The impact of expression of BER proteins on therapeutic effect of combining temozolomide with methoxyamine in melanoma cells

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Abstract

Differential expression of key components of repair systems in melanoma cell lines

Results

Base excision repair (BER) is a highly coordinated cellular biochemical system essential to cell survival. Variability of expression of BER proteins may affect the dynamics of the repair pathway or change the repair rate that determines the cellular sensitivity to the cytotoxicity exerted by alkylating agents. In the present study, we examined the relationship between the expression of BER proteins and therapeutic effect of temozolomide (TMZ) and the combination of TMZ and methoxyamine (MX), a BER inhibitor, in melanoma cells in vitro and in xenografts. The levels of BER proteins (including MPG, UDG, APE, and Pol β) were measured by western blotting assay in 4 cell lines, SkmCI-1, WM164, A375, and WM9. Results revealed that BER proteins were coordinately and differentially expressed in these melanoma cell lines, higher in A375 and much lower in WM164 cells. There were no significant differences in MGMT and MLH1-MSH2, two well known TMZ resistant factors in these two cell lines. To determine the role of BER proteins in TMZ cytotoxicity, clonogenic survival studies were performed in A375 and WM164 cells. The sensitivity to TMZ alone in these two cell lines was assessed by IC90 value produced by survival assay, showing 490 µM in A375 and 570 µM in WM164 cells. When cells were treated with the combination of TMZ and MX, MX efficiently potentiated TMZ cytotoxicity by 3 folds in A375 but failed to enhance the killing effect of TMZ in WM164 cells. Similar results were observed in xenografts. In xenograft study, MX enhanced TMZ antitumor effect, resulting in tumor growth delays of 13 days in mice receiving the combination of TMZ (80 mg/kg) and MX (2 mg/kg) as daily for five days in comparison to 6 days in mice treated with TMZ alone. No enhancement of TMZ-antitumor activity by MX was observed in mice carrying WM164 melanoma xenografts. The results suggest that the failure of TMZ-potentiation by MX in WM164 cells was related to the low activity of BER proteins, particularly low levels of MPG DNA glycosylase. Deficiency of the BER proteins would decrease the formation of AP sites, the targets for MX action. Therefore, these results indicate that BER proteins may be differentially expressed in tumor cells, which particularly impact the therapeutic effect of combining TMZ with MX.
Enhancement of decitabine cytotoxicity by methoxyamine via inhibition of base excision repair

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Abstract

Decitabine (5-aza-2’-deoxycytidine) is a nucleoside analog used for the treatment of hematological malignancies. Previously, it was shown that the cytotoxic effect of low dose decitabine treatment is due to incorporation of its active metabolite, 5-aza-2’-deoxycytidine triphosphate, into DNA leading to inhibition of DNA methylation by binding irreversibly to DNA methyltransferases. We hypothesized that incorporated 5-aza-2’-deoxycytidine (or its deaminated analog, 5-aza-2’-deoxouridine) into DNA is also recognized and processed by the base excision repair (BER) pathway. In this case, inhibition of BER by methoxyamine (MX) would potentiate the cytotoxicity of decitabine. We evaluated role of BER in decitabine cytotoxicity in colon cancer, melanoma cells, and primary acute myelogenous leukemia (AML) cells. Decitabine-induced abasic sites (AP-sites) were increased proportionally with dose and duration of exposure. MX reduced the number of available AP-sites up to 80% indicating formation of stable MX-bound AP-sites that have the potential to interrupt BER pathway. A similar correlation between decitabine dose and the AP-sites formed was observed after in vitro exposure of primary AML cells to increasing concentrations of decitabine. MX was able to bind a significant percentage (up to 69%) of these AP sites. Decitabine cytotoxicity was potentiated by MX. Cell survival assays demonstrate a 4-fold decrease in the IC50 for decitabine when cells were co-treated with MX (IC50 dec = 4 µM and IC50 Dec+ MIX = 1 µM). Apoptotic cell death measurements using Annexin V staining showed a 5-fold increase in cell death following decitabine and MX treatments. These events were accompanied by a concomitant increase in cleavage of PARP, and in γH2AX. Moreover, MX enhanced decitabine-induced antitumor effect in mice bearing A375 human melanoma xenografts, as measured by tumor growth delay: 7 days (0.5 mg/kg decitabine) versus 14 days (0.5 mg/kg decitabine plus 2 mg/kg MX). These studies not only suggest for the first time the role of BER in the processing of incorporated decitabine, but they also provide insights into a new and promising cancer therapeutic strategy of combining decitabine with MX to block BER.

Results

Background and Hypotheses

Hypothesis 1:
Incorporated 5-Aza deoxyctydine is a substrate for base excision repair pathway which process it as an abnormal base.

Hypothesis 2:
Methoxyamine blocks BER response and enhances cytotoxicity of Decitabine.

MX augments therapeutic effect of Decitabine in human xenografts

Increase sensitivity of CLL lymphocytes to Decitabine plus MX

MX enhances DNA strand breaks induced by Decitabine (mechanism for cell death)

Summary:
- MX binds AP sites formed by Decitabine
- MX enhances DNA strand breaks formed by Decitabine
- MX augments antitumor effect of fludarabine in cancer cell lines, primary cells and human xenografts
Induction of uracil DNA glycosylase (UDG) in human cancer cells in response to antitumor treatments and methyamines

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ABSTRACT

Background: Antimetabolites are used as cancer therapy to disrupt RNA and DNA production and induce cell death. Many DNA lesions created by antimetabolite chemotherapy are repaired by base excision repair (BER), including uracil-DNA glycosylase (UDG) which catalyzes the hydrolytic removal of uracil from DNA. Inhibiting UDG exacerbates DNA damage, depletes the thymine pool and induces cell death. Many DNA lesions created by antimetabolite chemotherapy are repaired by base excision repair (BER). We have previously reported that methoxyamine (MX), which inhibits BER by binding and stabilizing abasic (AP) sites after glycosylase removal of abasic sites, enhances the cytotoxicity of the antimetabolite fludarabine, a potential strategy in human leukemia (Jurkat) cells. This MX-potentiated cytotoxicity was also observed in lung, breast and colon cancer cells after treatment with pemetrexed, an antifolate antimetabolite. Cytotoxicity of pemetrexed was enhanced with MX co-treatment.

RESULTS SUMMARY

UDG induction in leukemia (Jurkat) cells treated with 5uM Fludarabine and 200uM Alimta, 6mM MX for 24 hr

- Protein and mRNA induction of UDG is observed in human leukemia cells following fludarabine treatment and is enhanced with methyamine co-treatment.
- Induction of UDG is observed at the protein and mRNA level following pemetrexed treatment in human lung cancer cells (H460) and is enhanced with methyamine co-treatment.
- Moderate fluctuations in UDG expression with cell cycle stage progression are observed in control and MX treated cells.
- Pemetrexed induction of UDG corresponds to concomitant increases in expression of thymidylate synthetase (TS) with a concomitant rise in γH2AX, cleaved PARP and Topoisomerase IIα.

INDUCTION OF UDG IN HUMAN CANCER CELLS

UDG expression correlates with in vitro enzyme cutting activity

- Induction of UDG in leukemia (Jurkat) cells treated with 200uM Alimta and 6mM MX for 24-72 hours.
- Modest fluctuations in UDG expression with cell cycle stage progression are observed in control and MX treated cells.
- Pemetrexed induction of UDG in leukemia (Jurkat) cells treated with 5uM Fludarabine and 200uM Alimta, 6mM MX for 24 hr.
- Therefore, UDG induction functioned as a mechanism of cell protection and resulted in enhanced DNA repair.

UDG induction is accompanied by enhancement of strand break formation and caspase activation as measured by PARP cleavage.

- Increased apoptosis and cytotoxicity was also observed in lung, breast and colon cancer cells after treatment with pemetrexed, an antifolate antimetabolite. Cytotoxicity of pemetrexed was enhanced with MX co-treatment.

Conclusions:

UDG induction is induced as a consequence of normal cell cycle progression. However, induction also appears to contribute to increased AP site damage at G1 and S phase.

REFERENCES


ACKNOWLEDGEMENTS

Medical Scientist Training Program NH T32 GM072730
Research Oncology Training Program NC T32 CA093286-15