U.23 - Evaluation of Angiogenic Capacity of Human Adenocarcinoma Cell Line Knockout for NF-κB1 Protein

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INTRODUCTION: Renal cell carcinoma (RCC) is the most common adult renal epithelial cancer. The most frequent subtype of RCC is clear cell (ccRCC). Most of ccRCC patients have a mutation in the Von Hippel-Lindau (VHL) tumor suppressor gene. The VHL gene encodes a protein, the VHL, which can up-regulate a series of intracellular proteins, including the hypoxia inducible factor (HIF). The transcription factor NF-κB is increased in the ccRCC. OBJECTIVES: To evaluate the impact of the NF-κB1 gene knockout on the VEGF and IL6 expression in the human RCC cells under normoxia and hypoxia. MATERIALS AND METHODS: The CRISPR/Cas-9 technique was used to obtain 786-0 cells knockout for the NF-κB1 protein. Western Blot assay was used to selected the clones. A hypoxia-inducing humid chamber was used and its effectiveness was validated its effectiveness was certified by the analysis of HIF-2α expression levels. The quantification of VEGF and IL-6 levels was measured using Real Time-PCR and MILLIPLEX assay. DISCUSSION AND RESULTS: The VEGF gene expression in the clones was significantly lower than that presented by the control both in normoxia (786-0-sg1 99.68±0.09%, 786-0-sg2 78.55±0.85%, 786-0-sg3 91.70±0.87%) and in hypoxia (786-0-sg1 98.30±1.49%, 786-0-sg2 75.21±4.14%, 786-0-sg3 98.44±0.18%). The expression of IL-6 gene was also significant lower in normoxia (786-0-sg1 49.03±0.80%, 786-0-sg2 76.59±12.43%, 786-0-sg3 66.98±10.89%) and in hypoxia (786-0-sg1 95.85±0.36%, 786-0-sg2 96.45±0.49%, 786-0-sg3 91.08±1.42%). The MILLIPLEX results show that there was a significant reduction of both VEGF and IL-6 in the culture medium of cells knocked out in normoxia and hypoxia compared to control group. CONCLUSION: Suppression of p50 expression in the clones resulted in the reduction of VEGF and IL6 in both conditions. The reduction in the IL-6 relative expression hypoxia/normoxia demonstrates a change in cellular responsiveness to decreased levels of oxygen.
Keywords: clear cell renal carcinoma (RCC), nf-κb1, hypoxia
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U.24 - Impact of Neuroinflammation on Epigenetic Transcriptional Control of SHH Pathway Members in the Central Nervous System

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INTRODUCTION: Sonic Hedgehog signaling plays a fundamental role in the development of the central nervous system, and alterations in this signaling can lead to neurological disorders that are often irreversible. Although well studied, little is known about its regulation of Shh signaling in the adult brain and its influence on diseases that affect humans. OBJECTIVES: In this study, we investigated the contribution of DNA methylation in the transcriptional control of members of the Hedgehog signaling pathway and the impact of neuroinflammation in the gene expression of DNA-modifying enzymes. MATERIALS AND METHODS: Through inducing a murine model of neuroinflammation by single-dose i.p. of LPS (0.33mg/kg) we determined the profile of gene expression and methylation of Hedgehog signaling pathway members by qPCR. DISCUSSION AND RESULTS: We were able to show that in the adult brain, the methylation of CpG-promoting regions of the members of the Hedgehog pathway was fundamental determining the differential transcription pattern observed between distinct brain regions. Another result was that neuroinflammation differentially modulates the gene expression of DNA-modifying enzymes, revealing the basal transcriptional profile of DNMTs and TETs-modifying enzymes in the central nervous system and demonstrating the effect of neuroinflammation on the transcriptional control of members of the Hedgehog pathway in the central nervous system in the adult brain. CONCLUSION: Altogether, our results support the hypothesis that epigenetic mechanisms, such as DNA methylation, might be involved in determining the endogenous expression pattern of Hedgehog pathway members, in the adult brain and that neuroinflammation modulates both gene expression of DNA-modifying enzymes and the Hedgehog pathway members, but only the reduction in Sufu gene expression was accompanied by increased DNA methylation levels of the promoter region.
Keywords: DNA methylation, Neuroepigenetics, Sonic Hedgehog / **Supported by:** Capes