High LET Radiation Enhances Nocodazole Induced Cell Death in HeLa Cells through Mitotic Catastrophe and Apoptosis

Ping Li1,2,3, Libin ZHOU1, Zhongying DA1,2,3, Xiaodong JIN1,3, Xinguo LIU1,3, Yoshitaka MATSUMOTO4, Yoshiya FURUSAWA4 and Qiang LI1,3*

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To understand how human tumor cells respond to the combined treatment with nocodazole and high LET radiation, alterations in cell cycle, mitotic disturbances and cell death were investigated in the present study. Human cervix carcinoma HeLa cells were exposed to nocodazole for 18 h immediately followed by high LET iron ion irradiation and displayed a sequence of events leading to DNA damages, mitotic aberrations, interphase restitution and endocycle as well as cell death. A prolonged mitotic arrest more than 10 h was observed following nocodazole exposure, no matter the irradiation was present or not. The occurrence of mitotic slippage following the mitotic arrest was only drug-dependent and the irradiation did not accelerate it. The amount of polyploidy cells was increased following mitotic slippage. No detectable G2 or G1 arrest was observed in cells upon the combined treatment and the cells reentered the cell cycle still harboring unrepaired cellular damages. This premature entry caused an increase of multipolar mitotic spindles and amplification of centrosomes, which gave rise to lagging chromosomal material, failure of cytokinesis and polyploidization. These mitotic disturbances and their outcomes confirmed the incidence of mitotic catastrophe and delayed apoptotic features displayed by TUNEL method after the combined treatment. These results suggest that the addition of high-LET iron ion irradiation to nocodazole enhanced mitotic catastrophe and delayed apoptosis in HeLa cells. These might be important cell death mechanisms involved in tumor cells in response to the treatment of antimitotic drug combined with high LET radiation.

INTRODUCTION

DNA-damaging agents in the form of ionizing radiation and chemotherapeutic drugs are the main components of most current cancer treatment regimens. Antimitotic drugs that disrupt the function of mitotic spindle by altering polymerization dynamics of microtubules have been extensively used for cancer therapy. Among these drugs vinca alkaloids and taxanes showed much success in clinical use against a number of cancers. The most striking phenotype of cells treated with these types of drugs is a cell cycle arrest in mitosis (metaphase), called mitotic arrest. Sustained mitotic arrest mainly leads to two types of cell fates. One is ‘mitotic cell death’, which means cells die in mitosis. Another pathway is that metaphase cells return to interphase as a 4N cell, a process known as mitotic slippage. In some cases, subsequent cell death or interphase arrest can also be elicited following mitotic slippage. In other cases, cells continue to proliferate, implying the resistance to antimitotic drugs.

Ionizing radiation is known to exert effective cell death on tumor cells. Studies have provided mounting evidence to indicate that the biological effects of high linear energy transfer (LET) radiation are more severe than those of low LET radiation. Accelerated iron ions are high LET, and this enhances their relative biological effectiveness (RBE), or ability to cause cell damage and death. G1 and G2-M checkpoints may be activated by irradiation to prevent the damaged DNA to be replicated and transmitted to the next generation. In this radiation-induced defense mechanism, p53 is one of the pivotal molecules involved in safeguarding cell cycle progression and genome stability. On the one hand, p53 is known to induce the synthesis of p21, which is an inhibitor of cyclin dependent kinase that is required for
the arrest of cells in G, on the other hand, p53 blocks cells at the G2 checkpoint involves inhibition of Cdc2, the cyclin-dependent kinase required to enter mitosis. Therefore, mutations in p53 may impair the activation G1 and G2 checkpoint after irradiations.

Apoptosis is a significant mechanism of tumor cell death due to chemotherapy, and resistance to this treatment has been linked to some cancer cell lines with a low propensity for apoptosis. There is more and more evidence that cells can also die through non-apoptotic mechanisms such as necrosis, autophagy, mitotic catastrophe, and so on. Mitotic catastrophe, known as mitotic death, has been widely described in p53 mutated tumor cells. This process has been suggested to be highly dependent on DNA-damage and the subsequent failure of cell cycle arrest in G2-M phase and is apparently selected as an alternative to rapid apoptosis. To detect the occurrence of mitotic catastrophe, both morphological characteristics (such as polyplody giant cells and micronuclei cells) and the presence of mitotic defects (such as incomplete nuclear condensation, chromosome alignment defects, unequal DNA separation or mitosis in the presence of DNA damage) are used.

Human cervix carcinoma HeLa cell line is one of the most used cell models in vitro. It is known to lack a functional p53 due to infection with human papillomavirus 18, which encodes the oncprotein E6/E7 responsible of p53 degradation via the ubiquitin in proteasome, thus making them insensitive to apoptosis induced by many conventional chemotherapeutic agents.

Nocodazole (Noco) is a well-known antimitotic agent, which is widely used in cell biology and in clinical practice. Induction of cell cycle disturbance and apoptosis by using Noco together with radiation has been reported separately. However, the combined effects of Noco and radiation on cancer cells have seldom been studied thoroughly, especially for high LET radiation. In this study we examined how the use of high LET iron ions promotes Noco-induced cell death. In addition, cell cycle alterations, DNA damages and mitotic disturbance in HeLa cells treated with the combination of Noco and high LET radiation were also investigated.

MATERIALS AND METHODS

Cell culture and treatments

HeLa cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) with 100 units/ml penicillin, 100 μg/ml streptomycin and 10% foetal bovine serum (FBS, Biowest, France). All the media and antibiotics were provided by Gibco, Life Technologies, Inc., Grand Island, NY, USA. Cell cultures were performed in a 5% CO2 atmosphere at 37°C. Depending on the experiments, the cells were seeded either in 25 cm² flasks or on 18 mm square coverslips placed into 35 mm Petri dishes (both purchased from Becton Dickinson, USA).

Noco (Sigma) stock solution in DMSO (1 mg/ml) was prepared. Treatments with DMSO did not alter cell growth or cell-phase distribution. The final concentration of DMSO was 0.0065% (v/v) in medium. Noco treatment was achieved by pretreating HeLa cells with a concentration of 65 ng/ml for 18 h. In the present experiments, metaphase cells were shaken off from a Noco arrested population of cells to obtain a pure preparation of mitotic cells for subsequent analysis and irradiation.

Irradiation

Cells were irradiated with iron ions accelerated by the Heavy Ion Medical Accelerator in Chiba (HIMAC) at the National Institute of Radiological Sciences (NIRS), Japan. There is a horizontal beam line for biological sample irradiation with heavy ions at HIMAC. The initial energy of the iron ion beam was 500 MeV/u and the LET value approximated to 200 keV/μm when traversing cell samples. Doses of 0 and 2 Gy were applied in this study and independent triplicate experiment was performed. All the irradiations were performed at room temperature.

Fluorescence flow cytometry

(1) Cell cycle analysis: For DNA content measurements, cells were trypsinized, centrifuged at 1500 rpm for 5 min in PBS and then fixed overnight in 75% cold ethanol. After centrifugation, the cells were resuspended in 1 ml of fresh PBS and then 50 μl of 100 μg/ml RNase (Sigma) was added. Cells were incubated for 30 min with RNase solution and then put on ice. Immediately prior to measurement, DNA was stained by adding 100 μl of 50 μg/ml propidium iodide (PI, Sigma). Measurements were taken on a flow cytometer cell sorter (BD FACS-Calibur, USA) with an argon laser line at 488 nm and complemented with appropriate filters.

(2) MPM-2 staining: Monoclonal antibody MPM-2 (Millipore) was used to quantify mitotic cells, with specificity for mitosis-specific and cell cycle-regulated phosphoproteins. Labeling was done using an indirect immunofluorescence technique modified from Eriksson et al. Briefly, freshly trypsinized cells were fixed in pre-cold 75% ethanol overnight. Fixed samples were blocked in 1% (m/v) BSA solution and incubated for 2 h with MPM-2 (1:200 dilution). Then the samples were washed by centrifugation and resuspended in secondary Alexa fluor 488-conjugated goat anti mouse IgG (Beijing Zhongshan Biotechnology Co., 1:200 dilution) for 1 h. Finally, the cells were suspended in 10 μg/ml PI.

DNA damage analysis

Detection of γ-H2AX as a marker to determine DNA double-strand breaks (DSBs) with flow cytometry assay was performed. Fixation and blocking were performed by referring to and modifying MacPhail et al. Subsequently, the cells were incubated for 2 h with mouse anti-gamma H2AX...
monoclonal antibody (Abcam, 1:200 dilution). After wash and centrifugation, the cells were resuspended in Alexa 488 secondary antibody the same as above for 1 h. Finally, the cells were suspended in PI solution as described above. Data from three independent experiments were collected with a flow cytometer (BD FACS-Calibur, USA) and the results were analyzed using the FlowJo7.6.1 software program (Tree Star, Inc).

Mitotic cell morphology

α-tubulin was visualized to identify the mitotic spindle in HeLa cells. The method was from a modified edition referring to Eriksson et al. 23 After irradiation, cells were fixed with 4% paraformaldehyde in PBS at room temperature and subsequently immunostained for α-tubulin detection. Briefly, the fixed cells were permeabilized with 0.5% Triton-X-100 and subsequently blocked with 10% goat blocking serum for 60 min. Anti-α-tubulin mouse monoclonal primary antibody (Sigma) was used at a dilution of 1:3000 in 1% (m/v) BSA in PBS and incubated at room temperature for 2 h. The slides were further incubated with 488-conjugated secondary antibody as described above (1:2000 dilution) for 60 min at room temperature. Nuclei were counterstained with mounting medium with DAPI (1.5 μg/ml, VECTASHIELD Mounting Medium, Vector Lab, Inc., United States). Coverslips were mounted to slides and viewed using a BX51 fluorescent microscope (Olympus, Tokyo, Japan). γ-tubulin was visualized for detection of altered amplification of centrosome numbers. Cells were fixed, blocked and stained as described above. Anti-γ-tubulin mouse monoclonal primary antibody was purchased from Sigma (clone GTU-88).

Polyploid cells analysis

To obtain the fraction of cells displaying more than 4N DNA content, polyploid cell formation was detected with fluorescence flow cytometry on PI stained cells after treatment as described above.

Assessment of apoptosis

TUNEL was used to detect apoptotic cell death. DNA content of the subpopulation of apoptotic cells was determined by a flow cytometry protocol with the TUNEL assay (Fluorescein FragEL™ DNA Fragmentation Detection Kit, Merck, Germany). 23 Briefly, trypsinized and floating cells were fixed in 4% paraformaldehyde at room temperature. Then the cells were permeabilised with 20 μg/ml proteinase K, incubated with TdT equilibration buffer and TdT labeling reaction mixture, and subjected to the TUNEL reaction. The cells were analyzed with the flow cytometer equipped with a 488 nm argon ion laser source.

Statistics

To compare the difference between the results of the control and treated cells, statistical significance was analyzed by the student-t test. The difference was considered to be significant at \( p < 0.05 \).

RESULTS

Induction of G2-M arrest

Figure 1 shows the cell cycle progression of the untreated HeLa cells (control) in comparison with irradiated and/or Noco treated cells. The treatments of Noco or/and the iron ion irradiation caused a severe alteration of the cell cycle. The FACS analysis indicates that more than 90% of the collected mitotic cells, which were obtained from incubation in 65 ng/ml Noco for 18 h, were arrested at M phase before irradiation and release (Fig. 1A). In contrast with the DNA content of the control (untreated) cells, cell cycle profiles of the irradiated cells (2 Gy) were changed by a transient G2-M arrest at 20 h post-irradiation (Fig. 1D). A shift from the G1 phase and S phase to the G2-M phase could be seen (Fig. 1B, C, D). The fraction of the irradiated cells at G2-M phase returned to the pretreatment level after 44 h (Fig. 1D). In contrast, Noco alone and the combined treatment mainly caused an accumulation of the treated cells at M phase for more than 10 h, namely mitotic arrest (Fig. 1D). At 8 h following irradiation and drug release, there were nearly 23.0 ± 2.3% of the untreated control cells at G2-M phase, whereas the numbers were 27.3 ± 6.3% and 27.8 ± 5.3% for the cells upon the treatments by Noco alone and the combination with the ionizing radiation (Fig. 1D). At 20 h, the G2-M fraction of the cells treated by Noco alone displayed a slight increase up to 29.7 ± 5.1% before a continuous decline, while no detectable G2-M arrest was observed in the cells treated by combination of Noco and irradiation after the mitotic arrest (Fig. 1D). The percentages of the cells at G2-M phase were reduced from 29.7 ± 5.1% to 17.8 ± 2.9% and from 25.6 ± 7.4% to 14.4 ± 2.0% during the time course of 20 h to 68 h in response to Noco alone and the combined treatment, respectively. The decline sustained for at least 60 hours rather than occurred transiently in the cells upon the combined treatment (Fig. 1D). The relative levels of the cells treated by Noco alone and the combination at G1 phase tended to increase after an initial decline (Fig. 1B). A shift from G1 and G2-M phase to S phase could be observed during first 20 h (Fig. 1C). Parallelly with the decrease of the cells at G1 and G2-M, the fraction of the cells treated by Noco alone and in combination with the iron ion irradiation at S phase reached to a maximum at 20 h before an uninterrupted reduction with time lapse (Fig. 1C).

These results are in accord with those obtained from the dual staining with the MPM-2 antibody and propidium iodide (see Fig. 2). The irradiated cells showed an initial G2-M arrest after irradiation. This arrest was transient and the cells reentered the cell cycle and progress into mitosis, increasing the mitotic index. The frequencies of mitoses in the cells treated by Noco alone and the combination were
decreased from top values (Noco: 6.6 ± 0.4%; Noco + 2 Gy: 6.9 ± 0.4%) to the minimums (Noco: 0.6 ± 0.1%; Noco + 2 Gy: 0.7 ± 0.5%) in the time interval of 8 h to 20 h, indicating that the treated cells undergone mitotic slippage and the occurrence was not accelerated by the irradiation. A lasting and compensatory increase of mitotic index was observed in the following time, approaching the frequency of the untreated control cells.

**DNA damage**

Taking phosphorylation of H2AX as a marker of DNA damage, DSBs occurred after the different treatments were
investigated. As shown in Fig. 3, the percentage of γ-H2AX positive cells in the untreated control cells sustained a steady level, whose median fraction amounted to 4.6% (range: 4.3–4.7%). Unlike the control cells, all the treated cells kept high levels of DSBs at 8 h (2 Gy: 51.7 ± 4.4%; Noco: 35.0 ± 5.4%; Noco + 2 Gy: 62.8 ± 4.4%). As the time went on, an inapparent increase of DSBs was observed in the Noco treated cells at 20 h, while the frequency showed an obvious and persistent decline in the time interval of 20 h to 68 h. The percentage of γ-H2AX positive cells in the cells treated by Noco together with the iron ions remained a higher level than that in the cells irradiated alone all the time, even if they kept a similar decrease pattern with time. During the whole process of damage and repair, the combinatorially treated cells possessed more DNA damages all along than the other populations, showing that severe damages, which were difficult to be repaired, were induced by the combined treatment.

**Effects of the combined treatment on mitotic cells**

The combination of Noco and the iron ion irradiation...
induced mitotic and nuclear aberrations as shown in Fig. 4 and Fig. 6. The typical pattern observed was an increase of multipolar mitotic spindles (Fig. 4A). Stained with the antibody against α-tubulin, the spindles could be visualized to reveal multipolar aberration patterns as exemplified by tripolar, tetrapolar divisions (Fig. 4A). γ-tubulin staining of the treated cells with three centrosomes is shown in Fig. 4B. In addition, an increase in the fraction of mitotic cells containing more than two centrosomes was seen in the treated cells (Fig. 5). No significant difference was observed between treatments of Noco alone and the combination of Noco with irradiation (Noco: 10.0 ± 3.3%; Noco + 2 Gy: 12.5 ± 2.3%, p > 0.05). In contrast to the iron ion irradiation alone, the combined treatment significantly (2 Gy: 8.1 ± 1.3%; Noco + 2 Gy: 12.5 ± 2.3%, p < 0.05) increased the frequency of cells with more than two centrosomes. These cells with amplification of centrosome frequently contained more than one nucleus. This increased number of centrosomes and multipolar metaphases should correspond to nuclear abnormality, showing typical morphological features. Some giant cells appeared with multiple nuclei of different sizes (Fig. 6, thick arrow), some of which presented micronuclei or binucleated features (Fig. 6, arrowhead or thin arrow). The origin of these giant interphase cells could be the wrong resolution of abnormal metaphases.

**Polyploid cells**

Flow cytometric analyses were performed to examine the relative amount of polyploidy cells in all the cells surviving the treatment of Noco or/and the irradiation. Measurements (Fig. 7) were taken at 8 h, 20 h, 44 h and 68 h following irradiation and drug release. Approximately 20 h after treatment, the number of polyploidy cells observed was significantly higher in all the treated cells at 44 h compared with that in the untreated control cells (Fig. 7). This increase was most pronounced in the cells upon the combined treatment (Fig. 7). After a gradual increase with time up to 44 h, the frequency of polyploid cells tended to decline in all the treated cases.

**Cell death**

To confirm the occurrence of cell death in HeLa cells following Noco treatment and the iron ion irradiation, TUNEL assay was performed to measure the delayed type of apoptosis by flow cytometry. Observations were made at 20 h, 44 h and 68 h after the different treatments for 48 h. As shown in Fig. 8, DNA fragmentation almost did not occur in the untreated cells and the median DNA fragmentation rate was only 4.4% (varied from 4.3 ± 0.3% to 4.5 ± 0.4%). The cells treated with Noco alone, observed from day 1 to 3 after drug release, had DNA breaks and kept the median DNA fragmentation in a range of 29.0 ± 4.2% to 34.8 ±

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**Fig. 7.** Noco or/and irradiation induced polyploidy in HeLa cells. Fluorescence flow cytometry on cells exposed to the different treatments (Noco or/and 2 Gy irradiation) and stained with propidium iodide was used.
leukemia/lymphoma cell lines with different p53 status.1) Noco with X-ray irradiation were also reported in B-cell subsequent events were pursued. Combined treatments of study focused only on the initial 4 hours after treatment, no diated cells (from 17.6 \pm 1.4\%) as well as at G2-M (from 27.8 \pm 5.2\% to 14.4 \pm 2.0\%) and S (from 11.8 \pm 1.1\% to 7.1 \pm 1.0\%) phases.

DISCUSSION

A combination of different anti-tumoral modalities is advantageous in limiting the non-specific toxicity often observed by an exceedingly high dose of a single regimen.10) Previous studies in regard to combined effects of Noco with low-LET radiation on cancer cells have been reported. Huang et al.26) found that mitotic catastrophe with a range of different nuclear morphologies from binucleated to multanimicronucleated cells was induced in HeLa cells treated by combination of Noco with \(\gamma\)-rays of 10 Gy. However, their study focused only on the initial 4 hours after treatment, no subsequent events were pursued. Combined treatments of Noco with X-ray irradiation were also reported in B-cell leukemia/lymphoma cell lines with different p53 status.1) The outcome suggested that X-ray irradiation of 4 or 6 Gy protected against the apoptosis-inducing effects of the microtubule inhibitor in cell lines defective in p53. The reason why the apoptotic cell death was not enhanced by the combined treatment is supposed that cells defective in p53 have some error(s) in the signaling pathway inducing apop-

tosis after irradiation, and the arrest in G2 prevents the cells from entering mitosis and from apoptosis in the presence of microtubule inhibitors. Therefore, the status of p53 plays an important role in cell death induction. In the present study, the effects of Noco combined with high LET radiation on HeLa cells, especially alterations in cell cycle, mitotic disturbances and cell death, were investigated.

A postmitotic G1 damage checkpoint and a sustained G2-M arrest after DNA damage, required for correct DNA damage reparation following irradiation, have been shown to require p53. Cells p53-deficient may initiate a G2-M arrest but may also rapidly escape from this arrest.23,27) The p53 status in HeLa cells is wild type. In this sense, postmitotic G1 damage checkpoint and G2-M checkpoint should be expected. However, neither detectable G1 nor G2-M checkpoint was observed in cells following the combined treatment of Noco with irradiation (Fig. 1B &1D). Actually, a transient and slight G2-M arrest was observed in cells irra-
diated alone and treated with Noco alone (Fig. 1D), respec-
tively. HeLa cells are known to be infected with human papillomavirus, which expresses E7, a protein derived from a viral oncogene and directly interacting with both p21 and retinoblastoma proteins, thereby inactivating their functions.29) Furthermore, severe damages induced by high LET radiation to metaphase cells may impair the functions of the proteins responsible for G1 and G2-M checkpoints. These might be the reasons why postmitotic G1 and G2-M arrests were failed to be induced in HeLa cells upon the combined treatment.

MPM-2 staining indicates that cells undergone mitotic slippage after the prolonged arrest at M phase (Fig. 2), implying that cells, still harboring unrepaired cellular dam-
gages, escaped to interphase and subsequently endoreduplication. This obviously has profound effects on the behavior of the cells in following cell cycle progression. Because no effective G1 and G2-M checkpoints were activated, cells harboring unrepaired damages might continue to replicate DNA and transmit them to next generation, suggesting the potential of mitotic disturbance. In addition, the iron ions had no impact on the occurrence of mitotic slippage, which seemed to mostly depend on the use of the drug. It has been reported that in the presence of numerous unattached microtubules, mitotic slippage could be accelerated over a range of spindle poison concentrations that allow microtubule assembly because the spindle assembly checkpoint (SAC) becomes satisfied on abnormal spindles.19) This suggests that addi-
tional DNA damage agents cannot accelerate mitotic slippage when the SAC is in an unfulfilled status. In our study, immediate introduction of the iron ion irradiation is sup-
posed not to accelerate mitotic slippage since spindles were unsatisfied due to exposure to Noco incubation.

Immunolabelled centrosomes correspond to the number of spindle poles. The frequency of multiple centrosomes displayed that no significant difference was observed between
cell populations treated by Noco alone and the combination. This indicates that the irradiation of 2 Gy iron ions did not effectively enhance the amplification of centrosomes. Both antimitotic drugs and radiation are capable of inducing the amplification of centrosomes,\(^{18,23}\) but Noco played a more important role in amplification of centrosomes in the present study.

Nuclear abnormalities are induced by mitotic disturbance (Fig. 6). Cells upon combined treatment often contain one or more micronuclei, formed by nuclear membrane formation around lagging chromosomes or chromosomal material. It has previously been shown that amplification of centrosomes leads to a failure in cytokinesis and abnormality of subsequent cell cycle progression.\(^{29,30}\) A failed cytokinesis results in binucleated cells.\(^{23}\) The cell cycle analysis in the present study reveals that polyploid cells were amplified (Fig. 7), especially the population treated with the combination of Noco and irradiation. The formation of polyploid cells can be initially explained by several mechanisms: either by mitotic slippage or by binucleated cells from a failure of cytokinesis, or by combination of the two processes.\(^{3,23}\)

Since the combined treatment was able to induce formation of amplification of centrosomes, polyploidy cells and multinucleated cells, these results suggest that mitotic catastrophe was involved in HeLa cell death. Delayed death, referred to as mitotic catastrophe, can occur in p53-deficient cells and is described as the main form of cell death induced by ionizing radiation.\(^{31}\) Until now, there is no broad consensus on the classification of mitotic catastrophe, although some reports suggest that it shares several biochemical hallmarks of apoptosis.\(^{32}\) In spite of these descriptions, important differences between the two cell death modalities have emerged. Mitotic catastrophe is characterized by multinucleated giant cells with the formation of nuclear envelopes around individual clusters of missegregated, undifferentiated chromosomes, while apoptosis results in cytoplasmic shrinkage and chromatin condensation.\(^{14,33}\) Moreover, mitotic catastrophe can also typify a “survival phenotype” representing an important response of p53-mutated tumors to DNA damaging agents and provides tumors with a mechanism of restitution and endocycle to be resistant to such treatments.\(^{34}\) Mitotic catastrophe can also be triggered by treatment with microtubule-damaging agents, such as taxol and Noco. In this study, after the treatments of Noco alone and single irradiation, polyploidy cells could also be observed, although the frequency was not as high as those in cells upon combined treatment (Fig. 7). Besides, the fraction of cells at each cell cycle phase sustained a relatively high level in contrast with those by combined treatment (Fig. 1 & Fig. 2). These results indicate that the mitotic catastrophe induced by Noco alone or the 2 Gy iron ions was not so effective to kill cells. Cells were inclined to survive and restitute. Accordingly, the high-LET iron ions intensified the production of polyploidy giant cells in the population treated by combination than Noco or irradiation alone.

Apoptosis is a cell death mechanism involved in the development and progression of cancer, as well as in the cytotoxicity or resistance induced by anticancer drugs. The TUNEL assay displayed a delayed form of apoptosis in the present study (Fig. 8). At 20 h, the combined treatment did not show any advantage than the Noco treatment in apoptosis induction. But the situation totally changed as the delayed apoptosis induced by the combined treatment increased dramatically with time lapse. The delayed apoptosis, therefore, might be a result of the mitotic disturbance and polyploidization.

In summary, we found that combined treatment with Noco and high-LET iron ions could enhance the cell death response of HeLa cells than Noco alone. Mitotic catastrophe and delayed apoptosis seem to contribute to the enhanced cell death response. High LET radiation played a crucial role in leading to the DNA DSBs and polyploidization, which contributed to nuclear aberrations. Definitely, further investigations in understanding the molecular mechanisms underlying the processes described above are required.

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