Conclusion- MTH1 inhibitor has a great potential for the treatment of cisplatin resistant tumors in patients. Elevated level of Pol κ in cisplatin resistance tumor agument the effectiveness of MTH1 inhibitor and it can be used as potential biomarker for MTH1 therapy.

Introduction

Fig 1. Cisplatin resistant Bladder cancer NTUB1/P cells are more sensitive to MTH1 depletions in comparison to parental NTUB1 cells

Fig 2. (A) and (C) Parental NTUB1 and Cisplatin resistant NTUB1/P bladder cancer cells were treated with Cisplatin, MTH1 inhibitors TH588 and Karonudib for 72 h then cell viability was measured using resazurin. (D) and (E) NTUB1 and NTUB1/P cells were treated with indicated dose of Cisplatin and MTH1 inhibitors for 24 h, then western blotting was done for indicated proteins. (F) Representative images showing higher expression level of indicated gene in NTUB1/P cells in comparison to NTUB1 cells. Both cells were treated with MTH1 inhibitors for 24 h, then annexine V staining was carried out to determine apoptotic cells. (G) Quantitative data

3. In response to MTH1 inhibitors, higher incorporation of 8-oxodG into DNA of NTUB1/P cells in comparison to NTUB1 cells

4. Therapeutic efficacy of MTH1 inhibitor Karonudib (TH1579) in Cisplatin resistant bladder cancer xenograft and Cisplatin resistant Ovarian cancer PDX.

Fig 4 NTUB1/P xenografted and Cisplatin resistant ovarian cancer PDX mice were treated with Vehicle or Karonudib (90 mg/kg) twice a day for every alternate day for 6 week. (A) Tumor growth curve. (B) Body wt in grams. (C) Kaplan-Meier survival plot. (D) Ultrasound images of ovarian cancer PDX. (E) Tumor growth curve

5. Cisplatin resistant NTUB1/P cells have high expression of Translesion Polymerase (TLS) which can incorporate 8-oxodG into to DNA

Fig 5 mRNA expression of indicated genes were measured using qPCR in NTUB1 and NTUB1/P cells. (A) To (C) different DNA Translesion Polymerases (TLS). (D) Efflux ABC transporter (E) MTH1. (F) and (G) Pol κ was knocked down using siRNA at the end of 72 h. mRNA expression of Pol κ was measured in both NTUB1 and NTUB1/P cells

6. Decreased apoptosis by MTH1 inhibitors in Pol κ depleted NTUB1/P cells

Fig 6 Pol κ was depleted in NTUB1/P cells for 72 h using siRNA followed by treatment with MTH1 inhibitors for 24 h. (A) Decrease in Annexin V Positive population in Pol κ depleted NTUB1/P cells in comparison to Non Target siRNA control cells in response to Karonudib.

7. Level of Pol κ modulates the sensitivity MTH1 inhibitors To cisplatin resistant cells.

Fig 7 Pol κ was depleted in NTUB1/P cells for 72 h using siRNA followed by treatment with MTH1 inhibitors for 72 h. (A and B) Significant difference in survival of NT-siRNA vs Pol κ depleted NTUB1/P cells in response to MTH1 inhibitors (C-E). Over expression of Pol κ in NTUB1 or NTUB1/P cells causes further sensitization to MTH1 inhibitors. GFP overexpressed Pol κ cells were treated with TH588 and TH1579 for 72 h and cell viability was measured using resazurin. (F) Moderate effect of TH1579 on cisplatin sensitive PDX PH321. (G) Growth inhibition of cisplatin resistant PDX PH35 by MTH1 inhibitor TH1579 (h) Expression level of Pol κ was measured by RNA seq on PDXs.