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ORIGINAL PAPER

Relationships between EGFR-initiated signalling, DNA double-strand break rejoining and survival in X-irradiated human glioma M059 cells

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Abstract. The aim of this study was to investigate the effect of signalling inhibition on survival and double-strand break (DSB) rejoining in cells differing in sensitivity to inhibitors, X-rays and bleomycin. Human glioma M059 cells, K (relatively radioresistant) and J (radiosensitive, defective in DSB rejoining for lack of DNA-dependent protein kinase catalytic subunit, DNA-PK_{cs}) were pretreated with signalling inhibitors: tyrphostin AG 1478, specific for epidermal-growth-factor-receptor (EGFR) kinase or PD 98059, specific for kinase MEK 1/2 (mitogen-activated, extracellular signal-activated kinases 1 and 2). Subsequently, the cells were X-irradiated or treated with bleomycin. Cell survival was determined by clonogenicity test. DSB rejoining was monitored with the use of pulsed-field gel electrophoresis (PFGE). We found that in X-irradiated M059 K cells EGFR kinase activity was necessary for efficient DSB rejoining and the kinase inhibitor, tyrphostin AG 1478, acted as radiosensitizer in the dose range that reduced cell survival to 0.7–0.8. Inhibition of EGFR kinase, however, did not decrease survival or affect DSB rejoining in DNA-PK_{cs}-deficient M059 J cells. These results indicated that the decrease in cell survival was due to a disturbed DSB rejoining by the DNA-PK dependent system. In contrast, inhibition of MEK 1/2 kinase on EGFR downstream signalling pathway by PD 98059 did not affect DSB rejoining in either cell line and exerted a radioprotective effect.

Key words: human glioma M059 K and J cells • DNA-dependent protein kinase (DNA-PK) • radiosensitivity • DNA double-strand break (DSB) rejoining • epidermal-growth-factor-receptor (EGFR) • signalling inhibitors: tyrphostin AG 1478 and PD 98059

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Introduction

Agents that target epidermal-growth-factor-receptor (EGFR) potentially exert anti-tumour effects by inhibiting cell proliferation and survival. Hence, effects of inhibitors of EGFR tyrosine kinase are of practical interest; moreover, combined X-irradiation and treatment with specific inhibitors of EGFR tyrosine kinase or inhibitors of EGFR-linked signalling pathways give promising results (e.g. [5, 17, 20, 21, 23, 25, 26, 33]).

Activation of EGFR takes place in a ligand-dependent or independent way and is followed by internalization. Ligand-independent activation of EGFR occurs in cells exposed to ionizing radiation [9, 28, 29] and most probably consists in generation of reactive oxygen species that inactivate redox-sensitive cysteine residues in the active sites of tyrosine phosphatases [24]. In consequence, the balance between active (phosphorylated) and inactive (dephosphorylated) forms of the EGF receptor is shifted towards the active form [27, 31]. Its internalization is through caveolae, followed by nuclear translocation and is linked to DNA-repair processes ([9], review in [29]). In contrast, ligand-induced EGFR internalization is through clathrin-coated pits; the signalling continues from within the cell until the receptor

molecules are degraded or recycled. Nevertheless, also radiation-induced, EGFR-linked MAPK (mitogen-activated protein kinase) signalling takes place and results in up-regulation of genes involved in DNA repair, e.g. XRCC1 (X-ray repair cross-complementing group 1), ERCC1 (excision repair cross-complementing 1) [32] and PCNA (proliferating cell nuclear antigen) [3]. Such response develops later than nuclear translocation and is reflected in residual DNA damage and survival.

In order to assess the contribution of the immediate, DNA-repair targeted (linked to nuclear translocation of EGFR) and the delayed, usually proliferation targeted (due to MAPK signalling) effects of EGFR activation, we have used two signalling inhibitors, tyrphostin AG 1478 – a specific EGFR tyrosine kinase inhibitor and PD 98059 that inhibits MEK 1/2 kinase downstream of EGFR activation, on the MAPK signalling pathway (see Fig. 1). The first inhibitor may affect all signalling pathways presented in the diagram and in some cell models this would impede discerning the specific effects of blocking the signal initiated by EGFR activation. In particular, EGFR activation is connected with transcription of the immediate early response genes, PKC-dependent [16] or PKC-independent [30]. These genes are nuclear signal transducers important for cell survival [15].

We used two closely related human glioma cell lines: M059 K and M059 J [1], the latter lacking the catalytic subunit of DNA-dependent protein kinase, DNA-PK_{cs}. The immediate effect of the inhibitors, estimated from DSB rejoining after X-irradiation was examined previously [13]. The results of that series of experiments indicated that in X-irradiated in M059 K cells the D-NHEJ (DNA-PK-dependent non-homologous end-joining) system of DSB repair is activated by EGFR and proceeds with nuclear translocation of the receptor. Autophosphorylation of the receptor, but not MAPK signalling, is essential for this process. Neither effect on DSB rejoining nor translocation of EGFR to the nuclei is observed in M059 J cells in which the targeted repair system is missing.

In this report, we attempted at evaluating to what extent the disturbance in DSB rejoining caused by signalling inhibition is reflected in cell survival.

Materials and methods

Cell cultures and treatment

Human glioma M059J and M059K cells, purchased from American Type Culture Collection were maintained in DMEM : Ham's F12 (1:1) medium, supplemented with 10% foetal calf serum and antibiotic-antimycotic solution (Gibco), at 37°C in a humidified 5% CO_2 incubator.

Stock solutions of signalling inhibitors: tyrphostin AG 1478 (5 mM, Sigma) and PD 98059 (20 mM, Promega) were prepared in DMSO; bleomycin sulphate (17.5 U/ml, Lundbeck, Copenhagen) and recombinant human epidermal growth factor, rhEGF (500 μg/ml, Biochrom, Germany) were dissolved in PBS. All the reagents were stored in small aliquots at –20°C and freshly dissolved in the medium before use.

X-irradiation was carried out at 0–4°C, with the use of an ANDREX defectoscope (Holger Andreasen, Denmark) operating at 200 kV and 5 mA, with 3 mm Al filtration, at a dose rate of 1.5 Gy/min.

Bleomycin, diluted to the appropriate concentration in the medium, was added to cell cultures for 1 h at 37°C; subsequently, cells were placed in the fresh medium.

Clonogenicity tests

The influence of the inhibitors: tyrphostin AG 1478 and PD 98059 on the cellular sensitivity to bleomycin and to X-radiation was determined from the loss of the colony forming ability. The cells were seeded onto 60-mm cell culture dishes, 300–10,000 cells per dish, and pre-incubated (or sham pre-incubated) with the inhibitors for 30 min. The inhibitors were used at the concentrations that reduced the cell survival to about 0.7 when used alone (paper submitted and [13]), i.e. 5 μM or 7 μM tyrphostin AG 1478 and 70 μM or 30 μM PD 98059 for M059 K or J cells, respectively. Subsequently, the cell cultures were cooled on ice and exposed (or sham exposed) to a range of X-ray doses or incubated with a range of bleomycin concentrations at 37°C. The inhibitors were present until the end of the experiments. After 10–14 days of growth, the cells were fixed with 10% formaldehyde and stained with Giemza. Colonies consisting of about 50 cells were counted.

Estimation of DNA double-strand break (DSB) rejoining by pulsed-field gel electrophoresis (PFGE)

A non-radioactive PFGE based assay [12] was used to monitor the rejoining of DNA double-strand breaks (DSBs) in M059 K and J cells after X-irradiation. The cell cultures (ca. 3×10^5 cells per dish) were pretreated (or not) for 30 min with tyrphostin AG 1478 or PD 98059. Concentrations used were: 1.5 μM and 5 μM tyrphostin AG 1478 for M059 K and J cells respectively and 20 μM PD 98059 for both cell lines. The cells were then cooled on ice and irradiated with X-rays at a dose of 10 Gy. Subsequently, the cells were either directly processed for PFGE (initial damage samples) or incubated further for 30, 60 or 180 min at 37°C (repair samples). The PFGE assay conditions were essentially as in [12]. In brief, the cells were detached from the dishes by trypsinisation, embedded in 1% low melting point agarose and incubated in agarose blocks in a lysing buffer (1 mg/ml proteinase K, 0.2% sodium deoxycholate, 1% N-lauroylsarcosine-sodium salt and EDTA, pH 8.0) for 20 h at 50°C. Then, the blocks were washed with 20 mM Tris/HCl and 50 mM EDTA, pH 8.0, equally sized agarose plugs were cut from the blocks and applied to the wells in a 0.8% chromosomal grade agarose gel. Electrophoresis was performed in a CHEF-III apparatus (BioRad), in 0.7 × TBE buffer (62 mM Tris/borate, 1.4 mM EDTA, pH 8.3) at 1.7 V/cm and 106° reorientation angle, at 14°C. The run comprised three 31-h blocks of increasing switch times: 50–200 s, 200–600 s and 2000–2200 s. After electrophoresis, DNA in the gel was stained with SYBR-Gold (Molecular Probes) and visualized under UV-light, followed by the

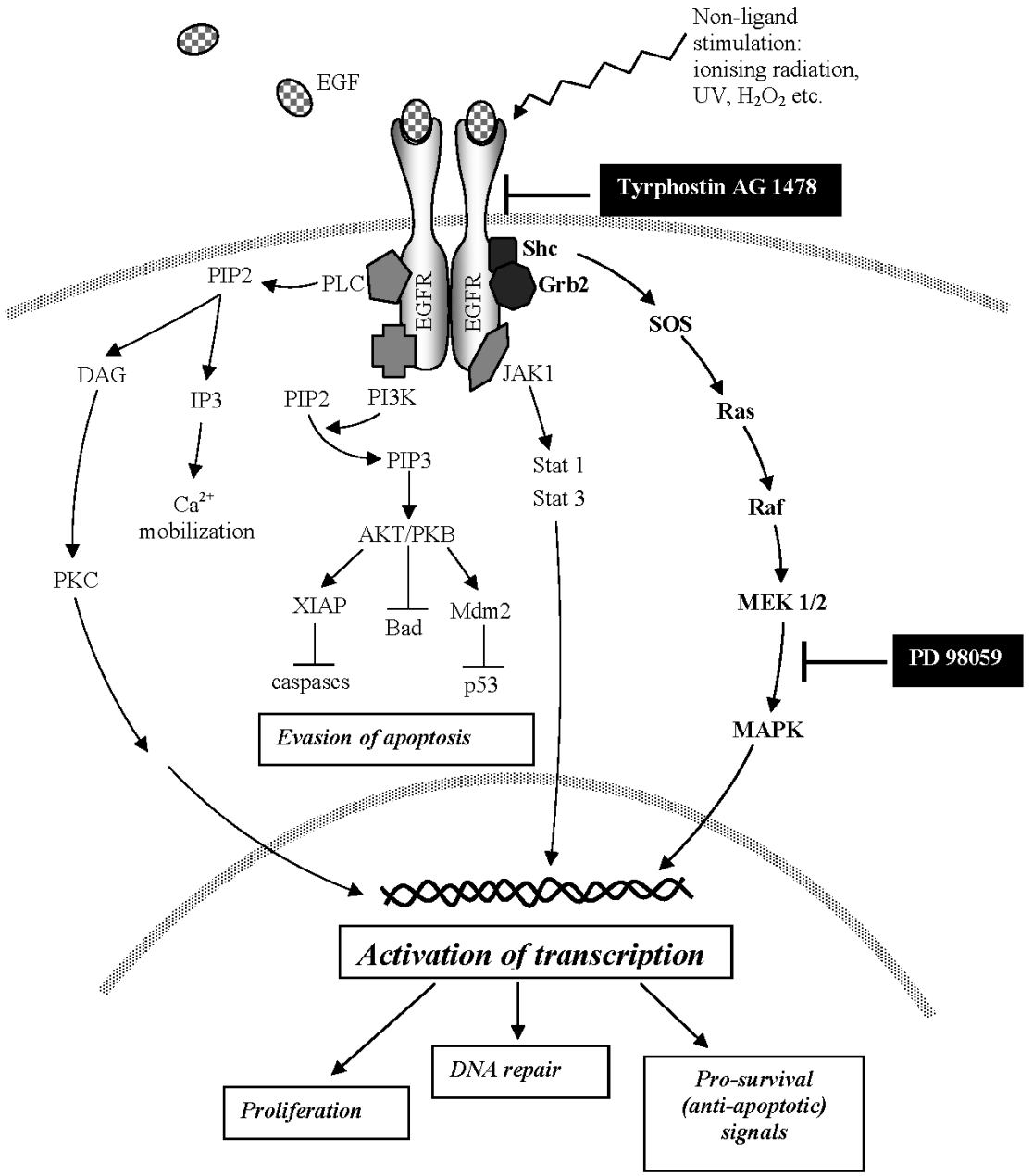

Fig. 1. Schematic presentation of signalling pathways originating upon epidermal-growth-factor-receptor (EGFR) activation; marked are the sites of action of the inhibitors: tyrphostin AG 1478 and PD 98059. The possible direct EGFR translocation to the nucleus was not shown on the picture. Abbreviations used: AKT/PKB, protein kinase B; Bad, proapoptotic protein belonging to Bcl-2 family; DAG, diacylglycerol; EGF, epidermal growth factor; IP3, inositol triphosphate; Grb, phosphotyrosine-binding adaptor protein; JAK1, Janus kinase 1 (protein tyrosine kinase); MAPK, mitogen-activated protein kinase; Mdm2, negative regulator of p53 tumor suppressor; MEK 1/2, mitogen-activated, extracellular signal-activated kinases 1 and 2; PIP2, phosphatidylinositol-biphosphate; PIP3, phosphatidylinositol-triphosphate; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PLC, phospholipase C; Raf, serine/treonine kinase; Ras, GTP binding protein; Shc, phosphotyrosine-binding adaptor protein; SOS, guanine nucleotide exchange protein; Stat 1,3, signal transduction and transcription proteins; XIAP, X-linked inhibitor of apoptosis protein.

analysis of the image negative with the use of GelScan software (Kucharczyk electrophoretic techniques, Poland). Fraction of DNA released to the gel (FR) was defined by the DNA content in the gel (measured as integrated optical density) divided by the total DNA amount applied (assessed in non-electrophoresed plugs with the use of Pico-Green, Molecular Probes). DSB level was defined as a product of FR and a number of DNA fragments in the gel, and normalized to a percentage above control value.

Statistics

Data sets were analysed using commercially available software (Microsoft Excel 2002, Microsoft Corporation). A mean of measured values ± SD was calculated for all groups. The Student's t-test was used to determine validity of the difference between means and $p \leq 0.05$ was considered significant.

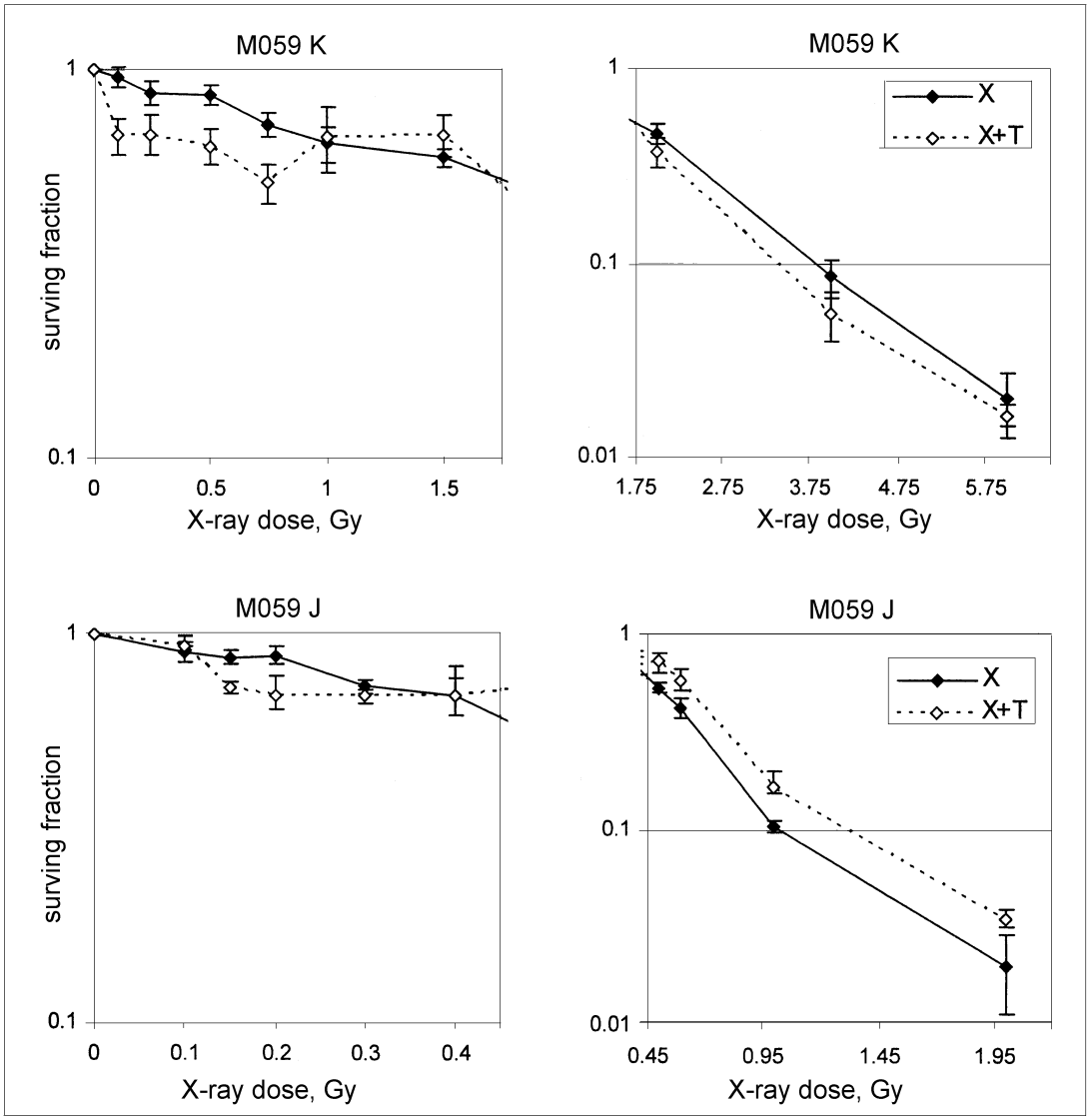

Fig. 2. Clonogenicity assays: the effect of continuous treatment with tyrphostin AG 1478 (T) on M059 K and J cell survival after X-irradiation. Concentration of the inhibitor was 5 μM for M059 K cells and 7 μM for M059 J cells. Under these conditions, the inhibitor, when used alone, reduced the cell survival to about 0.7 (paper submitted and [13]). The results of the X-ray treatment alone or the combined X-ray plus inhibitor treatment were normalized to their respective non-irradiated controls. Points represent means ± SD of 5–7 experiments.

Results

Survival experiments, X-irradiated cells

Two inhibitors were used for interrupting the signalling initiated at the plasma membrane: tyrphostin AG 1478, specific for EGF receptor kinase and PD 98059, specific for kinase MEK 1/2-downstream on the EGRF signalling pathway (Fig. 1). Our aim was to determine the effect of signalling inhibition on survival of X-irradiated cells as well as to assess how survival is related to the efficiency of the DNA-PK-dependent (M059 K) and DNA-PK-independent (M059 J) repair systems.

The effects of M059 cells treatment with X-rays and the signalling inhibitors were examined with the use of clonogenicity tests. Shown are the results for X-rays alone and for combined treatment with X-rays and tyrphostin AG 1478 (Fig. 2) or PD 98059 (Fig. 3). Both inhibitors were used at concentrations that reduced survival to 0.7 when added as single agents to the cell cultures [13]. In agreement with the previously reported data [1], the intrinsic radiosensitivity of M059 J cells is much higher than that of M059 K cells (note the difference in X-ray dose range between upper and lower panels in Figs. 2 and 3).

Upon combined treatment with tyrphostin AG 1478 and X-rays (Fig. 2) radiosensitization was clearly seen in M059 K cells at low radiation doses that reduced cell survival to 0.7–0.8 (Fig. 2, upper left panel). In M059 J cells this effect was much less pronounced and noted at only two doses (Fig. 2, lower left panel). At higher X-ray doses, the inhibitor exerted an additive (M059 K) or even a protective effect (M059 J; Fig. 2, lower right panel) on the irradiated cells.

In PD 98059+X treated M059 K cells (Fig. 3), only a protective effect of the inhibitor could be observed: at the highest doses applied that reduced survival to 0.1 or below – in M059 K cells (Fig. 3, upper right panel) and in the low dose range (up to 0.6 Gy) – in M059 J cells (Fig. 3, lower left panel).

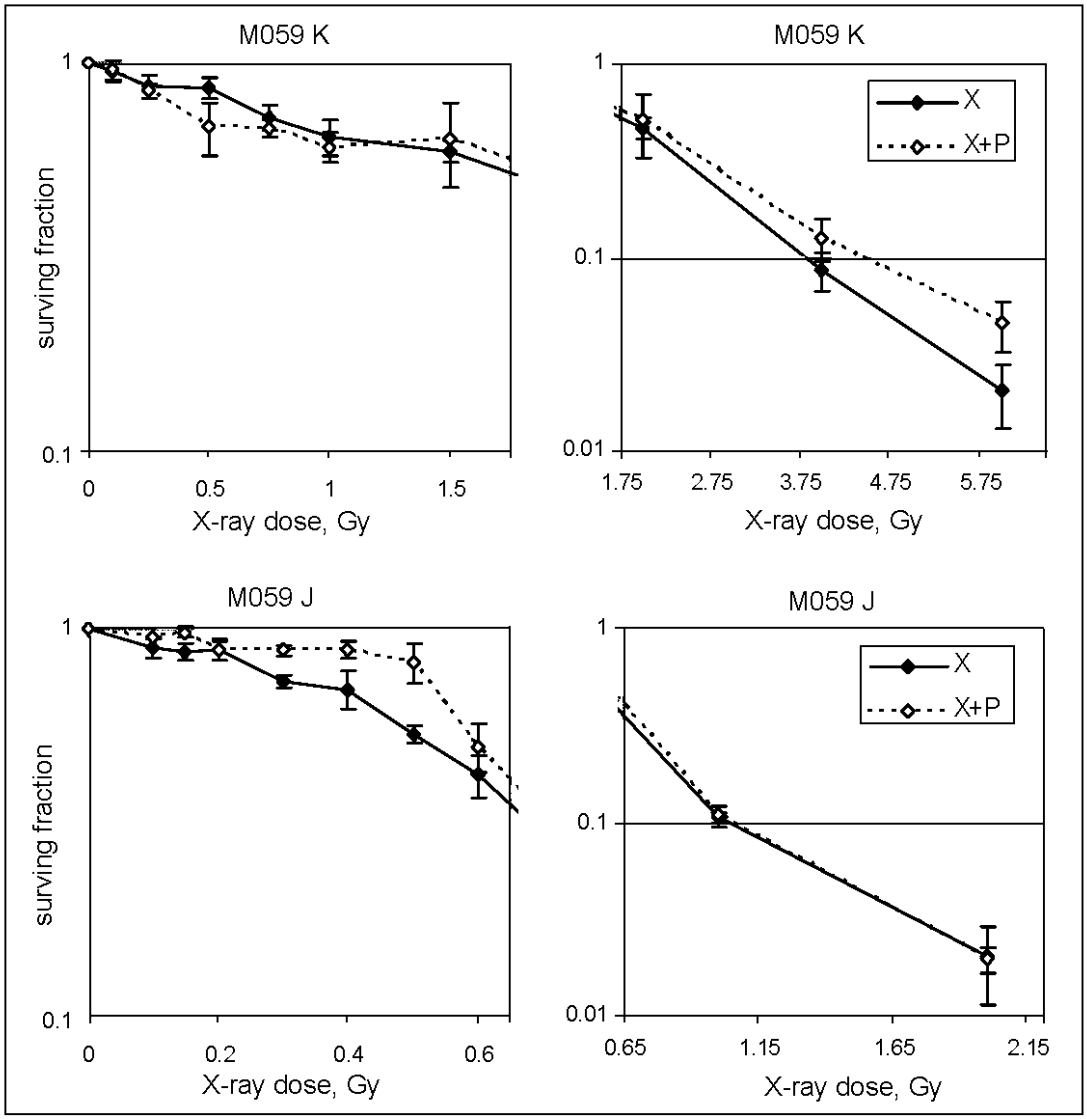

Fig. 3. Clonogenicity assays: the effect of continuous treatment with PD 98059 (P) on M059 K and J cell survival after X-irradiation. Concentration of the inhibitor was 70 μM for M059 K cells and 30 μM for M059 J cells. Under these conditions, the inhibitor, when used alone, reduced the cell survival to about 0.7 (paper submitted and [13]). The results of the X-ray treatment alone or the combined X-ray plus inhibitor treatment were normalized to their respective non-irradiated controls. Points represent means ± SD of 5–7 experiments.

DSB rejoining after X-irradiation

It was already reported that DNA-PK$_{cs}$ deficient M059 J cells show slower DSB rejoining than DNA-PK$_{cs}$ proficient M059 K cells [2, 7, 13, 20]. Recently, we reported the influence of the signalling inhibitors on DSB rejoining after X-irradiation in the two glioma M059 cell lines [13]: tyrphostin AG 1478 markedly slowed down DSB rejoining in M059 K cells, but had no statistically significant effect in M059 J cells. For PD 98059, there was no effect on post-irradiation DSB rejoining in either M059 cell line. Inhibitor concentrations applied were chosen so, as to exert only a little inhibitory effect on cell survival: at higher concentrations, a substantial increase in DSB levels, induced by the inhibitors alone, made difficult the interpretation of the PFGE results.

The effects of tyrphostin AG 1478 and PD 98059 are illustrated by Fig. 4, which shows the levels of DSB (expressed as % of initial damage) for cells irradiated with 10 Gy X-rays, measured 30 min (M059 K) or 60 min (M059 J) after irradiation. The timing takes into account the difference in the rejoining rate between M059 lines. Only tyrphostin AG 1478+X data for M059 K cells significantly differ from X alone, in agreement with the functional D-NHEJ being the target for X-ray-activated EGFR.

Survival experiments, bleomycin-treated cells

Figure 5 shows the difference in sensitivity of M059 cells to bleomycin alone, whereas Fig. 6 presents the results of single agent- and combined treatments with bleomycin and tyrphostin AG 1478. In this experiment, the choice of concentration of bleomycin and tyrphostin AG 1478 was according to cell sensitivity (see legend to Fig. 6). Survival of bleomycin + tyrphostin AG 1478 treated M059 K cells was lower than expected for additive effect, whereas in M059 J cells there was an additive effect. Thus, tyrphostin AG 1478 exerted sensitizing effect in bleomycin treated M059 K but not in

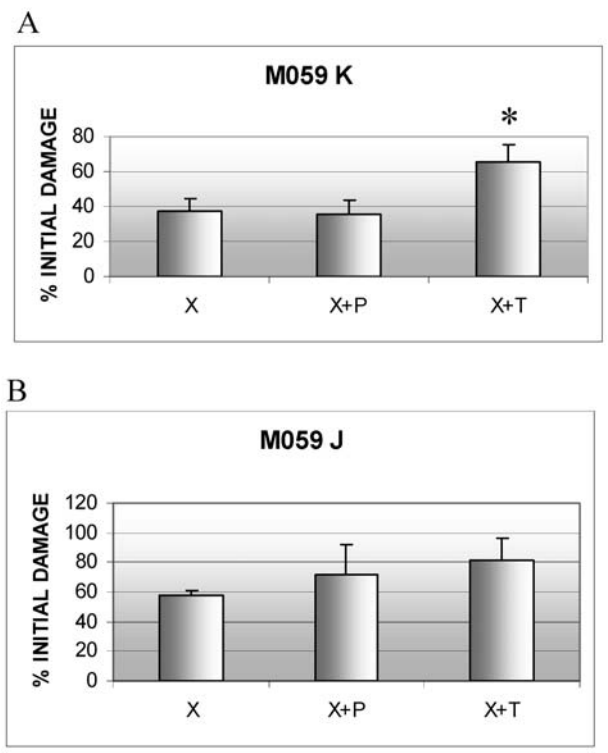

Fig. 4. PFGE: effects of continuous treatment of M059 K and J cells with tyrphostin AG 1478 (T) and PD 98059 (P) on DSB rejoining after X-irradiation (10 Gy). We used 1.5 μM and 5 μM tyrphostin AG 1478 for M059 K and J cells respectively and 20 μM PD 98059 for both cell lines. Bars are mean values ± SD of 3 experiments, asterisk marks statistically significant difference $p < 0.01$ (t-test).

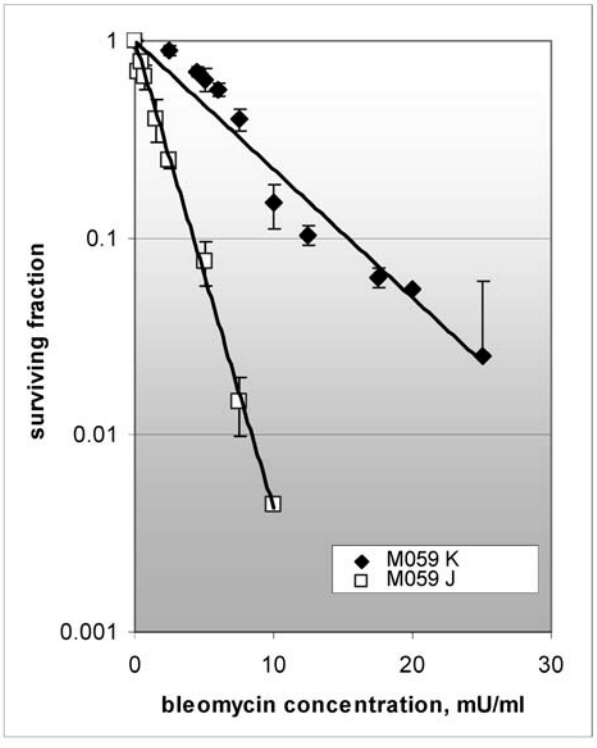

Fig. 5. Sensitivity of M059 cells to bleomycin estimated from the clonogenicity assays. Shown are the effects of 1-h treatment of the cells with different concentrations of bleomycin at 37°C. Points represent means ± SD of 3 experiments.

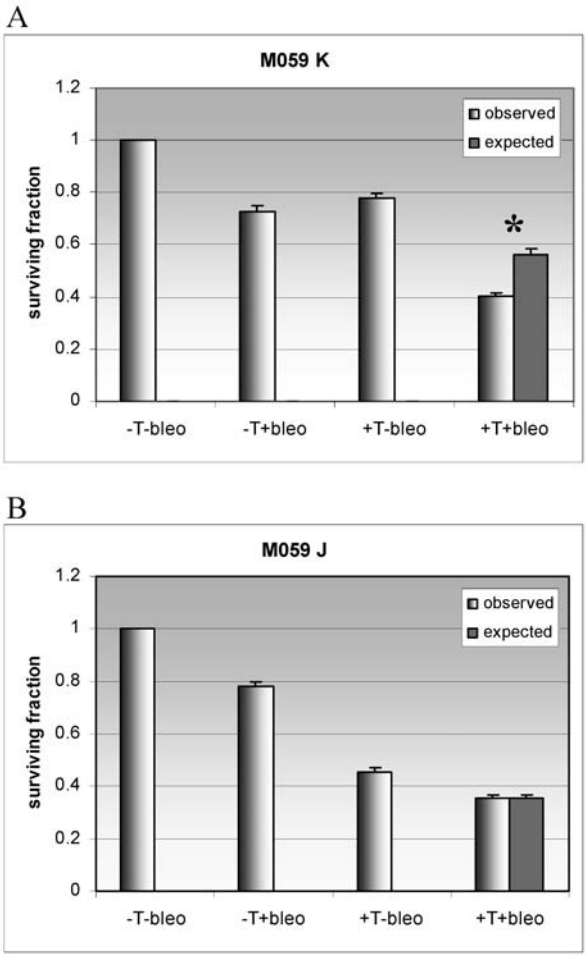

Fig. 6. Clonogenicity assays: the effect of continuous treatment with tyrphostin AG 1478 (T) on bleomycin (bleo)-treated M059 K and J cells. Concentrations used were: 4.5 mU/ml bleomycin and 5 μM tyrphostin AG 1478 for M059 K; 0.75 mU/ml bleomycin and 7 μM tyrphostin AG 1478 for M059 J. Bars represent means ± SD of 3 experiments. Asterisk marks statistically significant difference, $p < 0.01$ (t-test).

M059 J cells, according to the ability of this inhibitor to affect the post-irradiation DSB rejoining in these cells (Fig. 4).

Discussion

The connection between cellular signalling, DSB repair and survival is of considerable interest, as its knowledge may be applied for radiosensitivity modulation *in vivo*. Studies on the effect of blocking the signalling generated at the receptors of the EGFR family involved estimation of the radiosensitivity increase from cloning or from experiments on tumour-bearing animals (reviewed in [17, 18, 25]). The experiments of Huang and Harari [19] with the C225 antibody blocking the initiation of signalling by EGFR indicated an effect on sublethal and potentially lethal damage repair but did not identify the target repair system.

Our experiments were carried out on two human glioma cell lines M059 differing in radiosensitivity and sensitivity to bleomycin, as shown in Figs. 2, 3 and 5. Previously [13], we found a difference in their sensitivity to the signalling inhibitors applied, tyrphostin AG 1478

and PD 98059. The level of EGFR, as determined by ELISA was about 4 times higher in M059 J cells than in M059 K cells, in agreement with the difference in sensitivity to tyrphostin AG 1478 between the cell lines examined.

Enhanced lethality of M059 K cells subjected to combined (tyrphostin AG 1478+X) treatment was found only in the lower X-ray dose range (Fig. 2). Since the applied timing of treatment excluded the possibility of decrease in the expression of repair enzymes, this effect apparently was due to inhibition of the nuclear translocation of EGFR and hence, impaired D-NHEJ, as shown previously by Dittmann *et al*. [9] and by us [13]. This result may seem disappointing, however, as recently discussed by Connell *et al*. [6], in experimental systems, the combined modality treatments consist of a single radiation dose in the high lethality range, whereas in therapy, fractionated irradiation with low to moderate doses is applied. Therefore, such a response to tyrphostin AG 1478+X may present an advantage in combined modality treatment.

D-NHEJ is not functional in M059 J cells. In these cells there was a small but statistically significant effect of tyrphostin AG 1478 on the response to X-rays, with survival (Fig. 2) as end-point, but no effect on DSB repair [13] (Fig. 4). Hence, we could confirm D-NHEJ as the target DSB repair system for EGFR initiated signalling. Interestingly, we found a similar pattern of response to tyrphostin AG 1478+X and bleomycin + tyrphostin AG 1478 treatments (compare Figs. 2 and 6). Since bleomycin is capable of producing electron-deficient species such as hydroxyl radicals, superoxide, and hydrogen peroxide [22], it may act as EGFR activator on a similar basis to that of ionizing radiation.

Relative radiosensitivity of the M059 lines corresponded with DSB rejoining rate, as determined by PFGE (cf. Fig. 4). PD 98059 had little influence on DSB rejoining rate whereas tyrphostin AG 1478 significantly delayed this process in M059 K cells. Nevertheless, the level of residual damage at 3 h after irradiation was the same within detection limits. Consistently, slower DSB rejoining in these cells had not much effect on survival.

Altogether, there are more numerous reports on survival correlation with the initial or residual damage than with the repair rate. Survival correlation with the residual DNA damage was described, e.g. by Dikomey *et al*. [8], Chavaudra *et al*. [4], but El-Awady *et al*. [10] found that radiosensitivity of a series of human tumour cells is correlated with the induction but not with the repair of DSB. On the other hand, the rate of DSB rejoining was related to radiosensitivity in 89 out of 101 tumour cell lines examined by Foray *et al*. [11].

Lack of effect of delayed DSB rejoining on survival in M059 K cells can be due to several factors. In parallel to the stimulating effect on DNA repair, EGFR in X-irradiated cells may to some extent initiate the MAPK pathway that activates transcription factors and the PI3K/AKT pathway; the latter is the source of anti-apoptotic signals. Both pathways are linked to cell cycle control [21]. Moreover, Hagan *et al*. [14] described the impact of EGFR-MAPK-MEK signalling on the extent of poly(ADP-ribose) polymerase (PARP) activation in irradiated human prostate cancer cells, LNCaP and DU145. They noted that increase in PARP activation following irradiation was associated with survival decrease and both could be manipulated through inhibition of EGFR, MEK or PARP. In particular, inhibition of MEK decreased PARP activity and had a radioprotective effect. This observation may be pertinent to the survival increase of M059 cells subjected to combined (PD 98059+X) treatment (Fig. 3).

Altogether, signalling interruption brings about multiple effects, seen as proliferation inhibition, increase in apoptosis proneness and impaired DNA repair. These effects (see Fig. 1) may be reflected in survival to different extent, depending on radiation dose and cell type. The standard approach presented in this paper can be accepted as a preliminary study, but apparently is not sufficient to find all pre-requirements for a given cell type to be radiosensitized by treatment with signalling inhibitors.

Conclusions

EGFR kinase activity is necessary for efficient DSB rejoining in X-irradiated M059 K cells and the kinase inhibitor acts as radiosensitizer in the low dose range. Inhibition of EGFR kinase, however, does not decrease survival or affect DSB rejoining in DNA-PK_{cs}-deficient M059 J cells. This result is consistent with survival decrease being due to disturbed DSB repair by the DNA-PK dependent repair system. In contrast, inhibition of the downstream MAPK signalling does not slow down the DSB rejoining in either cell line and exerts a radioprotective effect.

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References

1. Allalunis-Turner MJ, Barron GM, Day RS 3rd, Dobler KD, Mirzayans R (1993) Isolation of two cell lines from a human malignant glioma specimen differing in sensitivity to radiation and chemotherapeutic drugs. Radiat Res 134:349–354
2. Allalunis-Turner MJ, Zia PK, Barron GM, Mirzayans R, Day RS 3rd (1995) Radiation-induced DNA damage and repair in cells of a radiosensitive human malignant glioma cell line. Radiat Res 144:288–293
3. Amorino GP, Mikkelsen RB, Valerie K, Schmidt-Ullrich RK (2003) Dominant-negative cAMP-responsive element-binding protein inhibits proliferating cell nuclear antigen and DNA repair, leading to increased cellular radiosensitivity. J Biol Chem 278:29394–29399
4. Chavaudra N, Bourhis J, Foray N (2004) Quantified relationship between cellular radiosensitivity, DNA repair defects and chromatin relaxation: a study of 19 human tumour cell lines from different origin. Radiother Oncol 73:373–382
5. Chinnaiyan P, Huang S, Vallabhaneni G *et al*. (2005) Mechanisms of enhanced radiation response following epidermal growth factor receptor signalling inhibition by erlotinib (Tarceva). Cancer Res 65:3328–3335

6. Connell PP, Kron SJ, Weichselbaum RR (2004) Relevance and irrelevance of DNA damage response to radiotherapy. DNA Repair 3:1245–1251
7. DiBiase SJ, Zeng ZC, Chen R, Hyslop T, Curran WJ Jr, Iliakis G (2000) DNA-dependent protein kinase stimulates an independently active, non-homologous, end-joining apparatus. Cancer Res 60:1245–1253
8. Dikomey E, Dahm-Daphi J, Brammer I, Martensen R, Kaina B (1998) Correlation between cellular radiosensitivity and non-repaired double-strand breaks studied in nine mammalian cell lines. Int J Radiat Biol 73:269–278
9. Dittmann K, Mayer C, Fehrenbacher B *et al*. (2005) Radiation-induced epidermal growth factor receptor nuclear import is linked to activation of DNA-dependent protein kinase. J. Biol Chem 280:31182–31189
10. El-Awady RA, Dikomey E, Dahm-Daphi J (2003) Radiosensitivity of human tumour cells is correlated with the induction but not with the repair of DNA double-strand breaks. Br J Cancer 89:593–601
11. Foray N, Arlett CF, Malaise EP (1997) Radiation-induced DNA double-strand breaks and the radiosensitivity of human cells: a closer look. Biochimie 79:567–575
12. Grądzka I, Iwaneńko T (2005) A non-radioactive, PFGE-based assay for low levels of DNA double-strand breaks in mammalian cells. DNA Repair 4:1129–1139
13. Grądzka I, Sochanowicz B, Buraczewska I, Szumiel I (2006) Participation of the EGF receptor in the response to X-irradiation in human glioma M059 K and J cells (abstract). In: Proc of the Int Conf Gliwice Scietific Meetings 2006, 17–18 November 2006, Gliwice, Poland, p 42
14. Hagan MP, Yacoub A, Dent P (2007) Radiation-induced PARP activation is enhanced through EGFR-ERK signaling. J Cell Biochem 101:1384–1393
15. Hallahan DE, Dunphy E, Virudachalam S, Sukhatme VP, Kufe DW, Weichselbaum RR (1995) C-jun and Egr-1 participate in DNA synthesis and cell survival in response to ionizing radiation exposure. J Biol Chem 70:30303–30309
16. Hallahan DE, Sukhatme VP, Sherman ML, Virudachalam S, Kufe D, Weichselbaum RR (1991) Protein kinase C mediates X-ray inducibility of nuclear signal transducers EGR1 and JUN. Proc Natl Acad Sci USA 88:2156–2160
17. Harari PM, Huang SM (2000) Modulation of molecular targets to enhance radiation. Clin Cancer Res 6:323–325
18. Harari PM, Huang SM (2004) Combining EGFR inhibitors with radiation or chemotherapy: will preclinical studies predict clinical results? Int J Radiat Oncol Biol Phys 58:976–983
19. Huang SM, Harari P (2000) Modulation of radiation response after epidermal growth factor receptor blockade in squamous cell carcinomas: inhibition of damage repair, cell cycle kineticss, and tumor angiogenesis. Clin Cancer Res 6:2166–2174
20. Macann AM, Britten RA, Poppema S *et al*. (2000) DNA double-strand break rejoining in human follicular lymphoma and glioblastoma tumor cells. Oncol Rep 7:299–303
21. Maher EA, Furnari FB, Bachoo RM *et al*. (2001) Malignant glioma: genetics and biology of a grave matter. Genes Dev 15:1311–1333
22. Mahmutoglu I, Scheulen ME, Kappus H (1987) Oxygen radical formation and DNA damage due to enzymatic reduction of bleomycin-Fe(III). Arch Toxicol 60:150–153
23. Park JS, Jun HJ, Cho MJ *et al*. (2006) Radiosensitivity enhancement by combined treatment of celecoxib and gefitinib on human lung cancer cells. Clin Cancer Res 12:4989–4999
24. Salmeen A, Barford D (2005) Functions and mechanisms of redox regulation of cysteine-based phosphatases. Antioxid Redox Signal 7:560–577
25. Sartor CI (2003) Epidermal growth factor family receptors and inhibitors: radiation response modulators. Semin Radiat Oncol 13:22–30
26. Schmidt-Ullrich RK, Mikkelsen RB, Dent P *et al*. (1997) Radiation-induced proliferation of the human A431 squamous carcinoma cells is dependent on EGFR tyrosine phosphorylation. Oncogene 15:1191–1197
27. Schmidt-Ullrich RK, Valerie K, Fogleman PB, Walters J (1996) Radiation-induced autophosphorylation of epidermal growth factor receptor in human malignant mammary and squamous epithelial cells. Radiat Res 145:81–85
28. Stoker AW (2005) Protein tyrosine phosphatases and signalling. J Endocrinol 185:19–33
29. Szumiel I (2006) Epidermal growth factor receptor and DNA double strand break repair: the cell's self-defence. Cell Signal 18:1537–1548
30. Tsai JC, Liu L, Guan J, Aird WC (2000) The Egr-1 gene is induced by epidermal growth factor in ECV304 cells and primary endothelial cells. Am J Physiol Cell Physiol 279:C1414–1424
31. Valerie K, Yacoub A, Hagan MP *et al*. (2007) Radiation-induced cell signaling: inside-out and outside-in. Cancer Ther 6:789–801
32. Yacoub A, McKinstry R, Hinman D, Chung T, Dent P, Hagan MP (2003) Epidermal growth factor and ionizing radiation up-regulate the DNA repair genes XRCC1 and ERCC1 in DU145 and LNCaP prostate carcinoma through MAPK signaling. Radiat Res 159:439–452
33. Yacoub A, Miller A, Caron RW *et al*. (2006) Radiotherapy-induced signal transduction. Endocr Relat Cancer 13;Suppl 1:S99–S114