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REVIEW

DNA damage signalling guards against activated oncogenes and tumour progression

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DNA damage response (DDR), the guardian of genomic integrity, emerges as an oncogene-inducible biological barrier against progression of cancer beyond its early stages. Recent evidence from both cell culture and animal models as well as analyses of clinical specimens show that activation of numerous oncogenes and loss of some tumour suppressors result in DNA replication stress and DNA damage that alarm the cellular DDR machinery, a multifaceted response orchestrated by the ATR–Chk1 and ATM–Chk2 kinase signalling pathways. Such activation of the DDR network leads to cellular senescence or death of oncogene-transformed cells, resulting in delay or prevention of tumorigenesis. At the same time, the ongoing chronic DDR activation creates selective pressure that eventually favours outgrowth of malignant clones with genetic or epigenetic defects in the genome maintenance machinery, such as aberrations in the ATM–Chk2–p53 cascade and other DDR components. Furthermore, the executive DDR machinery is shared by at least two anticancer barriers, as both the oncogene-induced DNA replication stress and telomere shortening impact the cell fate decisions through convergence on DNA damage signalling. In this study, we highlight recent advances in this rapidly evolving area of cancer research, with particular emphasis on mechanistic insights, emerging issues of special conceptual significance and discussion of major remaining challenges and implications of the concept of DDR as a tumorigenesis barrier for experimental and clinical oncology.
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Introduction

Maintenance of genomic integrity is one of the fundamental features of life. The genome surveillance machinery evolved face to face with diverse genotoxic insults both from the environment, including ultraviolet or ionizing radiation and various chemicals, and from inside our cells, such as reactive oxygen species (ROS) produced during normal metabolic processes. The complex cellular network that collectively forms the DNA damage response (DDR) machinery encompasses a plethora of dynamic, hierarchically ordered and mutually coordinated pathways capable of detecting the DNA lesions and signalling their presence to many DDR proteins and protein complexes that promptly repair the damaged DNA (Hoeijmakers, 2001; Kastan and Bartek, 2004; Bartek and Lukas, 2007). When a cell encounters damage that is more difficult to repair, the DDR machinery delays cell-cycle progression (the so-called checkpoints) to provide more time for repair of the lesions (Lukas *et al*., 2004; Bartek and Lukas, 2007). Even failing to repair the damage does not usually result into fixation of potentially deleterious mutations, as such genetically altered cells are commonly eliminated from the proliferative pool. This can be accomplished through inducing either a permanent proliferative arrest known as cellular senescence or physical elimination of the potentially hazardous, genetically unstable cells through one of the several forms of cell death (Campisi and d'Adda di Fagagna, 2007; Finkel *et al*., 2007; Vousden and Lane, 2007).

The biological significance of such genome surveillance is perhaps best apparent from the important roles the DDR plays during development and tissue homeostasis, as aberrations in the DDR machinery may lead to developmental defects or genetic diseases including severe immunodeficiency and neurodegenerative syndromes, premature ageing as well as cancer. While the link between defective DDR and predisposition to cancer has been known for decades (Hoeijmakers, 2001; Shiloh, 2003), and numerous components of the DDR network including p53, BRCA1 or BRCA2 (breast cancer 1 or 2), for example, qualify as tumour suppressors (Kastan and Bartek, 2004; Finkel *et al*., 2007; Vousden and Lane, 2007), recent work uncovered a critical role of the DDR machinery as a candidate physiological barrier that guards against progression of tumours from their early stages into overtly malignant, invasive lesions (Gorgoulis *et al*., 2005; Bartkova *et al*., 2005a). It is this emerging role of the DDR as an anticancer barrier that we wish to highlight in this review article, focusing on conceptual aspects of these recent discoveries.

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DNA damage response as an anticancer barrier: initial observations and concept formulation

The idea that mammalian cells can respond to oncogene-mediated transformation by permanently blocking their own proliferation, or inducing cell death has long been appreciated, and so has the notion that the p53 tumour suppressor plays a major role in these phenomena (Campisi and d'Adda di Fagagna, 2007; Finkel *et al*., 2007; Vousden and Lane, 2007). What has been much less apparent is to what extent are such mechanisms relevant for human cancer development, and what kind(s) of signalling activate human p53 under (patho)-physiological conditions *in vivo*. Insights into both of these fundamental issues have recently been obtained and helped to formulate the concept of DDR activation in response to tumorigenic insults and a candidate biological barrier that delays or prevents cancer progression *in vivo*. Arguably, the key initial observations that inspired the DDR barrier hypothesis were those of constitutively activated DNA damage signalling in subsets of human cancer cell lines, especially those defective in p53 function, and a broadly analogous phenomenon of chronically active DNA damage checkpoints, as exemplified by the presence of Thr68-phosphorylated, activated form of the checkpoint kinase Chk2 in clinical specimens of large subsets of human breast and lung carcinomas (DiTullio *et al*., 2002). Given that the biopsy specimens from such tumours were surgically removed before the patients were exposed to any adjuvant treatment, such constitutive DNA damage checkpoint signalling was not attributable to radiotherapy or chemotherapy. Instead, these results suggested that some disease-associated events that specifically occur in cancer but not in corresponding normal tissues led to long-term activation of the DDR machinery, and that apparently both p53-mutant human cancer cell lines *in vitro* and advanced human invasive carcinomas *in vivo* can proliferate despite such ongoing DNA damage signalling. Although intriguing, these initial observations were not easy to interpret, as both long established, p53-mutant cancer cell lines and advanced tumours were known to harbour pronounced genetic instability, and it was impossible to deduce the cause and consequences of the identified checkpoint signalling.

One exciting and potentially plausible interpretation of the data reported by DiTullio *et al*. (2002) was that some, likely cell-autonomous, oncogenic events might cause DNA damage, apparently leading to DNA double-strand breaks (DSBs) to which the ataxia telangiectasia mutated (ATM)–Chk2 kinase module responds. Furthermore, such checkpoint activation might contribute to antitumour defence unless it is disabled through DDR defects such as p53 mutations. This notion seemed to be also consistent with the reported ability of the cMyc oncogene to evoke ROS that led to DNA damage in apparently non-proliferating cells *in vitro* (Vafa *et al*., 2002). To test the DDR–tumorigenesis barrier hypothesis, a combination of at least two major approaches was required: cell culture models of conditional oncogene activation in human cells, and analysis of a large panel of human tumour biopsies from various stages of cancer progression, especially from the early, premalignant and pre-invasive lesions (Gorgoulis *et al*., 2005; Bartkova *et al*., 2005a).

DNA damage signalling in early lesions and models of oncogene activation

The two studies by Bartkova *et al*. (2005a) and Gorgoulis *et al*. (2005) jointly provided evidence for the likely role of the DDR machinery as an inducible barrier against tumour progression. These authors found that in contrast to normal human tissues, including the highly proliferative intestinal epithelium, tumour cells in clinical specimens from various tissues often show constitutive activation of DNA damage signalling, as demonstrated by the presence of activated forms of checkpoint kinases ATM and Chk2, phosphorylated histone H2AX (γH2AX) and p53, and foci formation by the DDR proteins such as 53BP1 (Figure 1a). The observed DDR activation was at its peak level already in the early stages of human tumours, and it often persisted, although sometimes in an attenuated form (possibly reflecting defects in the DDR network acquired during cancer progression), also among the invasive, overtly malignant tumours (Gorgoulis *et al*., 2005; Bartkova *et al*., 2005a). Furthermore, the fact that in the early, pre-invasive lesions, the DDR activation preceded occurrence of mutations and/or loss of expression of the DDR components such as those in the ATM–Chk2–p53 checkpoint cascade was consistent with the idea that the activated DDR barrier selects for defects that allow to breach this defense before the early cancers can progress. The DDR activation was also recapitulated in several human cell culture models in response to regulatable expression of various oncogenes and in human xenografts *in vivo* (Gorgoulis *et al*., 2005; Bartkova *et al*., 2005a). Importantly, also the ATR/Chk1 signalling module was activated in a rather acute manner upon oncogene activation (Bartkova *et al*., 2005a).

On the basis of their results (Gorgoulis *et al*., 2005; Bartkova *et al*., 2005a), the following concept was formulated by the two groups. The ATR/ATM-regulated DDR machinery serves as an inducible barrier to constrain tumour development in its early stages by inducing cellular senescence or cell death. The activated checkpoints create environment that selects for mutations or epigenetic silencing of checkpoint genes. The selected DDR alterations, for example those in p53, 53BP1 (Figure 1b) or the kinases ATM or Chk2 may rescue proliferation of the incipient cancer cells and reduce cell death, at the expense of enhanced genomic instability and tumour progression.

DNA damage response and oncogene-induced senescence

Overexpression and/or activation of diverse oncogenes in mammalian cells induces the phenotypic hallmarks of cellular senescence, a state of permanent growth arrest

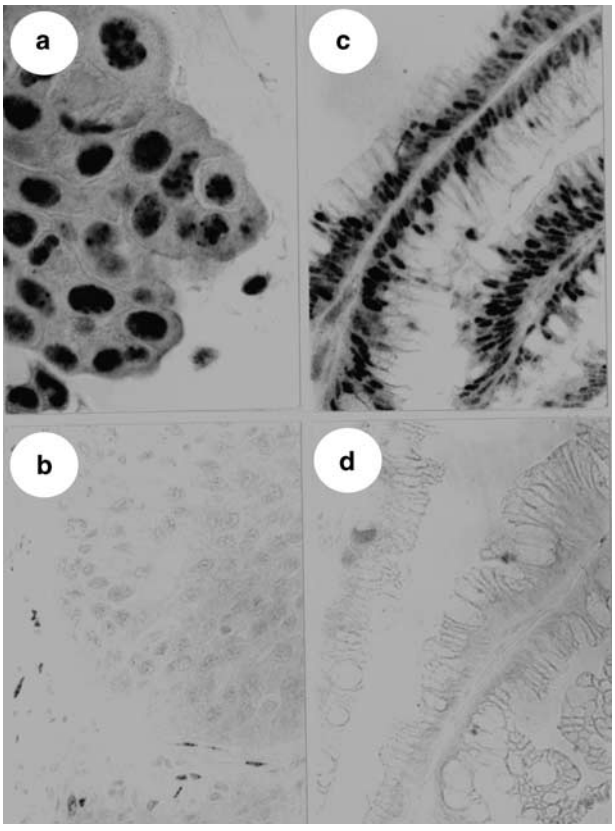

Figure 1 Examples of immunohistochemical detection of activated DNA damage signalling and loss of DDR factors in human tumours. (**a**) Focal pattern of the 53BP1 protein indicates activated DDR in a mucinous breast carcinoma. (**b**) Selective lack of 53BP1 protein in invasive squamous lung carcinoma cells, compared with preserved 53BP1 staining in the stromal cells, indicates acquired aberrant loss of this DDR factor during tumour progression. (**c**,**d**) Highly positive staining for the Thr68-phosphorylated, activated form of the Chk2 kinase in grade III colon adenoma (**c**) contrasts with the absence of Chk2 activation in a low-grade colon adenoma (**d**), despite the Chk2 protein was equally abundant in both lesions (data not shown).

refractory to physiological proliferation stimuli, with altered cell morphology and gene expression patterns, yet with preserved metabolic activity (Campisi and d'Adda di Fagagna, 2007; Finkel *et al*., 2007). Accumulating evidence indicates that senescence can be induced by diverse insults, including a range of DNA damaging agents and a wide spectrum of activated oncogenes, and by telomere shortening that causes the so-called replicative senescence (d'Adda di Fagagna *et al*., 2004; Campisi and d'Adda di Fagagna, 2007; Finkel *et al*., 2007). Most relevant to the topic of this review article, the phenomenon of cellular senescence also represents an emerging physiological tumour suppressive mechanism and a potential correlate of tissue ageing (Finkel *et al*., 2007).

Given that both the DNA damage checkpoints and cellular senescence are candidate biological barriers that limit cancer progression beyond the early, dysplastic stages, the question that arises is what is the relationship, if any, between these two emerging anticancer barriers. This important issue has been addressed by several recent studies that collectively document an intimate, causal link between the two mechanisms. Thus, it turned out that in human cell culture models of response to activated oncogenes such as ras, mos, cdc6, cyclin E or Stat5, and in ras-driven mouse epithelial tumours *in vivo*, the oncogene-triggered ATR/ATM-mediated DNA damage signalling is required for the downstream events that lead to the establishment of cellular senescence (Bartkova *et al*., 2006; Di Micco *et al*., 2006; Mallette *et al*., 2007). Experimental blockade of the oncogene-induced DNA damage signalling pathway, for example through siRNA-mediated knockdown of the ATM kinase, resulted in an escape from senescence and rescue of DNA synthesis to a degree equivalent to that achieved by analogous knockdown of p53, an established critical mediator of oncogene-induced senescence (Bartkova *et al*., 2006; Di Micco *et al*., 2006). Furthermore, in human clinical specimens from early stages of colorectal and urinary bladder tumours, markers of activated DNA damage checkpoints correlated well with markers of heterochromatinization characteristic for cellular senescence (Bartkova *et al*., 2006). Overall, these results indicate that the establishment of oncogene-induced cellular senescence is dictated, at least in part, by the upstream signalling from the DDR machinery. From a broader perspective, one could argue that the phenomenon of cellular senescence represents a long-term cell-cycle blockade imposed by chronic checkpoint signalling from persistent DNA damage.

Replication stress as the underlying trigger of oncogene-induced DNA damage response

Another key question related to the DDR-mediated tumorigenesis barrier function is the mechanistic basis of the oncogene-induced DNA damage. The so far limited studies of this complex issue implicate oncogene-induced DNA replication stress, including replication fork collapse and subsequent formation of DSBs, as the stimulus that evokes the observed response of the ATR/ATM-governed DDR network. The likely crucial role of DNA replication stress in response to oncogenes was postulated already in the two initial studies that formulated the concept of DDR as an anticancer barrier (Gorgoulis *et al*., 2005; Bartkova *et al*., 2005a), and further support for this notion has been provided by more recent works (Bartkova *et al*., 2006; Di Micco *et al*., 2006).

The initial evidence that inspired the formulation of such a model included the observed engagement of the ataxia-telangiectasia mutated and rad3-related/ATR interacting protein (ATR/ATRIP)-Chk1 kinase module, formation of single-stranded DNA stretches, phosphorylation and chromatin association of RPA, and delay in S and G_2 phases of the cell cycle in human cells conditionally exposed to oncogenes (Gorgoulis *et al*., 2005; Bartkova *et al*., 2005a). In addition, the idea of DNA replication stress occurring early during the human cancer development was consistent with the findings of preferential DNA breakage, manifested as loss of heterozygosity at the loci known as common fragile

sites, difficult to replicate regions of the genome that are prone to DSB formation under conditions of compromised DNA replication, in both clinical specimens of early human lesions and xenograft models of human epithelial hyperplasia (Gorgoulis *et al*., 2005; Bartkova *et al*., 2005a).

A more direct experimental support for oncogene-induced replication stress came from subsequent works that documented the colocalization of oncogene-evoked DDR foci with replication foci marked by the presence of proliferating cell nuclear antigen (PCNA) in S-phase cells, and the fact that formation of DSBs required the presence of the oncogene-expressing cells in S phase (Bartkova *et al*., 2006; Di Micco *et al*., 2006). Furthermore, whereas Di Micco *et al*. (2006) reported Ras-induced replication-associated stress marked by DNA re-replication, suggesting that some replication origins fired more than once, Bartkova *et al*. (2006) used the technique of DNA combing to assess the state of DNA replication forks and found oncogene-induced aberrant, premature termination of replication fork progression. Such premature fork arrest can result in fork collapse and DNA breakage (Branzei and Foiani, 2005), consistent with the observed DSBs and activated DDR under such conditions (Bartkova *et al*., 2006).

The available evidence for the role of replication stress in activation of the DDR barrier does not rule out a contribution of other potential sources of DNA damage in early stages of tumour development. Candidates for such additional events include telomere erosion, which is perceived by mammalian cells as a form of DSB (d'Adda di Fagagna *et al*., 2003; Takai *et al*., 2003), and ROS, known to be generated in cells transformed by various oncogenes and abundant in senescent cells (Mallette and Ferbeyre, 2007). The relative contributions of these alternative sources of DNA damage to the DDR activation observed in oncogene-transformed cells and early human lesions remain to be elucidated. For example, although the original report on Myc oncogene-induced DDR employed growth factor-starved cells and concluded that the observed DNA damage occurred in non-cycling cells due to ROS (Vafa *et al*., 2002), the most plausible explanation of such results is that the small fraction of the myc-expressing cells that showed the hallmarks of DNA damage reflected the subset of cells that did enter the S phase even under the starvation conditions, also consistent with the ability of over-expressed Myc to induce DNA synthesis. Thus, even in these experiments, it is likely that the observed Myc-induced DNA damage was attributable to DNA replication stress.

On the other hand, it is plausible that ROS can cause, or contribute to, the DNA damage observed in human tissues *in vivo*, both cancerous and inflammatory. For example, a recent immunohistochemical study of a wide spectrum of human tissues and tumours identified expression of the DDR activation markers also in some acute inflammatory tissues rich in infiltrating cells such as neutrophils, cells known to produce large amounts of inflammatory cytokines and ROS (Nuciforo *et al*., 2007). One conceptually important question with regard to the potential role of ROS in both oncogene-induced and inflammation-induced DDR is whether ROS acts largely indirectly, through stimulation of cell proliferation and hence possibly through replication stress. In any case, the role of the replication stress in oncogene-evoked DDR activation seems dominant in the few models in which the three sources of DNA damage have been considered so far, and replication stress also appears to be sufficient to induce the DDR and the resulting prolonged cell-cycle arrest even in experimental scenarios that do not result in detectable telomere shortening (Gorgoulis *et al*., 2005; Bartkova *et al*., 2005a; Di Micco *et al*., 2006).

DNA damage response as a candidate tumorigenesis barrier in different types of human tumours

One of the strong predictions in case the DDR barrier should be regarded as a biologically significant tumour suppressive mechanism is that evidence for the constitutive DNA damage signalling must be found in large fractions of early lesions from various human tissues. The presently available data indicate that the DDR machinery is commonly activated in major types of human melanocytic nevi and precursor dysplastic and adenomatous lesions in the lung, breast, colon, urinary bladder and prostate (Gorgoulis *et al*., 2005; Bartkova *et al*. 2005a; Fan *et al*., 2006; Tort *et al*., 2006; Nuciforo *et al*., 2007; Bartkova *et al*., 2007a). Importantly, the only types of normal human tissues that scored positive when examined immunohistochemically for the DDR activation markers were some lymphocytic cells in the bone marrow and spermatocytes in the adult testes (Bartkova *et al*., 2005b). These results are gratifying in the sense that the two positive cell types undergo physiological genome rearrangements accompanied by formation of DNA DSBs, during immunoglobulin gene rearrangements and meiotic recombination, respectively. In a similar vein, although human thymus tissue has not been examined so far, one might expect to find analogous physiological DDR signalling among thymic lymphocytes that undergo their antigen receptor rearrangements.

The spectrum of the types of human early lesions that may show the hallmarks of constitutive, therapy-independent activation of the DNA damage signalling will almost certainly broaden as additional tumour types are examined. This prediction is also supported by two additional pieces of evidence. First, recent findings of subsets of DDR marker-positive advanced cases among cancer types other than those listed above (Nuciforo *et al*., 2007) suggest that also in pre-malignant lesions from these additional tissues the DNA damage checkpoints will likely be activated. In fact, the fractions of early lesions in such tissues will probably show higher frequency of DDR barrier activation than in the corresponding malignant lesions, similar to the data based on different stages of progression among human lung, bladder and colon tumours, for example (Gorgoulis *et al*., 2005; Bartkova *et al*., 2005a; Nuciforo *et al*.,

2007). Second, cell culture and mouse model experiments in numerous laboratories demonstrated that activated oncogenes evoke a robust DDR activation in diverse cell types, including primary fibroblasts, lymphocytes and epithelial cells (Powers *et al*., 2004; Gorgoulis *et al*., 2005; Bartkova *et al*., 2005a, 2006; Di Micco *et al*., 2006; Frame *et al*., 2006; Pusapati *et al*., 2006; Reimann *et al*., 2007; Mallette *et al*., 2007).

One intriguing exception among the human tumours that have so far been examined for the presence of activated DNA damage signalling are the testicular germ-cell tumours (TGCTs), including their pre-invasive precursor lesion known as carcinoma *in situ* of the testis (Bartkova *et al*., 2007a, b). The lack of DDR activation in the TGCT lesions indicates that, in general, the oncogenic events that drive the molecular pathogenesis of TGCTs do not cause replication stress and/or DNA damage to the extent that would require activation of the DNA damage checkpoints or, alternatively, that these cells can repair the DNA breakage much more efficiently than cells in other tissues. One should also keep in mind the origin of these tumours, as TGCTs derive from precursor germ cells (early gonocytes) rather than from somatic cells, as do the other types of cancer that display the DDR activation phenotype; and this major biological difference may also account for the striking paucity of the DDR barrier activation during tumour development. What is potentially most relevant to the concept of the DDR anticancer barrier is the fact that TGCTs show an exceptionally low incidence of p53 mutations and virtually no loss of other DDR components such as the MDC1 (mediator of DNA damage checkpoint 1) or 53BP1 DNA damage mediators, again in sharp contrast to carcinomas (Bartkova *et al*., 2007a). Conceptually, the observed correlation of the presence (for example, in breast and lung carcinomas) versus absence (in TGCTs) of constitutive DNA damage signalling and DDR aberrations further strengthen the notion that the DDR machinery provides an inducible tumorigenesis barrier. Hence, in the absence of the barrier activation, as seen among TGCTs, there is also lower pressure to breach the DDR network by selection for defects such as those of p53, 53BP1, MDC1 or other DDR components during cancer progression.

Future challenges and potential implications for cancer management

As the concept of the DNA damage checkpoint activation as a biological barrier against tumour progression is only emerging, there are naturally still more questions than there are answers about many key aspects of this mechanism. One outstanding question is how many analogous inducible anticancer barriers are there in mammals, and what are the relative contributions of such alternative, and possibly redundant, mechanisms to the observed incidence of spontaneous tumours. Arguably, the most relevant additional barrier mechanisms include telomere attrition and the oncogene-induced expression of the alternative reading frame (ARF) tumour suppressor, another activator of the p53 pathway (Campisi and d'Adda di Fagagna, 2007; Vousden and Lane, 2007; Finkel *et al*., 2007). Although telomere shortening mimicks DNA breakage and evokes the DDR network activation in a manner broadly analogous to the oncogene-induced replication stress and DNA damage (d'Adda di Fagagna *et al*., 2004 and this article), the mutual relationship of these two convergent, DDR-mediated barriers with the ARF pathway remains to be elucidated. One obstacle in any immediate comparisons is that ARF becomes activated through transcriptional upregulation, and it is unknown what is the upstream signalling, and indeed the sensor mechanism, that leads to the observed engagement of ARF in response to oncogenic stress. It is possible that ARF and the DDR barriers respond to distinct classes of oncogenes or operate at different times during cancer development. It is also possible that there are significant interspecies differences, in that ARF might be a more prominent mechanism in mice, whereas the replication stress pathway and telomere attrition predominate in human tumorigenesis.

Given that at least some oncogenes are capable of activating both the replication stress response and ARF, it is also plausible to speculate that these barriers may be alarmed by distinct threshold levels and/or activities of a specific oncogene. For example, a recent elegant study indicates that quantitative changes in the abundance of the ras oncogene may be critical for shifting the balance between the ARF pathway remaining dormant or becoming activated (Sarkisian *et al*., 2007). In this mouse model, the data suggest that a priming lesion in the form of an activating ras mutation is necessary but not sufficient to induce cellular senescence, and that also a second 'hit' that causes increased levels of the mutant ras is required to trigger the senescence barrier (Sarkisian *et al*., 2007). Although in this particular model the role of the replication stress and DDR activation in the induced senescence phenotype was not examined, the DDR machinery seems important for ras-induced senescence in other model systems (Bartkova *et al*., 2006; Di Micco *et al*., 2006), and other experiments indicate that also the DDR barrier might respond differentially to distinct thresholds of oncogenic stress. For example, not all oncogenic insults are equal in terms of their relative abilities to cause replication stress. Whereas overexpression of the proto-oncogenic cyclin D1 and loss of the tumour suppressor p16ink4a seem to be unable to activate the DDR machinery, gene knockout or inactivation of the retinoblastoma tumour suppressor (Pickering and Kowalik, 2006; Tort *et al*., 2006) and overexpression of several oncogenes in the RB pathway, such as E2F1 or cyclin E, did evoke the replication stress barrier (Bartkova *et al*., 2005a, b).

Such differential responses of the tumorigenesis barrier mechanisms may be biologically meaningful, in that perhaps their activation is reserved only to the suprathreshold, potentially life-threatening oncogenic events that occur in the more risky, pre-malignant lesions, rather than responding indiscriminately to any initial benign growth. Consistent with such a scenario,

recent analysis of human colon adenomas of increasing histopathological grades (Tort *et al*., 2006) showed that in contrast to high-risk, grade III adenomas that commonly show multiple markers of activated DDR, the low-grade (grade I and II) adenomas showed only moderate or no detectable DDR activation (Figures 1c and d). As only grade III adenomas are recognized as *bona fide* pre-cancerous lesions, it appears that among early lesions of the same tissue origin, the DDR barrier becomes alarmed preferentially in those tumours that pose a greater threat of malignant progression. In any case, future studies should establish whether detection of constitutively activated DNA damage signalling might help in diagnostic or prognostic assessment of the individual tumours.

Among other immediate aims in this area of cancer research is the elucidation of the molecular basis for the observed DNA replication stress and the subsequent DNA breakage caused by diverse oncogenes, and what is the contribution of the replication stress to genomic instability that is observed in the majority of human tumours. On a more translational note, future work should focus on designing the ways to better predict responses of individual patients to DNA damaging therapies including radiation and various chemotherapeutics, based on the genetic and functional profiling of their specific tumours. This may facilitate selection of a proper modality or combination of treatment options on an individualized basis and also optimize the dose of such therapies according to the state of the DNA damage checkpoint and repair machineries of each patient. Given the promising results of poly(ADP-ribose) polymerase 1 (PARP1) inhibitors in the treatment of tumours defective in homologous recombination repair (Bryant *et al*., 2005; Farmer *et al*., 2005), additional smart strategies to exploit tumour-specific abnormalities within the DDR machinery will hopefully follow. In the meantime, perhaps the concept of the replication stress in the pre-malignant lesions might have some impact on PARP1 and analogous targeted treatments that take advantage of the synthetic lethal effects of combinations of endogenous tumour defects (such as in the DNA repair genes *BRCA1* or *BRCA2*) and the drug-targeted redundant repair pathways, such as those that require PARP1 function. Given that the replication stress distinguishes pre-malignant lesions from normal tissues, PARP1 inhibitors and analogous drugs might selectively affect such lesions that already display enhanced levels of DNA damage, and therefore higher demand for, and dependency on, efficient DNA damage signalling and repair. Such strategies might therefore preferrentially target malignancies and the risky pre-malignant lesions, while sparing cells in normal tissues which display only the normal, subthreshold levels of endogenous replication stress and DNA breakage.

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