DOI: 10.25205/978-5-4437-1843-9-294

COMBINED AND INDIVIDUAL IMMUNOMODULATORY EFFECTS OF COLORECTAL CANCER-DERIVED GALECTIN-1 AND -3 ON CD4⁺ T CELL FUNCTION *

V. S. Poletika[1], G. V. Reingardt[2], A. V. Kurnosenko[1,2], Yu. V. Kolobovnikova[1], O. I. Urazova[1]

[1] *Siberian State Medical University, Tomsk*
[2] *Tomsk Regional Oncology Center*

✉ vpoletika@yandex.ru

Abstract

This study investigates how colorectal cancer (CRC) cells modulate CD4⁺ T cell function via galectin-1 and -3 in transwell co-cultures with patient or healthy donor immune cells. Gal-1 consistently suppresses Th1/Th17 and promotes Treg activity, while Gal-3 exhibits context-dependent effects. Combined Gal-1/Gal-3 action additively enhances Treg function but redundantly suppresses Th1/Th17 in CRC.

Immune evasion by tumor cells is critical for colorectal cancer (CRC) progression. Galectin-1 (Gal-1) and galectin-3 (Gal-3), β-galactoside-binding lectins overexpressed in CRC, are potent immunomodulators that suppress anti-tumor T cell responses [1]. While their individual roles are established in various malignancies, the combined impact of CRC-derived Gal-1 and Gal-3 on key CD4⁺ T-helper subsets (Th1, Th17) and regulatory T cells (Treg) — particularly in diseased versus healthy immune contexts — remains poorly characterized. Furthermore, galectin functions exhibit significant plasticity across tumor types and immune cell states, hindering translational applications and targeted therapy development [2]. This study aimed to dissect the individual and combined effects of CRC-derived Gal-1 and Gal-3 on T cell function in an *in vitro* transwell co-culture model with peripheral blood mononuclear cells (PBMCs) from CRC patients or healthy donors.

The human CRC cell line COLO 201, expressing both Gal-1 and Gal-3, was co-cultured with PBMCs from treatment-naïve CRC patients or age-matched healthy donors using transwell systems (0.4 μm pore membrane), enabling soluble factor exchange without direct cell contact. PBMCs were activated using Phytohemagglutinin-P. Four conditions were tested: 1) intact co-culture (control), 2) co-culture with Gal-1 inhibitor OTX 008, 3) co-culture with Gal-3 inhibitor GB1107, and 4) co-culture with both Gal-1 and Gal-3 inhibitors. After 72 hours, PBMC supernatant levels of lineage-defining cytokines — IFNγ (Th1), IL-17A (Th17), and TGFβ1 (Treg) — were quantified by ELISA. Data was analyzed using non-parametric Friedman test with Dunn's post-hoc.

Selective Gal-1 inhibition in COLO 201-PBMC co-cultures significantly increased IFNγ and IL-17A production while decreasing TGFβ1 secretion by PBMCs from both CRC patients and healthy donors relative to controls. In co-cultures with patient-derived PBMCs, Gal-3 inhibition similarly enhanced IFNγ and IL-17A levels and reduced TGFβ1. However, in healthy donor PBMC co-cultures, Gal-3 blockade induced only a modest IFNγ increase but significantly suppressed IL-17A production and elevated TGFβ1. Combined Gal-1/Gal-3 blockade in patient PBMC co-cultures produced a greater reduction in TGFβ1 than individual inhibition (indicating additive stimulation of TGFβ1 secretion by both galectins), while IFNγ/IL-17A changes mirrored single-inhibitor effects (demonstrating functional redundancy in Th1/Th17 suppression). In healthy donor PBMC co-cultures, combined inhibition showed no synergistic effects beyond Gal-1 blockade alone.

In conclusion, CRC-derived Gal-1 acts as a consistent tolerogenic driver, suppressing IFNγ/IL-17A and promoting TGFβ1 secretion by PBMCs regardless of their source. In contrast, Gal-3 exhibits marked plasticity: it mimics Gal-1's tolerogenic role in the CRC context (patient PBMCs) but exerts pro-inflammatory effect on naïve immunity (healthy PBMCs), stimulating IL-17A while downregulating TGFβ1. The results of combined galectin inhibition indicate that Gal-1/Gal-3 additively drive TGFβ1 secretion but redundantly inhibit Th1/Th17 responses in CRC. These findings underscore the necessity of studying combinatorial galectin effects in disease-relevant contexts and highlight Gal-1 and -3 as compelling co-targets for CRC immunotherapy.

References

1. Thijssen V. L., Heusschen R., Caers J., Griffioen A. W. Galectin expression in cancer diagnosis and prognosis: A systematic review // Biochim. Biophys. Acta. 2015. Vol. 1855, No. 2. P. 235–247.

2. Kapetanakis N. I., Busson P. Galectins as pivotal components in oncogenesis and immune exclusion in human malignancies // Front. Immunol. 2023. Vol. 14.

* The study is supported by the Russian Science Foundation (project #25-25-20109) and a subsidy grant allocated by the Department of Scientific, Technological Development and Innovative Activities of Tomsk Region (agreement #02/3/2025).