725 **IMMUNOMODULATION BY TARGETING PDL-1 IN COLON CANCER USING NUCLEAR RECEPTOR 4A1 (NR4A1) ANTAGONISTS**

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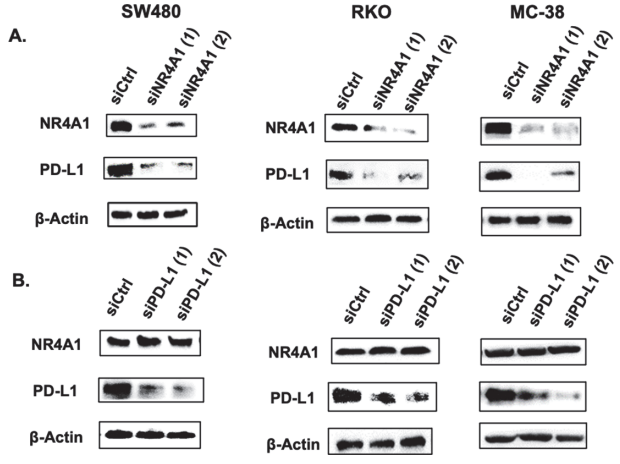
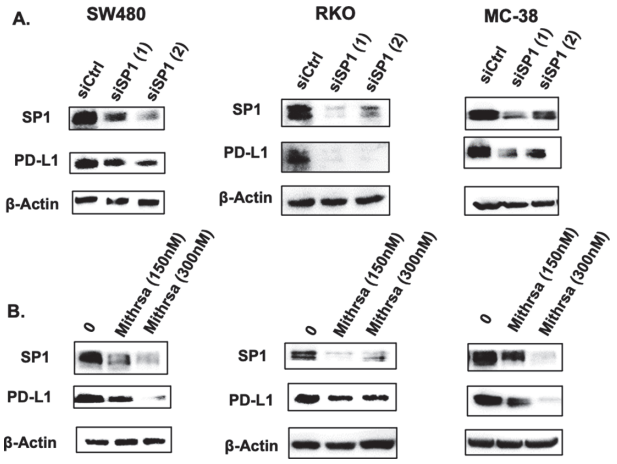
Background The nuclear orphan receptor 4A1 (NR4A1, Nur77, TR3) is overexpressed in multiple solid tumors including colorectal tumors and is a negative prognostic factor for patient survival.[1–3] NR4A1 is expressed in colon cancer cells and exhibit pro-oncogenic activity[4] and results of examination of several colon cancer cell lines show that PD-L1 expression is limited and NR4A1 and PD-L1 are co-expressed in SW480 and RKO colon cancer cell lines. Previous studies showed that PD-L1 was regulated by NR4A1 which activates transcription factor Sp1 bound to the PD-L1 gene promoter.[5–7] Knockdown of NR4A1 or Sp1 by RNA interference or treatment with mithramycin an inhibitor of Sp-mediated transcription decreased expression of PD-L1 in RKO and SW480 colon cancer cell lines.

Methods SW480, RKO and MC-38 cells were used in this study. Cells were treated for 24 hrs with DIM series of compounds.

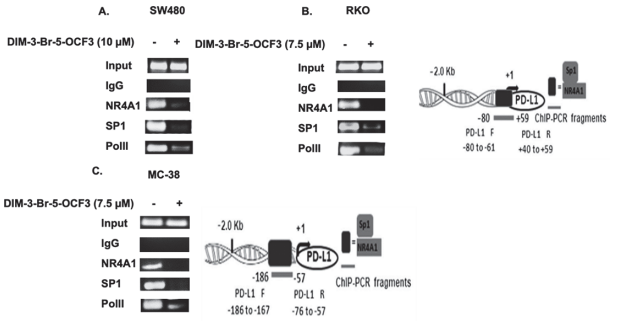
Results Current data coupled with ongoing gene expression and PD-L1 promoter studies demonstrate that PD-L1 expression is regulated by NR4A1/Sp1 in colon cancer cells (figures 1–3). Bis-indole derived NR4A1 ligand that act as receptor antagonists have been developed in this laboratory and these compounds block pro-oncogenic NR4A1-regulated genes/pathways. Treatment of RKO and SW480 colon cancer cell lines with a series of potent 1,1-bis(3′-indolyl)-1-(3,5-disubstituted-phenyl) analogs decreased expression of PD-L1. These results show that bis-indole derived NR4A1 antagonists act as small molecule mimics of immunotherapeutics that target PD-L1. In vivo applications of NR4A1 ligands that target PD-L1 and their effects on tumor growth and immune surveillance are currently being investigated.



Abstract 725 Figure 1 NR4A1 inactivation inhibits PD-L1 expression. SW480, RKO and MC-38 cells were transfected with siCtrl (non-specific oligonucleotide) and two oligonucleotides targeting NR4A1 (siNR4A1(1) and siNR4A1(2)) or PD-L1 (siPD-L1(1) and siPD-L1(2)) for 72 hrss. Protein expression from whole cell lysates were analyzed by western blots and effects on PD-L1 expression were determined



Abstract 725 Figure 2 Sp1 inactivation inhibits PD-L1 expression. SW480, RKO and MC-38 cells were transfected with siCtrl and oligonucleotides targeting Sp1 (siSp1(1) and siSp1(2)) for 72 hrs as well as treated with Mithrsamycin (150 and 300 nM) for 24 hrs. Protein expression from was analyzed by western blots and effects on PD-L1 levels were determined.



Abstract 725 Figure 3 Role of NR4A1/Sp in regulation of PD-L1. SW480, RKO and MC-38 cells were treated with DIM-3-Br-5-OCF3 for 24 hrss and protein interactions with the GC-rich PD-L1 promoter region were analyzed by ChIP using primers encompassing GC-rich region of the promoter

Conclusions Bis-indole derived NR4A1 antagonists inhibit PD-L1 expression. NR4A1/SP1 regulates PD-L1 and is inhibited by NR4A1 antagonist. NR4A1 ligands such as DIM-3-Br-5-OCF3 were among the most potent of the substituted DIM compounds and ongoing in vivo studies show that this DIM compound also inhibits tumor growth in a syngenic mouse model (data not shown). Data from this study demonstrate the pro-oncogenic activity of NR4A1 and show that the synthetic buttressed analog DIM-3-Br-5-OCF3 acts as an NR4A1 antagonist and inhibits PD-L1 expression. These drugs can be developed for future clinical applications.

REFERENCES

1. www.cancer.org/cancer/colon-rectal-cancer/about/key-statistics.html.
2. Garcia-Villatoro et al., Effects of high-fat diet and intestinal aryl hydrocarbon receptor deletion on colon carcinogenesis. *Am J Physiol Gastrointest Liver Physiol* 2020;**318**(3):G451–G463.
3. Safe S, Jin UH, Hedrick E, et al. Minireview: role of orphan nuclear receptors in cancer and potential as drug targets. *Mol Endocrinol* 2014;**28**(2):157–72.

4. Maxwell MA, Muscat GE. The NR4A subgroup: immediate early response genes with pleiotropic physiological roles. *Nucl Recept Signal* 2006;**4**:e002.
5. Lee SO, Li X, Hedrick E, et al. Diindolylmethane analogs bind NR4A1 and are NR4A1 antagonists in colon cancer cells. *Mol Endocrinol* 2014;**28**(10):1729–39.
6. Safe S, Kim K. Non-classical genomic estrogen receptor (ER)/specificity protein and ER/activating protein-1 signaling pathways. *J Mol Endocrinol* 2008;**41**(5):263–75.
7. Tao LH, Zhou XR, Li FC, Chen Q, Meng FY, Mao Y, et al. A polymorphism in the promoter region of PD-L1 serves as a binding-site for SP1 and is associated with PD-L1 overexpression and increased occurrence of gastric cancer. *Cancer Immunol Immunother* 2017;**66**(3):309–18.

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