RADIOSENSITIZATION OF NON-SMALL CELL LUNG CARCINOMA BY EGFR INHIBITION

by

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Molecular targeted cancer therapy is a promising treatment strategy. Considering the central role of the epidermal growth factor receptor in cell proliferation and survival, there are indications that targeted agents like tyrosine kinase inhibitors, *i. e.*, erlotinib, may enhance the antitumor treatment by radiation. The aim of this study is to analyze the inactivation effects of γ-rays and to test the radiosensitizing potential of erlotinib on human lung adenocarcinoma cells *in vitro*. Irradiations were performed with doses ranging from 1 Gy to 8 Gy. In order to increase the radiosensitivity of CRL-5876 lung adenocarcinoma cells, the cells were treated with a clinically relevant concentration of 2 μM erlotinib. The effects of single and combined treatments were monitored using clonogenic survival, cell viability and proliferation assays at different time points. For the detection and visualization of the phosphorylated histone H2AX (γ-H2AX), an important biological marker of DNA double-strand break formation, fluorescence immunocytochemistry, was performed. The response to the treatment was monitored at four time points: 30 min, 2, 6, and 24 h. Irradiations with γ-rays resulted in significant cell inactivation regarding all analyzed biological endpoints. Combined treatments revealed consistent cell inactivation. Moreover, compared to γ-rays alone, elevated levels of γ-H2AX foci were observed after pretreatment with erlotinib, indicating radiosensitization through impaired DNA repair.

Key words: human lung adenocarcinoma cell, γ-ray, DNA damage, erlotinib, radiosensitization

INTRODUCTION

Lung cancer is the most frequent cause of cancer mortality worldwide [1]. This aggressive disease can be subdivided as small-cell lung carcinoma (SCLC) and non-small-cell lung carcinoma (NSCLC) [2]. Within NSCLC, representing the majority of lung cancer cases, adenocarcinoma is the most frequently encountered histology [3]. The staging of lung cancer is essential for determining the appropriate therapeutic approach. Surgical intervention is the most common treatment for early stage NSCLC. When diagnosed close to 70% of patients are already in the advanced stage of the disease [4]. Radiotherapy and chemotherapy, alone or combined, remain the most common option for these patients [5]. Cancer cells are less effective in repairing the radiation-induced damage than normal cells, making them easier to be destroyed if radiation therapy is applied [6].

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In recent years, new generations of drugs, *i. e.*, targeted therapeutics were developed in order to block specific signaling pathways involved in cancer progression [7]. Certain members of the family of the epidermal growth factor receptor (EGFR) genes have been overexpressed or otherwise deregulated in almost all epithelial tumors, including NSCLC. This alone, as well as the findings on the importance of protein phosphorylation and the discovery that the first oncogene v-Src is a protein kinase, resulted in selecting EGFR as the primary target of molecular targeted therapy [8-10].

EGFR, a member of the ErbB family of receptor tyrosine kinases, is a transmembrane glycoprotein consisting of a single polypeptide chain and is found in majority of normal cells. The intracellular region of the EGFR is in charge of protein tyrosine kinase activity and plays an important role in the regulation of cell proliferation. EGFR family members are deregulated in cancers by the following three mechanisms: activation of gene mutations, increased number of gene copies (by amplification) and altered ligand expression [11]. Overexpression of EGFR is found in 40% to 80% of patients with NSCLC. It is associated with poor prognosis, since it also plays a specific role in the proliferation, invasion and metastasis of malignant cells [8, 12].

Most targeted therapies include anti-EGFR monoclonal antibodies and small-molecule tyrosine kinase inhibitors (TKI) [13]. Erlotinib hydrochloride (Tarceva®) is a quinazoline small-molecule inhibitor of the EGFR. It is an active and well-tolerated agent in advanced NSCLC [14]. Erlotinib is approved for the treatment of patients with locally advanced or metastatic NSCLC after failure of at least one prior chemotherapy regimen [15].

Radiation induces the expression of EGFR in cancer cells, possibly contributing to the resistance of those cells to therapy [16, 17]. Overexpression of EGFR by tumors is also associated with reduced local control after radiation [18]. Blockade of EGFR signalling *in vitro* has been proven to sensitize cells to the effects of radiation [16]. Considering the new insights into the role of EGFR in DNA repair, there is substantial interest in using EGFR inhibitors for sensitizing tumours to radiotherapy [17].

Several studies have analysed the combination of erlotinib with radiotherapy [16, 19, 20]. Clinical reports of NSCLC patients with brain metastasis exposed to whole-brain radiation therapy with a parallel administration of erlotinib demonstrate longer overall survival with particular benefits marked for patients with EGFR mutations. The rate of these mutations in the analysed group was much higher than expected [20].

Understanding cellular events and pathways underlying the enhancement of the radiation response by EGFR inhibition is important for further improvement of cancer treatment strategy. Therefore, in this *in vitro* study, γ-rays and erlotinib were combined in order to test the radiosensitising potential of erlotinib and, at the same time, improve anticancer effects. Combined effects of these agents were followed on the CRL-5876 human NSCLC lung adenocarcinoma cells. The chosen biological endpoints were: clonogenic survival, cell viability and proliferation. The level of radiation sensitivity is almost exclusively assessed using clonogenic assay (CA) considered as the gold standard. Colorimetric viability assays, such as sulforhodamine B (SRB) or 5-bromo-2'-deoxyuridine (BrdU) test are basically used for the assessment of cellular chemosensitivity. These tests are related to the total cell number or the corresponding proliferation capacity of the cells, while the clonogenic assay measures the survival of colonies [21]. All of the mentioned assays were specifically selected to support the comparison of cell inactivation effects produced by essentially different agents: radiation by γ-rays and radiosensitization via erlotinib. Phosphorylation of histone H2AX (γ-H2AX) was used for the detection of DNA double-strand breaks induced by ionizing radiation and was evaluated at different time points. This kinetic study enabled the detection of residual DNA damage at the level of individual cells. The relationship between the loss of clonogenic ability and the retention of γ-H2AX foci holds for drugs that damage DNA by different mechanisms [22]. Combining these experimental methods, additional data for the design of new therapeutic approaches will be obtained.

MATERIAL AND METHODS

Cell culture

Human NSCLC CRL-5876 cells were purchased from the American Type Culture Collection (ATCC, Manassas, Va, USA) and were cultured in the RPMI 1640 medium (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) supplemented with 10% foetal calf serum (FCS) (Sigma-Aldrich Chemie GmbH) and penicillin/streptomycin (Sigma-Aldrich Chemie GmbH). Cells were maintained in a humidified atmosphere of 5% CO_2 at 37 °C (Heraeus, Hanau, Germany).

Irradiation procedure and erlotinib preparation

CRL-5876 cells were exposed to γ-rays and/or erlotinib in the exponential phase of growth. Irradiations with ^{60}Co γ-rays were performed at the Vinča Institute of Nuclear Sciences, Belgrade, Serbia. The delivered single doses were in the range from 1 Gy to 8 Gy at the dose rate of ~1 Gy/min. For the assessment of γ-H2AX foci, cells were irradiated with a dose of 0.1 Gy at the same dose rate. All cell irradiations were performed at room temperature, except for the immunocytochemistry procedure involving irradiations performed at ~0 ºC. Erlotinib (Tarceva®) was purchased from Roche Diagnostics GmbH, Mannheim, Germany. The 10 mM stock solution was obtained by dissolution in dimethylsulphoside (DMSO; SERVA Electrophoresis GmbH, Heidelberg, Germany). That stock solution was then serially diluted in a cell culture medium. Considering that 2 μM of erlotinib correspond to clinically relevant doses, this concentration was chosen for the analysis of single and combined treatments with γ-rays. The final concentration of DMSO in cell samples did not exceed 0.1%. In accordance with data cited in literature, the drug was administered 1 h prior to radiation and maintained throughout the experiment [23].

Clonogenic assay

CA was performed according to the previously described protocol [24, 25]. Cells were harvested immediately after irradiation and/or erlotinib pretreatment and seeded at a suitable number in triplicate, in 6-well plates. A 7 day time point was chosen for the evaluation of radiobiological effects, since it enables at least six doubling times following irradiation [26]. After the incubation period of 7 days, cells were rinsed carefully with phosphate- buffered saline (PBS), fixed with methanol (Zorka Pharma, Šabac, Serbia), stained with 0.5% Crystal Violet (Allied Chemical, New York, USA) in 25% methanol for 10 min. After that, the crystal violet was removed, dishes were rinsed with tap water and left to dry at room temperature. Colonies consisting of 50 or more cells were counted under the inverted microscope. The survival fraction of treated cells was determined by comparing the number of colonies in treated samples with those in control. Digital images from 6-well plates were taken and colony sizes measured using ImageJ software [27].

Cell viability assay

The Sulforhodamine B (SRB) assay was used for cell density determination [28]. In order to check the results obtained by the clonogenic assay, these results were compared with SRB data [21]. To define the experimental conditions in the SRB assay, exponentially growing cells were treated with 2 μM erlotinib for 24, 48, and 72 h. The most pronounced single effect of erlotinib was obtained at the 72 h time point. Therefore, viability tests were performed 72 h and 7 days after the treatment of CRL-5876 cells. The SRB assay is based on the measurement of the cellular protein content. The solubilized dye, SRB, binds to the basic amino acids of cellular proteins. The colorimetric measurement of the bound dye provides data on the total protein content that is correlated with the cell number. Cells were seeded in 96-well plates at a density of 1000 cells per well and treated in the exponential phase of growth. Afterwards, chosen incubation period cells were fixed with 10% trichloroacetic acid and stained with 0.4% SRB (Sigma-Aldrich Chemie GmbH) for 15 min. The excess dye was removed by washing with 1% acetic acid. The protein-bound dye was dissolved in a 10 mM Tris base solution for absorbance determination, at 550 nm, using a microplate reader (Wallac, VICTOR2 1420 Multilabel counter, Turku, Finland).

Cell proliferation assay

The 5-bromo-2'-deoxyuridine (BrdU) assay (Roche Diagnostics GmbH) was used to measure cell proliferation by quantifying BrdU that is incorporated into the newly synthesized DNA of replicating cells. The assay was performed according to the manufacturer's instructions and at the same time points as the SRB assay (72 h and 7 days after treatment). The cells were incubated with the BrdU labeling solution for 2 h, fixed and incubated with the anti-BrdU-POD antibody. After removing the antibody, the substrate solution was added and incubated until the dye was developed (from 5 to 30 min). The reaction was stopped by adding the 1M H2SO4 solution. Absorbance was measured using a microplate reader (Wallac, VICTOR2 1420 Multilabel counter) at a test wavelength of 450 nm.

Immunofluorescence staining for detection of γ-H2AX foci

For the detection of γ-H2AX, the primary (direct) immunofluorescence procedure was used. Briefly, cells were grown on glass cover slips in 6-well dishes, overnight, in order to attach to the surface. At 0.5, 2, 6, and 24 h posttreatment, the medium was aspirated and cells washed with cold PBS. The cells were then fixed with ice-cold acetone-methanol (1:1) for 30 min at –20 ºC. Following fixation, the cells were washed again with PBS and blocked with a 5% bovine serum albumin (BSA, Fraction V; Sigma-Aldrich Chemie GmbH) in PBS for 1 h at room temperature. They were then incubated overnight at 4 ºC with primary Alexa Fluor® 488 anti-H2AX Phosphorilated (Ser 139) antibody (BioLegend, San Diego, Cal., USA) at 1:500 dilution. After incubation, the cells were washed 5 times, each for 10 min, with PBS Tween 20 (PBST) and counterstained with DAPI (4', 6-diamidino-2-phenylindole dihydrochlo- ride, 1 µg/ml; Sigma-Aldrich Chemie GmbH) in PBS. Following extensive washing in PBST, cover slips were mounted on glass slides with the Mowiol® 4-88 antifade mounting medium (Sigma-Aldrich Chemie GmbH). Slides were then sealed and examined using a confocal laser scanning microscope, Leica TCS SP5 II (Leica Microsystems CMS GmbH, Wetzlar, Germany) and LAS AF Lite software (Leica Microsystems CMS GmbH). The images were processed and the number of γ-H2AX foci was determined using CellProfiler image analysis software. Cells with more than 10 foci were considered as foci-positive. In each experiment, at least 50 foci-positive cells were examined.

Statistical analysis

During each experiment, measurements were made in triplicate and each experiment repeated three times. The statistical analysis was performed using independent Student's *t*-test and the value of $p < 0.05$

considered as statistically significant. The results were presented as the mean SEM (Standard Error of the Mean).

RESULTS

Survival of CRL-5876 cells after erlotinib and γ-rays

Clonogenic survival is the conventionally used criterion that illustrates the level of cellular radiosensitivity. The survival of CRL-5876 cells was analyzed after their exposure only to γ-rays, as well as after their combined exposure to erlotinib and γ-rays. Experimental data were fitted using the linear-quadratic equation: $S = \exp(-\alpha D - \beta D^2)$, where S is the surviving fraction for dose D, while α and β are the fitting parameters.

The best fit curves of the data points for two experimental setups obtained by clonogenic assay 7 days after irradiation are presented in fig. 1(a), while the corresponding radiobiological data are given in tab. 1. These results indicate a rather high level of radioresistance of the analyzed cells to lower doses of γ-rays (1 and 2 Gy). The estimated survival fraction at 2 Gy (SF2) is 0.61. Starting from 4 Gy, the response of CRL-5876 cells to γ-rays is major, showing significant cell inactivation, fig. 1(a). Pretreatment of the irradiated CRL-5876 cells with erlotinib causes higher inhibition of cell survival with respect to irradiation alone. The relative biological effectiveness at 2 Gy (RBE (2 Gy, γ)) is 3.12. It is defined as the ratio of a 2 Gy γ-ray dose and a γ-ray dose boosted by erlotinib that produces the same inactivation level as that given by 2 Gy of the reference γ-rays. Moreover, the reduction in colony number is accompanied by the reduction in colony size, as illustrated in fig. 1(b). Another commonly used radiobiological parameter is the D_{10} value which represents the radiation dose required to reduce survival to 10%. Pretreatment with erlotinib leads to a drop of D_{10} from 5.6 0.6 to 2.9 0.4. The radiosensitization potential of erlotinib is also illustrated by the sensitization enhancement ratio (SER) [29]. It is defined as the ratio of D_{10} without the sensitizer and D_{10} with the sensitizer, *i. e.*, in this case, erlotinib. The obtained SER of 1.9 indicates that the increase of sensitivity to γ-irradiation is induced by erlotinib (tab. 1).

Viability and proliferation of CRL-5876 cells after erlotinib and γ-rays

The SRB assay was performed to assess cell viability after irradiation without and with erlotinib pretreatment. According to the data obtained, 72 h after irradiation a major dose-dependent response to single γ-rays with respect to the control is observed, fig. 2(a).

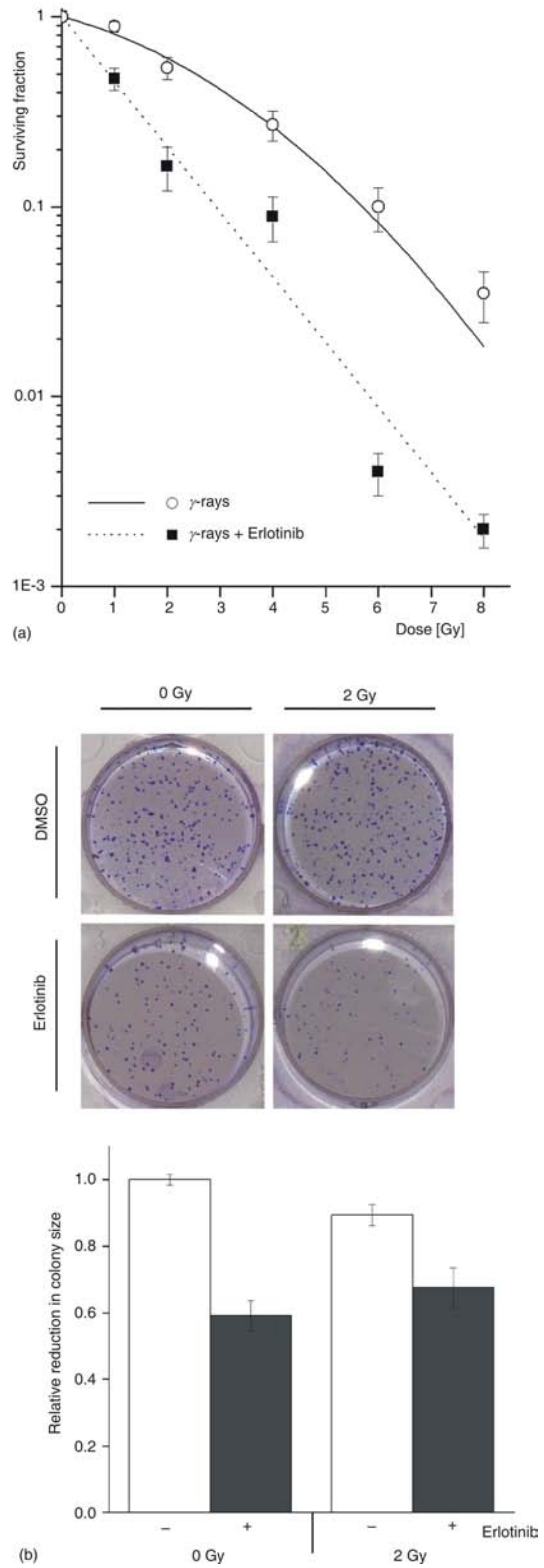

Figure 1. (a) Dose-dependent survival curves of human CRL-5876 cells after irradiation with γ-rays and after combined treatment with erlotinib and γ-rays, obtained by clonogenic assay. The curves represent the best fit of the survival data to the linear-quadratic equation. Irradiation doses are 1, 2, 4, 6, and 8 Gy. Concentration of erlotinib is 2 μM. Data obtained from 3 experiments are presented as mean SEM; (b) Relative reduction of the CRL-5876 colony size after single and combined treatment with 2 Gy γ-rays and 2 μM erlotinib

Table 1. Survival parameters, RBE and SER values for CRL-5876 cells

Treatment	α [Gy^{-1}]	β [Gy^{-2}]	SF2	RBE [2 Gy, γ]	D_{10} [Gy]	SER [D_{10}]
γ-rays	0.166 0.059	0.042 0.019	0.61 0.01	1.00	5.6 0.6	1
γ-rays + erlotinib	0.789 0.110	–	0.21 0.01	3.12 0.44	2.9 0.4	1.9 0.33

Data are presented as mean SEM

Viability is reduced for more than 50%. Both single and combined treatments show a statistically significant inhibition of cell viability compared to the control cells ($p < 0.001$). CRL-5876 cells reveal a considerably higher dose-dependent inactivation to combined treatment, as compared to single treatment with γ-rays. At higher doses of radiation, a slightly less pronounced effect of erlotinib is noticed ($p < 0.001$). Erlotinib alone also decreases the viability of the cells 72 h after administration ($p < 0.01$), fig. 2(a).

At 7 days of posttreatment with γ-rays, higher irradiation doses of 6 Gy and 8 Gy provoke a strong decline in cell viability. A better dose-dependent response with combined treatment is observed when compared to the 72 h time point. Moreover, at this time point, viability after combined treatments is significantly different with respect to each single treatment ($p < 0.001$ and $p < 0.01$ compared to γ-rays and erlotinib, respectively), fig. 2(b).

According to the results obtained by the BrdU assay, 72 h after exposure to single γ-rays, cell proliferation decreases from 86% to 4% of the control value. Combined treatments provoke an even stronger and statistically significant inhibition of proliferation, not exceeding 10% of the control value, fig. 3(a). Nevertheless, at the 7 days' time point, cells indicate proliferation recovery for all treatments, as compared to the 72 h time point, fig. 3(b).

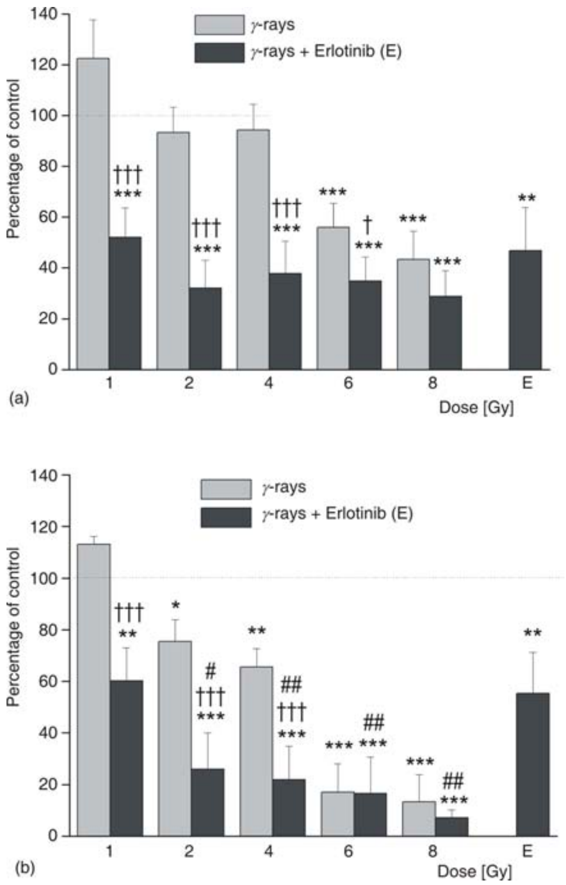

Figure 2. Viability of CRL-5876 cells after single and combined treatments with γ-rays and erlotinib determined by SRB 72 h (a) and 7 days (b) after irradiation. Irradiation doses are 1, 2, 4, 6, and 8 Gy, while the concentration of erlotinib (E) is 2 µM. Data obtained from 3 experiments are presented as mean SEM
(* – single or combined treatment *vs*. control, † – combined treatment *vs*. γ-irradiation, # – combined treatment *vs*. single drug treatment; $0.01 < p < 0.05$ (*, †, #), $0.001 < p < 0.01$ (**, ††, ##), $p < 0.001$ (***, †††, ###))

Kinetics of γ-H2AX foci formation

In order to evaluate the effect of erlotinib pretreatment on irradiation-induced DNA damage, immunofluorescent staining of phosphorylated H2AX and confocal image analyses were performed 0.5, 2, 6, and 24 h after irradiation. Representative micrographs of the cells containing γ-H2AX foci are shown in fig. 4(a). Immediately after exposure to 0.1 Gy γ-rays, the number of γ-H2AX foci increases and after reaching the maximum at 2 h, gradually decreases as the consequence of DNA repair. At 24 h postiradiation, the number of foci decreases to the control level. Although the induction and kinetics of the disappearance of γ-H2AX foci is similar in erlotinib pretreated cells, significant changes in the number of foci are observed only at 24 h posttreatment ($p < 0.05$), fig. 4(b).

DISCUSSION

In contemporary medical practice, radiation is being used as an effective modality in cancer treatment with the ultimate goal of providing the most optimal therapeutic effect. Over the past years, the main challenges in cancer cure were focused on treatment and delivery planning, with the aim of improving the therapeutic effects and minimizing corresponding, unintended, side effects. However, radiation oncology is faced with dose tolerance limitations and, if it is to progress further and deliver better clinical outcomes, it

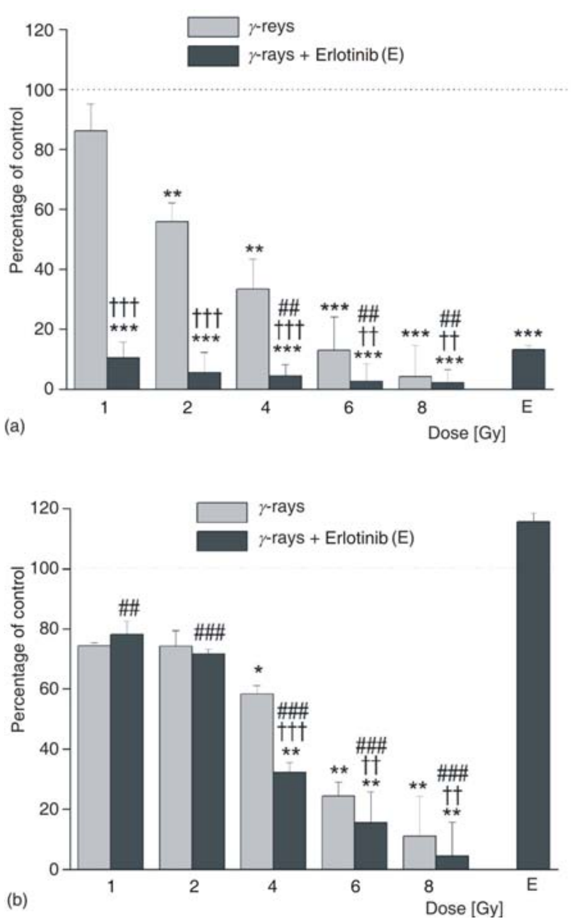

Figure 3. Proliferation of CRL-5876 cells after single and combined treatments with γ-rays and erlotinib revealed by BrdU 72 h (a) and 7 days (b) after irradiation. Irradiation doses are 1, 2, 4, 6, and 8 Gy, while the concentration of erlotinib (E) is 2 μM. Data obtained from 3 experiments are presented as mean SEM (* – single or combined treatment *vs*. control, † – combined treatment *vs*. γ-irradiation, # – combined treatment *vs*. single drug treatment; $0.01 < p < 0.05$ (*, †, #), $0.001 < p < 0.01$ (**, ††, ##), $p < 0.001$ (***, †††, ###)

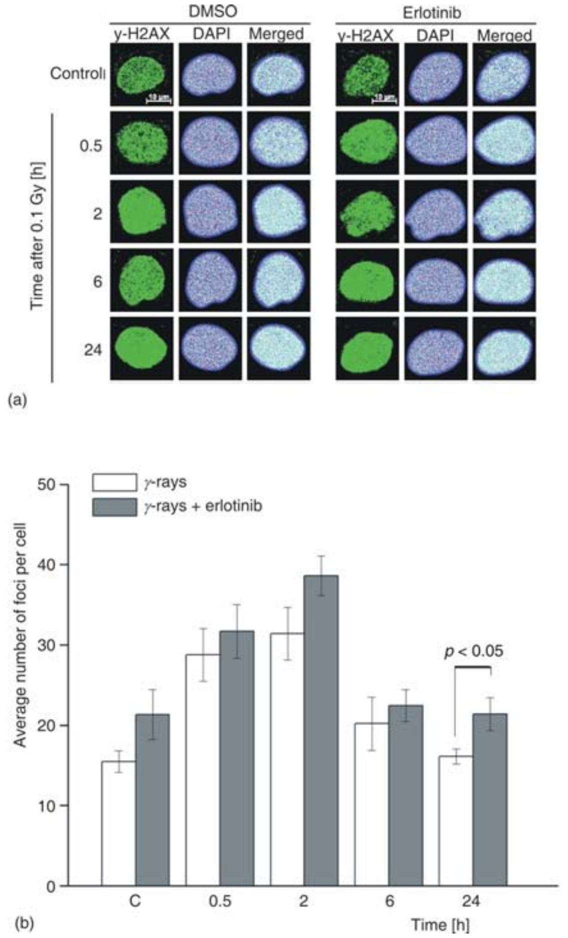

Figure 4. (a) Distinctive micrographs of the cells containing γ-H2AX foci after single and combined treatments with γ-rays and erlotinib, obtained by confocal laser scanning microscope in order to reveal kinetics of γ-H2AX foci; (b) Kinetics of irradiation-induced γ-H2AX foci formation after single and combined treatments with γ-rays and erlotinib. γ-H2AX foci are determined at the time points of 0.5, 2, 6, and 24 h. The irradiation dose is 0.1 Gy while the concentration of erlotinib is 2 μM. Concentration of DMSO corresponding to that added with erlotinib solution

needs to integrate biological innovations into the radiation therapy [30]. Molecular targeted drugs as modern radiosensitizing agents receive much attention. Preclinical *in vitro* and *in vivo* model systems may be used to examine mechanisms underlying tumor radiosensitization by these drugs [31]. The blockade of the wild-type EGFR has also been demonstrated to reduce radiation resistance through three separate mechanisms: by reducing DNA repair, by inhibiting antiapoptotic pathways and by reducing proliferation [16]. This study describes the ability of the EGFR tyrosine kinase inhibitor, erlotinib, to modulate the radiation response in human CRL-5876 NSCLC adenocarcinoma cells.

Several preclinical and clinical studies are underway to evaluate the combination of erlotinib with radiotherapy [20, 32]. Since there is a lack of basic research in this field, recent *in vitro* studies have been designed with erlotinib given before and/or after radiation, but the optimal way of administering these agents needs yet to be established [33]. In this experimental setup, erlotinib was added 1 h before irradiation, with prolonged incubation after irradiation when the assays were performed. A similar experimental setup was reported by Wang and coworkers [23].

The biological endpoints used in this work, *i. e*., clonogenic survival for the assessment of radiation damage and colorimetric assays enabling the follow up of cellular viability after administration of different antitumour agents, were chosen to allow for the complementary analyses of the combined treatment that was applied. All assays were performed at appropriate time points, thus enabling comparative investigation of obtained data. In addition, the kinetic study of appearance and disappearance of γ-H2AX foci, particularly extended to the time point of 24 h, was aimed to correlate the loss of clonogenic ability detected by CA, with the retention of DNA damage-induced foci, therefore providing a complementary approach [22].

As demonstrated by CA, with SF2 being 0.61, the CRL-5876 cells can be considered as radioresistant, fig. 1(a). In combined treatment, erlotinib increased the radiosensitivity of CRL-5876 cells, reaching 0.21 for SF2, whereas the D_{10} value also changed from 5.6 to 2.9 Gy, thus giving a SER of 1.9 (tab. 1). The decrease in colony number was accompanied by a decrease in colony size, fig. 1(b). A similar conduct of radiosensitivity as a function of dose after treatments with γ-rays alone and in combination with erlotinib was observed when cells were analyzed by other viability tests, such as SRB and BrdU, figs. 2 and 3. Nevertheless, CA showed a stronger inactivation of CRL-5876 cells than SRB and BrdU assays. This can be explained by the fact that the clonogenic assay measures only the capacity of individual cells to form macroscopic colonies, whilst viability assays measure the total protein content (SRB assay) or DNA replication (BrdU assay). Moreover, recent data indicate that radiation and erlotinib can cause growth arrest by inducing accelerated cell senescence [23]. Senescent cells remain metabolically active for an extended time (up to 7 days after treatment) and therefore would be scored as "survivors" in viability assays [34].

Most of the cancer therapeutic agents, including radiation therapy, directly or indirectly induce DNA double-strand breaks (DSB). If not properly repaired by the cellular repair machinery, these injuries are considered lethal. One of the first cellular responses to these damages is phosphorylation of histone H2AX, resulting in the formation of distinct foci within minutes after the initial damage [35]. Due to its capability to distinguish microscopically visible foci in single cells, γ-H2AX assay is widely applied in monitoring the effectiveness of radiation sensitizers [36]. Exploiting the high sensitivity of this assay, the impact of erlotinib on DSB repair kinetics after radiation was tested. To avoid foci overlapping and, thus, the possible underestimation of their number at high doses, a low irradiation dose, *i. e*., 0.1 Gy, was chosen for the investigation of the extent of the radiosensitizing effect of erlotinib. The results obtained show a significantly higher number of foci in erlotinib-pretreated cells 24 h after radiation. It was previously reported that this retention of γ-H2AX foci is to be associated with the loss of clonogenic potential [37]. Several studies have shown that repair-deficient cell lines preserve more foci when analyzed 24 h after irradiation [38, 39]. Cells that retained unrepaired foci are probably the cells that are intended to die. The percentage of cells that preserved γ-H2AX foci 24 h after irradiation was correlated with the percentage of cells that lost clonogenicity, thus making it possible to use the fraction of cells with residual foci as a way to estimate sensitivity to killing by ionizing radiation [22, 40].

EGFR is involved in several critical processes in DNA repair that include impact on translocation, transcription and phosphorylation of key proteins and genes responsible for DNA repair [41]. Therefore, this multiple role of EGFR in the DNA repair process may be the cause of the radiosensitization effects of erlotinib on analyzed CRL-5876 cells. The molecular mechanisms by which erlotinib regulates DSB repair need further in-depth investigation.

CONCLUSION

In summary, results reveal that γ-rays inactivate CRL-5876 cells in a dose-dependent way. Pretreatment with erlotinib sensitizes the cells to γ-rays, thus making this agent valuable in cancer treatment when used in synergy with radiation. The *in situ* detection of γ-H2AX foci was used in monitoring the effectiveness of pretreatment with the radiation sensitizer. The estimation of an optimal therapeutic schedule for the administration of erlotinib synchronized with radiation treatment would be beneficial for a combined therapy approach.

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РАДИОСЕНЗИБИЛИЗАЦИЈА НЕСИТНОЋЕЛИЈСКОГ КАРЦИНОМА ПЛУЋА ЧОВЕКА ИНХИБИЦИЈОМ ЕГФР-а

Молекуларно циљана терапија канцера је важан приступ за лечење ове болести. Имајући у виду улогу коју рецептор за епидермални фактор раста има на пролиферацију и преживљавање ћелија, постоје назнаке да циљани агенси, као што су инхибитори тирозин киназа, нпр. ерлотиниб, могу да повећају ефикасност зрачења у елиминацији тумора. Циљ ове студије је анализа ефеката различитих доза гама зрачења као и тестирање могућности ерлотиниба да повећа радиоосетљивост ћелија хуманог аденокарцинома плућа у *in vitro* условима. Примењене су дозе гама зрачења од 1 Gy до 8 Gy. У намери да се повећа радиоосетљивост CRL-5876 аденокарцинома плућа, ћелије су третиране клинички релевантном дозом ерлотиниба од 2 μM. Ефекти појединачних и комбинованих третмана су праћени помоћу клоногеног преживљавања ћелија, као и њихове вијабилности и пролиферације у различитим временским тачкама. За детекцију и визуелизацију фосфорилисаног хистона H2AX (γ-H2AX), који је важан биомаркер за праћење стварања дволанчаних прекида ДНК, коришћена је метода флуоресцентне имуноцитохемије. Одговор на третмане је праћен у четири временске тачке: 30 минута, 2, 6 и 24 часа. Озрачивање гама зрацима довело је до значајне инактивације ћелија на нивоу свих праћених биолошких параметара. Комбиновани третмани су показали конзистентну ћелијску инактивацију. Такође, повећан број γ-H2AX једараца је примећен након претретмана ерлотинибом у поређењу са самим гама зрачењем. Ово указује на повећање радиоосетљивости ћелија до које је дошло услед нарушене ДНК репарације.

Кључне речи: ћелија аденокарцинома плућа човека, гама зрачење, ДНК оштећење, ерлотиниб, радиосензибилизација