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Yin Yang 1 as a prognostic factor

In this issue, Castellano et al.[1] focus on the clinical relevance of Yin Yang 1 (YY1) overexpression in various types of cancer. YY1 is a ubiquitously expressed transcription factor that acts as an activator for some genes and a repressor for others, depending on the promoter sequences surrounding the YY1 binding sites and its interactions with other regulatory proteins.[2] In addition to playing a regulatory role in normal biological processes, several strands of evidence indicate that YY1 expression and activation is associated with tumorigenesis. The putative role of YY1 in tumorigenesis is also supported by its known interaction with cell cycle regulation.[3] Castellano et al.,[1] by computational analysis, demonstrate that YY1 is expressed at different transcript levels in several types of cancer, including melanoma, prostate, ovarian, breast, colon, liver and lung cancer, leukemia and Hodgkin's and non-Hodgkin's lymphoma. Using the proprietary Ingenuity Pathway Analysis software, the authors indicate that YY1 may be involved in B cell transformation, which gives rise to high-grade lymphomas, and may also be implicated in the regulation of the cell cycle.[1] Furthermore, YY1 transcript levels were significantly higher in cancer tissue compared to relative normal tissue for each tumor type.[1]

The clinical relevance of YY1 overexpression in the cancer types is summarized by Castellano et al.[1] YY1 overexpression was found to be associated with an increased malignant phenotype and poor outcome in breast cancer, cervical neoplasea, osteosarcoma, myeloid leukemia and Hodgkin's and non-Hodgkin's lymphoma. In contrast, higher YY1 protein levels were associated with a favorable prognosis in prostate and ovarian cancers. Overall, these findings confirm the contradictory role played by YY1 in cancer biology as a transcriptional activator and repressor.[2] Additionally, the potential utility of YY1 in cancer therapy is demonstrated, as the inhibition of YY1 induced the chemosensitization of tumor cells.[4] Consequently, YY1 appears to be a prognostic factor for several tumors, as well as an important therapeutic target.

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Apostolos Zaravinos and Demetrios A. Spandidos; Laboratory of Clinical Virology; Faculty of Medicine; University of Crete; Heraklion, Crete, Greece

BRAF and RKIP aberrations in actinic keratosis and non-melanoma skin cancers

This issue of *Cell Cycle* presents an important article on the mutation and expression status of the genes BRAF and RKIP in pre-cancerous lesions, such as actinic keratosis (AK) and non-melanoma skin tumors such as cutaneous squamous cell carcinoma (SCC). In their article entitled "BRAF and RKIP are significantly decreased in cutaneous squamous cell carcinoma," Zaravinos et al.[1] indicated that BRAF does not appear to be frequently mutated in AK and SCC. BRAF is a kinase that activates the RAF/MEK/ERK signal transduction cascade and gene mutations, especially the T to A missense transversion at nucleotide 1799 (leading to a V600E amino acid change in the BRAF protein), thereby causing constitutive activation of the BRAF kinase activity, independently of RAS activation, by converting BRAF into a dominant transforming protein.[2] Thus, BRAF gene mutations have been proposed to contribute to cancer development.[2] T1799A mutations have been observed in 80% of the malignant melanoma tumors and cell lines. Although, we have previously investigated the incidence of BRAF gene mutations within exons 11 and 15 in basal cell carcinoma (BCC), no mutations were detected.[4] The results of the study of Zaravinos et al.,[1] in combination with ours, confirm that BRAF is mutated exclusively in melanoma, but not in non-melanoma skin tumors or pre-cancerous lesions. Moreover, Zaravinos et al.[1] showed that BRAF and RKIP exhibit decreased mRNA expression levels in SCC, compared to the adjacent normal skin tissue.[1] Intriguingly, the two genes were found to be negatively correlated in SCC. The reduction of RKIP mRNA levels in tumors and in metastasis indicates that this gene may be useful as prognostic marker and target for therapeutic treatment in cutaneous SCC.

RKIP blocks the RAF-induced phosphorylation of MEK via direct interaction with RAF-1 kinase, and consequently, the activation of ERK.[3] It also has a weak binding affinity to MEK-1 and ERK-2, interfering with downstream phosphorylation events. In addition to its modulation of RAF signaling, RKIP inhibits NFκB activity by interacting with upstream NFκB activators such as the NFκB-inducing kinase (NIK) and TGF-B-activated kinase 1 (TAK1). Another study showed a decrease of RKIP expression in malignant melanoma and the absence of RKIP expression in melanoma metastases.[5] Zaravinos et al.[1] suggested that in the case of cutaneous lesions, RKIP follows a similar pattern in that the normal skin tissue and pre-cancerous skin lesions, such as AK, express the highest levels of RKIP. Moreover, the cancerous tissue (SCC and/or BCC) expresses significantly reduced mRNA levels. Consequently, its expression is diminished in metastatic melanoma.[5]

In conclusion, these new findings expand the understanding of the BRAF and RKIP aberrations in actinic keratosis and non-melanoma skin cancers, and suggest that RKIP is a potentially useful prognostic marker and potential target for therapeutic interventions of SCC.

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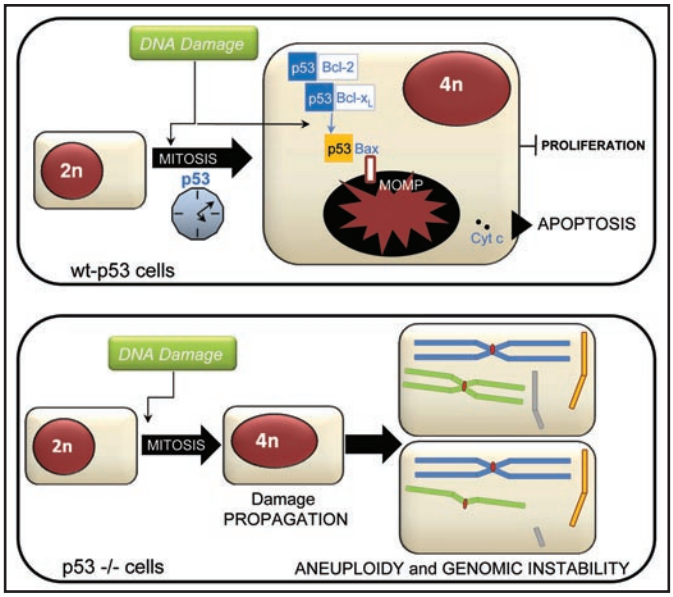
Massimo Libra and Elena Torrisi: Department of Biomedical Sciences; University of Catania; Catania, Italy; Email address: mlibra@unict.it

Tetraploidy triggers mithocondria

Since 1991, when p53 was discovered to promote apoptosis, an unexpected and unarrested flow of findings depicting the molecular details and the fine tuning of this process has taken place.[1] Soon after, p53 was shown to be pivotal in the execution of apoptosis induced by many of the commonly used anticancer therapies; loss of wt-p53 was reported to be a key determinant of chemo-resistance that often leads to cancer relapse.[2] Indeed, given its broad-range roles in apoptosis, as well as in growth arrest, genomic stability, cell senescence and differentiation, p53 has been often identified as potential attractive target for cancer pharmacological intervention.

Most of the work devoted to p53 and apoptosis has been performed in cancer cells. In this issue of *Cell Cycle*, work performed by Kroemer's group elegantly and convincingly shows that p53 integrity is essential in keeping properly the DNA content of a given normal cell. Indeed, mouse mammary epithelial cells derived from $p53^{-/-}$ mice exhibit features of tetraploidy and centrosomes enrichment when compared to their wt-p53 counterparts. The idea that p53 loss may represent a permissive input to polyploidization fits the hypothesis identifying in p53 a master sensor of mitotic clock.[3] If mitosis is abnormally prolonged in normal p53-proficient cells upon stress, p53



Tetraploidy triggers mithochondria. Wild type p53 triggers mitochondria-dependent apoptosis of tetraploid cells, whereas its loss represents a permissive input to aneuploidy and genomic instability onset and propagation.

accumulates to drive cell cycle arrest at mitotic exit and to prevent tetraploidy. Cell division failure that produces genetically unstable tetraploid cells would otherwise predispose normal cells to transformation, by facilitating the development of aneuploid malignancies in absence of p53.[4] Molecular effectors controlling the duration of mitosis seem crucial in cell fate determination. Mitotic arrest culminates in mutually exclusive outcomes regulated by opposite mechanisms, likely driven by mitotic inhibition of transcription. Following mitotic slippage, transcription resumes and drives cells to diverse destinations: aneuploidy, cell senescence or cell death.[5]

Enhanced resistance to apoptosis in damaged cells is a master feature underlying tumorigenesis. Work from Kroemer's group pictures a link between death susceptibility and polyploidization in p53-proficient cells. Loss of p53 renders tetraploid mouse mammary epithelial cells less prone to die in response to stress than their diploid precursors. Unlike their p53$^{-/-}$ counterparts, normal cells display early mitochondria apoptotic features as soon as they turn into tetraploid.

It is well established that the pro-apoptotic functions of p53 are not restricted to its nuclear transcriptional activity but also rely on its localization in the cytoplasm. Post-translational modifications of p53 may be involved in its subcellular distribution. Phosphorylation on serine 15 upon cell damage leads to stabilization and enhancement of p53 transcriptional activity.[6] Notably, serine 15 is also located within the N-terminal nuclear export signal of p53. Either loss or altered regulation of p53 phosphorylation may result in enhanced nuclear export and subsequent cytoplasmic accumulation.[7] Outside from the nucleus, p53 is sequestered by Bcl-2 and Bcl-XL, but its pro-apoptotic functionality can be released by BH-3 proteins, such as PUMA, in response to DNA damaging stimuli. This results in the activation of either Bax or Bak and eliciting of the mitochondria death machinery.[8] The ratio of either Bcl-XL/PUMA or Bcl-XL/p53 in the cytosol may therefore be a pivotal outcome of p53 pro-apoptotic effects.

Acute tetraploidization may mimic a severe damage. In p53 proficient cells, tetraploidy activates p53 and drives it to ignite mitochondria outer membrane permeabilization (MOMP), concomitant cytochrome c release and downstream caspase activation.[9] Suppression of apoptosis by inhibition of MOMP in p53 deficient cells appears to be permissive for survival and propagation of tetraploid cells. MOMP indeed has been reported to be specifically disabled in cancer cells. Hence, pharmacological agents that target mitochondria to subvert oncogenic MOMP inhibition or directly induce MOMP are being evaluated as therapeutic approaches for cancer treatment.[10]

All these findings highlight a critical role of wt-p53 in opposing cell transformation since its early genetic abnormalities. It appears that p53 might sense mitotic duration and guardian cell genome by preserving its integrity at different stages along the process of tumorigenesis. If so, the timing, the molecular targets and the subcellular localization of p53-mediated apoptosis might be closely related to the specific tumor type. This might contribute to explain why p53 inactivation occurs at different stages along specific processes of tumorigenesis. Accordingly to the data shown by Kroemer's group, it is reasonable to speculate that aberrant ploidy might contribute to generate selective pressure for p53 mutation. Increasing polyploidy in transformed cells can be considered a stress effector from which a complex signaling network, that includes cell cycle kinases, DNA-damage response proteins, stress kinases, is turned on.[11] Does such chain of events take place in primary tetraploid cells? For instance, we have learned that p53 family members such as p73 and p63 are capable to recapitulate most of wt-p53 activities when over-expressed in p53-null tumor cells. Here the absence of wt-p53 activity emerges to be so detrimental for acquiring tetraploid features that other p53 family members might be unable to exert any effect in avoiding or reducing the formation of a tetraploid sub-population. Additional knowledge calls for further experimental evidence.

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Barbara Benassi, Sabrina Strano and Giovanni Blandino; Regina Elena Cancer Institute; Via Elio Chianesi 53; 00144 Rome, Italy; Email: blandino@ifo.it or gblandino@activep53.eu

Joint surveillance of the replication foci by PCNA and CtIP

CtIP (CtBP-interacting protein) is emerging as a key new player in the maintenance of genome stability. CtIP was discovered as a cofactor of the ubiquitous transcriptional corepressor CtBP.[1] By virtue of its association with CtBP, it is presumed to function in transcriptional regulation. CtIP also interacts with the pRb family proteins and BRCA1 and has been linked to the transcriptional repression activities mediated by these tumor suppressors. While interaction of CtIP with pRb appears to be important for G_1/S regulation,[2] CtIP activity is also required for G_2/M DNA damage checkpoint via transient association with BRCA1 and Chk1 activation during treatment with ionizing radiation.[3] Recently, orthologs of CtIP that participate in DNA double strand repair have been identified in yeast, fungi, worm and plants.[4-7] Specifically, CtIP and its orthologs have been shown to be critical for dsDNA break repair by homologous recombination. These orthologs share a distinct region of homology with the C-terminal region of CtIP. CtIP and its orthologs have been shown to associate with

the Mre11/Rad50/NSB1 (MRN) complex and mediate DNA end resection to generate ssDNA intermediates for recombination. While CtIP orthologs appear to function primarily in DNA repair, the vertebrate CtIP appear to be a multifunctional modular adapter protein recruiting multiple protein complexes to mediate concerted cell cycle regulation and DNA damage checkpoint control and DNA repair.

In the current issue of *Cell Cycle*, Gu and Chen[8] report that CtIP is targeted to DNA replication foci by direct interaction with PCNA. The interaction between PCNA and CtIP is mediated by a 42-amino acid region of CtIP known as Replication Foci Targeting Sequence (RFTS) that contains a consensus PCNA-interacting motif.[9] Endogenous CtIP and that expressed by transient transfection colocalized with PCNA in replication foci and mutations in the RFTS abolished such colocalization. Interfering with the interaction between CtIP and PCNA via overexpression of an RFTS-GFP chimeric construct (dominant negative) induced cell cycle arrest at S/G_2 transition and cessation of cell proliferation. Cells expressing the chimeric RFTS construct induced DNA damage and activation of DNA damage checkpoint while a mutant RFTS construct defective in interaction with PCNA did not. These results suggest that the CtIP-PCNA complex might play a role in stabilization of the stalled replication forks. Since CtIP plays a role in DNA repair, it is possible that the normal function of the PCNA-CtIP complex might be involved in surveillance of the replication forks for potential stalling. Considering the established role of CtIP in DNA repair and the present results that PCNA directs CtIP to the replication foci, new investigations on the role CtIP in repair of DNA damage at the replication fork should be forthcoming.

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G. Chinnadurai; Institute for Molecular Virology; Saint Louis University Medical Center; St. Louis, MO USA; Email: chinnag@slu.edu

Oxygen availability sHIFts the cell cycle

The report by Hackenback et al. addresses the interface between cell cycle control and one of the key external requirements for normal cell function—oxygen. They have explored the effect of activating Hypoxia-Inducible Factor (HIF), a specific transcriptional response to low oxygen, on cell cycle progression. Using an elegant system to express constitutively active versions of HIF-1α and HIF-2α under conditions of normal oxygenation, they establish that either of these is sufficient to arrest NIH3T3 cells in G_1.

Oxygen matters because it is necessary for mitochondrial respiration, and in its absence the ability to generate ATP is massively reduced. Oxygen is also necessary for many other enzymatic reactions in the cell. Monooxygenases, which transfer one oxygen atom to the substrate and usually one to water, and dioxygenases, which transfer both oxygen atoms to substrates, are responsible for a very wide range of metabolic reactions. The K_m for oxygen of these reactions is generally substantially higher than that of cytochrome c oxidase so that as oxygenation is reduced their reaction rates will change before respiration is compromised. Examples of oxygenases include the extensive cytochrome P450 family, and notable pathways requiring oxygen include cholesterol synthesis and collagen assembly. Thus oxygen is necessary for many aspects of normal cellular function. Because its diffusion is limited, maintaining oxygenation at the level of cells, tissues and organisms is a fundamental biological challenge. Our experience as humans can lead us to assume that the outcome of reducing oxygen is binary; below a critical level irreversible harm and death will occur rapidly, while above this level variations are irrelevant. But what actually happens at a cellular level is that variations in oxygen are continuously monitored across the physiological range, and this information is used to adjust a broad range of aspects of cellular behavior including glucose uptake, glycolysis, mitochondrial respiration and production of angiogenic growth factors. This is achieved largely through the HIF transcription control system—a remarkable piece of molecular engineering in which the regulatory α subunit (HIF-1α or HIF-2α) is hydroxylated by specific dioxygenases, the prolyl hydroxylase domain enzymes (PHD1-3) and Factor Inhibiting HIF (FIH-1).[1] The prolyl hydroxylation provides a signal for their capture by the von Hippel Lindau protein (VHL), leading to ubiquitylation and destruction. The overall result is that the activity of HIF increases progressively as oxygenation falls, and this activates gene expression by interacting with hypoxia responsive elements in the vicinity of specific genes. There is also complex interplay beetween HIF and other transcription control systems—including MYC, Notch and p53.[2-4]

Given its central role in metabolism it is hardly surprising that the cell cycle responds to changes in oxygenation. Preventing cell cycle progression at lower oxygenation will immediately reduce energy expenditure, may avoid conditions in which key processes could become more error prone, and from a longer term perspective reduces the biomass requiring oxygen and metabolic substrates. However, the literature describing effects on the cell cycle of altering oxygenation or manipulating oxygen-response pathways is hard to synthesize, presumably because these manoeuvres have direct and indirect effects on a number of signalling pathways, and in turn these interface with different aspects of cell cycle control.

Hackenback et al. have deconvoluted this complexity by testing whether switching on an active HIF molecule without altering oxygen supply is sufficient to influence cell cycle control. They used HIFα molecules which were mutated at the three known oxygen acceptor sites, making them active regardless of cellular oxygenation. The answer is clear—active HIF1α or HIF2α has a major impact on cell cycle progression. One reason why this is important is that hypoxia and HIF activation are a striking feature of most solid tumors. Understanding how HIF influences the cell cycle will give insight into selection mechanisms that operate during the evolution from normal cells to cancer. This is especially the case in the kidney where the commonest form of cancer usually shows constitutive activation of HIF through loss of function of VHL.[5] An interesting aspect of the Hackenback et al. study is that the effects of HIF-1α and HIF-2α in this setting were equivalent, although it is clear they have biologically distinct roles and there are differences in their downstream effects in many settings.[6] A second reason for the effect of HIF on the cell cycle being important is that small molecules which activate HIF are being pursued as potential strategies for treating anaemia and ischaemic conditions, and this knowledge is likely to be important in anticipating undesirable on-target effects.

Notably, this study examines the effect of dialling HIF straight to a maximal "ON" signal. In real life HIF provides a very sensitive analogue signal, which has important effects on cell behavior even in normally oxygenated cells and tissues, and is progressively activated as oxygenation is reduced. Understanding how this analogue signal interfaces with cell cycle control in different cells will be a challenge. From a systems perspective it is an interesting paradigm in which complex analogue signals are synthesized to provide a critical binary outcome.

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Patrick H. Maxwell; Rayne Institute; University College London; 5 University Street; London, UK; Email: p.maxwell@ucl.ac.uk