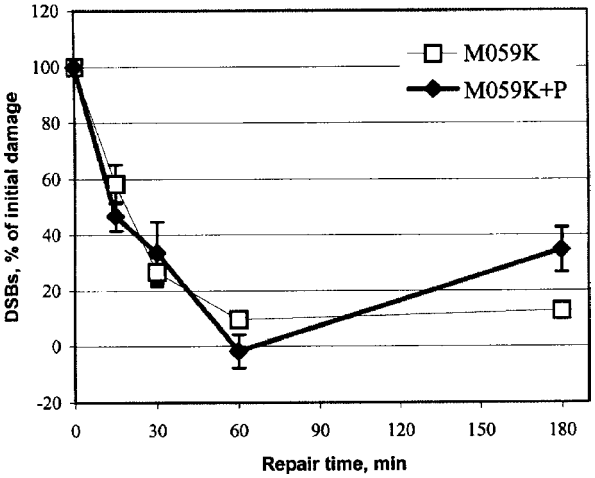

Fig.1. Effect of continuous treatment of M059 K cells with the MEK1/2 kinase inhibitor, PD 98059 (P, 20 μM) on DSB rejoining after X-irradiation (10 Gy) as determined by PFGE. Data points are mean values ± SEM.

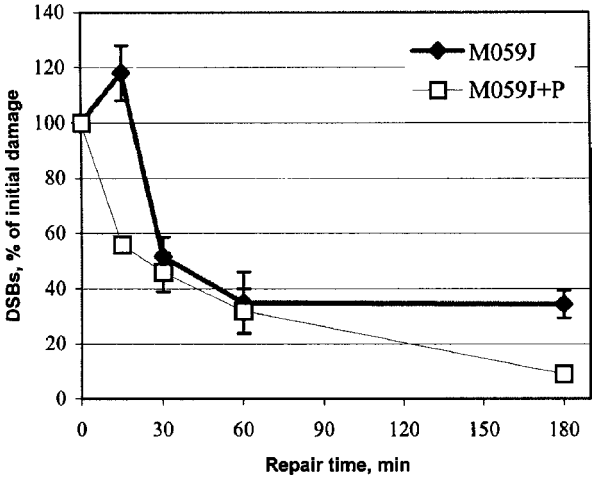

Fig.2. Effect of continuous treatment of M059 J cells with the MEK1/2 kinase inhibitor, PD 98059 (P, 20 μM) on DSB rejoining after X-irradiation (10 Gy) as determined by PFGE. Data points are mean values ± SEM.

Human glioma M059 cells [2], K (normal radiosensitivity) and J (radiosensitive, with defective DNA-PK catalytic subunit) were X-irradiated and treated with signalling inhibitor, PD 98059, specific for kinase MEK1/2. DSB rejoining was determined with pulse field gel electrophoresis (PFGE). M059 J cells are much more sensitive to X-radiation than M059 K cells; this correlates with lower initial DSB rejoining rate in the M059 J cell line as determined by PFGE (*cf.* Figs.1 and 2). M059 K cells are more sensitive to cell signalling inhibitor: PD 98059 as compared to M059 J cells. In contrast to the effects on cell survival, PD 98059 has little influence on DSB rejoining rate, with the exception of 3 h repair time for M059 cells, as shown in Fig.2. The apparent discrepancy between survival and DSB rejoining after combined PD+X-ray treatment may be due to PD effect on apoptosis.

Supported by the State Committee for Scientific Research (KBN) – grant No. 4 P05A 022 15.

References

[1]. Amorino G.P., Hamilton V.M., Valerie K., Dent P., Lammering G., Schmidt-Ullrich R.K.: Mol. Biol. Cell, 13, 2233-2244 (2002).

[2]. Allalunis-Turner M.J., Barron G.M., Day R.S. 3rd, Dobler K.D., Mirzayans R.: Radiat. Res., 134, 349-354 (1993).

REPAIR OF DNA DOUBLE STRAND BREAKS IN DIFFERENTIALLY RADIOSENSITIVE GLIOMA CELLS X-IRRADIATED AND TREATED WITH TYRPHOSTINE AG 1478

Iwona Grądzka, Iwona Buraczewska, Barbara Sochanowicz, Irena Szumiel

Signalling pathways are a potential target in cancer radiotherapy [1]. Of special interest are pathways initiated by EGFR (epidermal growth factor receptor) [2,3]. The signal is generated at the receptor that – upon ligand binding – acquires tyrosine kinase activity. The outcome of such signalling is activation of specific transcription factors and expression of specific genes, including those that code DNA repair enzymes.

We determined the effects of signalling inhibition by using tyrphostine AG 1478, specific for EGFR tyrosine kinase, which is activated both by the specific ligand, EGF, and X-rays [4]. The effects were examined on survival (not shown) and on double strand break (DSB) rejoining. In order to establish whether the DNA-PK-dependent repair system is the target of the signalling pathway initiated at the EGFR, we used two differentially radiosensitive cell lines, human glioma M059 [2]. Activity of DNA-PK (DNA-dependent protein

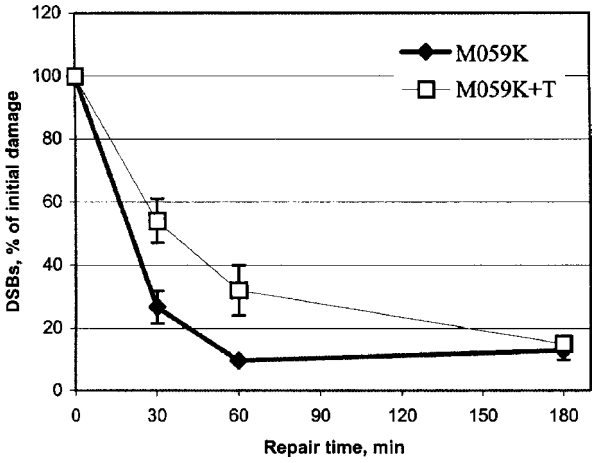

Fig.1. Effect of continuous treatment of M059 K cells with tyrphostine AG 1478 (T, 5 μM) on DSB rejoining after X-irradiation (10 Gy) as determined by PFGE. Data points are mean values ± SEM.

kinase) determines the function of the non-homologous rejoining of DSB (NHEJ), the main repair system of mammalian cells.

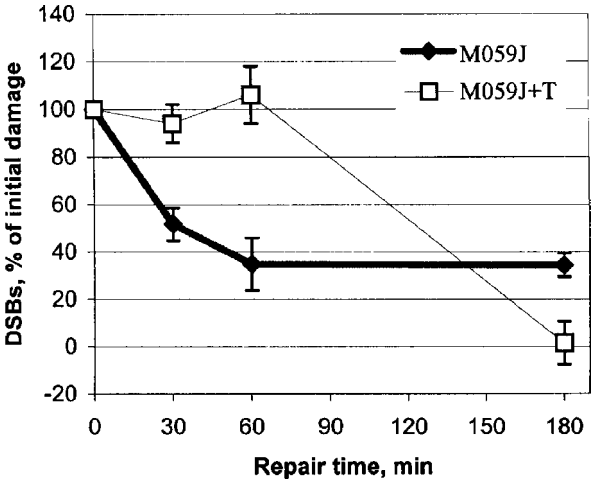

Fig.2. Effect of continuous treatment of M059 J cells with tyrphostine AG 1478 (T, 5 μM) on DSB rejoining after X-irradiation (10 Gy) as determined by PFGE. Data points are mean values ± SEM.

M059 K cells (normal radiosensitivity) and M059 J cells (radiosensitive, with defective DNA-PK catalytic subunit) were X-irradiated and treated with tyrphostine AG 1478. DSB rejoining was determined with pulse field gel electrophoresis (PFGE). M059 K cells are considerably more sensitive to tyrphostine AG 1478 than M059 J cells. This is explained by the difference in EGFR levels [5]. Tyrphostine AG 1478 has a marked influence on DSB rejoining rate (Figs.1 and 2) and the effect is considerably stronger in M059 J than in M059 K cells. Since M059 J cells with defective DNA-PK-dependent NHEJ so strongly respond to signalling inhibition, this observation suggests its preferential action on homologous recombination repair or DNA-PK independent non-homologous end-joining. The effect of tyrphostine AG 1478 on DSB rejoining is considerably stronger in M059 J than in M059 K cells. Since M059 J cells with defective DNA-PK-dependent NHEJ respond to signalling inhibition, this observation suggests its preferential action on homologous recombination repair or DNA-PK independent non-homologous end-joining (B-NHEJ according to the term proposed by Wang *et al.* [6]).

Supported by the State Committee for Scientific Research (KBN) – grant No. 4 P05A 022 15.

References

[1]. Amorino G.P., Hamilton V.M., Valerie K., Dent P., Lammering G., Schmidt-Ullrich R.K.: Mol. Biol. Cell, 13, 2233-2244 (2002).
[2]. Milas L., Mason K.A., Ang K.K.: Int. J. Radiat. Biol., 79, 539-545 (2003).
[3]. Sartor C.I.: Semin. Radiat. Oncol., 13, 22-30 (2003).
[4]. Allalunis-Turner M.J., Barron G.M., Day R.S. 3rd, Dobler K.D., Mirzayans R.: Radiat. Res., 134, 349-354 (1993).
[5]. Grądzka I., Sochanowicz B.: Epidermal growth factor receptor activation in X-irradiated glioma cells M059 K and M059 J. In: INCT Annual Report 2004. Institute of Nuclear Chemistry and Technology, Warszawa 2005, pp.111-112.
[6]. Wang H., Perrault A.R., Takeda Y., Qin W., Wang H., Iliakis G.: Nucleic Acids Res., 31, 5377-5388 (2003).

EPIDERMAL GROWTH FACTOR RECEPTOR ACTIVATION IN X-IRRADIATED GLIOMA CELLS M059 K AND M059 J

Iwona Grądzka, Barbara Sochanowicz

In studies concerning new potential targets in cancer radiotherapy of special interest are signalling pathways initiated by EGFR (epidermal growth factor receptor) [1,2]. The signal is generated at the receptor that – upon ligand binding – acquires tyrosine kinase activity. It is further transmitted through the MAPK/ERK (mitogen-activated protein kinase/extracellular signal-regulated kinase) pathway. EGF receptor also is activated by ionising irradiation [3], usually 1-5 Gy of X- or γ-rays. This is the dose range applied in cancer radiotherapy. The outcome of such signalling is activation of specific transcription factors and expression of specific genes, including those that code DNA repair enzymes.

To complete our studies on the effects of signalling inhibition on double strand break (DSB) rejoining in M059 cells [4], we determined the level of EGFR and its activation by X-rays. Human glioma M059 cells [5], K (normal radiosensitivity) and J (radiosensitive, with defective DNA-PK catalytic subunit) control and X-irradiated (10 Gy) served for preparation of extracts. EGFR was determined with ELISA.

ELISA kit for human EGFR (Biosource International, Inc., USA) was used. With the use of specific primary antibodies, polyclonal antibody against

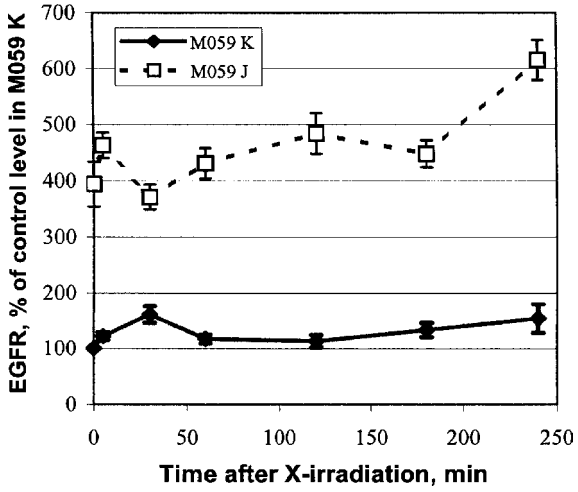

Fig.1. Change in the level of EGFR in M059 K and M059 J cells after irradiation with 10 Gy X-rays, determined with ELISA test. Data points are mean values ± SD.