Role of Base Excision Repair in temozolomide-induced DNA damage

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Since the efficiency of chemotherapy in cancer treatment is counteracted by the action of DNA repair pathways, it is often necessary to combine anticancer drugs with specific DNA repair inhibitors. Temozolomide (TMZ) is a DNA alkylating agent used in the treatment of glioblastoma (GBM), an aggressive form of brain tumour with a low survival rate due in great part to resistance to TMZ. The main lesion induced in DNA by TMZ is N7-meG, harmless by itself but prone to generate abasic (AP) sites that are highly cytotoxic and mutagenic. AP sites are repaired through the Base Excision Repair (BER) pathway initiated either by AP endonucleases or by AP lyases. Recently, it has been shown that in *Arabidopsis thaliana,* the AP sites generated from N7-meG are processed through an AP lyase/DNA phosphatase pathway mediated by the lyase FPG and the phosphatase ZDP. In human cells, the homologous protein of ZDP is the polynucleotide kinase 3'-phosphatase, PNKP. In the present work, we aimed to study the role of PNKP in the repair of AP sites induced by TMZ and, in doing so, highlight its relevance to the cellular sensitivity to this agent. We have used TMZ-resistant and sensitive GBM cell lines to specifically block the activity of PNKP by a specific inhibitor (PNKPi) and to deplete its expression by small interference RNA (siRNA). Using such approach, we examined the impact of PNKP in TMZ sensitivity (by clonogenic and survival assays), in the repair of TMZ-dependent breaks (by alkaline comet assays) and in cellular apoptosis (by flow cytometry). Our data show that the depletion of PNKP activity (by PNKPi or siRNA) sensitizes GMB cells to TMZ treatment, impairs the repair of TMZ-induced breaks and increases cellular apoptosis. Our results may help to identify novel therapeutical targets in TMZ- treated tumours.

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