A NOVEL NUCLEAR RECEPTOR 4A1 (NR4A1) ANTAGONISTS ATTENUATES T-CELL EXHAUSTION IN COLORECTAL CANCER

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Background Colorectal cancer (CRC) is a highly complex disease with multiple risk factors and both genetic and environmental components contribute to disease incidence.¹⁻² Cancer immunotherapy using immune-checkpoint blockades represents a major advance in treatment strategy.³⁻⁴ The orphan nuclear receptor 4A1 (NR4A1) is overexpressed in lung, colon, liver and breast cancers and in Rhabdomyosarcoma and is a negative prognostic factor for cancer patient survival.⁵⁻⁸ Previous studies in breast cancer cells showed that PD-L1 was regulated by NR4A1 which activates transcription factor Sp1 bound to the PD-L1 gene promoter. Genome-wide studies have identified NR4A1 as a key mediator of T-cell dysfunction and NR4A1 also plays an important role in regulating genes which are involved in tumor-induced T-cell exhaustion.⁹ Bis-indole derived NR4A1 ligand that act as receptor antagonists have been developed in this laboratory and these compounds block pro-oncogenic NR4A1-regulated genes/pathways.

Methods Immune competent C57BL/6 mice and mouse MC-38 colon cancer cells were used and tumor Infiltrating Lymphocytes (TILs) were isolated from mice either untreated or treated with CDIM/NR4A1 antagonists. FACS analysis and Real-Time PCR were performed to determine expression of exhaustion markers in these tumor T-cell population.

Results

Two compounds: 1,1-bis(3-indolyl)-1-(3-bromo-5-trifluoromethoxyphenyl)methane (DIM-3-Br-5-OCF₃) and 3,5-dichlorophenyl analog (DIM-3,5-Cl₂) inhibited tumor growth and weight at doses of 2.5 and 7.5mg/kg/day (figure 1). Tumor CD8+ T-cells isolated from mice treated with DIM-3,5-Cl₂ and DIM-3-Br-5-OCF₃ exhibited decreased mRNA expression of NR4A1 and high mobility group – box transcription factors NFAT, TOX and TOX2 and increased mRNA levels of Interferon- γ (IFN γ), granzyme β (GzB) and perforin compared to control animals (figure 2). As TOX and TOX2 cooperate with NR4A1 to modulate CD8+ T-cell exhaustion, we investigated the expression of several inhibitory receptors of T-cell exhaustion in CD8+ TILs, including PD-1, 2B4, TIGIT and TIM3. Following treatment with DIM-3,5-Cl₂ or DIM-3-Br-5-OCF₃, there was a significant decrease in the percentage of PD1 and 2B4 cells and a decrease in TIGIT and TIM3 (figure 3). These results indicate that NR4A1 antagonists reverses T-cell exhaustion.

Conclusions NR4A1 plays a critical role in T-cell dysfunction, and this includes T-cell exhaustion.¹⁰⁻¹¹ Our results demonstrate that the NR4A1 antagonists reverse many markers of T-cell exhaustion including activation of cytokines. The combined effects of NR4A1 antagonists in both tumors and T-cells result in inhibition of colon tumorigenesis by targeting pathways/genes in tumor cells and by enhancing immune surveillance via reversal of T-cell exhaustion.

Abstract 732 Figure 1 CDIM/NR4A1 antagonists inhibit colon tumor growth. Model for regulation of genes with GC-rich promoters by NR4A1/SP1 (A). C57BL/6 mice bearing MC-38 cells as xenografts were treated for 21 days with corn oil (control), DIM-3-Br-5-OCF₃ (2.5 and 7.5 mg/kg/d), DIM-3,5-Cl₂ (2.5 and 7.5 mg/kg/d) and effects on tumor volume (B), and tumor weights (C) were determined.

Abstract 732 Figure 2 CDIM analogs alter transcription factors expression. FACS analysis and CD4 and CD8 – specific antibodies were used to determine T-cell population in tumors of mice treated with corn oil (control), DIM-3-Br-5-OCF₃ (2.5 and 7.5 mg/kg/d) and DIM-3,5-Cl₂ (2.5 and 7.5 mg/kg/d) (A). Real time PCR was used to determine expression of nuclear factors (B) and cytokine (C) mRNA levels in CD8+ T-cells isolated from tumors. Results are expressed as means $\pm$ SD replicates from each treatment group and significant ($p < 0.05$) induction or inhibition is indicated (*).

Abstracts

Abstract 732 Figure 3 NR4A1 ligands decreases T-cell exhaustion markers. FACS analysis in tumors derived from mice treated with corn oil (control), DIM-3-Br-5-OCF3 (2.5 and 7.5 mg/kg/d) and DIM-3,5-Cl2 (2.5 and 7.5 mg/kg/d) using specific antibodies was carried out to determine percentage of CD8+ T-cells expressing T-cell exhaustion markers - PD1 (A), 2B4 (B), TIGIT (C) and TIM3 (D). Significant ($p < 0.05$) induction or inhibition is indicated (*) and results are expressed as means $\pm$ SD for at least 4 separate mice per treatment group.

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Ethics Approval All animal studies were carried out according to the ethical procedures approved by the Texas A&M University Institutional Animal Care and Use Committee. Approval number is 2020-0138.

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