Complex engagement of DNA damage response pathways in human cancer and in lung tumor progression

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Tumor initiation and progression provide a multitude of occasions for the generation of DNA damage and the consequent activation of the DNA damage response (DDR) pathway. DDR signaling involves the engagement of key factors such as ATM, CHK2, 53BP1 and the phosphorylation of histone H2AX (γ-H2AX). The systematic study of DDR in human tumors and normal tissues by high-throughput tissue microarrays revealed that ATM and γ-H2AX were engaged in cancer but the extent of their activation was strongly affected by the organ and cell type involved, whereas 53BP1 loss was the most consistent feature among the tumor studied. Unexpectedly, we also observed activated DDR markers in morphologically normal tissues, also in association with inflammation. Analysis of the dynamic engagement of DDR along the different stages of lung tumorigenesis showed that 53BP1 loss occurs early at the transition from normal to dysplastic change whereas the activated forms of ATM and CHK2, but not γ-H2AX, initially accumulate in pre-invasive lesions and are then lost during tumor progression. In individual lung tumors, the activation of ATM, CHK2 and the presence of 53BP1 were consistently correlated, whereas γ-H2AX did not correlate with activated ATM. Finally, the study of associations between critical clinicopathological parameters and activated DDR factors highlighted a statistically meaningful correlation between reduced local tumor extension and the phosphorylation of ATM, CHK2 and the presence of 53BP1, whereas no significant correlations with parameters such as survival or relapse of early-stage lung carcinomas were found.

Introduction

Cells respond promptly to the generation of DNA damage by launching a coordinated set of actions, collectively known as the DNA damage response (DDR) (1). This program has the dual function of activating the mechanisms devoted to DNA damage repair and of halting cell cycle progression. The latter checkpoint function can act either transiently, providing the time to repair the damage, or permanently, leading to a prolonged cell cycle arrest called cellular senescence or to a cell suicide program termed apoptosis. The DDR is act either transiently, providing the time to repair the damage, or permanently, leading to a prolonged cell cycle arrest called cellular senescence or to a cell suicide program termed apoptosis. The DDR is initiated by the recognition of DNA damage by a set of sensors that activate the protein kinase activity of ATM, when the trigger is a DNA double-strand break (DSB), or ATR, when single-strand DNA has been exposed (2). ATM and ATR can modify chromatin at the site of DNA damage, by phosphorylating S139 of histone H2AX, an event that facilitates DDR enforcement. Activated ATM and ATR signal to two more downstream kinases, CHK1 and CHK2, which modify additional elements of the DDR cascade. Signaling between upstream and downstream kinases is facilitated by a set of mediators of the DDR which include 53BP1, whose accumulation at the site of damage is mediated by chromatin modification events (3,4). DDR pathways are not strictly linear and redundant signaling occurs, making the DDR network more robust.

Tumors are the result of clonal amplification of cells carrying genetic alterations that drive their unrestrained proliferation. Tumorigenesis provides various occasions for DDR activation. These include telomere shortening (5,6), reactive radical species accumulation (7), chromatin alterations (8), hypoxia (9) and rampant genome instability. In addition, oncosene activation per se is sufficient to engage the DDR machinery (10,11) and markers of an activated DDR have been detected in early tumors (12,13).

Genetic evidence for an important tumor suppressive role of DDR genes in vivo has been provided by the observation of a tumor-prone condition of patients carrying mutations in various DDR genes such as ATM, NBS1, ATR, MRE11, CHK2, BRC1 and 2 and in knockout mice for several DDR genes (14,15). In addition, mutations and loss of DDR gene expression have been reported in tumors (16–23). Recently, we and others have shown that a mechanism through which DDR genes can exert their tumor suppressive functions is by enforcing cellular senescence, thereby preventing the proliferation of damaged cells (10,11).

It is still unclear how widespread DDR activation is among human tumors of different histotypes and at different stages during cancer progression. Here, we report on the engagement of key components of the DDR-signaling cascade in a broad collection of human tumors of different histotypes. In addition, we show in detail the differential degree of activation of individual DDR factors during lung tumor progression. In these same samples, we also analyze the correlations among activated DDR factors and the potential association between the activation of individual DDR factors and various clinicopathologic parameters.

Materials and methods

Samples and tissue microarray preparation

Normal skin for antibody validation (Figure 1) was from a female patient who underwent reductive mammoplasty. After surgical excision, the sample was cut into two and one part only was ex vivo X-rays irradiated (20 Gy). After 30 min incubation in phosphate-buffered saline, both parts were fixed in buffered formalin and included in paraffin. Normal lung samples (Figure 2C) were derived from three patients who underwent a surgical procedure for a non-neoplastic pathology. Tissue samples were provided by Istituto Europeo di Oncologia (Milano).

Three multitumor tissue microarrays (TMAs) were specifically designed for the screening (Table 1), and prepared as previously described in ref. 24 with minor modifications. Briefly, two representative normal (when available) and tumor areas (diameter 0.6 mm) from each sample, previously identified on hematoxylin–eosin-stained sections, were removed from the donor blocks and deposited on the recipient block using a custom-built precision instrument (Tissue Arrayer—Beecher Instruments, Sun Prairie, WI). Two micron sections of the resulting recipient block were cut, mounted on glass slides and processed for immunohistochemistry (IHC). Overall, multitumor TMAs contained tissue samples from breast (11 normal, 27 fibroadenomas and 43 carcinomas), lung (64 normal and 107 carcinomas), colon–rectum (15 normal and 18 carcinomas), kidney (15 normal and 18 carcinomas), larynx (20 normal and 28 carcinomas), stomach (24 normal and 28 carcinomas), hematopoietic system (20 non-Hodgkin’s and 8 Hodgkin’s lymphomas), skin (14 nevi and 20 melanoma), soft tissues (18) and bone (10 osteogenic sarcomas) and central nervous system (4 meningiomas and 20 gliomas). Importantly, care has been taken to avoid proximity to a resected margin as we have observed that thermal damage during surgery may induce a strong DDR (Nuciforo PG, unpublished observation).

Specimens were provided by the Pathology Departments of Ospedale Maggiore (Novara), Presidio Ospedaliero (Vimercate) and Ospedale Sacco (Milano).

Lung-specific TMA (Figure 3) was made of 98 lung squamous cell carcinomas that were diagnosed and treated from 1987 to 1992 at the Verona City Hospital (Novara), Presidio Ospedaliero (Vimercate) and Ospedale Sacco (Milano).

Abbreviations: DDR, DNA damage response; DSB, double-strand break; HGD, high-grade dysplasia; IHC, immunohistochemistry; LGD, low-grade dysplasia; SCC, squamous cell carcinoma; TMA, tissue microarray.

†These authors contributed equally to this work.

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Carcinomas, four lung carcinomas and two stomach carcinomas) with Triazol.

Total RNA was isolated from frozen tissues (one normal breast, four breast
high proliferation, respectively.

positive cells for p53 and Ki67 was used to establish protein accumulation and
cut-off of

redistributed signals in

unequivocal positivity. When scoring for 53BP1, the presence of diffuse and/or

scores 2 and 3 were considered to represent an

53BP1, CHK2 pT68 and p53 become apparent after treatment in both keratinocytes and stromal cells. Magnifications ×40 and ×60 of the same sample are shown.

Major Hospital (Italy). Criteria to be enrolled into the study included p-stage I

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RNase-free DNase (Invitrogen, Carlsbad, CA) and anti-53BP1 (1:15, gift from T. Halazonetis). A semi-

Biotechnology, Lake Placid, NY), anti-p53 (1:200, DO1, FIRC Institute of Molecular

Biotechnology, Lake Placid, NY), anti-ATM pS1981 (1:400, Rockland Immuno-
optical slides. When staining the carcinomas, cut-open sections of each tissue sample used as calibrator.

Statistical analysis

Fisher’s exact test was used to calculate statistical significant differences in

protein expression between normal (N) and tumor (T) counterparts (Table I)
and between the different stages (normal, LGD, HGD, pT1 and pT2) of lung

squamous cell neoplastic disease (Figure 3). Relationship between genes and

correlation of each gene with clinical, histological and biological parameters in

lung squamous cell carcinoma (SCC) (Table II and data not shown) were

assessed using the Pearson’s Chi-square. Survival analysis was performed

using Kaplan–Meier method and curves were compared by the log-rank test

data not shown). All throughout our analyses, differences were judged signif-

icant at confidence levels ≥95% (P ≤ 0.05).

Results

Antibody validation on fixed material

DDR activation in mammalian cells consists in the nuclear activation by

phosphorylation of a set of DDR proteins that are cytologically detectable by immunostaining, often in the form of nuclear foci. Although the immunological reagents commonly used for these studies have been extensively validated in vitro on cultured cells, their use in complex tissue specimens has been limited so far. We therefore validated a set of these reagents by testing their ability to specifically

Method (Invitrogen, Corporation, Carlsbad, CA). One microgram of total RNA

was reverse transcribed (PowerScriptRT, Clontech, Laboratories Inc., Mountain

View, CA) with 100 ng random primers (Invitrogen). Preparations without

the enzyme were used as negative controls. Real-time polymerase chain re-

action was carried out for each sample in triplicate on the 7900 HT Fast Real-

Time Polymerase Chain Reaction System (Applied Biosystems) using the

TAQMAN Assay on Demand specific for 53BP1 (HS00996818_M1) in a final

volume of 15 µl with the Eurogentech Master mix 2 ×. The reaction was carried

out for 2 min at 50°C, and then a step of 10 min at 95°C, followed by 40 cycles

of 15 s at 95°C and 60 s at 60°C. To normalize the amount of total RNA present

in each reaction, we amplified the housekeeping gene 18S. All the analyzed

tumors were treated as N-fold 53BP1 mRNA relative to a normal breast

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detect a DDR by IHC on formalin-fixed, paraffin-embedded normal human skin, a part of which had previously been X-rays irradiated ex vivo. Figure 1 shows that antibodies raised against phosphorylated S139 of histone H2AX (\(\gamma\)-H2AX) (25), autophosphorylated and therefore activated ATM pS1981 (8), activated CHK2 pT68 (26) and p53 (27), all detect a strong nuclear staining exclusively in the irradiated sample that is absent in the untreated portion. An antibody against 53BP1 generates a diffuse nuclear staining in the non-irradiated sample that becomes stronger and punctuated upon irradiation. A weak ATM pS1981 cytoplasmic staining was also occasionally observed, but it was not further considered in this analysis. Overall, the observed nuclear-staining mirrors that previously reported in cultured cells and we concluded that these are reliable reagents that specifically detect the activation of distinct components of the DDR in archival material by IHC.

A wide survey of DDR in human tumors of different histotypes

We screened a total of 383 human tumors of different common histotypes and 147 paired normal tissues using multitumor TMAs (see Materials and methods for composition) to assess the engagement of the DDR machinery in tumors and in their normal counterparts, when available. TMA is a reliable and representative technology that allows the \textit{in situ} analysis of tumoral and normal tissue samples in a high-throughput and internally controlled format. In this initial screen, we chose to interrogate the DDR-signaling cascade at three nodal points: ATM pS1981, the activated form of a key upstream regulator that controls most DDR events; 53BP1, a central mediator of the DDR and \(\gamma\)-H2AX, a downstream chromatin modification event strongly linked to the generation of DSBs. Results are summarized in Table I and representative pictures are shown in Figure 2A.

The extent of ATM pS1981 accumulation varied both in normal and tumor samples, depending on the tissue and cell type. ATM activation was absent or low (\(<\)20% of positive samples) in all normal tissues and in gastrointestinal, kidney and lung cancers (SCC and large cell carcinomas subtypes), whereas higher levels of ATM pS1981 were observed in the other tumor histotypes being significantly up-regulated in breast and larynx cancers compared with their normal counterparts (Fisher’s exact test, \(P = 0.04\) and \(P = 0.03\), respectively). The same TMAs were scored for the presence or absence of 53BP1. While the majority of normal tissue samples stained positive for it (with a diffuse nuclear-staining pattern reminiscent of the non-active form), we observed a significant dramatic loss of 53BP1 signal in many tumor types such as lung (\(P = 0.001\)), kidney (\(P = 0.002\)), larynx (\(P = 0.007\)) and stomach (\(P = 0.004\)) carcinomas. A subset of the same tumors analyzed by IHC–TMA (\(n = 10\), six 53BP1 IHC positive and four 53BP1 negative) was subsequently analyzed by quantitative polymerase chain reaction to assess if 53BP1 protein loss correlates with its transcript. 53BP1 mRNA was indeed generally lower in samples scored negative in IHC than in positive ones (Figure 2B). This suggests that altered 53BP1 accumulation in tumors can be the result of transcriptional modulation. In addition, in one-third of larynx carcinomas, 53BP1 loss was achieved by nuclear exclusion and cytoplasmic retention. Importantly, all tumors retaining nuclear 53BP1 displayed a focal nuclear pattern of staining, highly suggestive of its activation (Figures 2 and 3).

Similar to other DDR genes, the study of \(\gamma\)-H2AX-positive cells revealed a tissue type-specific pattern of accumulation. With the only exception of lung, no \(\gamma\)-H2AX accumulation was found in normal cells. In contrast, half of all kidney tumors stained positive, (\(T\) versus \(N\), \(P = 0.007\)) and nearly one non-small cell lung carcinoma in four...
stained γ-H2AX positive—the percentage of positive cases varying among different histotypes and it was significantly higher in SCCs followed by large cell carcinomas and adenocarcinomas (Table I), in agreement with the reported different genomic instability (28,29). Our analysis also revealed that sarcomas, osteogenic sarcomas in particular, were characterized by high levels of both γ-H2AX and ATM pS1981 (7 out of 10 strongly positive osteogenic sarcomas) and presence of 53BP1. Nevertheless, tumors from the breast, larynx and the gastrointestinal tract very rarely showed γ-H2AX accumulation. Notably, in breast tumors and glioblastomas, we clearly observed ATM activation in the absence of γ-H2AX. This intriguing observation suggests that other events distinct from DSB generation, possibly related to chromatin changes (8), may contribute to ATM activation in cancer.

As mentioned above, a limited number of samples from normal tissues flanking the tumors stained positive for γ-H2AX in lung and for the activated form of ATM in lung, kidney and the gastrointestinal tract. To assess whether DDR activation in normal tissues was an indirect consequence of the tumor condition, we stained a separate set of large portions of normal lung samples from non-neoplastic patients (see Materials and methods) for γ-H2AX. We discovered that also in these samples γ-H2AX-positive bronchial cells were clearly detectable but only near acutely inflamed areas defined by a pathologist according to morphological criteria such as the presence of neutrophil granulocytes in the bronchial epithelium (Figure 2C). These novel observations (and others not shown) suggest that markers of an activated DDR, such as γ-H2AX, accumulate also in acutely inflamed tissues, therefore, in the absence of frank pre-neoplastic and neoplastic condition.

Overall, the screen of multiterritorial TMA revealed that although tumorigenesis can be associated with the activation of the factors studied, ATM, 53BP1 and γ-H2AX, the extent of their engagement is strongly affected by the organ and the cell types involved. All but one (colon) tumor types showed at least one of the three DDR markers studied differentially regulated between invasive tumors and normal tissues. Indeed, some tumors lack bona fide markers of DSB accumulation such as γ-H2AX. Instead, loss of 53BP1 is the most reproducible observation among different cancers analyzed. Furthermore, unanticipatedly, we observed that morphologically normal tissues flanking a tumor or in association with an ongoing inflammatory process contain activated components of the DDR apparatus.

**Fig. 3.** DDR in lung SCC progression. (A) Percentage of positive samples is shown on the vertical axis. The horizontal axis describes the stage of the lesion. Normal lung includes also bronchial respiratory epithelia from multiterrorial TMA. DDR gene activation is shown on the left line-graph; p53, ki67 and caspase 3 activation are on the central line-graph. Statistical differences among groups are calculated with Fisher’s exact test and shown in the right table; Blank cells indicate lack of statistical significance. N, normal bronchial epithelium. (B) Representative images of lung-specific TMA sections stained for γ-H2AX, ATM pS1981, 53BP1, CHK2 pT68 and p53. Normal (N), dysplastic (D, including both LGD and HGD), pT1 and pT2 tumor areas are shown. (C) High-magnification images of 53BP1 nuclear pattern in normal lung (N), dysplasia (D) and invasive tumor (T): note 53BP1 redistribution in foci in transformed cells (as shown by arrows) compared with normal epithelium.
disease stages with a broader range of DDR markers. We therefore followed DDR engagement during lung squamous cell tumorigenesis in normal (N), LGD and HGD, pT1 and pT2 stage I SCC tissues from patients with long-term follow-up. In addition to γ-H2AX, ATM pS1981 and 53BP1, we analyzed these specimens for the presence of the activated forms of CHK2 (CHK2 pT68), together with caspase 3 active, p53 and Ki67.

The results of this analysis, summarized in Figure 3, strengthened those observed in the first screening and increased its resolution. Consistent with our previous set of samples, we observed a progressive and dramatic loss of detectable 53BP1 during tumor progression (Figure 3A, left panel; right panel for P values) from as early as the N to LGD transition. ATM pS1981 and CHK2 pT68 are present in a small percentage of normal bronchial cells and they progressively increase up to HGD (N versus HGD, P values = 0.05 for ATM pS1981 and 0.01 for CHK2 pT68) to dramatically drop in pT2 (HGD versus pT2, P values = 0.02 for ATM pS1981 and 0.01 for CHK2 pT68). Differently, although a slight, non-significant, increase in γ-H2AX positivity was observed from normal to pre-neoplastic/neoplastic condition, γ-H2AX levels remain similar in all samples analyzed, independently from the disease stage. Tumor progression was associated with an initial accumulation of activated caspase 3 (from N to LGD, P = 0.009) and a subsequent loss, coincident with p53 inactivation by mutation, as suggested by its increased stability (N versus HGD, P = 0.0001) (30). Overall, stepwise 53BP1 loss, p53 mutation and ATM/CHK2 inactivation lead to a progressive increase of Ki67-positive cells (Figure 3A, central panel; right panel for P values) and it is consistent with a tumor suppressive role of DDR components.

### Table I. Multitumor TMA analysis of DDR activation

<table>
<thead>
<tr>
<th>Organ</th>
<th>Histotype</th>
<th>ATM pS1981</th>
<th>53BP1</th>
<th>γ-H2AX</th>
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<tbody>
<tr>
<td>Lung</td>
<td>Normal</td>
<td>11% (63)</td>
<td>86% (64)</td>
<td>13% (52)</td>
</tr>
<tr>
<td></td>
<td>SCC</td>
<td>15% (59)</td>
<td>27% (62)</td>
<td>33% (60)</td>
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<td></td>
<td>AC</td>
<td>23% (34)</td>
<td>33% (36)</td>
<td>6% (32)</td>
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<td></td>
<td>LCC</td>
<td>0% (9)</td>
<td>33% (9)</td>
<td>22% (9)</td>
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<td>Breast</td>
<td>N/FC</td>
<td>0% (10)</td>
<td>91% (11)</td>
<td>0% (8)</td>
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<td></td>
<td>FA</td>
<td>48% (23)*</td>
<td>100% (23)</td>
<td>0% (27)</td>
</tr>
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<td></td>
<td>IDC</td>
<td>36% (39)*</td>
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<td>5% (43)</td>
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<td>Colon–rectum</td>
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<td></td>
<td>Carcinoma</td>
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<td>Kidney</td>
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<td>87% (15)</td>
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<tr>
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<td>7% (28)</td>
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<td></td>
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<td>21% (28)*</td>
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<tr>
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<td>GBL</td>
<td>68% (19)</td>
<td>100% (20)</td>
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</tbody>
</table>

Data are shown by organ of origin and histotype. The percentage of positive samples next to the total number of samples studied is shown—see Materials and methods for scoring criteria. In the lung, the normal tissue was represented by both bronchial and alveolar epithelium. N/FC, normal breast/fibrocystic disease; FA, fibroadenoma; IDC, infiltrating ductal carcinoma; CCC, clear cell carcinoma; AC, adenocarcinoma; LCC, large cell carcinoma; NHL, non-Hodgkin’s lymphoma; HL, Hodgkin’s lymphoma; STT, soft tissue tumor and OGS, osteogenic sarcoma. Asterisks indicate statistically significant differences between normal and tumor samples as assessed by Fisher’s exact test.

### Table II. Statistical correlations among individual activated DDR components and cell-cycle markers as measured by a Pearson’s Chi-square test

<table>
<thead>
<tr>
<th></th>
<th>γ-H2AX</th>
<th>ATM pS1981</th>
<th>53BP1</th>
<th>CHK2 pT68</th>
<th>p53</th>
<th>Caspase 3 act</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATM pS1981</td>
<td>0.2</td>
<td>0.1</td>
<td>&lt;0.0001</td>
<td>0.008</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>53BP1</td>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>CHK2 pT68</td>
<td>0.02</td>
<td>0.02</td>
<td></td>
<td>0.008</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>p53</td>
<td>0.9</td>
<td>0.6</td>
<td></td>
<td>0.7</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Caspase 3 act</td>
<td>0.6</td>
<td>0.6</td>
<td></td>
<td>0.6</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Ki67</td>
<td>0.9</td>
<td>0.8</td>
<td></td>
<td>0.9</td>
<td>0.8</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Positive correlations are indicated in italic.
histotypes. Our results are consistent with the emerging evidence that DDR components are misregulated in tumorigenesis (16–22). Nevertheless, differently from published reports, our data demonstrate that the scenario is complex and suggest a word of caution before drawing a univocal and exclusive correlation between tumorigenesis and full DDR activation. In fact, we observed that individual DDR factors are activated or, in the case of 53BP1, lost with different frequencies according to the tumor histotypes. Although DDR in tumorigenesis is a dynamic process and therefore the extent of the detectable DDR activation depends on the stage of the tumor, our data are consistent with a potentially dissimilar ability of different oncogenic events to preferentially activate/inactivate individual DDR pathways to variable extents. The reason for the observed differences may also lie in the reported different ability of some cell types to mount a DDR (32), possibly also in relation to the different subcellular localization of some DDR factors (19,33,34) and the ability of some pathways to impact on DDR activation (35).

Of note, we detected a DDR in a number of different normal tissue samples, including a subset of lung samples in which normal bronchial epithelial cells showed activation of ATM, CHK2 and H2AX. This may reflect the cell response to DNA damage generated by exposure to exogenous agents and endogenous cellular events. Interestingly, we found that acute inflammatory processes may be associated with a DDR. This is intriguing, because a link between inflammation and transformation has been proposed (36) and our data, together with reports of increased loss of heterozygosity in normal epithelium next to lung carcinomas (28) and in inflamed tissues (26,37), support the hypothesis that a non-oncogenic or pre-oncogenic process, such as inflammation, may engage components of the DDR machinery. In this scenario, the selective pressure for the loss of DDR may stimulate the neoplastic growth. However, it is also possible that the observed DDR activation in normal tissues next to tumors may result from a bystander effect (38).

Our vertical analysis of lung SCC highlights the dynamic and complex involvement of DDR-signaling pathway. 53BP1 loss is the earliest event observed, occurring in the transition from normal to LGD; this observation suggests a pivotal sentinel role of this DDR factor in lung tumor progression. Differently, ATM and CHK2 activations peak in HGD and they coincide with p53 inactivation. As expected, loss of p53 functions reduce the fraction of apoptotic cells, as assessed by a decrease in active caspase 3. Later, cancer progression toward frank tumor formation is associated with progressive loss of the activated forms of ATM and CHK2, with the most significant reduction at the pT1-pT2 step. Differently, γ-H2AX does not seem to be significantly modulated among the samples studied.

Therefore, 53BP1 loss, p53 mutation and ATM/CHK2 inactivation are three stage-specific events that, once integrated, provide a conceptual framework to explain the otherwise progressive increase in ki67-positive, and therefore proliferating, cells and tumor evolution.

These data suggest that transformation exerts a tremendous selective pressure against the accumulation of these proteins and strongly point to a tumor suppressive function of DDR components, 53BP1 in particular. This is consistent with the lymphoma-prone phenotype of 53BP1 knockout mice (39). Although the molecular mechanisms of 53BP1 tumor suppressive functions are not fully known, its inactivation leads to escape from the proliferative block associated with oncogene-induced cellular senescence (10) and to the impairment of p53-dependent apoptosis (13).

Our results may also have potentially important consequences for the use of small molecule inhibitors of ‘druggable’ DDR components (40). These agents are pursued with the idea of sensitizing tumors to therapy or in the hope that the loss of a DNA repair pathway may render cancer cells more susceptible to the inactivation of a parallel pathway (41,42). Our results may help choosing tumor histotypes that preferentially activate, or not, a given DDR pathway and may therefore respond differently to this approach. Furthermore, as the activation of many DDR proteins is lost during tumor progression, the stage of the tumor should also be considered. Finally, in the light of the tumor suppressive functions of some DDR factors, the use of DDR inhibitors in the absence of additional DNA-damaging agents may, in fact, accelerate tumor growth. Therefore, our results suggest that the use of DDR modulators should greatly benefit from a careful analysis of the DDR activation pattern of the individual tumor before treatment. Furthermore, since we noticed that some DDR components are activated in normal tissues, DDR inhibitors may have unexpected side effects in non-neoplastic tissues.

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**References**


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