Expression and function of Regulator of G Protein Signaling (RGS) proteins in pathogenesis of colitis-associated colorectal cancer

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Introduction:

Colorectal cancer (CRC) is one of the most common neoplasms world widely. One of the types of CRC is colitis-associated cancer (CAC), which originates from inflammatory bowel diseases (IBD). G-protein coupled receptors (GPCR) affect crucial elements of oncogenesis and its prevention, such as regulation of cell cycle, responses to medications, angiogenesis and forming metastases. The magnitude of GPCR signalling is controlled by regulators of G-protein signaling (RGS). Typically, RGS proteins possess GTPase activity which facilitates GTP to GDP transformation and thus termination of G-protein signaling. AXIN is an atypical RGS without GTPase. In fact, its main role is involvement in β-catenin destruction complex through which AXIN antagonizes prooncogenic canonical Wnt signaling pathway (Figure 1).
Recently attention has been paid to the role of cannabinoids and their potential tumor-suppressive properties. Cannabinoids exert their physiological effects through $G\alpha_{i/o}$-type GPCR – CB1 and CB2, that eventually lead to apoptosis.
The aim of this project is to examine the expression and function of AXIN in CRC cell lines and mouse model of CAC. Moreover, the therapeutic potential of simultaneous targeting of AXIN and CB receptors by KYA1797K and WIN 55,212-2 was tested.

Methods (2nd year of doctoral studies):

Cell line of healthy enterocytes: CCD 841 CoN and CRC: Caco-2, SW480 were cultured. Cytotoxicity of KYA1797K (1; 10; 100, 300 μM), WIN 55,212-2 (1; 10; 100 μM) and combined therapy (equal conc. 1; 10; 100, and 100+10 μM, respectively) was assessed using MTT test. Genes' expression was evaluated using quantitative real-time PCR and Western blot methods. Statistical analysis included Shapiro-Wilk test, one-way ANOVA with post-hoc Dunnett's and Tukey's tests.

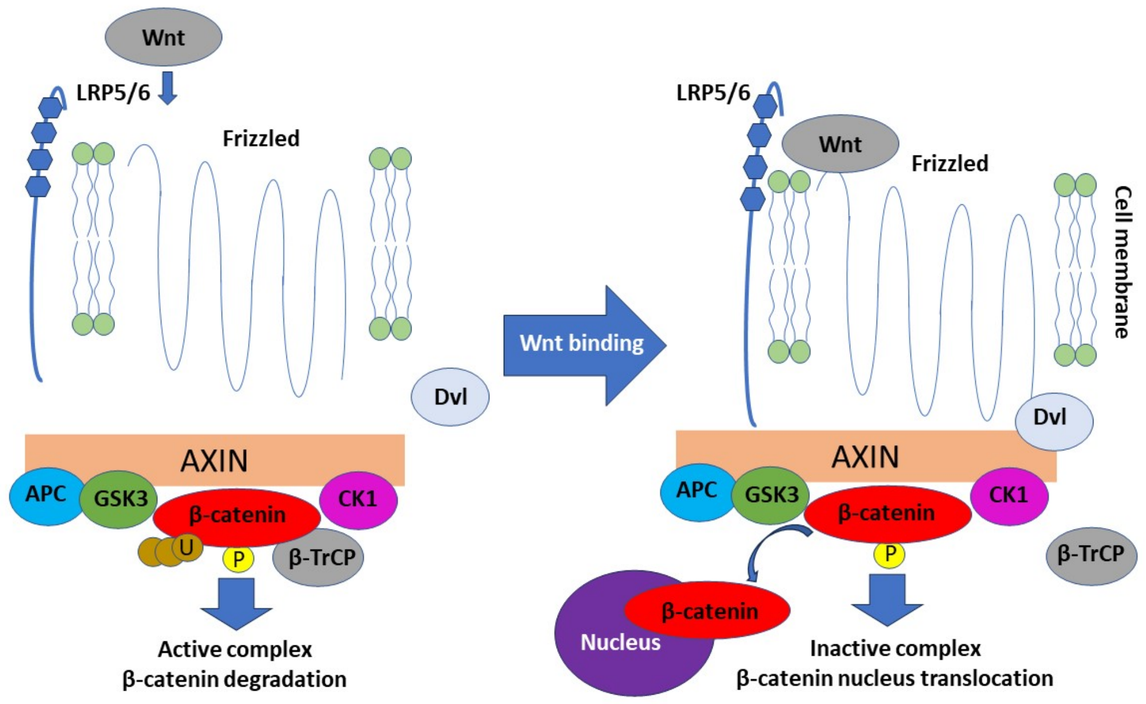

Fig.1 β-catenin destruction complex function without and upon Wnt binding. LRP5/6 - low-density lipoprotein receptor-related protein 5/6, APC - adenomatis polyposis coli, GSK3 - glycogen synthase kinase3, CK1 - casein kinase1, and β-TrCP - E3 ubiquitin ligase component TrCP1 .

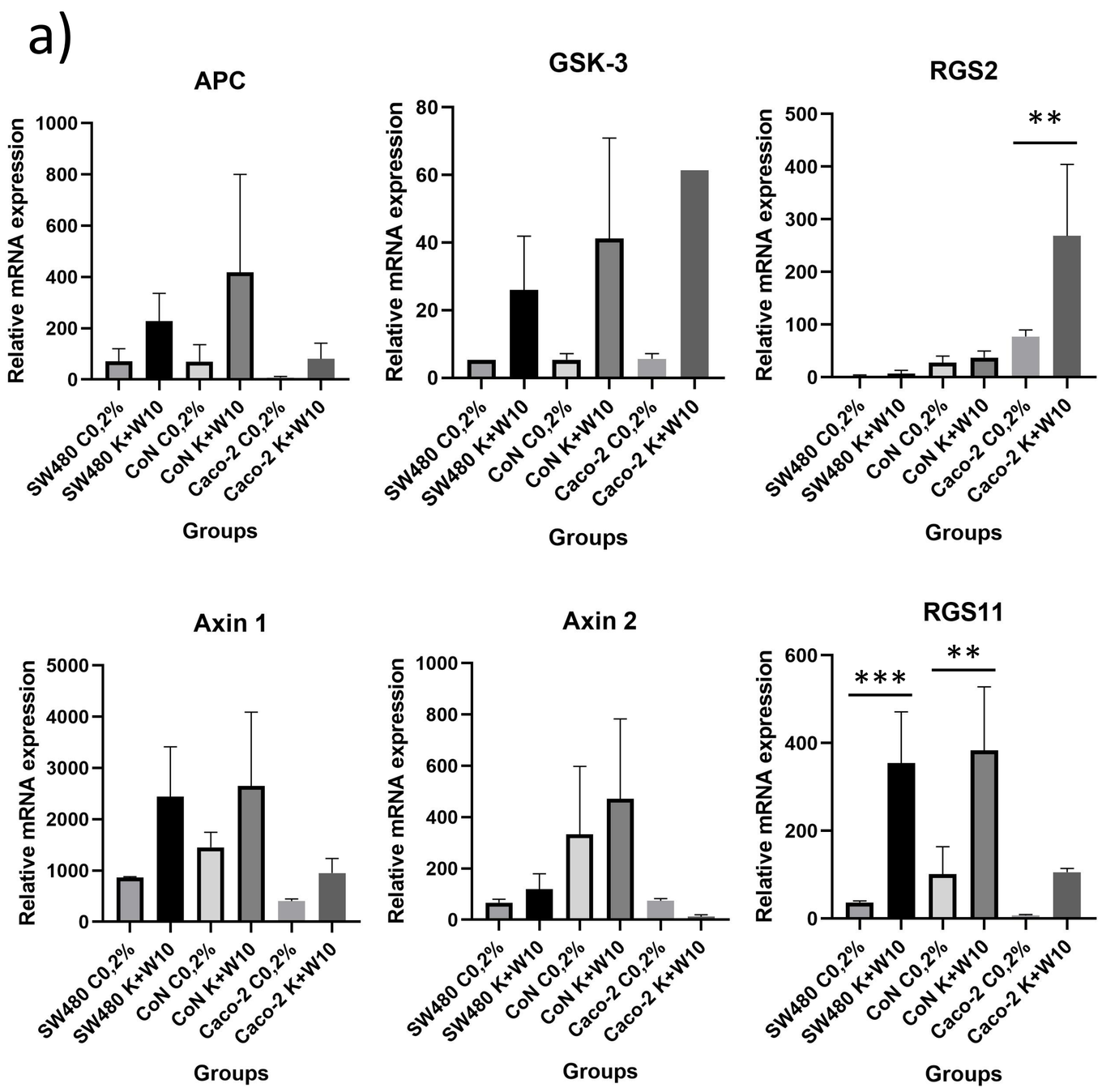

Fig.2 The results obtained during 2023/24 academic year. A) real-time PCR results for respective genes' expression relating to HPRT x1000. B) MTT results for the most promising treatment – K+W 10. c) Western blot results for examination of β-catenin postranslational expression d) Bands from β-catenin and GAPDH (reference) Western blot examination. *Legend: CoN – CCD 841 CoN cells; GAPDH - glyceraldehyde 3-phosphate dehydrogenase; HPRT - hypoxanthine-guanine phosphoribosyltransferase; K+W10 – combination of KYA1797K and WIN 55,212-2 in concentration of 10* μM; *p values: * (<0,05); ** (<0,01); *** (<0,001).*

Results:

In previous experiments we found that treatment with K+W10 causes significant cytotoxicity in cancerous SW480 cells but not in normal enterocytes CCD 841 CoN. Since K100+W10 treatment caused overall higher cytoxicity of CCD 841 CoN cells, K+W10 remained the most promising combination (Fig 2b). Moreover, K+W10 treatment resulted in increased expression of AXIN 1 and 2 in each cell line except AXIN 2 in Caco-2, in which opposite effect was observed. The combined treatment decreased CB1 receptors' expression in all examined cell lines, whereas opposing tendency was observed for CB2. Furthermore, K+W10 treatment led to increased expression of RGS2, 11, APC and GSK-3 (Fig.2a). At the protein level K+W10 treatment unsurprisingly tended to decrease β-catenin relative expression (Fig.2c and 2d). CB2 receptors were significantly more expressed in SW480 than in CCD 841 CoN that was consistent with cytotoxicity results.

Conclusions:

AXIN stabilization is a promising new approach in CRC treatment. AXIN stabilization does not weaken CB1 and 2-dependent effects. The combined treatment of AXIN stabilizer and CB agonist may be especially important as life-prolonging therapy for patients with advanced CRC.