

The Role of AMPK in Metabolic Changes in Pancreatic Cancer Cells through High Protein Diet

Kiana Khazaei

Biotechnology Program, Institute of Parasitology, McGill University, Saine-Anne-de-Bellevue, Quebec H9X 3V9, Canada

Abstract: The present study investigates the contribution of AMPK response and mTOR (mammalian target of rapamycin) pathways through high protein diet to the pancreas cancer susceptibility. The hypothalamic modulation of the AMPK/ACC and mTOR signaling pathways induce by HPD and are followed by assaying the tumorigenicity. The results demonstrate that AMPK and mTOR cooperated in the hypothalamus during HPD and resulted in reducing AMPK activity, and increasing mTOR activity.

Key words: AMPK, pancreas cancer, HPD, tumor suppressor, tumor promoter.

Abbreviations

AMPK: AMP-activated protein kinase
HPD: high-protein diets
ATP: adenosine triphosphate
AMP: adenosine monophosphate
CaMKK β : Ca⁺²/calmodulin-dependent protein kinase β
TAK1: TGF- β -activated kinase-1
miRNAs: microRNAs
HIF1a: hypoxia-inducible factor 1-alpha
ACC: acetyl-CoA carboxylase
PAGE: polyacrylamide gel electrophoresis

1. Introduction

AMPK (AMP-activated protein kinase) plays a vital role in homeostatic control of cellular energy balance [1]. AMPK, a serine/threonine protein kinase which is a principle metabolic sensor for metabolic systems and signaling networks [2], belongs to the yeast SNF1 (Sucrose Non-Fermenting 1) subfamily [1]. AMPK consists of several sectors such as regulatory and catalytic subunits which can serve as mediator to moderate the functions of Serine/threonine kinase 11 (STK11) which is also known as liver kinase B1 LKB1. The mediation would take place under the close interaction between energy regulators and cancer pathogenesis. The presence of variety of subunits in

AMPK implies the particularity of the tissues which can further contribute to growth of tumor cell and its independent reproduction [3].

During metabolic stress situations, such as hypoxia and glucose deprivation, any decrement in ATP (adenosine triphosphate) and increment in AMP (adenosine monophosphate) results in energy homeostasis restoration by AMPK at cell and organismal levels. Furthermore, AMPK can also restore energy by acting as an energy sensor under certain circumstances such as muscle contraction that ATP consumption is accelerated and ADP (adenosine diphosphate) and AMP level are improved [2]. Another function of AMPK is to retain the energy balance of the cell. This goal can be achieved by supplying anabolic pathways (like lipid biosynthesis) and activating catabolic pathways (like autophagy) both of which are performed directly by phosphorylation of metabolic enzymes or/and within phosphorylation of transcription factors and coactivators [2]. There have been recently numerous studies on AMPK activity and its relationship with stress resistance and survival in tumor cells. Thus, AMPK has been linked to the regulation of tumorigenesis. AMPK includes three upstream kinases namely the tumor suppressor LKB1, CaMKK β (Ca²⁺/calmodulin-dependent protein kinase β) and

TAK1 (TGF- β -activated kinase-1) [3]. Regardless of the energy status of the cells, AMPK is stimulated during energy stress formed by LKB1 but CaMKK2 activate by increased amount of intercellular Ca^{2+} [3]. The activation of AMPK is function of its phosphorylation of upstream kinases and dephosphorylation of phosphatases. The tumor suppressor LKB1 which is located in upstream, and other tumor suppressors such as TSC2 and p53 which are located downstream of AMPK, regulate its function [4]. Hence, AMPK can potentially be considered as a tumor suppressor due to proximity to the established aforementioned tumor suppressors. AMPK has vital roles in several tumor types and can act as pro-tumorigenic or anti-tumorigenic in cancerous tumors [3] (Fig. 1). It can also influence the cell growth and proliferation by cooperating with oncogenic drivers to reprogram tumor cell metabolism. The other advantage of AMPK activation is regulating cellular metabolic plasticity which enables tumor cells by the flexibility to adapt to metabolic stress.

Regardless of genomic modifications, the AMPK regulation mechanism can also affect its activity level in the cancer [6]. For instance, microRNAs (miRNAs) are a possible mechanism to regulate AMPK. miRNAs can promote tumor growth. miRNAs that target LKB1-AMPK signaling may promote dynamic transcriptional/translational regulation of this energy-sensitive pathway independent of genomic alterations [5].

1.1 AMPK as Tumor Suppressor

The fundamental mechanism for AMPK to act as suppressor reveals under the ability of the kinase to exert an “anti-Warburg” effect by downregulating hypoxia-inducible factor 1-alpha (HIF1a) and its downstream glycolytic genes [2]. Additionally, inhibition of unchecked mTORC1 activity and de novo lipogenesis, results in applying metabolic tumor-suppressor role of the AMPK. It is of worth mentioning that both mTORC1 activity and de novo

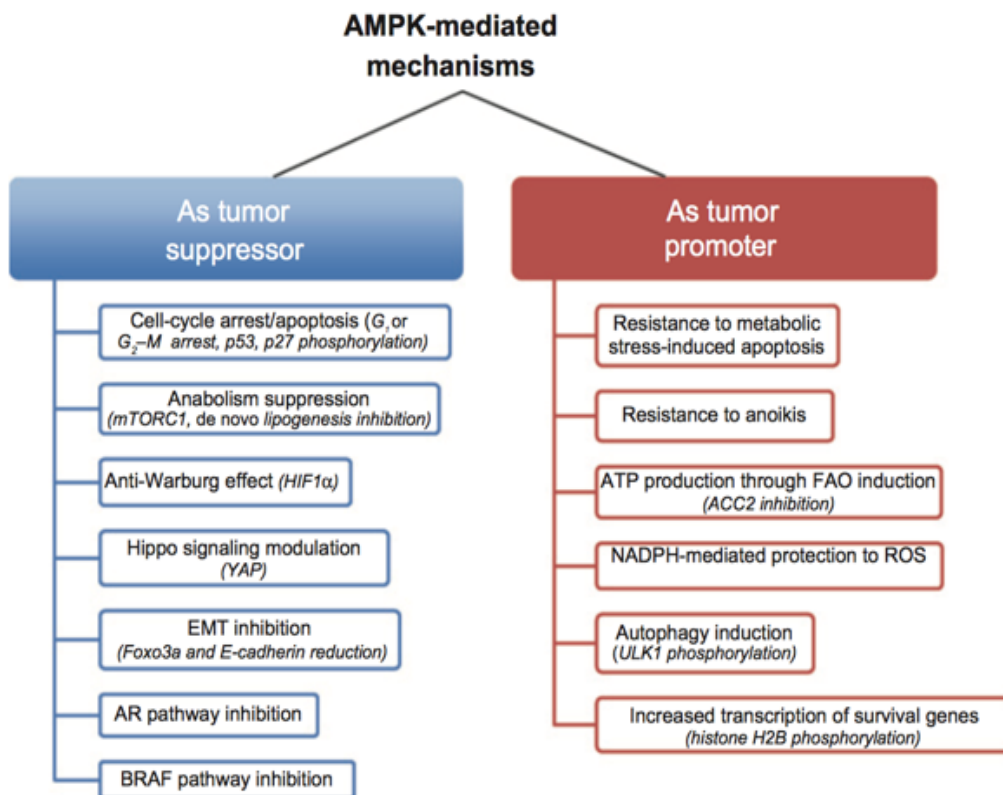


Fig. 1 The dual role of AMPK on cancer incidence [3].

lipogenesis are required during G1-S and G2-M phases [3]. It is proved that there is an inverse relationship between de novo FA synthesis concomitant and phosphorylation of its major target ACC1, prior to cytokinesis initiation. Therefore, inhibition of de novo FA synthesis and FA incorporation into membranes would prevent cells from completing mitosis and results in arresting them at a “lipogenic”G2-M checkpoint under direct supra-physiological activation of AMPK. It is of importance to indicate the role of direct metabolic-independent in cell-cycle regulation.

1.2 AMPK as Contextual Tumor Promoter

Although LKB1/AMPK pathway can act as a tumor suppressor due to its ability to restrain tumor growth, it can also behave as “tumor promoter”, allowing tumor cells to be more resistant to metabolic stress [6]. A bright example of such case is when tumor growth exceeds the capacity of its blood supply to deliver oxygen and nutrients.

1.3 High Protein Diet

The effects of HPDs (high-protein diets) on energy expenditure have dragged a great deal of attention within the past few decades. The weight of evidence is an indicator that proves high-protein meals potentially result in reduced subsequent energy intake. Additionally, HPD can impact AMPK as long as it acts as a sensor of cellular energy charge. In fact, AMPK phosphorylates ACC (acetyl-CoA carboxylase) switches on energy-producing pathways at the expense of energy-depleting processes [7]. The mTOR (mammalian target of rapamycin) catalytic activity is another target molecule for the control of energy homeostasis. mTOR has been suggested to be influenced by the phosphatidylinositol 3-kinase/akt pathway [8]. mTOR signaling is inhibited under conditions of low nutrients, such as glucose and amino acids, and low intracellular ATP levels. Due to the roles of AMPK and mTOR pathway in cancer, and

also the effect of high protein diet on both, we sought to determine whether the response of the AMPK and mTOR pathways in high protein diet could contribute to cancer.

Researchers studied over 6,000 people aged 50 and older for 18 years and they figured out that people who had high protein diet rich in animal proteins during middle age were more than four times as likely to die of cancer versus those who had low protein diet. Hence, a population of mice was considered to find a link between protein consumption and cancer. For this purpose, melanoma cancer cells were injected to mice [9]. Levine et al. [9] claimed that the level of IGF-1, a growth hormone, was increased and tumors survived in mice which fed by high level of proteins diet. Researches claimed that increasing amount of IGF-1 may be a reason that high protein diet helps normal cells become cancer-like cells [9]. This study is trying to find another probability to find a reason for this phenomenon. Therefore, the link between amount of AMPK in HPD and cancer is considered in this study.

Besides, we undertook the present study to further evaluate the hypothesis that the likelihood of cancer incidence could be enhanced by using high-protein diet which results in decreasing AMPK and increasing mTOR activity. Due to the role of pancreas in digestion of proteins by the pancreatic enzymes trypsin, chymotrypsin, and carboxypeptidase, pancreas cancer is considered as the main target of this research.

2. Methodology

2.1 Protein Intake and Cancer in Mice

To recognize the mechanism that links high protein diet to cancer, effects of a variety of protein intake are considered. The 30 female C57BL/6 mice (8-12 weeks old) are fed continuously for 39 days with different levels of protein diets. Ten mice are fed with high protein level diet, 10 mice with low protein level, and the last 10 mice are not feed with protein diet. To investigate the effect of different protein levels on

survival and develop of newly formed tumor, all mice are shaved and injected in the right flank with 200 μ L single cell suspension containing 1.0×10^6 panc-02 cells [10]. The panc-02 cells are injected subcutaneously in the lower back of the mice. Tumor incidence is determined by palpation of the injected area and tumor size, and measured using a digital vernier caliper two weeks after tumor implantation, at which point the mean tumor diameter was approximately 5 mm.

2.2 Protein Intake and AMPK and mTOR

2.2.1 Western Blot Analysis

We use rabbit to produce antibodies against AMPK alpha1. AMPK alpha1 antibody is against the peptide based on the amino acid sequence for the residues 346-360 (TSPDSDLDDHLTR) [2]. In cysteine residue at the N-terminus, the peptide produces and joins to keyhole limpet hemocyanin. In order to make rabbits immune, a polypeptide including complete sequence of AMPK alpha2 cDNA is expressed as a His-tagged protein, purified by Ni-column chromatography and PAGE (polyacrylamide gel electrophoresis). For western blotting, polyclonal anti-AMPK alpha1 and alpha2 antisera are collected. The mTOR antibodies are purchased from Thermo Fisher. The western blot analysis in the hypothalamic tissue of 30 mice is assayed according to the Johns Hopkins University procedure [11].

2.2.2 Confocal Microscopy

A double-staining confocal microscopy is served to determine co-expression of AMPK with mTOR. Paraformaldehyde-fixed hypothalamic sectioned (5 μ m) and used in regular single- or double-immunofluorescence staining anti-AMPK, anti-mTOR, and anti-phospho-ACC antibodies [8]. The confocal microscopy is performed according to a previously described protocol [12]. An LSM 510 laser confocal microscope is used for further analysis and photo documentation of results. Also, the anatomical correlation is made according to the landmarks given

in a stereotaxic atlas [13].

3. Results and Discussion

According to the research conducted by Roppelle et al. [8] to identify the role of AMPK and mTOR in HPD in weight lost, it is expected that hypothalamic ATP level increases and AMP-to-ATP ratio decreases in HPD. Furthermore, their results demonstrated that AMPK and mTOR cooperate in the hypothalamus during HPD and resulted in reducing AMPK activity, and increasing mTOR activity. On the other hand, Levin et al. [9] conclude that high protein intake can increase the likelihood of cancer incidence. Their findings support the hypothesis of this work which tries to correlate the effect of high protein diet and likelihood of cancer incidence by reducing amount of AMPK. It is of worth mentioning that the AMPK is not only a sensor of cellular energy, but also acts as a tumor suppressor. In fact, reduce in amount of LKB1 and AMPK in tumor cells can potentially improve mTOR activity by increasing HIF1 α -driven glucose and glutamine metabolism and bypassing metabolic checkpoints which control cell growth in low nutrient conditions. In previous studies B16 melanoma was used for research as a model [9]. However, in the present study, PAN 02 is used to investigate on whether there is an association between HPD and pancreatic cancer. This was mainly due to the relevance of pancreas function to protein digestion and high protein diet. It is expected that amount of AMPK decreases and mTOR activity increases for the mice which used to have high protein diet.

4. Conclusion

AMPK has a complex role in cancer and its positive or negative role depends on the degree and/or mechanism of AMPK activation, the particular expression of AMPK isoforms, AMPK subcellular localization, the activity of other signaling networks in the cell, and extracellular environmental conditions. More studies and genetic manipulations are required

to find out the particular role of AMPK in cancer.

Acknowledgment

The author would like to thank Dr. Edris Madadian for sharing his pearls of wisdom during the course of this research, and for comments that greatly improved the manuscript.

References

- [1] Hawley, S. A., Davison, M., Woods, A., Davies, S. P., Beri, R. K., Carling, D., and Hardie, D. G. 1966. "Characterization of the AMP-Activated Protein Kinase from Rat Liver and Identification of Threonine 172 as the Major Site at Which It Phosphorylates AMP-activated Protein Kinase." *Journal of Biological Chemistry* 271 (44): 27879-87.
- [2] Kato, K., Ogura, T., Kishimoto, A., Minegishi, Y., Nakajima, N., Miyazaki, M., and Esumi, H. 2002. "Critical Roles of AMP-Activated Protein Kinase in Constitutive Tolerance of Cancer Cells to Nutrient Deprivation and Tumor Formation." *Oncogene* 21 (39): 6082.
- [3] Zadra, G., Batista, J. L., and Loda, M. 2015. "Dissecting the Dual Role of AMPK in Cancer: From Experimental to Human Studies." *Molecular Cancer Research* 13 (7): 1059-72.
- [4] Jones, R. G., Plas, D.R., Kubek, S., Buzzai, M., Mu, J., Xu, Y., Birnbaum, M.J., and Thompson, C. B. 2005. "AMP-Activated Protein Kinase Induces a p53-dependent Metabolic Checkpoint." *Molecular Cell* 18 (3): 283-93.
- [5] Faubert, B., Vincent, E. E., Poffenberger, M. C., and Jones, R. G. 2015. "The AMP-activated Protein Kinase (AMPK) and Cancer: Many Faces of a Metabolic Regulator." *Cancer Letters* 356 (2): 165-70.
- [6] Zhao, G., Zhang, J.-G., Liu, Y., Qin, Q., Wang, B., Tian, K., Liu, L., Li, X., Niu, Y., and Deng, S.-C. 2013. "Mir-148b Functions as a Tumor Suppressor in Pancreatic Cancer by Targeting AMPK α 1." *Molecular Cancer Therapeutics* 12 (1): 83-93.
- [7] Kahn, B. B., Alquier, T., Carling, D., and Hardie, D. G. 2005. "AMP-Activated Protein Kinase: Ancient Energy Gauge Provides Clues to Modern Understanding of Metabolism." *Cell Metabolism* 1 (1): 15-25.
- [8] Ropelle, E. R., Pauli, J. R., Fernandes, M. F. A., Rocco, S. A., Marin, R. M., Morari, J., Souza, K. K., Dias, M. M., Gomes-Marcondes, M. C., and Gontijo, J. A. 2008. "A Central Role for Neuronal AMP-Activated Protein Kinase (AMPK) and Mammalian Target of Rapamycin (mTOR) in High-protein Diet-Induced Weight Loss." *Diabetes* 57 (3): 594-605.
- [9] Levine, M. E., Suarez, J. A., Brandhorst, S., Balasubramanian, P., Cheng, C.-W., Madia, F., Fontana, L., Mirisola, M. G., Guevara-Aguirre, J., and Wan, J. 2014. "Low Protein Intake Is Associated with a Major Reduction in IGF-1, Cancer, and Overall Mortality in the 65 and Younger but Not Older Population." *Cell Metabolism* 19 (3): 407-17.
- [10] Kashiwagi, H., McDunn, J. E., Simon, P. O., Goedegebuure, P. S., Vangveravong, S., Chang, K., Hotchkiss, R.S., Mach, R.H., and Hawkins, W.G. 2009. "Sigma-2 Receptor Ligands Potentiate Conventional Chemotherapies and Improve Survival in Models of Pancreatic Adenocarcinoma." *Journal of Translational Medicine* 7 (1): 24.
- [11] Quintero, M., Colombo, S. L., Godfrey, A., and Moncada, S. 2006. "Mitochondria as Signaling Organelles in the Vascular Endothelium." *Proceedings of the National Academy of Sciences* 103 (14): 5379-84.
- [12] Araujo, E. P., Amaral, M. E., Souza, C. T., Bordin, S., Ferreira, F., Saad, M. J., Boschero, A. C., Magalhães, E.C., and Velloso, L.C.A. 2002. "Blockade of IRS1 in Isolated Rat Pancreatic Islets Improves Glucose-Induced Insulin Secretion." *FEBS Letters* 531 (3): 437-42.
- [13] Mai, J., Assheuer, J., and Paxinos, G. 1997. *Atlas of the Human Brain*. New York: Academic Press San Diego.