CA102N suppresses the growth of mouse colon cancer by inhibiting PI3K pathway and immune modulation

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Introduction

COX-2 inhibitors have the immense possibility in treating colorectal cancer (1). Nevertheless, the cardiovascular toxicity of COX-2 inhibitors hampered further development of these inhibitors in cancer management (2). CA102N is a covalently bound conjugate of the biological polymer sodium hyaluronate (NaHA) and nimesulide (Nim) aiming to deliver Nim directly to the tumor tissues to limit the systemic toxicity . Our laboratory has utilized allograft CT-26 syngeneic Balb/c mouse colon cancer model (3) to understand whether CA102N suppresses the development of colorectal cancer. We tested the inhibitory effect of CA102N on the tumor growth of colon cancer and its relevant molecular mechanism via targeting COX-2 pathway and immune modulation (4,5). We also comprehend whether urinary COX-2 metabolites could serve as a surrogate biomarker for CA102N using mouse syngeneic colon cancer CT26 model.

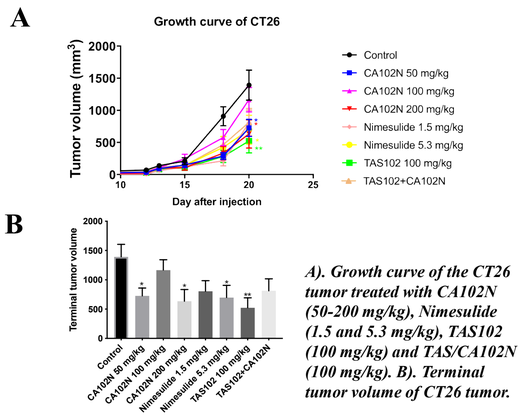
Methods

- **All animal experiments were approved by The University of Texas MD Anderson Cancer Center Animal Care and Use Committee. Mouse CT26 allograft colon cancer model was utilized to investigate the effect of CA102N on mouse tumor progression. Briefly, Six-week-old Balb/C mice were inoculated with CT-26 cells (5 x 10^5). When the tumor volumes reached between 25-50 mm^3, mice bearing the CT26 tumor were randomized to vehicle control, CA102N (50-200 mg/kg, i.v. twice per week), 2 doses of Nimesulide (1.5 and 5.3 mg/kg, oral daily), TAS102 (100 mg/kg), and TAS 102 plus CA102N (100 mg/kg). The tumor size was measured three times per week.**
- **Intratumor eicosanoids and their urinary metabolites were measured by LC/MS/MS method (6)**
- **Serum cytokines were measured by Mutliplex Mouse Cytokine kits (Meso Scale Discovery).**
- **Immune modulation was determined by profiling tumor infiltrating lymphocytes with flow cytometry.**
- **Cell signaling proteins relevant to PI3K/mTOR pathways were determined by western blot in CA102N treated tumor tissues.**

Acknowledgement

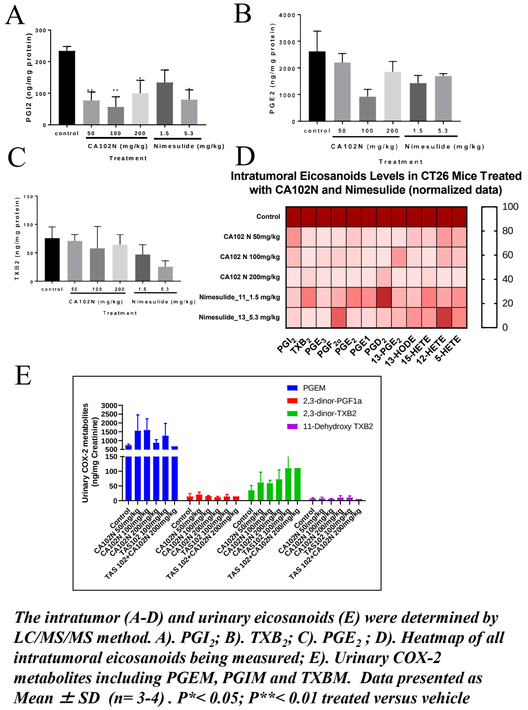
This study was supported by Holy Stone Healthcare Corporation.

CA102N Suppressed the Growth of CT26 Tumor



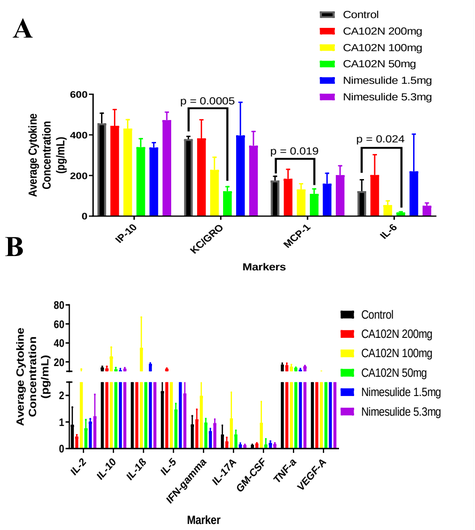
A). Growth curve of the CT26 tumor treated with CA102N (50-200 mg/kg), Nimesulide (1.5 and 5.3 mg/kg), TAS102 (100 mg/kg) and TAS/CA102N (100 mg/kg). B). Terminal tumor volume of CT26 tumor.

Tumoral and urinary eicosanoid levels in mice bearing CT26 tumor



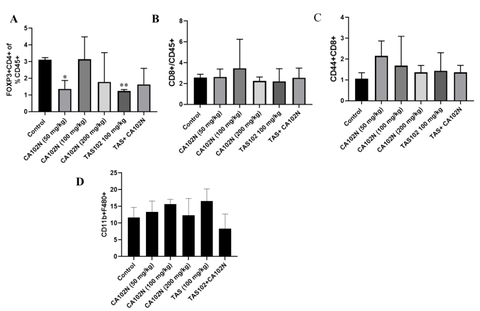
The intratumor (A-D) and urinary eicosanoids (E) were determined by LC/MS/MS method. A). PGI_2; B). TXB_2; C). PGE_2 ; D). Heatmap of all intratumoral eicosanoids being measured; E). Urinary COX-2 metabolites including PGEM, PGIM and TXBM. Data presented as Mean ± SD (n= 3-4) . P*< 0.05; P**< 0.01 treated versus vehicle control.

Serum cytokines in CA102N and Nimesulide treated mice bearing CT26 tumor



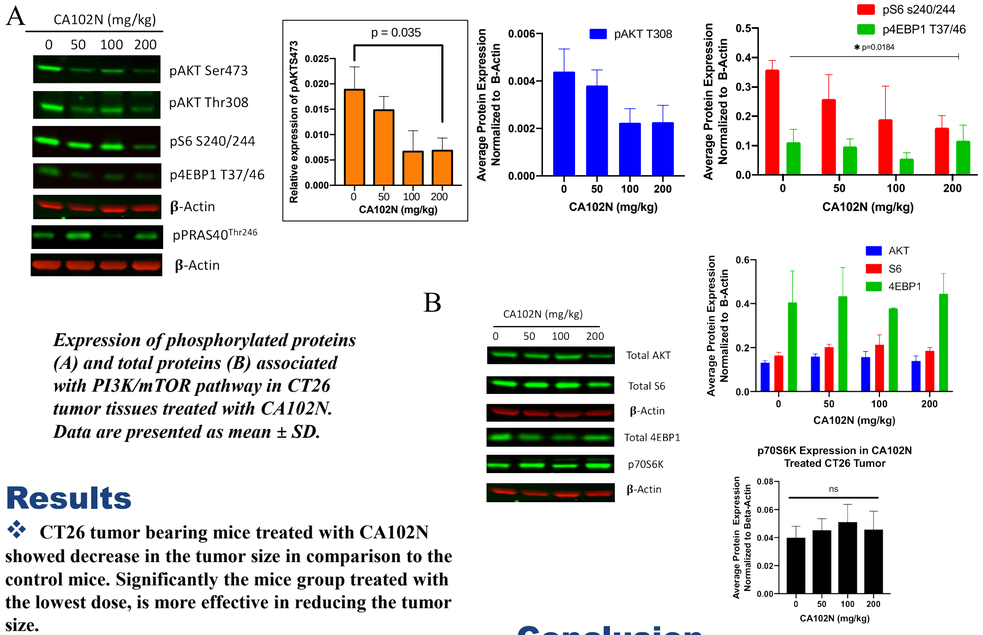
A-C: Serum cytokine analysis suggested that levels of MCP-1 (109.9 ± 24.3 pg/ml) and IL6 (18.0 ± 3.8 pg/ml) in CA102N (50 mg/kg) treated mice were significantly lower than that of control group (175.4 ± 21.0 pg/ml) and (123.3 ± 55.8 pg/ml), respectively (p < 0.05) (B).

Immune modulation within the tumor of mice with CT26



A). T-reg cell population. B). Cytotoxic T-cells population. C). Cytotoxic T-cells population and CD44 positive immune cells. D). mouse macrophage cell populations. Data presented as mean ± SD. * P < 0.05, ** P < 0.01 versus control.

Effect of CA102N treatment on AKT/PI3Kinase/mToR pathway protein expression in CT26 mouse colon tumor



Expression of phosphorylated proteins (A) and total proteins (B) associated with PI3K/mTOR pathway in CT26 tumor tissues treated with CA102N. Data are presented as mean ± SD.

Results

❖ **CT26 tumor bearing mice treated with CA102N showed decrease in the tumor size in comparison to the control mice. Significantly the mice group treated with the lowest dose, is more effective in reducing the tumor size.**

❖ **Intratumor COX-2 metabolite analysis show that CA102N treated mice have reduced levels of both PGE_2 and PGI_2 showing COX2 inhibitory effect.**

❖ **Levels of proinflammatory chemokine and cytokine MCP-1 and IL6 levels in CA102N (50 mg/kg) treated mice were significantly lower than that of control group.**

❖ **TILs profiling showed FOXP3+ T-regulatory immune cell population was reduced by almost 63% in CA102N (50 mg/kg) treated mice whereas the number of $CD8^+$ T cells in CA102N treated tumors was about 2-fold higher than the control group, suggesting the antitumor efficacy of lower dose of CA102N might be mediated by immune modulation.**

❖ **Western blot analysis of pAkt and pS6 in CA102N (200 mg/kg) treated tumor tissues were significantly downregulated by more than 50% compared to that of control group, suggesting antitumor activity of higher dose of CA102N is associated with down-regulating PI3Kinase pathway.**

Conclusion

We report that CA102N treatment implies a promising new approach to colon cancer treatment. Our data suggests that lower dose of CA102N (50mg/kg) exerts antitumor activity in mouse colon tumor model by affecting tumor microenvironment whereas higher dose of CA102N (200mg/Kg) suppressed tumor growth through directly targeting tumor cells PI3kinase pathway.

References

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