using p53-shRNA. Furthermore, p53- induced apoptosis was mediated by trans-activation of PUMA, a bone-fide target of p53 during apoptosis, suggesting that p53 transcriptional activity was restored. In addition, casapase 9 cleavage was evident indicating that a mitochondrial apoptosis pathway was initiated. To substantiate their in vitro findings, the authors utilized an

in vivo xenografts model and found that PRIMA-1^{met} inhibited the growth of wild-type but not the truncated or depleted p53-expressing tumors. Measuring the expression of p53 pro-apoptotic targets in the dissected tumors corroborated the in vitro findings; namely, p53 exhibited typical trans-activation activity in the onset of apoptosis.

The findings presented herein, suggest that both wild-type and mutant p53- bearing tumors might respond to PRIMA-1^{met} treatment, thus extending the spectrum of this drug in terms of tumor stage and type (**Fig. 1**). Therefore, this study invokes a new concept in which drugs may target both the mutant and the inactive wild-type forms of p53. Moreover, it is tantalizing to speculate that additional drugs, which alter the conformation of other tumor-suppressor mutant forms at large, will yield similar results. Future studies will decipher the detailed mechanism by which PRIMA-1^{met} restores wild-type p53 activity.

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Linking oncogenes to invasion in thyroid cancer

Comment on: Nowicki TS, et al. Cell Cycle 2011; 10:100–7

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Thyroid cancer prognosis, like that of many solid tumors, is predicted by the presence or absence of distant metastasis, gross local invasion, tumor size, patient age and tumor histology.[1] Local invasion is a particularly vexing issue for patients with large papillary thyroid cancers, as grossly invasive tumors can be difficult to treat and are associated with an aggressive course. Moreover, it has been recognized increasingly that radioactive iodine therapy, the most frequently used systemic treatment for recurrent or residual thyroid cancer following initial surgery, is often ineffective when the residual cancer invades muscle or trachea. Aggressive completion surgery and external beam radiation therapies are associated with difficult side effects and are often not curative. For these reasons, there has been interest in defining the mechanisms by which locally aggressive papillary thyroid cancers progress to enable more effective initial and salvage therapies.

Over the past several years, a series of studies have examined, either globally or in a targeted manner, signaling pathways that may be involved in thyroid cancer progression. A number of investigators have demonstrated, for example, that PTCs with activating mutations in *BRAF* have a greater predilection for local invasion and aggressive tumor behavior (reviewed in ref. 2). This feature of *BRAF* mutation-positive thyroid cells has been confirmed using elegant in vitro and in vivo model systems.[3,4] Moreover, an important role for late stage activation of phosphoinositide 3 (OH) kinase signaling by gain of mutations or gene amplification of signaling molecules and other mechanisms has been recognized to be important in the process of invasion and dedifferentiation for thyroid cancers.[5,6] The precise mechanisms for these associations had not been clearly delineated; however, a series of recent studies are beginning to shed light on these data creating a conceptual framework for how thyroid cancers progress in different tissue niches.

Using a global gene expression approach, Vasko et al., identified a gene signature for the invasive fronts of a small group of T4 grossly invasive PTCs that was consistent with epithelial to mesenchymal transition (EMT), with particular representation of integrin and TGF beta signaling.[7] This work was consistent with data suggesting a role for matrix metalloproteinases in *BRAF* V600E induced EMT in vitro.[4] Additional studies have demonstrated functional roles for TGF β,[8] focal adhesion kinase (FAK),[9] fibronectin regulators,[10] thrombospondin-1[11] and p21 activated kinase[12] in thyroid cancer invasion and progression. Together, these papers and many others have created a molecular picture of local PTC invasion by which a program resulting in cell invasion through matrix degradation, stromal interactions and cell shape changes occurs at the invasive fronts. Whether this process is initiated and maintained by the cancer cells, by cells recruited to the microenvironment or by other stromal elements is uncertain. Moreover, how or if these pathways are directly coupled to initiating oncogenes such as *BRAF* V600E is not certain.

Nowicki et al. extend these observations by adding another upstream regulator to the story, the urokinase plasminogen activator receptor (uPAR).[13] uPAR is a multifunctional receptor that, when bound to urokinase plasminogen activator (uPA), results in matrix degradation and also binding to and activation of integrins enabling binding to extracellular matrix, cell morphology changes and proliferation.[14] Intriguingly, uPAR expression is induced by ERK signaling, suggesting it may link *BRAF* V600E to integrin pathway activation.[15] Nowicki et al. demonstrate in the *BRAF* V600E mutant-positive human PTC cell line BCPAP that uPAR loss using siRNA results in inhibition of cell invasion and motility, reduced FAK and Akt signaling, reduced proliferation and increased cell senescence.[13] While the data are limited to a single cell line and confirmatory in vitro as well as human tumor and in vivo studies are required, they provide important evidence of a potential direct mechanistic link between ERK, integrin and PI3K that can regulate thyroid cancer cell invasiveness.

Studies evaluating the mechanisms of local invasion in the thyroid/neck microenvironment are crucial in defining pathways that may be exploited for better treatment of locally aggressive thyroid cancers. How they relate to mechanisms involved in the development and progression of distant metastases, the most important determinant of thyroid cancer survival, is uncertain. Indeed, recent models have challenged the notion that an EMT is required for tumor metastases, and clinical data in thyroid cancer suggest that distant metastases can occur either early or late in the lifetime of an individual tumor.[16] Nonetheless, the ability of cancer cells to bind to and

degrade extracellular matrix likely is necessary for local progression whether the cancer cells are located in the primary tumor site or in metastatic niches.[17] Further studies linking thyroid cancer cells to matrix degradation and proliferation will likely shed important mechanistic light into how thyroid cancers survive, grow and progress in a variety of microenvironments, with potential to identify new therapeutic targets.

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SAHF, to senesce or not to senesce?

Comment on: Kosar M, et al. Cell Cycle 2011; 10:457–68

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Cellular senescence has been defined as a state of permanent replicative arrest.[1] It can be induced by short telomeres, in which case it is called replicative senescence, or it can be triggered prematurely, i.e., before telomeres become eroded, by DNA double strand break (DSB)-inducing agents, activated oncogenes and certain bacterial toxins. The ATM checkpoint kinase, a sensor of DNA DSBs, and the p53 tumor suppressor protein, a substrate of ATM, are central to induction of senescence. Short telomeres and the agents that induce premature senescence all activate the ATM-p53 pathway. Induction of senescence is also facilitated by enhanced expression of the cell cycle inhibitor p16INK4A (p16), which regulates the activity of cyclin-dependent kinase 4 (CDK4) and phosphorylation of the retinoblastoma (Rb) tumor suppressor protein.

Several markers of cellular senescence have been identified, including lack of 5-bromo-2′-deoxyuridine (BrdU) incorporation, senescence-associated beta-galactosidase activity (SA-β-gal), persistent DNA damage-induced nuclear foci and heterochromatin formation.[1] The latter, manifested as increased methylation of histone H3 on Lys9 (H3K9me) and formation of senescence-associated heterochromatin foci (SAHF), has been considered critical for induction of senescence, as it was postulated that heterochromatin formation was responsible for silencing genes that drive cell cycle progression.[2] Studies by Kosar et al., published in a recent issue of *Cell Cycle*, and by Di Micco et al. now challenge this notion.[3,4]

While the phenomenon of cellular senescence has been recognized for several decades now,[5] molecular insights are much more recent. A landmark study showed that the Ras oncogene induces senescence when expressed in human fibroblasts[6] and permitted the identification of molecular markers of senescence.[2] One of these markers was widespread heterochromatin formation in the nucleus of senescent cells. Heterochromatinization was evident by the presence of histone H3 methylated on Lys9 and, interestingly, also by the presence of nuclear foci, referred to as SAHF, that stained intensely with the DNA dye 4′,6′-diamidino-2-phenylindole (DAPI). Because of their apparent higher DNA content, SAHF are thought to correspond to condensed chromatin. In this first study showing chromatin compaction in senescent cells, the induction of SAHF was dependent on increased levels of p16. Since senescence is also dependent on p16, the logical conclusion was that heterochromatin facilitates induction of senescence. It was further suggested that heterochromatin was silencing E2F target genes.[2] However, the evidence linking the SAHF to gene silencing was mostly correlative.

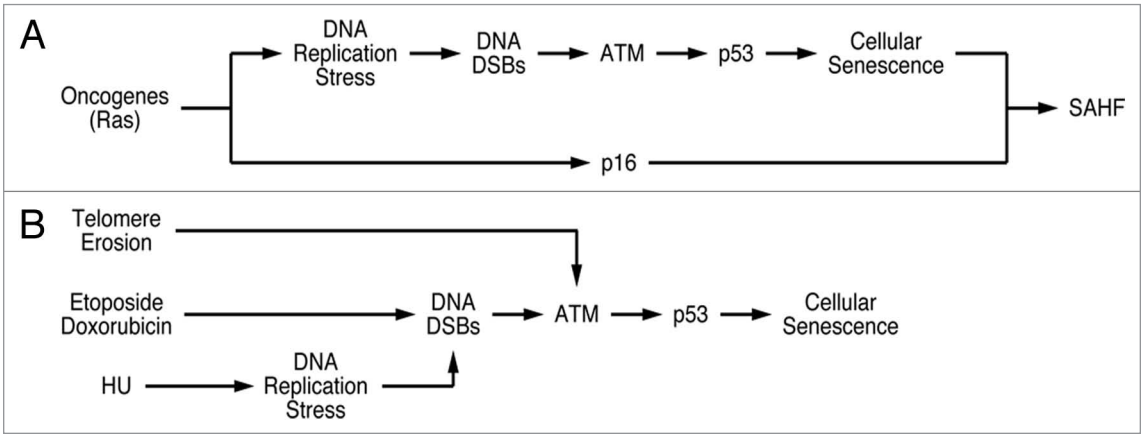

Figure 1. Two pathways leading to senescence. (A) Oncogene-induced senescence is associated with induction of p16 and formation of SAHF. (B) Induction of senescence by telomere erosion or DNA damaging agents is not accompanied by p16 induction and SAHF. HU, hydroxyurea.

Kosar et al. and Di Micco et al. examined for the presence of senescence markers, including SAHF, in various cells undergoing replicative or premature senescence.[3,4] Whereas SAHF were evident in cells that became senescent in response to Ras, cells that became senescent in response to telomere erosion, DNA damage or bacterial toxins did not have SAHF. However, all types of senescent cells shared the other markers of senescence, such as cell cycle arrest, SA-β-gal and DNA damage-induced foci. Consistent with the original study linking increased heterochromatin formation to p16 levels, only the cells that became senescent in response to Ras had high levels of p16.

Based on what we already knew about induction of cellular senescence[1] and on the results of the new studies, one can propose two variant pathways leading to cellular senescence. In the first variant, exemplified by Ras as the inducing agent, oncogene-induced DNA replication stress leads to formation of DNA DSBs, activation of ATM and p53 and induction of senescence. In parallel, through poorly understood mechanisms, Ras leads also to increased expression of p16, which in the context of cellular senescence, induces heterochomatin formation and SAHF (**Fig. 1A**). In the second variant, telomere erosion and DNA damaging agents lead to senescence in the absence of p16 induction and in this case, cellular senescence is not associated with SAHF (**Fig. 1B**).

Cellular senescence is more than an in vitro curiosity. Senescent cells have been observed during aging,[7] and oncogene-induced senescence is present in human precancerous lesions, where it serves as a barrier to cancer development.[8,9] In human precancerous lesions, senescence is associated with heterochromatic histone modifications, such as histone H3 Lys9 methylation, and with increased levels of the heterochromatic protein HP1γ.[3,10] However, Kosar et al. could not observe SAHF in these lesions.[3] This apparent discrepancy may reflect variations in the degree of heterochromatinization present in senescent cells in vitro and in vivo. In vitro, chromatin compaction may be more extensive than it is in vivo, leading to formation of SAHF.

After an eight year marriage, the apparent separation between heterochromatin formation and senescence leaves open a number of questions. What is the mechanism by which expression of cell cycle genes is repressed in senescent cells? Previously, the repression was attributed to heterochromatin formation and the SAHF,[2] but the ability of cells to senesce without heterochromatin induction implicates a different mechanism. Second, what is the significance of heterochromatin formation in oncogene-induced senescence and what is the molecular mechanism leading to SAHF? The results of Kosar et al. are consistent with the earlier findings of Narita et al. in implicating p16 in heterochromatin induction,[2,3] yet we still need a much greater mechanistic understanding of this biological pathway that impacts important processes, such as cancer development and aging.

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A Janus molecule provides a rational reshape to a multi-functional drug

Comment on: Donia M, et al. Cell Cycle 2011; 10:492–9

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Since the discovery of the endogenous nitric oxide (NO) metabolic pathway, it was evident that, besides its fundamental function on cardiovascular homeostasis, NO was also playing a primary role in the host defense mechanisms against infective diseases and cancer. Because of its radical reactivity with strong pro-oxidant effect, most of the initial studies evaluated its role as a final effector molecule. However, evidence accumulated during the last decade has unveiled the primary role that NO plays also as an intra- and inter-cellular messenger for the triggering and regulation of the defense mechanism against cancer,[1] leading to several attempts aimed at designing novel anti-cancer drugs with increased therapeutic proficiency.[2]

The recent report by Donia et al.,[3] besides offering relevant insights into the molecular mechanisms by which NO modulates tumor cell response, also provides a very promising applicative approach. A novel drug candidate retaining a saquinavir-like protease inhibitory effect while harboring a NO-releasing moiety has demonstrated an innovative pharmacological profile not only in vitro but also in in vivo models of prostate cancer. Importantly, it is worth noticing that the mentioned data[3] refers to a p53-deficient, hormone-resistant prostate cancer, which better reflects the hormone-refractory prostate cancer phenotype to which most of patients progress following the first approach of palliative treatment based on androgen deprivation. In agreement with previous reports showing powerful and pleiotropic effects against multidrug-resistant cancer cells[4] as well as in two rodent models of melanoma,[5,6] data presented by Donia et al. further encourage considering Saq-NO as a plausible novel anti-cancer drug.

Though this is not the first work describing mechanisms involving NO in cancer biology,[1] nor the first proposing a dual action anti-cancer compound based on NO,[2] the data by Donia et al. uncovers a novel p53- and YY1-independent mechanism based on a TRAIL and DR5 upregulation which reinforces the relevance of NO in prostate cancer[7] and further completes the overall picture supporting the rational for designing novel anti-cancer drugs based on NO release for treating the hormone-refractory stage of prostate cancer.

Curiously, in apparent contradiction with Donia's data, a previous work by Nanni et al.[8] using androgen sensitive prostate cancer demonstrated strong positive correlation between the aggressive phenotype observed in clinical prostate cancer and the eNOS expression and translocation to the nucleus in association with estrogen receptor β and hypoxia inducible factor. Furthermore, they observed that inhibition of eNOS was able to significantly inhibit cancer cell proliferation, leading to a less aggressive cancer phenotype, and therefore encouraged strategies based on eNOS inhibition to treat prostate cancer.

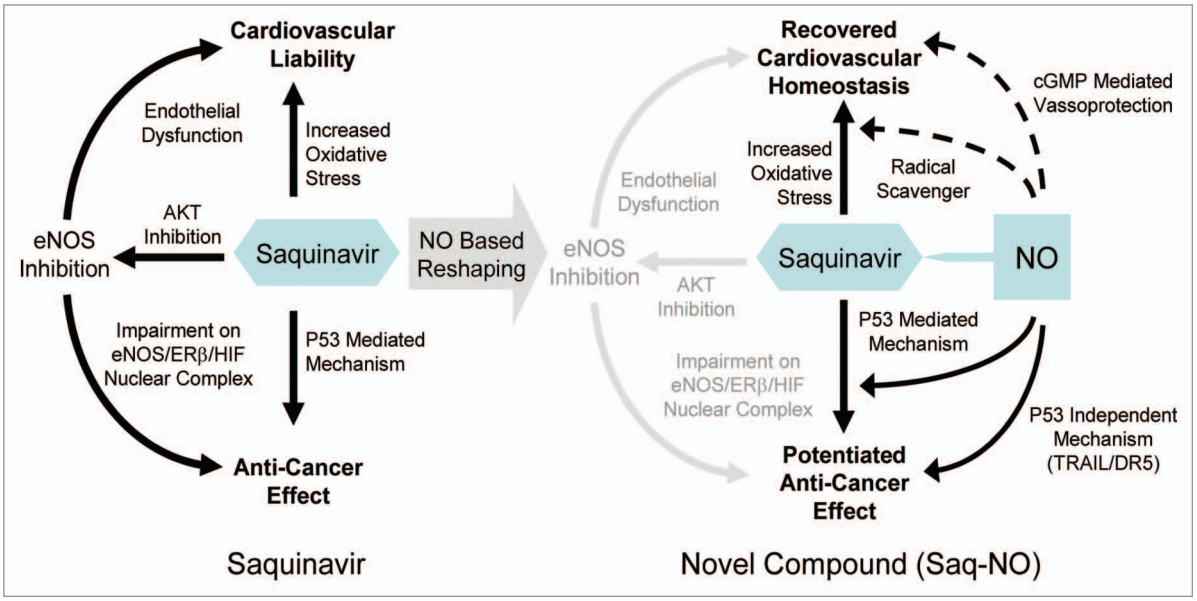

Figure 1. General scheme illustrating the NO-mediated shift in the molecular mechanisms conferring an improved therapeutic proficiency to a novel saquinavir-related compound.

Though a definitive explanation to reconcile these apparently opposite findings may require further investigations, it is important to underline that higher NOS expression and/or NOS activity does not necessarily translate into higher NO availability. A major distinction must be made between NO and other highly reactive radical species produced under specific compartment/micro-environmental conditions of oxidative stress and eNOS uncoupling.[9] Under certain circumstances, despite the increased eNOS expression, high oxidative stress resulting in low NO availability, might benefit from a moderate donation of exogenous NO aimed at reestablishing cell/tissue homeostasis.

In this regard, going back to Donia's paper, the novel compound retaining saquinavir-like activity while releasing moderate amounts of NO seems indeed a very promising approach. Whereas saquinavir, interferes with eNOS activity leading to endothelial dysfunction,[10] the novel dual-action compound, instead, provides exogenous NO. Such a peculiarity not only helps to explain a superior pharmacological antitumor effect, but it also sheds light on the observed advantageous safety profile.[5]

Two main objectives are envisioned when designing novel dual action molecules harboring NO releasing moieties. Firstly, improve the therapeutic benefit by associating two different but synergizing pharmacological actions. Secondly, counteract parent drug-related adverse side effects. Once some of saquinavirs most important adverse side effect have been definitively related to eNOS inhibition, meaning cardiovascular liability,[10] it is indeed reasonable to expect that concomitant exogenous NO support could counteract saquinavir-related toxicity.[5]

In our opinion this is a very promising, valuable approach deserving to be further exploited, not only to better understand the role of NO and eNOS in cancer biology but also for implementing more efficient and less toxic therapies for prostate cancer.

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