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**Aim:** The study aimed to compare the differences in activity of PI3K-Akt and Ras-Raf-MAPK pathways, and changes in the Ras-Raf-MAPK activity after PI3K-Akt silencing, between different cell lines and tissue samples from primary tumour sites of human CRC.

**Introduction:** Alterations in EGFR-related Ras-Raf-MAPK and PI3K-Akt pathways are involved in the pathogenesis of up to 55% and 15% colorectal cancers (CRC) respectively. The Ras-Raf-MAPK pathway mutations are assessed before introducing a standard anti-EGFR treatment, as they indicate lack of response. However, the autonomic activity of alternative PI3K-Akt pathway may also have an impact on the effectiveness of targeted therapy.

**Methods:** The study was carried out on three ATCC human CRC cell lines derived from primary tumours (COLO320, SW480 and HT29) and ten patient tissue samples. Cell lines were cultured according to the protocol. Genomic DNA and RNA were isolated, PCR and RT-PCR were performed. Restriction enzymes were applied. Primers for the following fragments of genome were used: KRAS (exons 2, 3, 4), NRAS (exons 2, 3, 4), and BRAF exon 15 for PCR; KRAS, NRAS, BRAF, PIK3CA for RT-PCR. Proteins were extracted, purified and Western Blot was conducted. siRNA for Akt and specific PI3K inhibitors were used to silence PI3K-Akt activity.

**Results:** The analyzed material presented variable profiles of pathways activity. Interestingly, high expression of Ras protein was positively correlated with Akt protein level. In case of low level of Ras, Raf protein was dominating whereas Akt expression was significantly decreased.

**Conclusion:** Ras and Akt can simultaneously present a high level of expression. Thus, as PI3K-Akt is an alternative pathway to Ras-Raf-MAPK for EGFR signaling and its autonomic activity may affect the efficacy of anticancer treatment, it has a potential to be taken into consideration while planning a treatment and developing new anticancer agents.<sup>1,2</sup>

## References

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### The role of the hypoxic tumor microenvironment on the macrophage-tumor cell interplay

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**Aim:** The aim of this work is to unveil the role of the hypoxic microenvironment on macrophage-tumor cell interplay, using colorectal cancer (CRC) as a model.

**Introduction:** Microenvironment, in most cases hypoxic, is composed by cancer cells, extracellular matrix, stromal and immune cells, that cooperate and affect each other activities. Macrophages are one of the most abundant immune cells at the tumor microenvironment, acting as tumor suppressors or promoters. Previous research had shown that both hypoxia and immunosuppressive macrophages are associated with tumor progression. Nevertheless, these studies did not focus on the interplay between hypoxia and macrophage-cancer cell crosstalk.

**Methods:** To achieve our goal co-cultures of CRC cells and human macrophages, both in normoxia and hypoxia, were established. Macrophages were characterized functionally and phenotypically and their potential to induce cancer cell invasion was evaluated.

**Results:** Our results suggest that hypoxia, and the presence of cancer cells, decreases the cell surface expression of an anti-inflammatory marker (CD163), however the mRNA expression was not altered. Nevertheless, hypoxia induced an increase in the mRNA expression of the macrophage pro-inflammatory marker (CCR7).

Macrophages metabolic activity was not altered by hypoxia but decreased when co-cultured with cancer cells. In addition, lactate production decrease in co-culture while glucose consumption increased. Notably, macrophages in normoxia presented a more rounded morphology while in hypoxia are more elongated with evident cellular protrusions, suggesting dynamic alterations at the actin cytoskeleton organization. Interestingly, MMP-2 and MMP-9 activity profiles were not altered by the presence of cancer cells or hypoxia. Nevertheless, cancer cell invasion ability increased in the presence of macrophages, suggesting that other MMPs might be involved.

**Conclusion:** Findings in normoxia regarding macrophage potential to induce cancer cell invasion are consistent with those previously described by our group. Interestingly, we demonstrate now that hypoxia potentiates the invasive behavior of cancer cells and also macrophage pro-invasive ability.

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### Ethnopharmacological use of *Cymbopogon citratus* (DC.) Stapf and *Cymbopogon schoenanthus* (L.) Spreng.: Anti-inflammatory potential of phenol-rich extracts



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**Aim:** The aim of this work consisted on expanding the knowledge on the chemical composition of different extracts from *Cymbopogon* spp., and on the evaluation of their anti-inflammatory potential in cell and cell-free systems.