Sulfasalazine could modulate the CD44v9-xCT system and enhance CDDP-induced cytotoxic effects in metastatic bladder cancer; A novel therapeutic strategy for metastatic bladder cancer

Koichiro Ogihara[1], Eiji Kikuchi[2], Toshikazu Takeda[1], Kazuhiro Matsumoto[1], Hideyuki Saya[3], and Mototsugu Oya[1]

1: Dept. of Urology, Keio University School of Medicine, Tokyo, Japan 2: Dept. of Urology, St. Marianna University School of Medicine, Kanagawa, Japan

3: Division of Gene Regulation, Institute for Advanced Medical Research, Keio University School of Medicine, Tokyo, Japan

Introduction and Aim

- The development of a new therapeutic strategy against cisplatin (CDDP) resistant metastatic bladder cancer is strongly warranted.
- Cancer stem cells (CSCs) are known to be key cells involved in the tumor growth, recurrence, metastasis and treatment resistance. CD44v9, a variant isoform of CD44 which is thought to be a CSC modulator, interacted with and stabilized xCT, a subunit of the cystine transporter, thereby promoting intracellular glutathione synthesis (GSH), which contributes to the protection against reactive oxygen species (ROS) generated by various cellular environmental stresses (Figure 1A). Recently, sulfasalazine (SSZ), widely used for the treatment of ulcerative colitis, has been reported to regulate CD44v9 and thereby to induce intracellular ROS production (Figure 1B).
- The aim of our study was to investigate 1) the functional role of SSZ in ROS production and its cytotoxic effects on MBT-2V cells, which were established from a parent MBT-2 tumor with multiple lung metastases and 2) the therapeutic effects of SSZ with or without cisplatin (CDDP) using a MBT-2V lung metastatic bladder cancer model.

Figure 1. The role of CD44v9 in CSCs

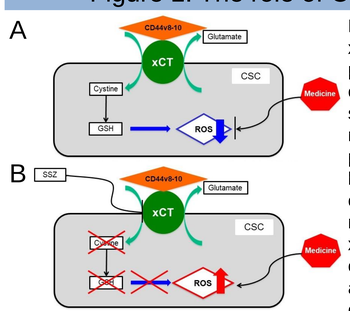

Figure 1. (A) CD44v9 stabilizes xCT at cell membrane and promotes the cellular uptake of cysteine and the consequent synthesis of GSH. GSH is a major antioxidant essential for protecting cells against ROS. It has a key role in resistance to cancer therapy and cancer metastasis. (B) SSZ inhibits the xCT activity. It contributes an oxidative stress to cancer cells and inhibits proliferation of cancer cells.

Summary of Findings

Figure 2. The effect of SSZ on mouse bladder cancer CD44v9 positive cell lines MBT-2V cells, which have highly lung metastasis potential

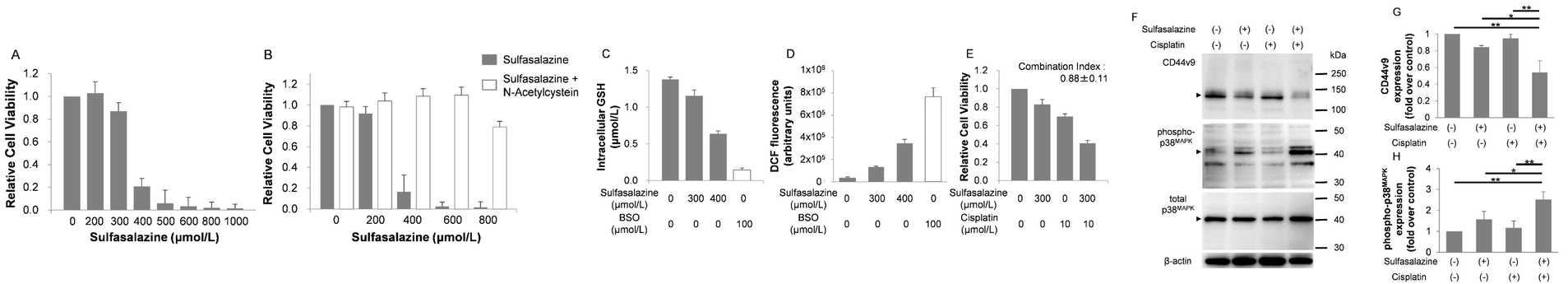

Figure 2: Sulfasalazine selectively inhibits cell proliferation, decreases GSH synthesis, increases ROS levels, and enhances cisplatin-induced cytotoxic effects in MBT-2V cells.
(A) Cytotoxic effects of sulfasalazine (SSZ) in MBT-2V cells. Cells were exposed to various concentrations of SSZ for 48 hours. (B) Cytotoxic effects of SSZ in the presence or absence of N-acetylcysteine (NAC, an antioxidant). Cells were exposed to various concentrations of SSZ with or without NAC (3 μM) for 48 hours. (C) Intracellular GSH levels of MBT-2V cells treated with the vehicle control, 300 and 400 μM of SSZ, and 100 μM of BSO for 24 hours. (D) Quantitative analysis of ROS production by MBT-2V cells treated with the vehicle control, 300 and 400 μM of SSZ, and 100 μM of BSO for 24 hours. (E) Cytotoxic effects of SSZ (300 μM), cisplatin (CDDP) (10 μM), and their combinations in MBT-2V cells for 48 hours. (F) The expression of CD44v9, phospho-p38^{MAPK}, and total p38^{MAPK} protein in MBT-2V cells treated with the vehicle control, SSZ alone (300 μM), CDDP alone (10 μM), and their combinations detected by Western blotting. (G and H) Signal intensities of CD44v9 and phospho-p38^{MAPK} protein expression in each group was quantified. All data are shown as means ± SE, * indicates p<0.01, ** indicates p<0.001.

Figure 3. The effect of SSZ and CDDP for murine lung metastasis model

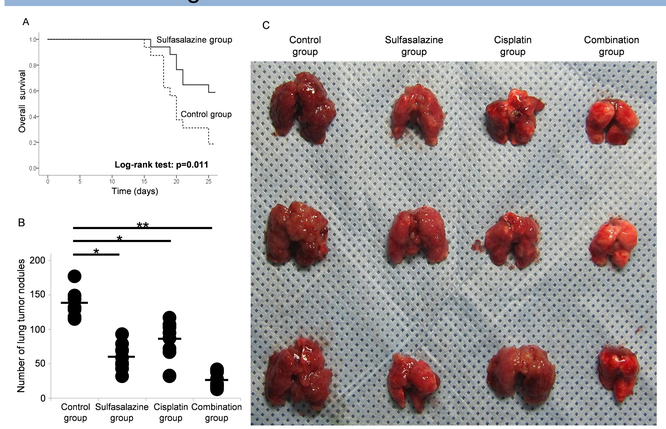

Figure 3: The survival analysis of sulfasalazine treatment and anti-tumor effects for lung tumor nodules of sulfasalazine alone, cisplatin alone, and their combinations in the murine lung metastasis model
(A) Lung tumor nodules were generated by injecting 2×10^5 MBT-2V cells into the tail veins of female C3H/HeN mice on day 0. The intraperitoneal administration (2 days on/1 day off) of SSZ (500 mg/kg) or the vehicle control of PBS was started on day 3 (n=18 in each group). Survival analysis was evaluated by Kaplan-Meier curve between the sulfasalazine (SSZ) treatment group and control group. (B) In the lung metastasis model, mice were classified into four groups: vehicle control, SSZ alone (500 mg/kg), cisplatin alone (2 mg/kg, every fifth day), and their combinations (n=10 in each group). Mice were sacrificed on day 15, and the number of lung tumor nodules was counted macroscopically. * indicates p<0.01, ** indicates p<0.001. (C) Representative lungs extracted from mice treated with the vehicle control, SSZ alone, cisplatin alone, and their combinations.

Figure 4. Representative microscopic findings

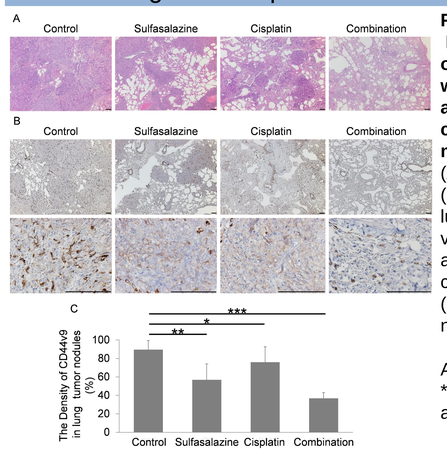

Figure 4:
Representative microscopic findings of lungs extracted from mice treated with the vehicle control, sulfasalazine alone, cisplatin alone, and their combinations in the murine lung metastasis model.
(A) H&E staining and
(B) immunostaining for CD44v9 in the lung tissue of mice treated with the vehicle control, sulfasalazine (SSZ) alone, cisplatin (CDDP) alone, and their combinations. The bar indicates 100 μm. (C) The density of CD44v9 in lung tumor nodules of the four treatment groups.

All data are shown as means ± SE, * indicates p<0.05, ** indicates p<0.01, and *** indicates p<0.001.

Conclusion

Sulfasalazine could induce ROS production and enhance CDDP-induced cytotoxic effects. The combination of Sulfasalazine with CDDP might be a novel therapeutic modality against metastatic bladder cancer.

COI disclosure information

First author: Koichiro Ogihara

The authors have no financial conflicts of interest to disclose concerning the presentation.