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

Cássio Dejair Fleming de Moraes, Dr. Wallace Martins de Araujo and Dr. José Andrés Morgado-Díaz
Grupo de Estrutura e Dinâmica Celular, Programa de Oncobiologia Celular e Molecular, CPq – INCa.
E-mail: jmorgado@inca.gov.br

ABSTRACT

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. In this context, the Wnt/β-catenin pathway is chronically active in CRC preventing the formation of the β-catenin destruction complex resulting in the accumulation of cytoplasmic β-catenin and consequently translocation to the nucleus, where it activates target genes of Tcf/Lef responsible for the events above described. Others signaling pathway such as PI3K is also activated during the progression of CRC. It is known that Insulin-like growth factor 1 (IGF-1) binds to its receptors activating RAS/MEK/ERK and PI3K/Akt signaling. Activated Akt phosphorylates GSK3, leading to its inhibition. GSK3 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 in different concentrations and times. After, protein expression was evaluated by Western blotting and Crystal violet assay was performed to measure cell proliferation. Furthermore, Wnt/β-catenin activity was assessed by measuring luciferase activity. **Results:** The first part of this project was to evaluate the ideal concentrations of IGF-1 and Wnt-3a on the activation of theirs respective signaling pathways. We observed that treatment with 50ng/mL and 100ng/mL of IGF-1, increased cell proliferation in both cell lines. This effect was seen in 24 and 48h for HT-29 cells but only in 48h for HCT cells. Western blot showed an increase in AKT activity after treatment wih 50ng/mL of IGF-1, showing a peak on short times such as 15min lasting until 60min. This effect, however, starts to decrease after 60min, indicating that the IGF-1-mediated AKT activity is a fast event. Different concentrations of Wnt3a (20, 50 and 100ng/uL) induced the activation of TCF/LEF in both cells lines. Concentration of 50ng/uL showed the best results. **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.

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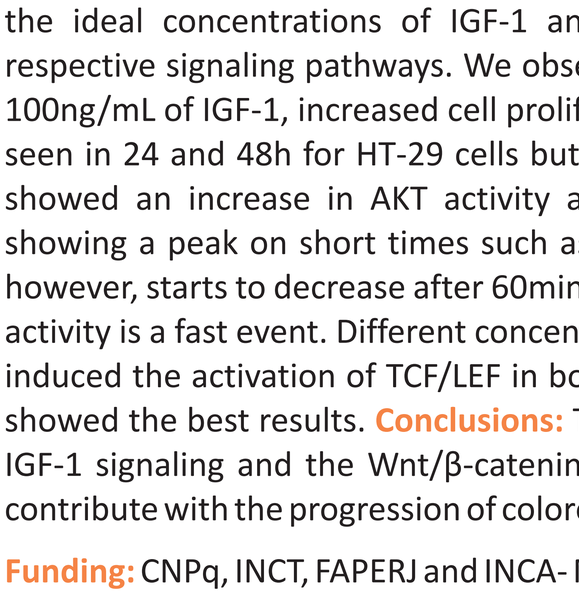


Figure 1: Cell growth response after IGF-1 treatment. (a) HT-29 and (b) HCT-116 cells were treated with 50 ng/ml and 100 ng/ml of IGF-1. The proliferation was measured by crystal violet assay after 24 and 48 hours. Results of three independent experiments, * p <0.05, ** p <0.01. ANOVA and Bonferroni post-test.

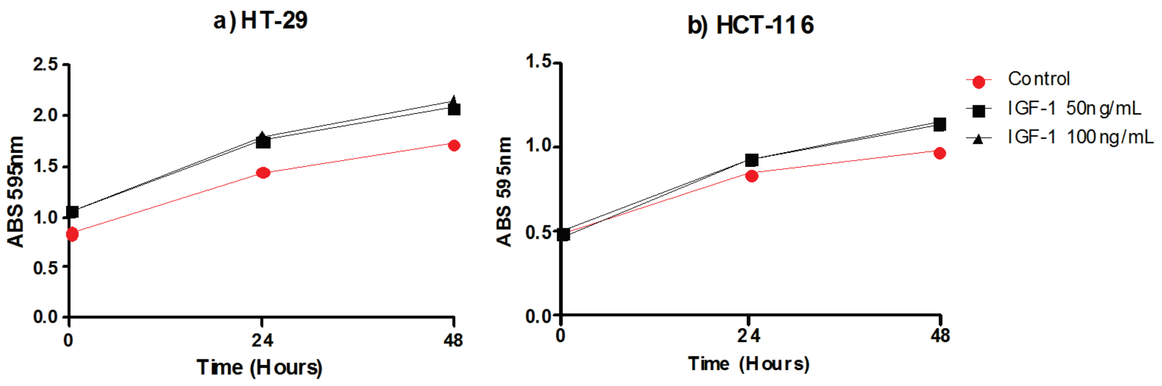
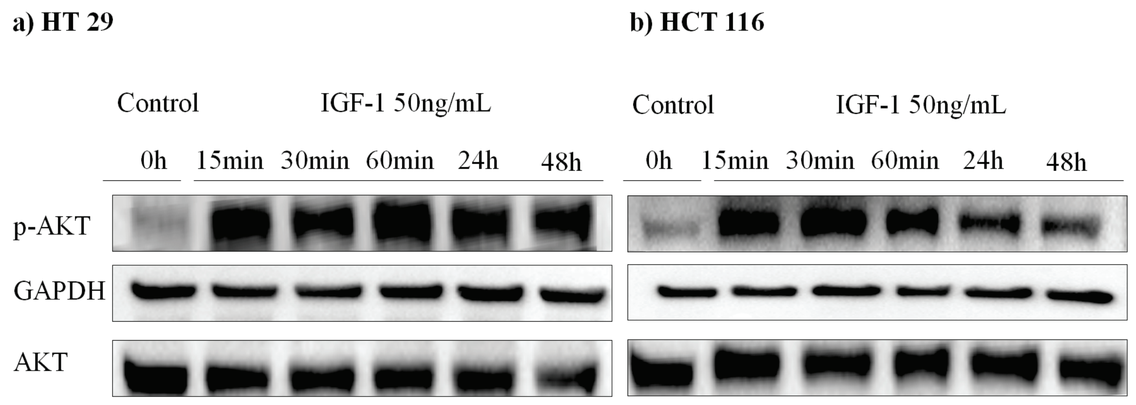


Figure 2: Curve of cell growth response after IGF-1 treatment. (a) HT-29 and (b) HCT-116 cells were treated with 50 ng/ml and 100 ng/ml of IGF-1. The proliferation was measured by crystal violet assay after 24 and 48 hours. Results of three independent experiments.



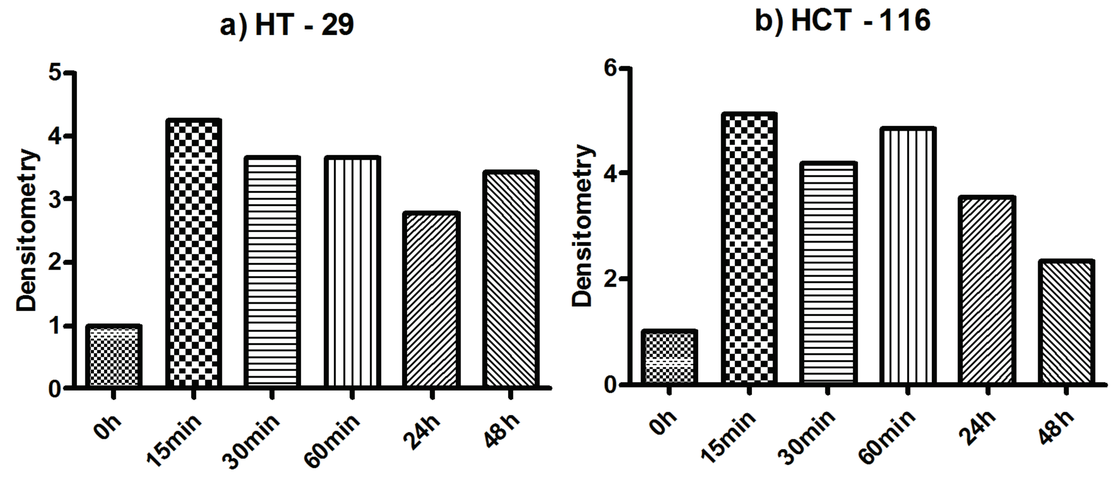


Figure 3: Kinects of IGF-1 on Akt activity. Western blot analysis of Akt-p and total Akt after the treatment with 50ng/mL of IGF-1 for 15min, 30min, 60min, 24h and 48h. GAPDH was used as the loading control. Result representative of only one experiment.

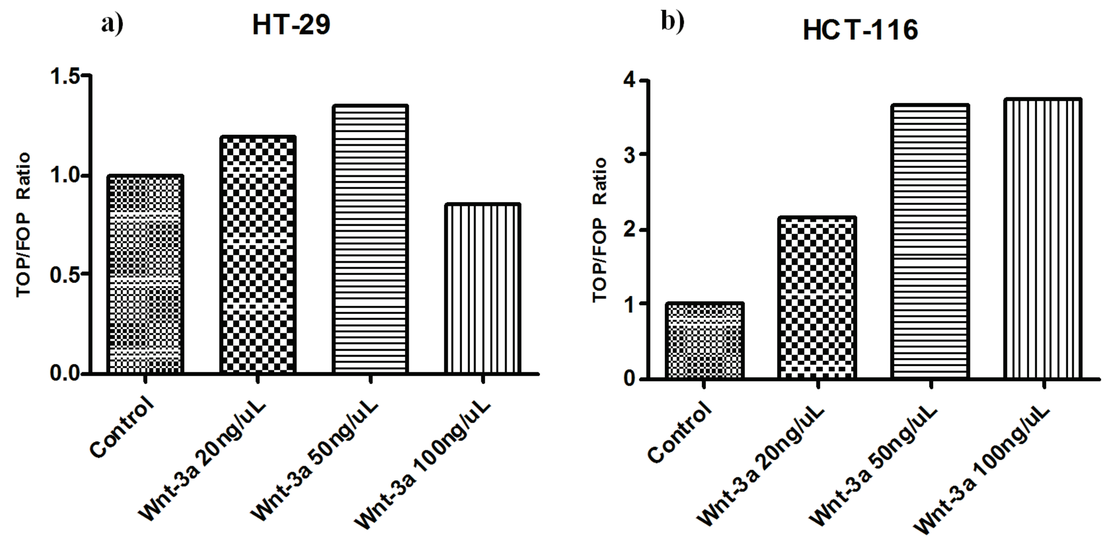


Figure 4: Effects of treatment with Wnt-3a for 24h on Luciferase Activity. a) HT-29 and (b) HCT-116 cells were transfected with TOP and FOP reporters and treated with 20ng/uL, 50ng/uL and 100ng/uL of Wnt-3a for 24h in 1% SFB. Result representative of only one experiment.

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