

925

### CHEMOKINE MALFUNCTION: CCL2-NITRATION MEDIATED DYSFUNCTION OF T CELLS IN BLADDER CANCER

Neelam Mukherjee\*, Niannian Ji, Zhen-Ju Shu, Zaineb Hassouneh, Robert S Svatek.  
University of Texas Health San Antonio, San Antonio, TX, USA

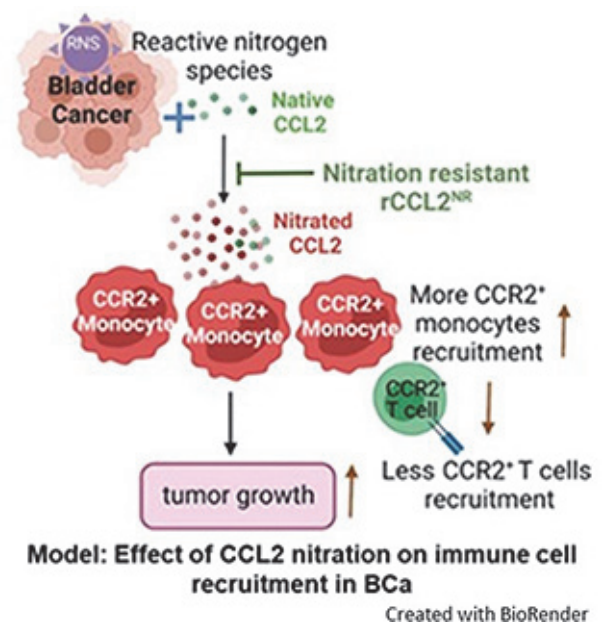
**Background** The chemokine CCL2 (C-C motif ligand 2) is best known for its ability to induce the trafficking of immune cells by binding its primary receptor, CCR2. The recruitment of immunosuppressive monocytes by CCL2 promotes cancer in several tumor types and anti-CCL2 is being tested for other solid tumors in clinical trials.

**Methods** To test CCL2 signaling in bladder cancer (BC), WT mice and mice deficient in CCL2 (CCL2KO) were given the urothelial carcinogen N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN). We also orthotopically challenged WT, CCL2KO, and CCR2KO mice (lacking the CCL2 receptor) with MB49 BC. We further used T cell adoptive transfer and T cell depletion strategies to delineate the role of T cells in CCL2's effect on BC. Intravesical recombinant CCL2 (rCCL2) either alone or in combination with intravesical gemcitabine were tested in MB49 and humanized models of BC. The effect of anti-CCL2 on BC growth was also measured in mice. Further, nitration of CCL2 was measured by mass spectrometry and confocal microscopy in BC and the effects of nitration on immune cells were investigated *in vivo*.

**Results** Tumor incidence and growth were higher in CCL2KO and CCR2KO mice demonstrating an unanticipated protective role for CCL2 signaling in different BC murine models. The depletion of T cells abolished this protective effect of CCL2. Adoptive transfer of CCR2<sup>+</sup> T cells into CCR2KO mice restored protection against MB49. CCR2<sup>+</sup> T cells were also more activated, functional, and tumor-specific compared to their CCR2<sup>-</sup> counterparts. We found that anti-CCL2 promotes BC growth highlighting a concern for the use of anti-CCL2 in BC. Moreover, intravesical rCCL2 either alone or in combination with intravesical gemcitabine reduced bladder tumor growth and improved the survival of mice with BC. Most studies researching chemokines, including CCL2, in cancer, assume the chemokine to be completely active and do not consider their different modified states which may be one of the critical reasons behind the failure of chemokines in clinical trials. We show for the first time that bladder tumors induce post-translational nitration of CCL2 and block T cell recruitment to the bladder (figure 1) which is restored by exogenous rCCL2 treatment. Chemical nitration of rCCL2 abolished this therapeutic efficacy of rCCL2 and decreased bladder T-cell infiltration and increased monocyte infiltration in BC.

**Conclusions** Bladder tumor protection by CCL2/CCR2 opposes the dogma of CCL2's role in cancer. We also unveiled the tumor-suppressive effect of posttranslational nitration on this T cell-mediated anti-tumor BC axis.

**Ethics Approval** The study obtained institutional IACUC approval (20150058AR).



Abstract 925 Figure 1

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