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

**Mikołaj Świerczyński**, MD, Department of Biochemistry
Supervisor: Maciej Sałaga, PhD, Associate Professor

## Introduction:

Colorectal cancer (CRC) is one of the most common neoplasms in both male and female populations 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 various endo- and exogenous stimuli (e.g. 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 within which it cooperates with adenomatis polyposis coli (APC), glycogen synthase kinase3 (GSK3), casein kinase1 (CK1), and the E3 ubiquitin ligase component TrCP1. Through this complex, axin antagonizes prooncogenic canonical Wnt signaling pathway that depend on β-catenin.
Recently attention has been paid to the role of cannabinoids and their potential tumor-suppressive properties. Cannabinoids exerts their physiological effects through $G\alpha_{i/o}$-type GPCR – CB1 and CB2, that eventually leads to apoptosis.
In this project we aim to examine expression and function of axin in CRC cell lines and mouse model of CAC. Moreover we will test the therapeutic potential of simultaneous targeting of axin and CB receptors by KYA1797K and WIN 55,212,2.

## Hypotheses:

- RGS proteins have important role in CAC mouse model, including atypical RGS - axin.
- Small molecule axin stabilizers are effective in monotherapy and in combination with cannabinoids in CAC. Stabilization of axin does not weaken the effect of CB receptor ligands.

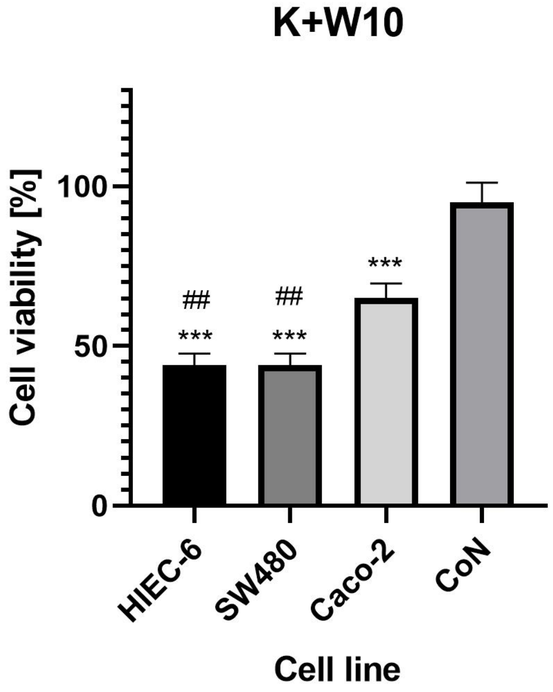


*Fig.2 The differences between cell viability in examined cell lines after incubation with KYA1797K and WIN 55,212,2 in equal concentrations 10 µM (K+W10). Statistical significance was observed for any possible comparison between the cell lines, besides HIEC-6 and SW480 (Tukey's test). Legend: CoN – CCD 841 CoN cells; p values: *** - p < 0.001 vs. CoN, ## - p < 0.01 vs. Caco-2.*

## Methods (1st year of doctoral studies):

Cell lines of healthy enterocytes: CoN, HIEC-6 and CRC: Caco-2, SW480 were cultured. Scientific material for future studies was obtained. Cytotoxicity of KYA1797K (1; 10; 100, 300 µM), WIN 55,212,2 (1; 10; 100 µM) and combined therapy (equal conc. 1; 10; 100 µM) was assesed using MTT test. Statistical analysis included Shapiro-Wilk test, one-way ANOVA with post-hoc Dunnett's and Tukey's tests.

## Results:

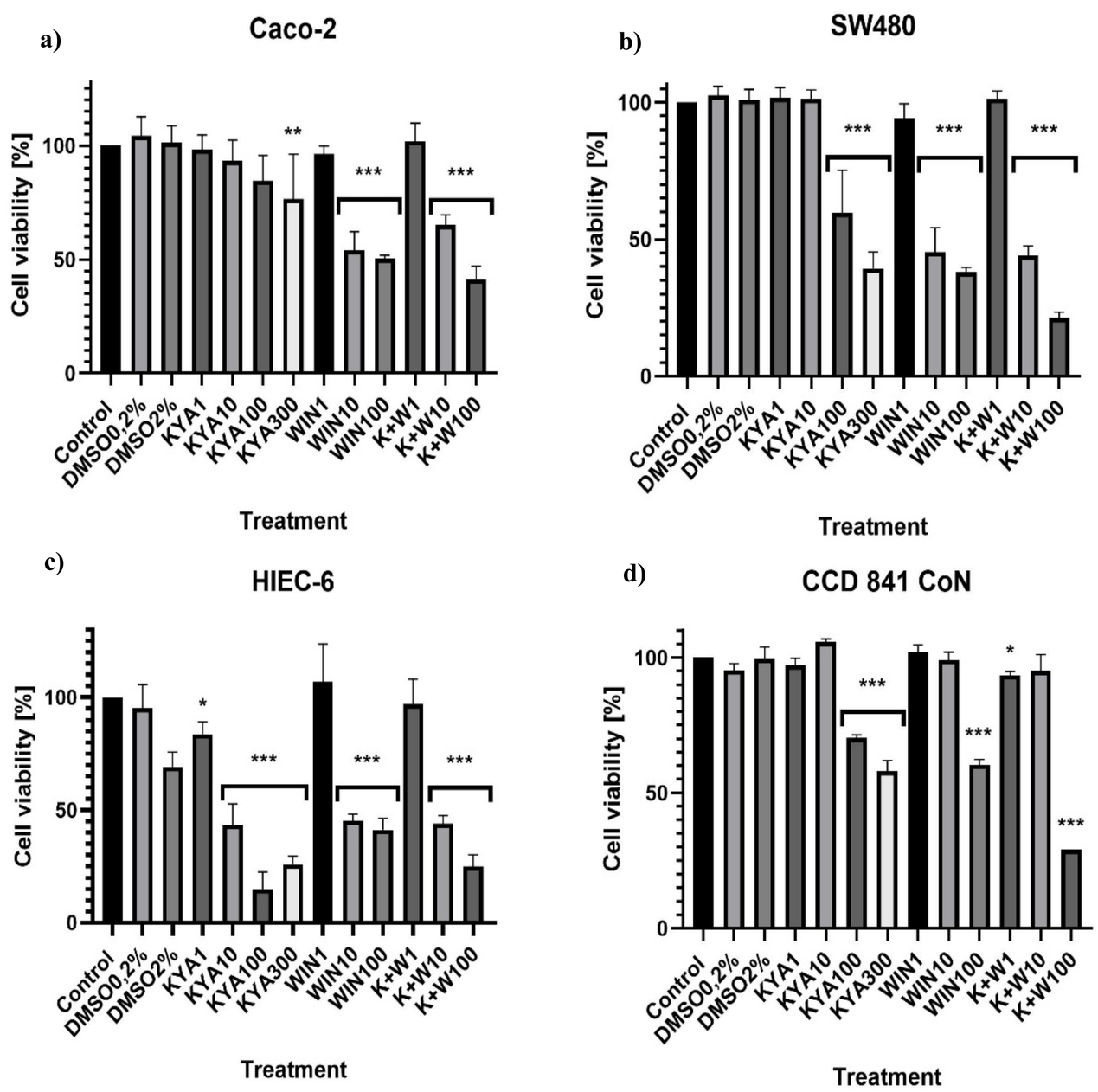


*Fig.1 MTT test results expressed as percentage of cell viability comparing to control. a) Caco-2, b) SW480, c) HIEC-6, d) CCD 841 CoN. Statistical significance comparing to control were calculated using Dunnett's test. P values marks: p: * (<0,05); ** (<0,01); *** (<0,001). KYA – KYA1797K with dose in µM, WIN – WIN 55,212,2 with dose in µM, K+W – combination of both drugs.*

Caco-2 cells are insensitive to KYA1797K in monotheraphy excluding concentration 300 µM, for which the viability differs significantly comparing to control. For WIN 55,212,2 in concentration of at least 10 µM in both mono- and combined therapy significant differences were observed. Similar profile of changes was observed in SW480 line, however the amplitude of observed changes and sensitivity to KYA1797K were higher. Helathy enterocytes, HIEC-6 cells had the highest sensitivity to used drugs. In CoN cells significant additive effect of KYA1797K and WIN 55,212,2 was observed for 100 µM with no changes observed for 10 µM. Comparing the effects of combined therapy with KYA1797K and WIN 55,212,2 (10 µM) significant changes in cell viability are observed between each of possible comparisions besides HIEC-6 and SW480 (Figure 2.).

## Conclusions:

Obtained results prove significant impact of KYA1797K and WIN 55,212,2 on survival of cancer cells. The most significant differences were observed after incubation with combination of the drugs in concentration 10 µM. Further studies will include examination of gene expression on transcipt level (qRT-PCR – in progress) and posttranslational level (ELISA, Western Blot).

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