Identification of Decatenation G2 Checkpoint Impairment Independently of DNA Damage G2 Checkpoint in Human Lung Cancer Cell Lines

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ABSTRACT

It has been suggested that attenuation of the decatenation G2 checkpoint function, which ensures sufficient chromatid decatenation by topoisomerase II before entering mitosis, may contribute to the acquisition of genetic instability in cancer cells. To date, however, very little information is available on this type of checkpoint defect in human cancers. In this study, we report for the first time that a proportion of human lung cancer cell lines did not properly arrest before entering mitosis in the presence of a catalytic, circular cramp-forming topoisomerase II inhibitor ICRF-193, whereas the decatenation G2 checkpoint impairment was present independently of the impaired DNA damage G2 checkpoint. In addition, the presence of decatenation G2 checkpoint dysfunction was found to be associated with diminished activation of ataxia-telangiectasia mutated in response to ICRF-193, suggesting the potential involvement of an upstream pathway sensing incompletely catenated chromatids. Interestingly, hypersensitivity to ICRF-193 was observed in cell lines with decatenation G2 checkpoint impairment and negligible activation of ataxia-telangiectasia mutated. These findings suggest the possible involvement of decatenation G2 checkpoint impairment in the development of human lung cancers, as well as the potential clinical implication of selective killing of lung cancer cells with such defects by this type of topoisomerase II inhibitor.

INTRODUCTION

Lung cancer currently claims more lives than any other cancers, placing it as the leading cause of cancer death in economically well-developed countries (1, 2). Lung cancer cells frequently exhibit complex chromosomal abnormalities, including multiple numerical (i.e., aneuploidy) and structural alterations including deletion, translocation, homogeneously staining region, and double minutes (3). Cell cycle checkpoints are a monitoring system that arrests cells at a particular phase of the cell cycle in response to a lack of appropriate conditions for progression, thereby maintaining genetic stability and ensuring a smooth progression through the cell cycle (4). Recent molecular and cellular biological studies have revealed that cell cycle checkpoints are often impaired in cancer cells, leading to the failure of normal growth control and the generation of genetic instability in cancer cells (5, 6). We previously reported on the pervasive presence of numerical chromosomal instability phenotype (7) and the frequent impairment of the mitotic spindle checkpoint in human lung cancer cell lines (8). We have also shown that DNA damage G2 checkpoint is often impaired in small cell lung cancer in a histological type-specific manner (9).

It has been proposed that cells monitor the status of intertwined daughter chromatids after DNA replication and actively delay mitosis until chromatids are sufficiently decatenated by topoisomerase II (10). Because complete chromatid decatenation is required for accurate chromatid segregation, it has been suggested that attenuation of decatenation G2 checkpoint function contributes to the acquisition of genetic instability in cancer cells (11). The bisdioxopiperazine, ICRF-193, sequesters topoisomerase II in a closed-clamp conformation without causing DNA damage and leads to the activation of decatenation G2 checkpoint, resulting in the blockade of topoisomerase II at a point in its catalytic cycle after strand passage and re-ligation but before release of the passed DNA and ATP hydrolysis (12, 13). With regard to the underlying mechanisms of decatenation G2 checkpoint, Deming et al. (11) previously reported that in contrast to DNA damage checkpoint, decatenation G2 checkpoint activation relies on ataxia telangiectasia and Rad3-related (ATR) activity and nuclear exclusion of cyclin B1 instead of ataxia-telangiectasia mutated (ATM)-dependent down-regulation of cdc2/cyclin B1 activity. They also showed that decatenation G2 checkpoint was not properly activated in a BRCA1-null cell line and that reconstitution with wt-BRCA1 restored ICRF-193-induced G2 arrest (11). To date, however, little is known about the potential involvement of decatenation G2 checkpoint impairment in human cancers, and virtually no data are available regarding its relation to the pathogenesis of lung cancers.

In this study, we examined a panel of human lung cancer cell lines and found for the first time that decatenation G2 checkpoint is impaired in a proportion of human lung cancer cell lines independently of the presence of the impairment DNA damage G2 checkpoint. In addition, initial attempts were made to elucidate the molecular mechanism of the impairment of decatenation G2 checkpoint in lung cancers.

MATERIALS AND METHODS

Cell Lines and Culture. ACC-LC-48, ACC-LC-49, ACC-LC-172, and ACC-LC-176 were established in our laboratory, whereas NCI-H460 and A549 were purchased from the American Type Culture Collection (Manassas, VA). QG56 and PC-10 were generously provided by Dr. Yoshihiro Hayata (Tokyo Medical University, Tokyo, Japan). This panel of lung cancer cell lines included three small cell lung cancers (ACC-LC-48, ACC-LC-49, and ACC-LC-172), an adenocarcinoma (A549), three squamous cell carcinomas (ACC-LC-176, QG56, and PC-10), and a large cell carcinoma (NCI-H460) cell lines. Normal human bronchial epithelial cells were purchased from Sanko Junyaku (Tokyo, Japan). These cell lines were tested with the TaKaRa PCR Myco- plasma detection kit (Takara Bio, Inc., Shiga, Japan) and were shown to be free of Mycoplasma contamination. Normal human bronchial epithelial cells were cultured essentially according to the manufacturer’s instructions. Other cell lines were cultured in RPMI 1640 supplemented with 5% fetal bovine serum (Invitrogen-Life Technologies, Inc., Grand Island, NY), 100 units/ml penicillin, and 100 μg/ml streptomycin.

Cell Cycle Synchronization. All synchronization was performed using optimized conditions for each cell line via the double thymidine/aphidicolin sequential block, except for PC-10 via single thymidine block. In brief, 24-h treatment with 2 mM thymidine was used to arrest exponentially proliferating cells in the early S-phase, except for ACC-LC-172, in which 36-h treatment was carried out. Cells were then released from the arrest by three washes in...
PBS and grown in fresh medium supplemented with 1 μM deoxyctydine for the following durations: 10 h in A549 and ACC-LC-172; 12 h in NCI-H460; 14 h in QG56 and ACC-LC-48; and 18 h in ACC-LC-49 and 20 h in ACC-LC-172. Five μM aphidicolin were added in all of the cell lines, except for QG56 (2 μM), followed by additional incubation for 14 h in A549, NCI-H460, QG56, and ACC-LC-176, 16 h in ACC-LC-48, and for 24 h in ACC-LC-49 and ACC-LC-172. Cells were washed three times with PBS and placed into normal medium. At this point, ~85% of the cells were found to be at the G1-S border by propidium iodide-flow cytometry analysis. The cells were then grown without any constraints for the following periods of time, which were predetermined in each cell line to attain optimal synchronization in the G2 phase: 6 h in A549 and NCI-H460; 7 h in QG56 and ACC-LC-176; 8 h in ACC-LC-48 and PC-10; 10 h in ACC-LC-172; and 12 h in ACC-LC-49. At this point, the following percentages of cells were found to be in the G2 phase of the cycle as evidenced by 4N DNA content with very few mitotic figures: 77% in A549; 55% in NCI-H460; 83% in QG56; 74% in ACC-LC-49; 87% in ACC-LC-172; 70% in ACC-LC-48; 64% in ACC-LC-176; and 72% in PC10.

**Antibodies.** The following antibodies were purchased from their respective companies: anti-cdc2 (sc-54), anti-cyclin B1 (GNS1), anti-ATR/FPR1 (N-19), and anti-BRCA1 (C-20) (Santa Cruz Biotechnology, Santa Cruz, CA); anti-p-H2AX (rabbit) and anti-Pik1 (Upstate Biotechnology, Lake Placid, NY), anti-ATM (Ab-3) (Bioch- em, La Jolla, CA); anti-α-tubulin (Sigma, St. Louis, MO); and anti-WRN (BD Transduction Laboratories, San Diego, CA). Anti-topoisomerase II/α (clone 1C5) and anti-53BP1 antibodies were generous gifts from Dr. Ryoji Ishida (Aichi Cancer Center Research Institute) and Dr. Thanos D. Halazonetis (Department of Molecular Genetics, The Wistar Institute, Philadelphia, PA), respectively.

**Measurement of Mitotic Indices.** Mitotic delay was examined by quantifying the mitotic index in replicate cultures of treated and control cells. To measure responses to ICRF-193 or MST-16, cells were treated with solvent alone or with 2 μM ICRF-193 (a generous gift from Dr. Ryoji Ishida) or with 100 μM MST-16 (Zenyaku Kogyo, Tokyo, Japan), whereas 1 G of irradiation using Hitachi MBR-1520R (Hitachi, Tokyo, Japan) was conducted to examine DNA damage-induced mitotic delay in G2. Sham-treated controls were subjected to the same movements into and out of incubators as treated cells. Cells were harvested by trypsinization, followed by fixation with 4% formalin in PBS for 30 min. Cells were then attached to glass slides by centrifugation using an Auto Smear CF-12D (Sakura, Tokyo, Japan) and stained with 0.1 μg/ml 4’,6-diamidino-2-phenylindole. To measure the mitotic index, at least 2000 cells were counted for each slide under a fluorescence microscope as described previously (14).

**Western Blot Analysis.** An equal amount of total cell lysate solubilized in Laemmli’s electrophoresis sample buffer was run on SDS-PAGE gels and transferred to Immobilon-P filters (Millipore Corp., Bedford, MA). The filters were first incubated with a primary antibody and then with horseradish peroxidase-conjugated secondary antibodies [antimouse IgG and antirabbit IgG, Cell Signaling Technology (Beverly, MA); antigoat IgG MBL (Nagoya, Japan); antimouse IgG and antirabbit IgG, Jackson ImmunoResearch Laboratories, West Grove, PA) and anti-rabbit IgG (Santa Cruz Biotechnology, Santa Cruz, CA). Horseradish peroxidase-conjugated secondary antibodies were used. Blots were developed using ECL (Amersham, Buckinghamshire, United Kingdom) and chemiluminescence detection kit (Perkin-Elmer, Boston, MA). The autoradiographs were digitized and analyzed with NIH Image software.

**Assay for Clamp Formation between Topoisomerase II and DNA.** DNA-PKcs was expressed and purified as described previously (15). 1 μg of DNA-PKcs was incubated with 2 μM ICRF-193 for 2 h and 1 μg of H2AX antibody and mouse monoclonal anti-53BP1 antibody (BD Transduction Laboratories, San Diego, CA). Anti-topoisomerase II/α (clone 1C5) and anti-53BP1 antibodies were generous gifts from Dr. Ryoji Ishida (Aichi Cancer Center Research Institute) and Dr. Thanos D. Halazonetis (Department of Molecular Genetics, The Wistar Institute, Philadelphia, PA), respectively.

**Identification of Impaired G2 Arrest in Response to ICRF-193**

**Mutational Analysis.** PCR amplification using random-primed first-strand cDNAs was performed with the aid of the following oligonucleotide primers: sense primer; 5′-GACAATCAAGCAACGGTCTTTC-3′ and antisense primer; 5′-GGACCACTCAGCAGAATT-3′. PCR products were separated on 1% agarose gel and stained with ethidium bromide. DNA bands were visualized and photographed with transilluminator. The visibility of DNA bands was quantified using the software of Quantity-One program (Bio-Rad Laboratories, Inc., Hercules, CA).

**Identification of Impaired G2 Arrest in Response to ICRF-193**

**Cell Proliferation Assay.** ICRF-193-induced cytotoxicity was determined by modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Briefly, cells were plated in 96-well plates and treated with various concentrations of ICRF-193 in a final concentration of 0.2% DMSO. After 48 h, the number of cells was measured with a colorimetric assay reagent, TetraColor One (Seikagaku, Tokyo, Japan), according to the manufacturer’s protocol. Five replicate wells were measured for each drug concentration. The IC50 values were calculated as the drug concentration that killed 50% of the cells.

**RESULTS**

**Identification of Impaired G2 Arrest in Response to ICRF-193 in Human Lung Cancer Cell Lines.** Eight human lung cancer cell lines were examined to investigate whether decatennation G2, checkpoint is perturbed in this common adult cancer, resulting in the identification of two distinct types of response. Five cell lines showed significant inhibition of entry into mitosis by the treatment with ICRF-193, a finding consistent with the presence of proficient decatennation.

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were synchronized to the G2 phase of the cell cycle and then incubated with solvent alone or with 2 μM ICRF-193. A, significant inhibition of the entry into mitosis in the presence of 2 μM ICRF-193 in five of eight lung cancer cell lines, a finding consistent with the presence of proficient decatenation G2 checkpoint. B, significantly perturbed G2 arrest in response to ICRF-193 treatment in three of the eight lung cancer cell lines, indicating impaired decatenation G2 checkpoint function. C, significant abrogation of G2 arrest in response to MST-16 treatment in ACC-LC-48 in contrast to inhibition of mitotic entry in ACC-LC-172. bars, ±SD.

Fig. 1. Impaired decatenation G2 checkpoint function in lung cancer cell lines. Cells were synchronized to the G2 phase of the cell cycle and then incubated with solvent alone or with 2 μM ICRF-193. A, significant inhibition of the entry into mitosis in the presence of 2 μM ICRF-193 in five of eight lung cancer cell lines, a finding consistent with the presence of proficient decatenation G2 checkpoint. B, significantly perturbed G2 arrest in response to ICRF-193 treatment in three of the eight lung cancer cell lines, indicating impaired decatenation G2 checkpoint function. C, significant abrogation of G2 arrest in response to MST-16 treatment in ACC-LC-48 in contrast to inhibition of mitotic entry in ACC-LC-172.

Identification of Distinct Responses to ICRF-193 or Ionizing Irradiation in Lung Cancers. We previously reported the presence of DNA damage G2 checkpoint impairment in lung cancer cell lines (9). In the study reported here, we noted that the impairment of decatenation and DNA damage G2 checkpoints were discordantly present in the panel of lung cancer cell lines (Table 1). We therefore examined whether ICRF-193-arrested cells exhibited a response similar to that to ionizing irradiation in terms of cyclin B1/cdc2 activity. The in vitro kinase assay demonstrated that marked reduction of cyclinB1/cdc2 kinase activity occurred in two of the eight cell lines, A549 and NCI-H460, whereas the other cell lines did not show any significant inhibition (Fig. 2). We wondered if this significant inhibition of cyclinB1/cdc2 kinase activity in A549 and NCI-H460 might have been caused by the ICRF-193-induced DNA double-strand breaks (DSBs) and subsequent activation of the DNA damage G2 checkpoint. Accordingly, subcellular localizations of γ-H2AX and 53BP1, both of which are thought to form nuclear foci at the sites of DNA DSBs (17, 18), were analyzed in the presence or absence of ICRF-193. In A549 and NCI-H460, the entire cell populations showed clear colocalization of γ-H2AX and 53BP1, uniformly forming marked nuclear foci when analyzed after 1 h-exposure to ICRF-193 (data shown for A549 in Fig. 3), which was further substantiated by the comet assay, which also detects the presence of DNA damage (data not shown). In contrast, no other cell lines exhibited ICRF-193-induced γ-H2AX-positive nuclear foci, and 53BP1 remained in a rather diffuse state in the presence of ICRF-193 (data shown for ACC-LC-172 and ACC-LC-49 in Fig. 3). Western blot analysis did not show significant differences in the amount of γ-H2AX in the presence or absence of ICRF-193 in any of the cell lines examined in this study (data not shown). These observations indicated that, in contrast to the previously held notion, ICRF-193 does induce DNA DSBs in certain cell lines such as A549 and NCI-H460, in which G2 arrest may be in part caused by the activation of DNA damage G2 checkpoint. By the same token, it was clearly indicated that QG56, ACC-LC-49 and ACC-LC-172 were properly arrested at G2 in response to ICRF-193 in the absence of DNA damage G2 checkpoint activation, indicating that decatenation G2 checkpoint was intact in these cell lines.

Intramolecular autophosphorylation of ATM on Ser1981 has been shown to be essential for turning the inactive ATM dimer into a potent protein kinase in response to the occurrence of DNA damage (19). We observed that ICRF-193 elicited autophosphorylation on Ser1981 in A549 and NCI-H460, a finding consistent with the occurrence of DNA DSB, leading to the nuclear foci formation of γ-H2AX and 53BP1 and inhibition of cyclin B1/cdc2 kinase activity (Fig. 4A). Of special interest was that QG56, ACC-LC-49, and ACC-LC-172 also exhibited autophosphorylation on Ser1981, although they did not form nuclear foci of γ-H2AX or 53BP1 and lacked down-regulation of cyclin B1/cdc2 activity (Fig. 4B). In contrast, ATM activation in response to ICRF-193 was significantly diminished in ACC-LC-48 and ACC-LC-176 with prominent decatenation G2 checkpoint impairment, whereas PC-10 with slightly less pronounced perturbation of decatenation G2 checkpoint also showed considerably reduced ATM activation, suggesting a possible link between diminished ATM activation and the decatenation G2 checkpoint impairment (Fig. 4C).

Hypersensitivity to ICRF-193 in Cell Lines with Decatenation G2 Checkpoint Impairment. We next investigated whether continuous exposure to ICRF-193 differentially induces growth inhibition
between lung cancer cell lines with and without decatenation G2 checkpoint impairment. Consequently, ACC-LC-48 and ACC-LC-176, both of which had markedly impaired decatenation G2 checkpoint with clearly perturbed ATM activation in response to ICRF-193, were shown to be hypersensitive to ICRF-193, whereas the respective IC50 values of these cell lines were 0.85 and 1.2 μM. In contrast, other cell lines did not reach IC50 even at 20 μM (Fig. 5A and Table 1). In addition, we measured the percentage of apoptotic cells after 48-h continuous exposure to 2 μM ICRF-193 using propidium iodide staining and flow cytometry to enumerate the sub-G1 population. As shown in Fig. 5, B and C, ACC-LC-48 and ACC-LC-176 showed a significant induction of apoptosis, an indication of hypersensitivity to ICRF-193, in comparison with other lung cancer cell lines and primary normal bronchial epithelial cells.

Search for Alterations in Potential Targets, Including the WRN Gene. A recent study demonstrated that decatenation G2 checkpoint requires WRN, a DNA helicase that is defective in the Werner syndrome, a familial cancer syndrome associated with premature aging (20). Thus, we searched for the mutations in the WRN gene in this panel of eight human lung cancer cell lines. We carried out direct sequencing analysis of the entire 4.3-kb coding region of WRN, resulting in the identification of five nucleotide substitutions, including those with amino acid substitutions at amino acids 1074 and 1367. However, none of the nucleotide substitutions were apparently associated with the presence of decatenation G2 checkpoint impairment, and all five substitutions could be found in a database of single nucleotide polymorphisms at the National Center for Biotechnology Information. In addition, Western blot analysis of WRN, ATR, PLK1, BRCA1, and DNA topoisomerase II did not show any apparent association between their expression levels and the presence of decatenation G2 checkpoint impairment (data not shown).

**DISCUSSION**

It is widely accepted that checkpoint impairment may contribute to the carcinogenesis and progression of human cancers. We previously

**Table 1** Summary of cellular and biochemical responses to ICRF-193 in human lung cancer cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Decatenation G2 checkpointa</th>
<th>DNA damage G2 checkpointb</th>
<th>cdc2/cyclinB1 inactivation</th>
<th>γ-H2AX foci formation</th>
<th>ATMc activation</th>
<th>IC50 (μM)d</th>
<th>p53 status</th>
</tr>
</thead>
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<tr>
<td>A549</td>
<td>N.A.ª</td>
<td>Normal</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>&gt;20</td>
<td>Wild-type</td>
</tr>
<tr>
<td>NCI-H460</td>
<td>N.A.ª</td>
<td>Normal</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>&gt;20</td>
<td>Wild-type</td>
</tr>
<tr>
<td>QG56</td>
<td>Normal</td>
<td>Impaired</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&gt;20</td>
<td>Mutant</td>
</tr>
<tr>
<td>ACC-LC-49</td>
<td>Normal</td>
<td>Impaired</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>&gt;20</td>
<td>Mutant</td>
</tr>
<tr>
<td>ACC-LC-172</td>
<td>Normal</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.85</td>
<td>Mutant</td>
</tr>
<tr>
<td>ACC-LC-48</td>
<td>Impaired</td>
<td>Impaired</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.2</td>
<td>Wild-type</td>
</tr>
<tr>
<td>ACC-LC-176</td>
<td>Impaired</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>±</td>
<td>&gt;20</td>
<td>Mutant</td>
</tr>
<tr>
<td>PC-10</td>
<td>Impaired</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>±</td>
<td>&gt;20</td>
<td>Mutant</td>
</tr>
</tbody>
</table>

ª Decatenation G2 checkpoint response was examined by treating synchronous cell cultures with ICRF-193 in G2 phase (See Fig. 1).

b DNA damage G2 checkpoint response was examined and classified according to mitotic indices 2 h after 1 Gy of irradiation. Normal, <10%; impaired, >30%. Except for NCI-H460 and QG56, data are from our previous study (9).

c ATM, ataxia-telangiectasia mutated; N.A., not available.

d IC50 values were determined by modified MTT assay in cells continuously exposed to various concentrations of ICRF-193 for 48 h.

ª Not available due to activation of DNA damage G2 checkpoint in response to ICRF-193 treatment.
reported on the frequent occurrence of DNA damage G2 checkpoint impairment (9), as well as of mitotic checkpoint dysfunction in human lung cancers (8, 15) in addition to G1 checkpoint impairment, because of frequent p53 inactivation (21). In the study reported here, we have provided for the first time direct evidence that decatenation G2 checkpoint is impaired in a proportion of human lung cancer cell lines, adding decatenation G2 checkpoint as another checkpoint mechanism perturbed in lung cancers. Although chromosome instability is very common in lung cancer cells (3), an intriguing link between inactivation of decatenation G2 checkpoint function and acquisition of chromosomal abnormality has been suggested. Deming et al. (22) reported that chromatid discohesion and subsequent constrictions and tangles were caused by overriding ICRF-193-induced G2 arrest with the use of an ATM/ATR inhibitor caffeine, which resulted in the eventual chromatid breaks and exchanges. Franchitto et al. (20) also suggested that inactivation of decatenation G2 checkpoint could contribute to chromosomal instability in conjunction with other failures.

To date, however, very little information is available on this type of checkpoint defect in human cancer cells. We note that during the preparation of this article, Doherty et al. (23) reported the identification of decatenation G2 checkpoint impairment by analyzing five bladder cancer cell lines. Although future studies of other types of human cancers are needed, it is possible that decatenation G2 checkpoint impairment may be a common event in various types of human cancers, playing a role in the processes of carcinogenesis and/or progression by inducing a chromosome instability phenotype.

This study also clearly shows that impairment of decatenation G2 checkpoint is not concordantly present with the perturbation of DNA damage G2 checkpoint in lung cancer cell lines, suggesting that the signaling pathways, which activate these two checkpoints, are at least in part distinct, although this does not preclude the possibility that these checkpoints share a common molecule(s) in their signaling pathways. In this study, we were able to show that three lung cancer cell lines, namely QG56, ACC-LC-49, and ACC-LC-172, retained their ability to properly arrest at G2 in response to ICRF-193 without causing appreciable DNA damage-induced reactions such as those observed by 1 Gy of irradiation in DNA damage G2 checkpoint-proficient cell lines. In addition, we did not observe phosphorylation of Chk1 at Ser345 in ICRF-193-treated QG56 (unpublished observation), which is consistent with the result of a previous study (11), supporting the notion that the observed delays in entry into mitosis in response to ICRF-193 was not caused by the imposition of replication stress. These findings are consistent with the previously held notion that ICRF-193 is a purely catalytic inhibitor of topoisomerase II, acting in the form of a closed protein clamp without inducing DNA damage (10, 13, 24, 25). However, we also observed the rather unexpected occurrence that ICRF-193-induced DNA DSBs and DNA damage checkpoint activation in two cell lines, i.e., A549 and NCI-H460. DNA DSBs observed in A549 and NCI-H460 are not attributable merely to the deficiency of decatenation G2 checkpoint function.
because ACC-LC-48, ACC-LC-176, and PC-10 did not form nuclear foci of γ-H2AX and 53BP1 without proper G2 arrest in response to ICRF-193 treatment. Our results suggest that ICRF-193 induces DNA DSBs in a certain cellular context by an as yet unknown underlying mechanism. In this connection, a similarly contradictory finding was recently reported by Hajii et al. (26) using the comet assay and pulsed-field gel electrophoresis analysis in cultured V79 and irs-2 Chinese hamster lung fibroblasts. These observations indicate that future studies of decatenation checkpoint need to pay careful attention to inadvertent activation of DNA damage checkpoint by a said catalytic, non-DNA-damaging topoisomerase II inhibitor such as ICRF-193.

As for a candidate molecule responsible for such a defect, it is notable that normal lymphoblast and fibroblast cells of patients with Werner syndrome have been shown to be unable to arrest before entering into mitosis when incubated with the bisdioxopiperazine Werner syndrome have been shown to be unable to arrest before ICRF-193.

catalytic, non-DNA-damaging topoisomerase II inhibitor such as to inadvertent activation of DNA damage checkpoint by a said catalytic, non-DNA-damaging topoisomerase II inhibitor such as ICRF-193.

In this connection, the findings of our study suggests a possibility of a potential link between decatenation G2 checkpoint impairment and diminished ATM activation in response to ICRF-193. Because it has been shown that ATM can be activated by hypotonic swelling or by treatment with chloroquine or trichostatin in the absence of a detectable DNA strand break (19), it is possible that activation of ATM in the absence of DNA DSBs may reflect changes in chromatin structure such as incompletely catenated chromatids because of the ICRF-193 treatment. It has been shown that ICRF-193-induced G2 arrest is ATM independent and relies on ATR activity by analyzing ataxia-telangiectasia cells and human fibroblast overexpressing a kinase-inactive ATR allele (11). The ATM activation by the ICRF-193 treatment observed in our study may accordingly be a mere reflection of activation of a molecule(s) upstream to ATM. It remains possible, however, that ATM might cooperate with ATR and redundantly play a functional, yet dispensable, role in the implementation of decatenation G2 checkpoint. Nevertheless, our findings strongly suggest that in a proportion of human lung cancer cell lines, the process of sensing incompletely catenated chromatids, which also triggers ATM activation in lung cancer cell lines as well as in primary normal human bronchioc epithelial cells (data not shown), is impaired.

Another interesting point of this study is a potential association between decatenation G2 checkpoint impairment and hypersensitivity to ICRF-193, although the small number of cell lines examined did not allow us to draw a definite conclusion. Both ACC-LC-48 and ACC-LC-176, which exhibited markedly impaired decatenation G2 checkpoint and significantly diminished ATM activation, exhibited hypersensitivity to ICRF-193. This notion that ICRF-193 may selectively render decatenation G2 checkpoint-defective cancer cells vulnerable to the induction of cell death is of great clinical interest because we may be able to take advantage of the presence of this association to confer cancer-specific killing activity with a catalytic circular cramp-forming topoisomerase II inhibitors. In this regard, catalytic topoisomerase inhibitors such as aclarubicin and sobuzoxane are being used as antineoplastic agents (27, 28), and novel agents can be expected to emerge soon.

We note that our results are also in line with those of a recently published study, which reported that continuous inhibition of topoisomerase II by ICRF-187 with activities similar to ICRF-193 induced apoptotic cell death in the WRN-defective normal cells of the Werner syndrome cases and that introduction of wild-type WRN cDNA restored decatenation checkpoint as well as resistance to ICRF-187-induced apoptosis (20). Furthermore, they showed that normal cells, which could escape the decatenation G2 checkpoint because of caffeine treatment and were treated continuously with ICRF-187, underwent apoptotic cell death only to a lesser extent. In this connection, we also observed that caffeine abrogated decatenation G2 checkpoint in lung cancer cell lines with proficient decatenation G2 checkpoint but did not significantly sensitize decatenation G2 checkpoint proficient cell lines to ICRF-193 (data not shown). These findings suggest that hypersensitivity to ICRF-193 is not the result of solely and directly from entering into mitosis with insufficiently catenated chromatids but rather from the combination of these effects with failure of an additional surveillance pathway(s). Additional studies are obviously necessary to clarify the molecular basis of hypersensitivity to catalytic topoisomerase II inhibitors, which may ultimately lead to the development of an attractive strategy for lung cancer treatment, i.e., selective killing of targeted cancer cells without causing prominent toxicity in normal cells.

In conclusion, our study clearly shows that decatenation G2 checkpoint is impaired in a proportion of lung cancer cell lines in association with diminished ATM activation and also potentially with hypersensitivity to ICRF-193. The observations reported here warrant further study aimed not only at a better understanding of the underlying mechanisms and biological consequences of decatenation G2 checkpoint impairment in lung cancers but also the development of novel targeted therapeutic approaches for this fatal cancer.

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