IGF-1 SIGNALING AND WNT/BETA-CATENIN PATHWAY INTERACTION DURING THE PROGRESSION OF COLORECTAL CANCER

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ABSTRACTS

Background: Colorectal cancer (CRC) is a public health problem worldwide. It results from mutations in oncogenes and tumor suppressor genes, which leads to the deregulation of different pathways responsible for events such as differentiation, proliferation, migration and survivor. The Wnt/β-catenin pathway is chronically active in CRC and results in the accumulation of cytoplasmic β-catenin and consequently translocation to the nucleus, where it activates target genes of Tcf/Lef. Others signaling pathway such as PI3K is also constitutivelly activated during the progression of CRC. It is known that Insulin-like growth factor 1 (IGF-1) binds to its receptors and activates the RAS/MEK/ERK and PI3K/AKT signaling. Activated AKT phosphorylates GSK-3β, leading to its inhibition. GSK-3β is also one of the protein in the β-catenin destruction complex, indicating a possible interaction between those two signaling pathways. **Aims:** To evaluate the interaction between the signaling mediated by IGF-1 and the Wnt/β-catenin pathway during the progression of CRC. **Methods:** HCT-116 and HT-29 CRC cells lines were incubated with IGF-1 and Wnt3a. After, protein expression was evaluated by Western blotting and Crystal violet assay was performed to measure cell proliferation. The localization of β-catenin was evaluated by immunofluoresce. Furthermore, Wnt/β-catenin activity was assessed by measuring luciferase activity. **Results:** We observed that treatment with IGF-1 increased cell proliferation in both cell lines but this increases was not seen when cells were treated with Wnt3a. Western blot showed an increase in AKT activity after treatment wih IGF-1. GSK-3β activity was also increased. Treatment with Wnt3a induced the activation of TCF/LEF in both cells lines. Surprising, treatment with Wnt3a increased the activity of GSK-3β, but not of AKT. Moreover, the treatment with Wnt3a increased the luciferase activity as well as the pertubed the localization of β-catenin. The combined treatment resulted in a decrease of AKT activity compared with IGF-1 treatment, but had no effects on GSK-3β. **Conclusions:** These preliminar experiments show that IGF-1 signaling and the Wnt/β-catenin pathway may interact in some way to contribute with the progression of colorectal cancer.

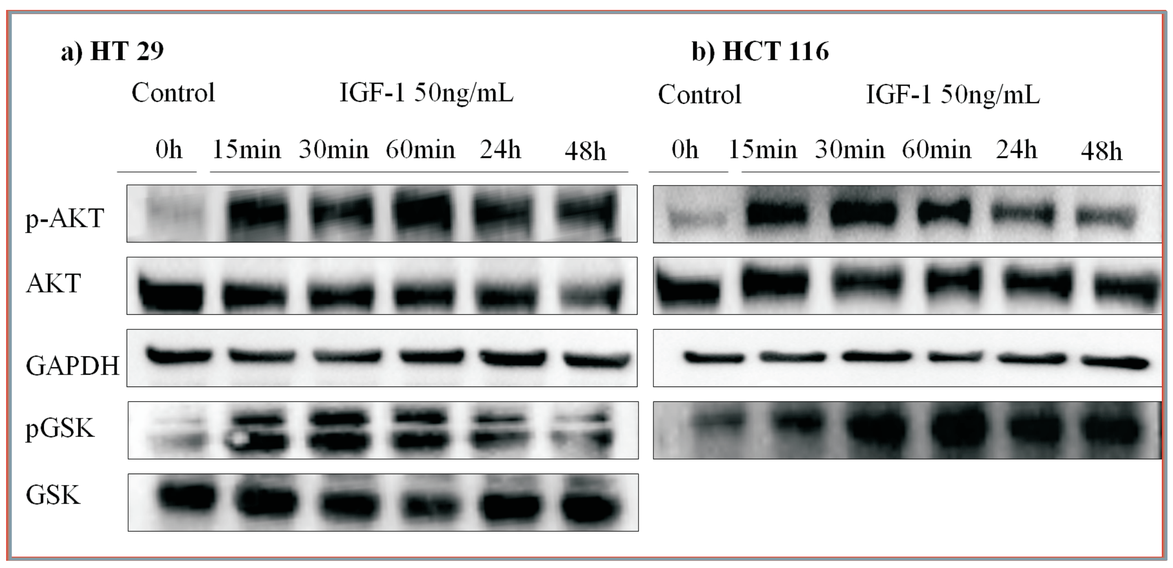

Figure 1: Activity of AKT/GSK after IGF1 treatment. Western blot analysis of pAKT, pGSK, total AKT and total GSK after the treatment with 50ng/mL of IGF-1 for 15min, 30min, 60min, 24h and 48h. GAPDH was used as the loading control. Figure representative of three independent experiments

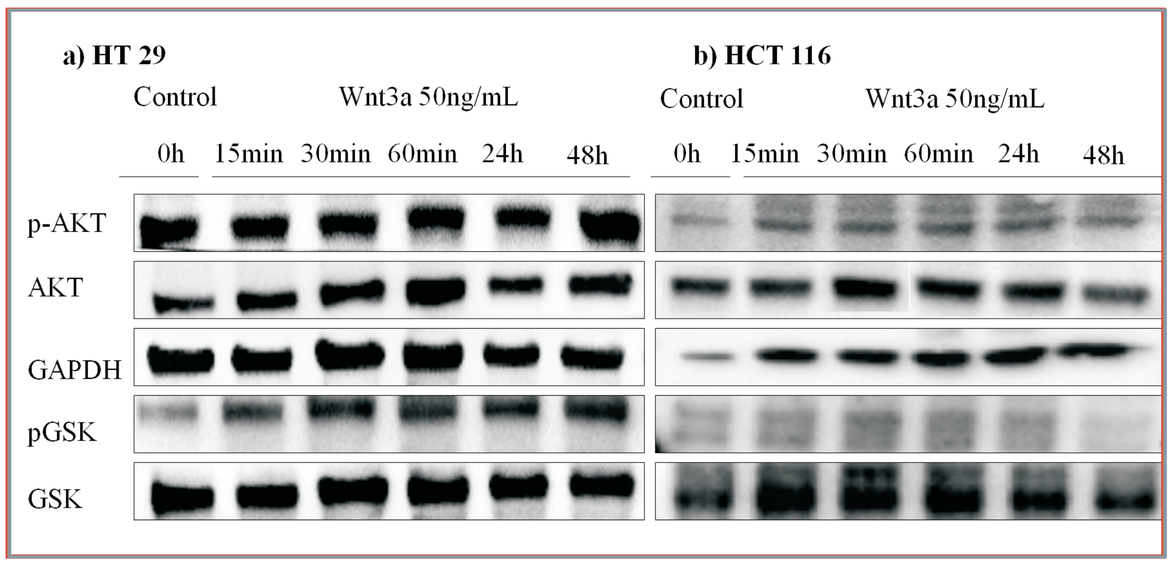

Figure 2: Activity of AKT/GSK after Wnt3a treatment. Western blot analysis of pAKT, pGSK, total AKT and total GSK after the treatment with 50ng/mL of Wnt3a for 15min, 30min, 60min, 24h and 48h. GAPDH was used as the loading control. Figure representative of four independent experiments.

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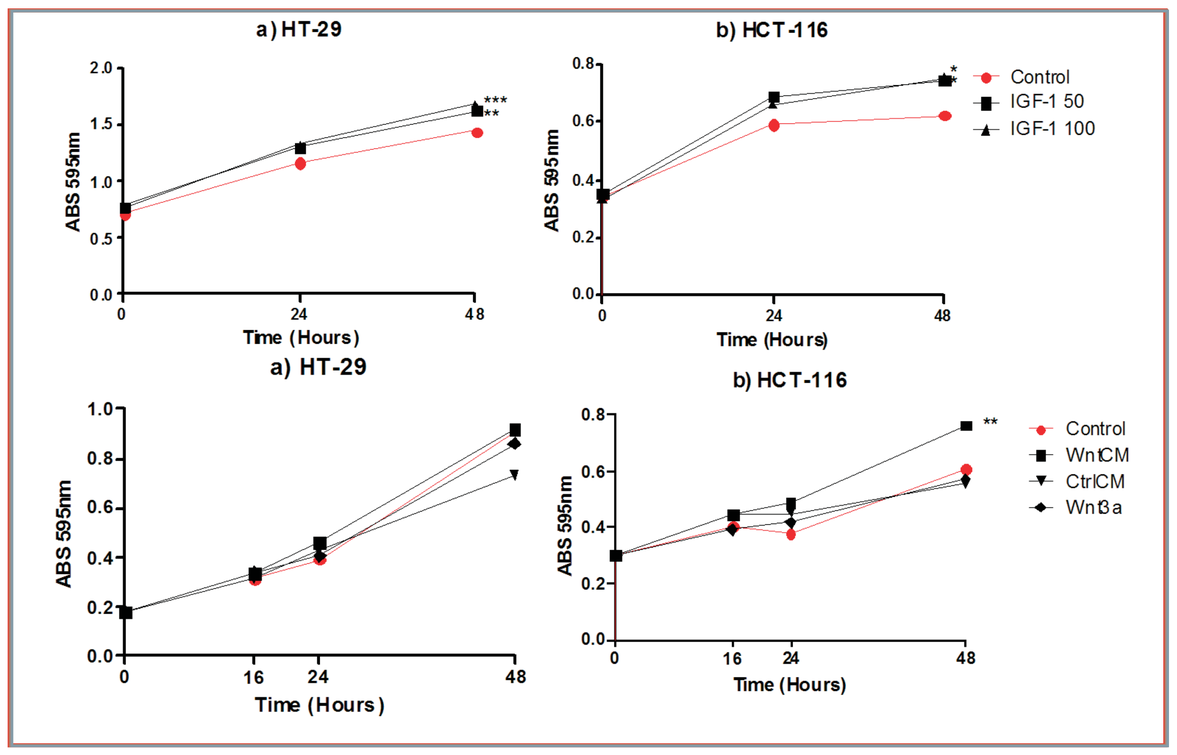

Figure 3: Cell growth response after IGF or Wnt3a treatment. (a) HT-29 and (b) HCT-116 cells were treated with 50 ng/ml of IGF1, 50ng/ml of Wnt3a, conditioned medium from L-cells overexpressing Wnt3a (WntCM) or conditioned medium from its parental cell (CtrlCM). The proliferation was measured by crystal violet assay after 24 and 48 hours. Results representative of four independent experiments. *p<0,05, **p<0,01, ANOVA and Bonferroni post test.

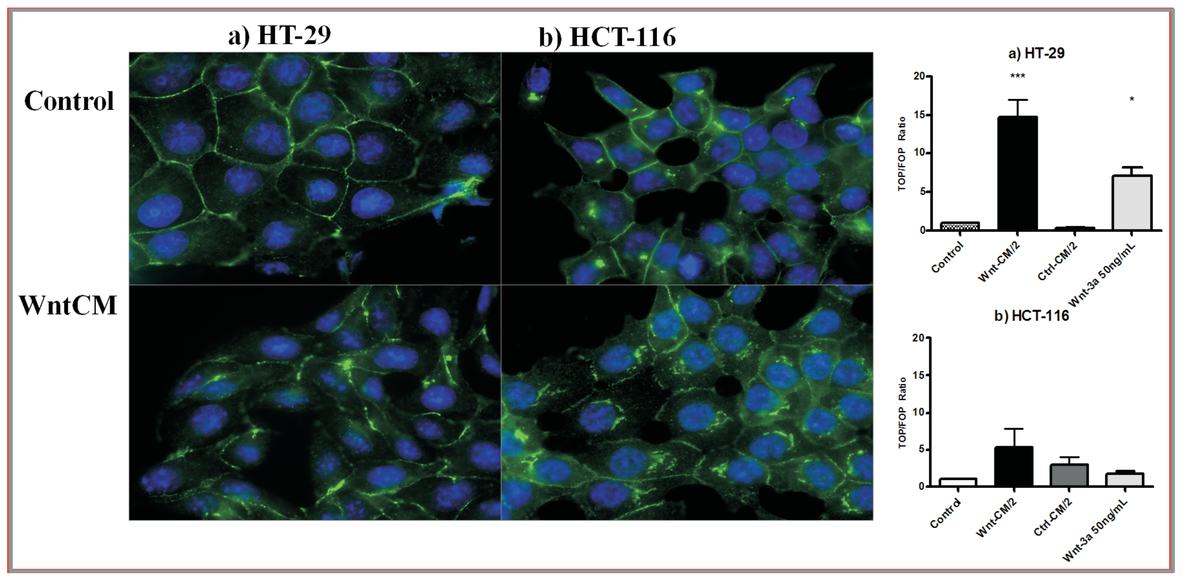

Figure 4: Effects of Wnt-3a on ß-Catenin localization and activity. (Right panel) (a) HT-29 and (b) HCT-116 treated with 50ng/ml of Wnt3a, conditioned medium from L-cells overexpressing Wnt3a (WntCM) or conditioned medium from its parental cell (CtrlCM) for 24h. Cells were fixed with Metanol and incubed with β-catenin (green) antibody overnight. DAPI is shown in blue. Images were taken on Zeiss Observer Z1 microscopy with 40x objective. *(*Left Panel) *C*ells were transfected with TOP and FOP reporters and treated as described above. Results representative of three independent experiments. *p<0,05, ***p<0,001, ANOVA and Bonferroni post test.

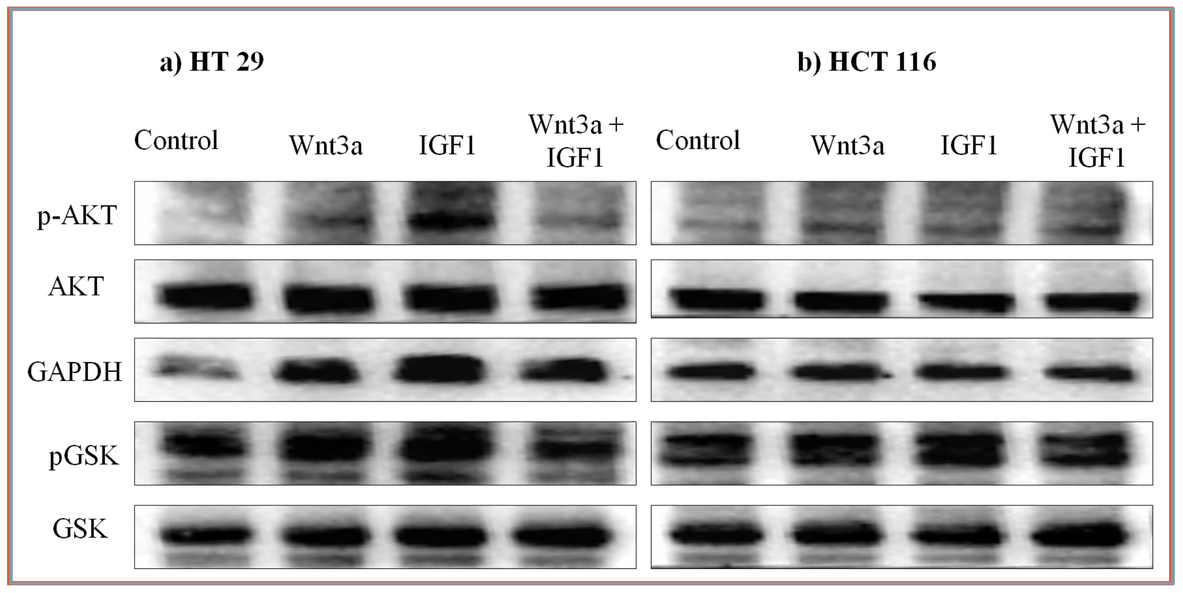

Figure 5: Activity of AKT/GSK after combined treatment. Western blot analysis of pAKT, pGSK, total AKT and total GSK after the treatment with 50ng/mL of Wnt3a, 50ng/mL IGF1 or both for 30min. GAPDH was used as the loading control. Figure representative of three independent experiments

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA

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