Role of an AP Lyase pathway in the repair of AP sites generated by DNA methylating agents

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Abasic (AP) sites are major DNA lesions generated during temozolomide (TMZ)–induced genotoxic stress in human cells. It is generally accepted that they are primarily repaired through the canonical base excision repair (BER) pathway initiated by AP endonucleases. In plants, AP sites derived from spontaneous loss of N7-methylguanine (N7-meG), the predominant lesion induced by TMZ and others DNA methylating agents, are processed through an AP lyase/DNA phosphatase pathway involving FPG and ZDP. The human homologs of these enzymes are the bifunctional glycosylases/AP lyases NEIL1 and NEIL2, and the polynucleotide kinase 3′-phosphatase (PNKP). Our aim was to investigate the role of the NEIL1/2-PNKP axis in the repair of TMZ-induced AP sites and its impact on cellular sensitivity to DNA methylating agents. We used human glioblastoma (GBM) cells to study AP site accumulation and repair upon TMZ treatment. To inhibit AP site repair, we used the BER inhibitor methoxyamine. PNKP activity was inhibited with A12B4C3 a selective small-molecule inhibitor, and NEIL1/2 were depleted by siRNA. TMZ sensitivity was then evaluated using cell viability assays in both TMZ-sensitive and TMZ-resistant GBM cell lines. Finally, DNA single-strand break (SSB) formation and repair kinetics were analysed using comet assay and immunofluorescence detection of poly-(ADP-ribosylation) (PARylation). We found that TMZ treatment induced AP site accumulation in GBM cells. Inhibition or depletion of PNKP significantly enhanced TMZ sensitivity and delayed SSB repair. Importantly, combined downregulation of NEIL1 and NEIL2 partially rescued TMZ resistance and reduced PARylated SSB levels in PNKP-inhibited cells. Collectively, our findings support a model in which NEIL1 and NEIL2 process TMZ-induced AP sites and generate SSB intermediates that require PNKP for efficient resolution of methylation-induced DNA damage.