Abstract:

Role of Dual Oxygenase 2 (DUOX2) in preventing PD-L1 upregulation and increasing radiosensitivity of stomach cancer cells.

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ABSTRACT

Human Stomach cancer cells (NCI-N87) expressing endogenous or downregulated DUOX2 levels were injected into the abdomen of nude mice which were then treated with five 15cGy fractions of Low Dose WART (LD-WART) for three consecutive days. Our data indicate that expression of DUOX2 increased the levels of protein oxidation in mice serum exposed to LD-WART and increased the odds of preventing cancer dissemination by ten-fold. Moreover, downregulation of DUOX2 leads to HIF-1 and PD-L1 up-regulation in human tumors grown in mice. We also observed the same correlation in mice stomach cancer cells (NCC-S192) when DUOX2 was knockdown by CRISPR/Cas9. The upregulation of HIF-1 indicates that these tumors are hypoxic, which contributes to radio-resistance. In addition, PD-L1, a downstream target of HIF-1, is also associated with radio-resistance. A mouse angiogenesis profiler array indicated that several angiogenetic factors were upregulated in the DUOX2 positive but not negative tumors in response to LD-WART. This could have contributed to increase reoxygenation in the DUOX2 positive tumor and increase radiosensitivity to low dose radiation. The role of LD-WART in the immune response was then investigated in immunocompetent C57BL/6 mice. The mice were exposed to the same regimen of 15cGy LD-WART twice a day for three consecutive days either once or for two consecutive weeks. Samples were then collected one, two, or three weeks later following the last radiation dose. Our data indicate that LD-WART significantly increased CD8+ T cells (Lag3-/PD1-) in the blood and spleen one week following LD-WART and gradually faded off. On the other hand, exposing the mice to two consecutive rounds of LD-WRT fail to upregulate the CD8+ T cells.

BACKGROUND

- **Treatment options are rather limited for gastrointestinal cancer patients whose disease has disseminated into the intra-abdominal cavity;**
- **Dual Oxidase 2 (DUOX2), an enzyme functioning in the production of hydrogen peroxide, is required to sensitize gastric cancer cells to low-dose radiation;**
- **This offers the possibility to revisit the concept of Whole Abdominal Radiation Therapy (WART) to decrease the intraabdominal recurrence of disseminated disease.**

Figure 1: DUOX2 expression and activity in human gastric cells.

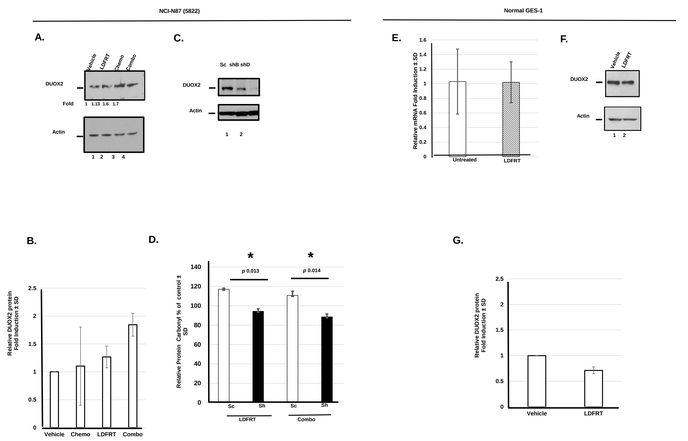

Fig.1: (A) Western blots analysis of gastric cancer NCI-N87 cells exposed to the indicated treatment. **(B)** Quantitation by densitometry of the NCI-N87 Western blots, **(C)** Down-regulation of DUOX2 in NCI-N87 cells, **(D)** Relative protein carbonyl content in the media of NCI-N87 cells expressing endogenous (Sc: Scrambled) or reduced (Sh) DUOX2, **(E)** DUOX2 mRNA expression in normal gastric epithelial GES-1 cells untreated or exposed to radiation (LDFRT), **(F)** Western blots of DUOX2 in GES-1 cells exposed to the indicated treatment, **(G)** Quantitation by densitometry of the GES-1 Western blots.

Figure 2: The effect of the treatments on cancer dissemination in the whole abdomen at different times following treatments.

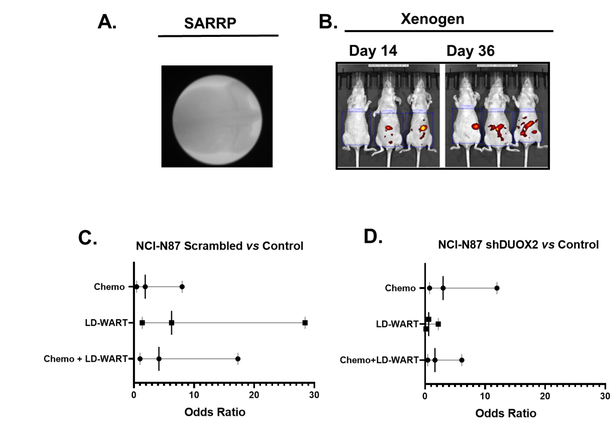

Fig.2: (A) Representative image obtained with a SARRP's portal camera to verify field placement of irradiated field (whole mouse abdomen). **(B)** Representative fluorescence image (closed abdomen) obtained with a Xenogen IVIS optical imager on days 14 and 36 post-injection of human gastric cancer cells stably transfected with Pgfp-C-shDUOX2 lenti. **(C,D)** Odds of reducing cancer dissemination as measured by fluorescence intensity being less than 25% of the intensity of day 1 at day 45. **(C)** Odds of a tumor expressing endogenous DUOX2 levels treated with the indicated treatment compared to untreated animals. **(D)** Odds of a tumor expressing reduced DUOX2 levels treated with the indicated treatment compared to untreated animals.

Figure 3: Effect of combined regimen of mDCF and LD-WART on angiogenesis and HIF-1 in vivo.

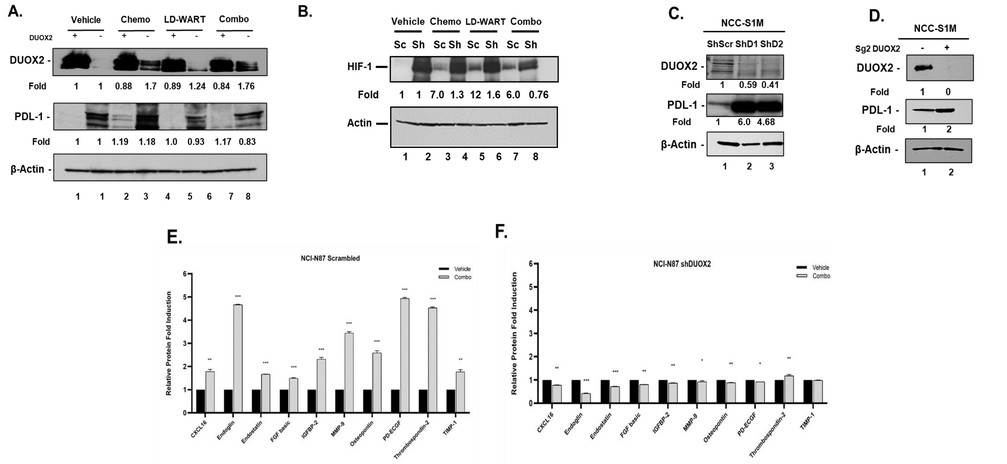

Fig.3: (A) Effect of Chemotherapy (mDCF) LD-WART and combined regimen on PD-L1, and **(B)** HIF-1.**(C-D)** DUOX2 downregulation in mouse gastric cancer cells triggers PD-L1 upregulation. **(E-F)** Mouse angiogenesis profiler array performed on tumors expressing endogenous DUOX2 levels.

Figure 4: CD8+ T cells population as measured by FACS analysis.

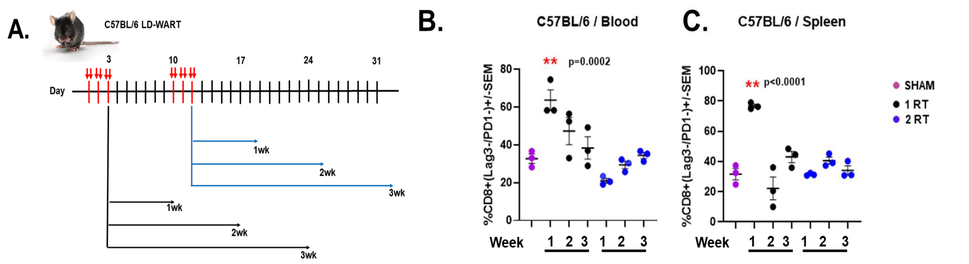

Fig.4: (A) Immunocompetent C57BL/6 mice exposed to the same regimen of 15cGy LD-WART twice a day for three consecutive days either once or for two consecutive weeks. **(B)** Blood or **(C)** Spleen of mice exposed for 3 consecutive days b.i.d. to LD-WART (0.15Gy/fraction) was extracted 1-.2- or 3-weeks post-treatment (1 RT: black circles). The same regimen was also repeated for two consecutive weeks (2RT: blue circles) and blood and spleen were extracted 1-.2- or 3-weeks post-treatment.

CONCLUSIONS

- **➔33% of human stomach cancer do not express DUOX2;**
- **➔ Expression of DUOX2 increases radiosensitivity and prevents HIF-1 and PD-L1 upregulation in tumors grown in mice;**
- **➔ Expression of DUOX2 in stomach cancer could thus help guide the potential application of LD-WART for disseminated stomach cancer and better understand tumor immune response to RT.**

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