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# ANALYSIS OF EXPRESSION OF P-AMPK IN COLORECTAL CANCER SURVIVAL PATTERNS

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**Abstract:**

***Introduction:*** *Colorectal cancer (CRC) is the third most common tumour worldwide and represents the second leading cause of cancer death in Europe and the USA.* ***Aims and objectives:*** *The main objective of the study is to analyze the expression of P-AMPK in colorectal cancer survival patterns.* ***Material and methods:*** *This descriptive study was conducted in CMH, Rawalpindi during April 2018 to October 2018. This study was done for the analysis of expression of P-AMPK in colorectal cancer patients. The data was collected from 50 cancer patients. Expression of P-AMPK was measured with immunoblotting. For a complete list of the cell lines, antibodies, and reagents, see the supplemental material. Proteins were resolved using SDS-PAGE and transferred to nitrocellulose membranes, blocked in Odyssey blocking buffer (Li-Cor), hybridized with primary and secondary antibodies in Tris-buffered saline (TBS)–0.1% Tween 20, and detected using an Odyssey imaging system. Significance of the data was calculated using SPSS version 19.0.* ***Results:*** *The data was collected from 50 colorectal cancer patients. Phosphorylated AMPK expression was associated with p-MAPK3/1 expression (*P*<0.0001) and inversely with high tumour grade (*P*=0.0009), MSI-high (*P*=0.0021) and CIMP-high (*P*<0.0001). In 16 of 20 sections, tumour centre and tumour invasive front showed concordant expression status, indicating that p-AMPK expressions in tumour centre and invasive front were not different in most cases.* ***Conclusion:*** *It is concluded that AMPK activation is associated with good prognosis among MAPK3/1-activated colorectal cancer patients, while AMPK activation is not associated with prognosis among MAPK3/1-inactive cancer patients.*

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## INTRODUCTION:

Colorectal cancer (CRC) is the third most common tumour worldwide and represents the second leading cause of cancer death in Europe and the USA. Approximately 20% of CRC patients will present distant metastasis (stage IV) at the time of diagnosis with 5-year survival <10%. Management of metastatic CRC (mCRC) has substantially evolved over the past decade with the introduction of new chemotherapy combinations and biological agents [1]. The US Food and Drug Administration approved the anti-vascular endothelial growth factor (VEGF) antibody bevacizumab for first-line treatment of patients with mCRC in 2004.

Since its approval, bevacizumab in combination with chemotherapy has become the standard first-line treatment option in mCRC, extending both progression-free survival (PFS) and overall survival [2]. Despite clinical use of bevacizumab for nearly a decade, however, important questions still remain, including the prediction and management of potential adverse effects and selection of the ideal patients' population. In this regard, predictive biomarkers of response are still not available, although a number of potential systemic, circulating, tissue and imaging biomarkers have emerged from recently completed clinical trials [3].

AMP-activated protein is a heterotrimeric serine/threonine protein kinase, which acts as a cellular sensor for energy balance status. AMP-activated protein kinase is phosphorylated by its upstream kinase STK11 (LKB1) in response to an increase in cellular AMP/ATP ratio [4]. A third of all human cancers, including a substantial percentage of colorectal, lung, and pancreatic cancers, are driven by activating mutations in Ras genes. Activating K-Ras mutations are present in 35 to 40% of colon tumors and are thought to be both drivers of tumorigenesis and determinants of therapeutic regimens [5]. Therapeutic disruption of Ras function has been clinically ineffective to date, but investigation of Ras pleiotropy continues to yield a diversity of downstream effectors with obligate roles in the maintenance and adaptation of Ras-driven tumors to changing environments. The Raf–MEK–extracellular signal-regulated kinase (ERK) signaling pathway is essential for the oncogenic properties of mutated K-Ras [6].

### Aims and objectives

The main objective of the study is to analyze the expression of P-AMPK in colorectal cancer survival patterns

## MATERIAL AND METHODS:

This descriptive study was conducted in CMH, Rawalpindi during April 2018 to October 2018. This study was done for the analysis of expression of P-AMPK in colorectal cancer patients. The data was collected from 50 cancer patients. Expression of P-AMPK was measured with immunoblotting. For a complete list of the cell lines, antibodies, and reagents, see the supplemental material. Proteins were resolved using SDS-PAGE and transferred to nitrocellulose membranes, blocked in Odyssey blocking buffer (Li-Cor), hybridized with primary and secondary antibodies in Tris-buffered saline (TBS)–0.1% Tween 20, and detected using an Odyssey imaging system. Significance of the data was calculated using SPSS version 19.0.

## RESULTS:

The data was collected from 50 colorectal cancer patients. Phosphorylated AMPK expression was associated with p-MAPK3/1 expression ($P<0.0001$) and inversely with high tumour grade ($P=0.0009$), MSI-high ($P=0.0021$) and CIMP-high ($P<0.0001$). In 16 of 20 sections, tumour centre and tumour invasive front showed concordant expression status, indicating that p-AMPK expressions in tumour centre and invasive front were not different in most cases. Furthermore, whole tissue section-based expression status and TMA-based expression status were concordant in 18 of 20 cases, indicating that expression status determined using TMA represented expression status of tumour as a whole in a vast majority of cases.

**Table 01:** P-AMPK expression in colorectal cancer patients

| **Clinical, pathologic** | **p-AMPK expression** | | |
|---|---|---|---|
| | **Negative** | **Positive** | **P-value** |
| BMI | | | 0.69 |
| <30 kg $m^{-2}$ | 254 (82%) | 340 (83%) | |
| ⩾30 kg $m^{-2}$ | 55 (18%) | 68 (17%) | |
| Family history: (−) | 236 (76%) | 318 (78%) | 0.66 |
| (+) | 73 (24%) | 91 (22%) | |
| Tumour Location: Proximal colon (cecum to transverse) | 155 (51%) | 192 (48%) | 0.61 |
| Distal colon (splenic flexure to sigmoid) | 89 (29%) | 131 (32%) | |
| Rectum | 61 (20%) | 79 (20%) | |
| Stage: I | 55 (18%) | 105 (26%) | 0.15 |
| II | 100 (32%) | 114 (28%) | |
| III | 91 (29%) | 113 (28%) | |
| IV | 45 (15%) | 56 (14%) | |
| Unknown | 18 (5.8%) | 21 (5.1%) | |
| Grade: Low | 269 (88%) | 386 (95%) | 0.0008 |
| High | 38 (12%) | 22 (5.4%) | |
| | | | |
| Tumour border: Expansile | 237 (86%) | 306 (85%) | 0.79 |
| Infiltrative | 38 (14%) | 52 (15%) | |
| (−) | 227 (80%) | 242 (63%) | <0.0001 |
| (+) | 57 (20%) | 145 (37%) | |
| 0.055 | | | |
| TP53 expression : (−) | 194 (63%) | 229 (56%) | 0.054 |
| (+) | 112 (37%) | 178 (44%) | |
| FASN expression: (−) | 267 (88%) | 330 (81%) | 0.021 |
| (+) | 38 (12%) | 76 (19%) | |

## DISCUSSION:

In human colon tumor cells dependent upon oncogenic Ras, KSR1 is required for anchorage-independent proliferation, survival, and in vivo growth. In nontransformed human colon epithelial cells (HCECs), KSR1 is dispensable for survival, consistent with the observation that KSR1 is not required for normal development [7]. Transient depletion of KSR1 results in about 30% cell death in Ras-driven cancer cells. Additionally, cells that survive KSR1 knockdown lose their ability to form anchorage-independent colonies in soft agar. These in vivo and in vitro analyses demonstrate that tumor-specific pathways are activated by oncogenic Ras in a KSR1-dependent manner [8].

Considering experimental data on the link between the STK11 (LKB1)-AMPK and MAPK3/1 pathways, the modifying effect of MAPK3/1 on AMPK may not be surprising. In colon cancer cells, AMPK potentially inhibits the MAPK3/1 pathway; inhibition of AMPK by expressing a dominant-negative form potentiates MAPK3/1 activation under glucose deprivation [9]. Selenium, an essential trace element, blocks the carcinogenic agent-induced MAPK3/1 activation via AMPK [6]. A study using melanoma cell has shown that the MAPK3/1 pathway phosphorylates STK11 on Ser325 and Ser428 and promotes the uncoupling of AMPK from STK11, which negatively regulates AMPK. Regulation of AMPK activity by the MAPK3/1 pathway, independent of STK11 Ser428 phosphorylation, has also been reported [10].

## CONCLUSION:

It is concluded that AMPK activation is associated with good prognosis among MAPK3/1-activated colorectal cancer patients, while AMPK activation is not associated with prognosis among MAPK3/1-inactive cancer patients.

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