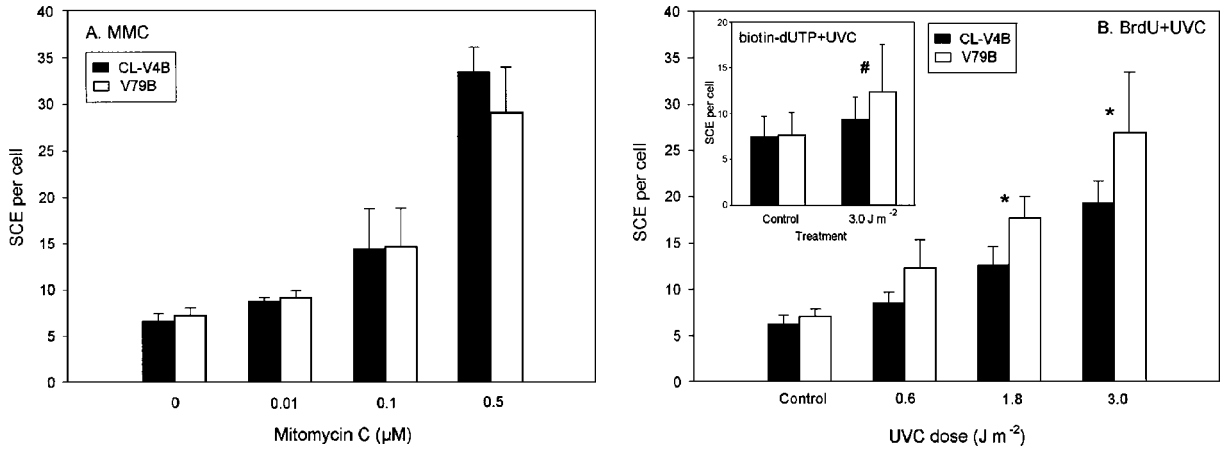

Fig.1. SCEs in first mitosis (M1) following treatment of CL-V4B and V79B cells with: A – MMC and B – BrdU+UVC. Twenty cells were scored per point in each experiment. Error bars represent standard deviations from mean values of three independent experiments (inset: treatment with biotin-dUTP+UVC; standard deviations from a single set of data). * – difference significant at $p<0.05$, two-sided, paired Student's *t*-test; # – difference significant at $p<0.05$, two-sided, unpaired Student's *t*-test.

removed by nucleotide excision repair. The reduced frequency of UVC-induced SCEs in CL-V4B cells could either be due to enhanced removal of photoproducts before the damaged DNA is replicated or due to reduced SCE formation by this type of damage.

This conclusion is not incompatible with the results of the MMC-induced SCE experiments because it is known that multiple recombination pathways exist which can lead to SCE formation [4]. Which pathway is triggered may depend on the type of DNA lesion, as recently suggested [5]. Also, it is not totally certain that the reduced SCE frequencies following exposure to UVC are a consequence of the Rad51C deficiency. CL-V4B cells may have other deficiencies apart from the mutated *Rad51C* gene. Thus, the genetic defect that may be responsible for the reduced SCE frequencies following exposure to UVC is somewhat uncertain.

As shown in the preceding report [6], mutation in Rad51C did not affect SCE formation after MMC treatment. Additionally, while the wt cells showed the same frequency of MMC-induced SCE in M1 and M2, in CL-V4B cells somewhat more SCE were observed in M2 than M1. This suggests that in Rad51C mutants ICL induced by MMC are either not removed completely or are transformed into another form of damage, which persists until the next cell cycle. In conclusion, our results indicate that homologous recombination repair is not involved in the SCEs induced by the DNA interstand-crosslinking agent MMC. In accordance with the model of Shafer [7], we suggest that SCEs may represent a mechanism to bypass an ICL during DNA replication.

Supported by the State Committee for Scientific Research (KBN) statutory grant for the INCT.

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REPAIR OF DNA DOUBLE STRAND BREAKS IN DIFFERENTIALLY RADIOSENSITIVE GLIOMA CELLS X-IRRADIATED AND TREATED WITH KINASE INHIBITOR PD 98059

Iwona Grądzka, Iwona Buraczewska, Barbara Sochanowicz, Irena Szumiel

Radiosensitisation caused by inhibition of cellular signalling has recently been subject of numerous studies. Signalling pathways are a potential target in cancer radiotherapy [1]. Of special interest are pathways initiated by EGFR (epidermal growth factor receptor). 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.

The aim of this study was to determine the effect of signalling inhibition on double strand break (DSB) rejoining as well as to establish whether the DNA-PK-dependent repair system is the target of the signalling pathway initiated at the EGFR.

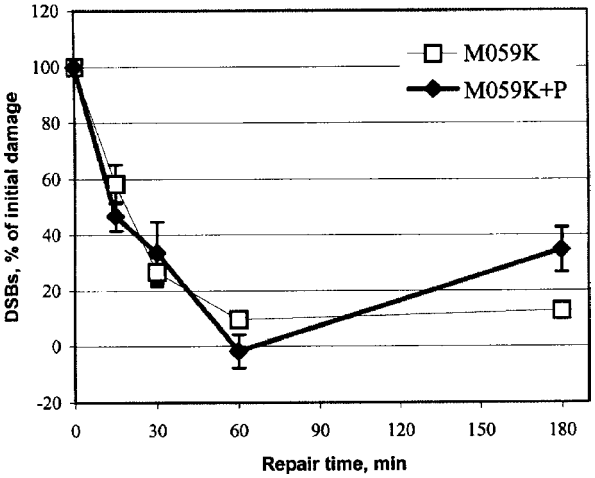

Fig.1. Effect of continuous treatment of M059 K cells with the MEK1/2 kinase inhibitor, PD 98059 (P, 20 μM) on DSB rejoining after X-irradiation (10 Gy) as determined by PFGE. Data points are mean values ± SEM.

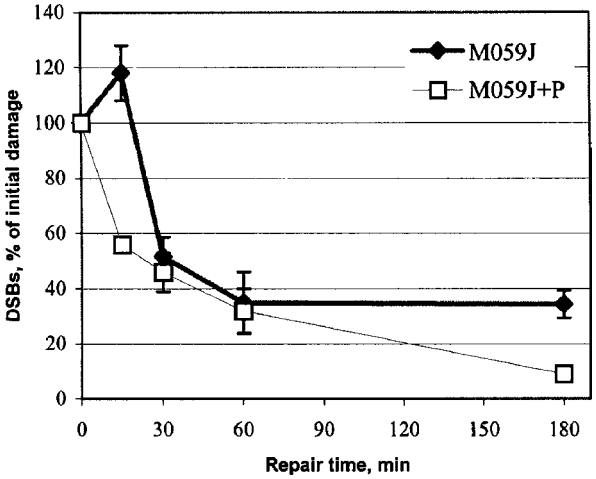

Fig.2. Effect of continuous treatment of M059 J cells with the MEK1/2 kinase inhibitor, PD 98059 (P, 20 μM) on DSB rejoining after X-irradiation (10 Gy) as determined by PFGE. Data points are mean values ± SEM.

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.

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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

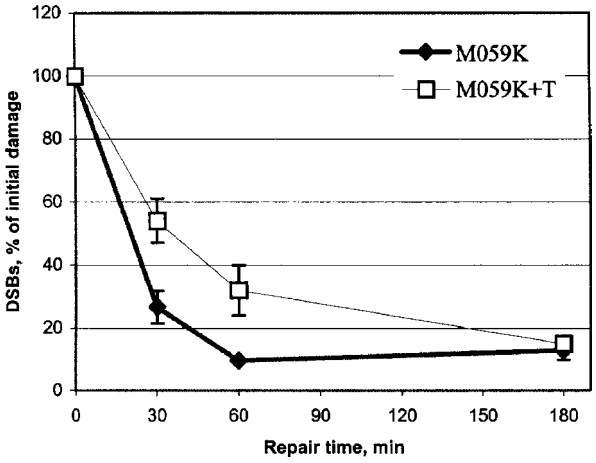

Fig.1. Effect of continuous treatment of M059 K cells with tyrphostine AG 1478 (T, 5 μM) on DSB rejoining after X-irradiation (10 Gy) as determined by PFGE. Data points are mean values ± SEM.