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Deciphering the Role of AMPK in Regulating Integrin Profile of Tumor-Derived Exosomes as a Potential Antimetastatic Strategy

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Abstract

Introduction: Cancer cell adapt their metabolic activity to survive in stressful environments with limited nutrients and oxygen. To sense and respond to nutrient cues, cells rely on a "fuel gauge" protein known as AMP-activated protein kinase (AMPK). While previous work has largely focused on AMPK's role in cell metabolism, its role in metastasis is poorly defined. Interestingly, AMPK also regulates the membrane trafficking of integrins – key proteins in cell adhesion and migration. Recent studies have also shown that circulating integrins in exosomes are predictive of metastasis. Therefore, understanding the biology of AMPK signaling on the exosomal integrin profile not only allow for better cancer treatments but also help developing a potential predictive biomarker. I hypothesize that AMPK activation alters the integrin profile of exosomes in breast and endometrial cancer.

Methods: Breast and endometrial cancer cells, MDA-MB-231 and HEC-1 respectively, will be cultured in full nutrient media conditions. 48 hours before exosome harvest, the media will be changed to minimal specialized media for extracellular vesicles (EV) collection. To induce AMPK activation, cells will be treated with limiting concentrations of glucose and glutamine, or with metabolic poisons 2-deoxyglucose (2-DG) and oligomycin. Total Exosome IsolationTM (TEI) solution will be used to enrich released exosomes that will be subjected to mass spectrometry to analyze the exosomal integrin profiles.

Anticipated Results: Exosomes secreted from MDA-MB-231 and HEC-1 cells are expected to contain different exosomal integrin profile in both basal and induced AMPK activation conditions. It is anticipated that integrin subunits that promote metastatic and survival signaling such as $\alpha_v\beta_5$, $\alpha_6\beta_4$, $\alpha_6\beta_1$, will be upregulated in exosomes following AMPK activation. This could suggest tumor cells adapting to nutrient insufficiency by AMPK activation may present a more aggressive exosomal integrin profile to support cancer progression.

Discussion: Understanding how metabolic cues such as nutrient insufficiency impact integrin content of tumour-derived exosomes can help identify novel metabolic drug targets to limit pro-survival and pro-metastatic signaling in primary breast tumors.

Conclusion: This study investigates how AMPK activation modulates the exosomal integrin profile in breast and endometrial cancer cells, potentially uncovering predictive biomarkers and therapeutic targets for metastatic cancers.

Keywords: exosomes; integrin; AMPK; cancer; breast; endometrial

Introduction

Cancer cells require a persistent supply of energy to fuel rapid proliferation rates and indefinite cell growth [1]. This useable energy is majority synthesized at the mitochondria (oxidative phosphorylation sites) in the form of adenosine triphosphate (ATP) and is heavily dependent on the catabolic breakdown of glucose, lipids and oxygen in the extracellular environment [2]. Typically, ATP is broken down to ADP and could be further converted to AMP. Under energy dysregulation, AMP-Activated Protein Kinase (AMPK) works as a critical regulatory complex that modulate metabolism according to nutrient availability [3].

Metabolic Stress and The Role of AMPK

Metabolic stress is when the rate of ATP consumption becomes higher than ATP synthesis in cells, resulting in high AMP:ADP ratios as well as ADP:ATP ratios [4] that activates AMPK. AMPK is also known to be activated separately by low cytosolic glucose concentration and glycolysis intermediates as well as low oxygen availability [5]. Activation of AMPK either by key metabolic nucleotides or upstream kinases namely Calcium–calmodulin (CaM)-dependent protein kinase II (CamKII) [6] leads to variety of processes that upregulate energy producing pathways like increased expression of GLUT4 transporters and downregulate energy demanding processes

like cell division and migration [7]. Overall, AMPK acts as a "fuel gauge" for sensing even subtle changes in metabolic activity of cells and remains of great interest in studying tumor microenvironments.

Tumor Microenvironments

Tumours often have unconventional vascularization such that some cancer cells within the tumour are close to blood vessels and receive sufficient oxygen and critical nutrients to generate ATP and survive while some other cancer cells within the tumour remain far away from vascularization, hence are constantly under metabolic stress [8]. Despite experiencing constant nutrient and oxygen insufficiencies, cancer cells can adapt their metabolic activity to survive and proliferate and even migrate to other locations in the body. It may be possible that cancer cells regulate the activity of AMPK differently to thrive in low-nutrient environments.

AMPK Regulation of Cell Surface Integrin

It has already been established that AMPK can regulate the abundance of the cell surface proteome through clathrin-mediated endocytosis (CME) to enhance energy production in cells and inhibit energy demanding process; a prime example being the glucose transporter, GLUT4. In 2015, *Ross et. al* showed that the activation of AMPK through allosteric activators, low glucose and oxygen insufficiencies, decreases the cell surface abundance of a key cell adhesion protein called integrin [9]. How AMPK regulates the abundance of integrin is poorly understood and so is the fate of integrins after their upregulated internalization. Upon internalization, these integrins might be entering a recycling pathway by fusing with a lysosome, being turned over and placed at a different location on the membrane or are fusing with a multi-vesicular body and are being exported out of the cell via exosomes.

Integrins and Their Diverse Roles in Cell Migration, Signalling and Growth

Integrins are heterodimeric trans-membrane receptors consisting of α and β subunits [10] and are best recognized as being cell adhesion and cell migration proteins, although they have other roles in cells. Different combinations of the many α and β subunit types of integrins gives rise to their unique properties. For example, some integrins promote cell adherence and quiescence (α1β1 and α2β1), while others, foster the breakdown of cell adhesions and remodeling of the extracellular matrix (α5β1 and αvβ3) [11]. Having diverse roles in cell survival, integrins remain to be greatly studied in the world of cancer cell proliferation and growth. Integrins are also key biomarkers of exosomes [12], extracellular vesicles (EV) released from the cell surface and can be transported to nearby or distant cells.

Exosomes and Their Integrin Content

Exosomes are the smallest type of EV (ranging from 30-150 nm in diameter) that are actively released by cells into the extracellular environment and thus readily available in bodily fluids such as blood. Despite being of great interest now, exosomes were previously best known for maintaining cellular waste and debris [13]. Enriched by numerous protein receptors, mRNAs, microRNAs and lipids, current literature highlights their ability to cause reprograming in nearby or distant cells and act as the central intracellular communication mediators [14]. Exosomes of different parental cell origin harbours diverse sets of proteins and lipids made them a specific biomarker for cancer prognosis and diagnosis. Recent research in the field reveales that tumor-derived exosomes determined future metastatic sites within the body mainly through differential integrin expression and intracellular transport [15]. Recent studies also suggests that the type and stage of various cancers, namely breast and ovarian cancer can be distinguished by assessing the integrin profile of the tumor-derived exosomes [16]. Although this offers great advantages in cancer prognosis, the underlying mechanism for the selective expression and assortment of integrins in exosomes is poorly understood.

The **objective** of this study is to determine the role of AMPK signaling on the integrin content of tumor-derived exosomes. AMPK activation has previously shown [9] to increase integrin internalization through CME, and we hypothesize that the endocytosed integrins are being packaged in exosomes to leave the cells and travel to different sites in the body to induce pro-metastatic signals. By inducing AMPK activation in breast and endometrial cancer cell lines followed by exosome harvest, we will profile the diverse integrin expression in these exosomes. This will allow us to understand the impact of AMPK on this preferential integrin selection and intracellular transportation through exosomes. Understanding the relationship of AMPK signalling and integrin transport will guide the development of therapeutic drugs inhibiting pro-metastatic signal distribution in disease progression.

Methods

Cell Culture

The triple-negative breast and endometrial cancer cell lines; MDA-MB-231 [17] and HEC-1 [18] respectively, and retinal pigment epithelium (RPE, non-cancerous control) cells will be cultured in full nutrient media conditions (Dulbecco's Modified Eagle Medium (DMEM) with fetal bovine serum (FBS), and pencillin/streptomycin (P/S)). The flask will be passaged at 90% confluency; the media will be aspirated, and cells washed with phosphate buffered saline (PBS). Cells were maintained in a humidified environment at 37°C under 5% CO_2.

AMPK Activation

Direct AMPK Activation will be induced 24 hours before exosome harvest by addition of 100 μM allosteric activator A-769662 [20]. Indirect AMPK activation with be induced 24 hours before exosome harvest by addition of

5 µM Oligomycin [21], 5 mM 2-Deoxyglucose [22]. The extent of AMPK Activation and the corresponding exosomal integrin profile will be assessed by introducing low-to-high concentrations of glucose (0 mM, 5 mM, 20 mM and 50 mM to cells [23]. Low and high glutamine concentrations will also be tested (0 mM, 2 mM, 4 mM, and 6 mM) [24].

Extracellular Vesicle Enrichment and Protein Extraction

Cells will be first seeded in regular medium, then 48 hours before exosome harvest, the media will be changed to minimal specialized media for EV collection consisting of EV-depleted FBS and P/S [19]. AMPK activation will be performed as previously described in section 2.2. EVs from cell-conditioned media will be centrifuged at 500 × g for 5 min, then at 2000 × g for 30 min, then incubated for 24 hours with Total Exosome IsolationTM consisting of a 1:1 volume of 2X PEG solution (16% (w/v) polyethylene glycol, 1 M NaCl). Thereafter, the sample will be centrifuged at 3000 × g for 60 min and pellets will be resuspended in PBS for an ultracentrifugation purification step (100,000 × g for 70 min). Final pellets will be lysed in lysis buffer (10% SDS, 120 mM Tris-HCl pH 6.8, 2.5% β-mercaptoethanol, and protease inhibitor) for complete breakdown of exosomal membrane. Snap-freeze will be performed to prepare the sample for mass spectrometry.

Data Processing and Analysis

Experiments will be carried out in three biological replicates and data is represented as mean ± standard error of mean (SEM). Spectral counts of alpha and beta integrin subunits secreted into extracellular vesicles (EVs) will be quantified. Comparison of spectral counts of EV-derived alpha and beta integrin subunits will be analyzed in AMPK activated and un-activated conditions across all the cell lines. A t-test will be performed when comparing stressed and unstressed cell exosomal integrin counts and a one-way ANOVA test followed by a post-hoc Tukey test will be performed to determine significance of data across the three different cell lines in metabolic stress conditions. A p value < 0.05 is considered statistically significant.

Results

Exosomes secreted from MDA-MB-231, HEC-1 and RPE cells (non-cancerous control) with induced AMPK activation are expected to contain an altered integrin profile compared to full nutrient conditions.

It is expected that spectral counts of the α and β subunits of integrins will be vary based on the cancer cell model used and with the activation of AMPK. The control cell model (non-cancerous) RPE, is likely to only exhibit common EV-derived integrins namely $\alpha_1\beta_1$, $\alpha_2\beta_1$, and $\alpha_3\beta_1$ in both basal and stressed conditions. Cancer cell lines (MDA-MB-231 and HEC-1) Under basal conditions will likely exhibit integrin content significantly different from RPEs such that HEC-1 may have a higher presence of the $\alpha_5\beta_5$ isoform [26] and the MDA-MB-231 cell line might have exosomes enriched in $\alpha_6\beta_4$ and $\alpha_6\beta_1$ isoforms.

Additionally, it is likely to observe that the spectral counts of EV-derived integrin isoforms for the cancerous cell models will vary significantly ($p<0.05$; in a t-test) in stressed versus unstressed cellular conditions whether based on type of isoform or the sum of isoform type found. This varying integrin portfolio is expected to be AMPK dependent.

Discussion

To interpret these results comprehensively, it will be crucial to delve into the specific integrin isoforms and subunits that contribute significantly to the observed variations. Correlating these findings with existing knowledge about integrin function and cancer biology could unveil novel mechanisms by which AMPK activation influences exosome-derived integrins under stress. The spectral counts of each isoform could inform a variety of aspects about the cancer cell; namely whether the cell is in a motile stage, experiencing quiescence, and sending pro-metastatic signals. The use of a t-test or ANOVA to establish significance ($p<0.05$) adds a statistical rigor to the analysis, suggesting that the stress-induced conditions have a discernible impact on the integrin composition of EVs. The identification of specific integrin isoforms that exhibit such variation could provide valuable insights into the adaptive responses of cancer cells to stress.

Currently, the integrin isoform $\alpha_5\beta_5$ has been revealed to be upregulated in endometrial cancer cell model (e.g. HEC-1) [26]. This isoform has also been associated with being a liver-specific metastasis. If AMPK activation enhances the abundance of the $\alpha_5\beta_5$ isoform, or alter it, this may lead to unveiling the role of nutrient insufficiency in deciphering the metastasis potential of a cancer cell. Similarly, presence of $\alpha_6\beta_4$ and $\alpha_6\beta_1$ integrins in breast cancer cell models has been reported to be involved in promoting lung metastasis [26, 27]. AMPK activation may have a role in this physiological response in terms of nutrient stress in tumor microenvironments.

Finally, the expectation of different spectral count top hits across various cell lines during AMPK activation adds complexity to the findings. This suggests that the activation of AMPK might modulate integrin expression in a cancer cell line-specific manner, emphasizing the need for a tailored approach when investigating the interplay between AMPK and integrins in the context of cellular stress.

Conclusion

Under metabolic stress, characterized by an imbalance in ATP consumption and synthesis, AMPK activation orchestrates a series of events that enhance energy-producing pathways and suppress energy-demanding processes. The significance of AMPK in tumor microenvironments becomes evident, where cells experience varying levels of nutrient and oxygen availability.

Tumors' unconventional vascularization patterns contribute to the diverse metabolic states of cancer cells within the same tumor, making the regulation of AMPK crucial for their survival and adaptation. Notably, AMPK's role in regulating the cell surface abundance of integrins, key players in cell adhesion and migration, adds complexity to its functions. Knowing the interplay of AMPK in integrin recycling & turnover raises questions about the significance of integrin isoform selection and packaging into exosomes for extracellular transport. If cancer cells exhibit different integrin content than non-cancer cells, and if this integrin selection is AMPK-dependent and has downstream effects like selecting future metastatic sites, then there is great potential for various cancer therapeutic routes.

AMPK suppressors targeted to cancer cells specifically can be used to suppress tumor survival and growth. Removing the adaptive regulator in cancer cells will leave them more vulnerable to chemotherapy and targeted therapies. Also, suppressing AMPK activity in cancer cells can allow preventing development of metastasis through the paracrine or distant signalling to other niche through integrins packaged in exosomes.

To better understand the pathway that connects AMPK with exosomal integrin selection, other methods need to be used namely ultra-ID [28]. This method could allow tracking of specific proteins as they are transported between different intracellular compartments and into exosomes that are secreted. The spatial & temporal role of AMPK in determining the fate of integrins and their packaging into exosomes will develop our understanding of this novel pathway and drug targeting therapies for cancer cells.

List of Abbreviations
ADP: adenosine diphosphate
AMP: adenosine monophosphate
AMPK: adenosine monophosphate activated protein kinase
ANOVA: analysis of variance
ATP: adenosine triphosphate
CamKII: Ca^{2+} / calmodulin-dependent protein kinase II
CME: clathrin mediated endocytosis
CO_2: carbon dioxide
DMEM: Dulbecco's modified eagle medium
EV: extracellular vesicles
FBS: fetal bovine serum
GLUT4: glucose transporter type 4
HCl: hydrochloric Acid
HEC-1: human endometrial cancer -1
MDA-MB-231: M.D. Anderson model breast cancer - 231
mRNA: messanger ribonucleic acid
NaCl: sodium chloride
P/S: penicillin-streptomycin
PBS: phosphate buffered saline
PEG: polyethylene glycol
RPE: retinal pigment epithelium
SDS: sodium dodecyl sulfate
SEM: standard error of mean

Conflicts of Interest
The author declares that they have no conflict of interest.

Ethics Approval and/or Participant Consent
This is an in-vitro study, and no ethical approval or participant consent was required except acquiring the cell lines from key suppliers.

Authors' Contributions
AM: Substantial contribution to the concept and design of the study. Designed the experimental model, objectives, paths of analysis, workflow and data acquiring and interpretation. Drafted the manuscript and is accountable for the work submitted.

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References
[1] Zhao Y, Butler EB, Tan M. Targeting cellular metabolism to improve cancer therapeutics. Cell Death & Disease. 2013 Mar;4(3):e532-. https://doi.org/10.1038/cddis.2013.60
[2] Bonora M, Patergnani S, Rimessi A, De Marchi E, Suski JM, Bononi A, et al. ATP synthesis and storage. Purinergic Signalling. 2012 Sep;8:343–57. https://doi.org/10.1007/s11302-012-9305-8
[3] Cetrullo S, D'Adamo S, Tantini B, Borzi RM, Flamigni F. mTOR, AMPK, and Sirt1: Key players in metabolic stress management. Critical Reviews™ in Eukaryotic Gene Expression. 2015;25(1). https://doi.org/10.1615/CritRevEukaryotGeneExpr.2015012975
[4] Jeong EM, Liu M, Sturdy M, Gao G, Varghese ST, et al. Metabolic stress, reactive oxygen species, and arrhythmia. Journal of Molecular and Cellular Cardiology. 2012 Feb 1;52(2):454–63. https://doi.org/10.1016/j.yjmcc.2011.09.018
[5] Coughlan KA, Valentine RJ, Ruderman NB, Saha AK. AMPK activation: A therapeutic target for type 2 diabetes?. Diabetes, Metabolic syndrome and Obesity: Targets and Therapy. 2014 Jun 24:241–53. https://doi.org/10.2147/DMSO.S43731

[6] García-Prieto CF, Gil-Ortega M, Plaza A, Manzano-Lista FJ, González-Blázquez R, Alcalá M, et al. Caloric restriction induces H2O2 formation as a trigger of AMPK-eNOS-NO pathway in obese rats: Role for CAMKII. Free Radical Biology and Medicine. 2019 Aug 1;139:35–45. https://doi.org/10.1016/j.freeradbiomed.2019.05.016

[7] Song H, Guan Y, Zhang L, Li K, Dong C. SPARC interacts with AMPK and regulates GLUT4 expression. Biochemical and Biophysical Research Communications. 2010 Jun 11;396(4):961–6. https://doi.org/10.1016/j.bbrc.2010.05.033

[8] Fukumura D, Jain RK. Tumor microenvironment abnormalities: Causes, consequences, and strategies to normalize. Journal of Cellular Biochemistry. 2007 Jul 1;101(4):937–49. https://doi.org/10.1002/jcb.21187

[9] Ross E, Ata R, Thavarajah T, Medvedev S, Bowden P, Marshall JG, et al. AMP-activated protein kinase regulates the cell surface proteome and integrin membrane traffic. PLoS One. 2015 May 26;10(5):e0128013. https://doi.org/10.1371/journal.pone.0128013

[10] Ata R, Antonescu CN. Integrins and cell metabolism: An intimate relationship impacting cancer. International Journal of Molecular Sciences. 2017 Jan 18;18(1):189. https://doi.org/10.3390/ijms18010189

[11] Paolillo M, Schinelli S. Integrins and exosomes, a dangerous liaison in cancer progression. Cancers. 2017 Jul 26;9(8):95. https://doi.org/10.3390/cancers9080095

[12] Tai YL, Chen KC, Hsieh JT, Shen TL. Exosomes in cancer development and clinical applications. Cancer Science. 2018 Aug;109(8):2364–74. https://doi.org/10.1111/cas.13697

[13] Qin J, Xu Q. Functions and application of exosomes. Acta Pol Pharm. 2014 Mar;71(4):537–43. https://www.ptfarm.pl/pub/File/Acta_Poloniae/2014/4/537.pdf

[14] Burtenshaw D, Regan B, Owen K, Collins D, McEneaney D, Megson IL, et al. Exosomal composition, biogenesis and profiling using point-of-care diagnostics—Implications for cardiovascular disease. Frontiers in Cell and Developmental Biology. 2022 Jun 1;10:853451. https://doi.org/10.3389/fcell.2022.853451

[15] Hoshino A, Costa-Silva B, Shen TL, Rodrigues G, Hashimoto A, Tesic Mark M, et al. Tumour exosome integrins determine organotropic metastasis. Nature. 2015 Nov 19;527(7578):329–35. https://doi.org/10.1038/nature15756

[16] Hurwitz SN, Meckes DG. Extracellular vesicle integrins distinguish unique cancers. Proteomes 2019. https://doi.org/10.3390/proteomes7020014

[17] Chavez KJ, Garimella SV, Lipkowitz S. Triple negative breast cancer cell lines: One tool in the search for better treatment of triple negative breast cancer. Breast Disease. 2010;32(1-2):35. https://doi.org/10.3233%2FBD-2010-0307

[18] Kuramoto H, Hamano M, Imai M. HEC-1 cells. Human Cell. 2002 May;15(2):81–95. https://doi.org/10.1111/j.1749-0774.2002.tb00103.x

[19] Shelke GV, Lässer C, Gho YS, Lötvall J. Importance of exosome depletion protocols to eliminate functional and RNA-containing extracellular vesicles from fetal bovine serum. Journal of Extracellular Vesicles. 2014 Jan 1;3(1):24783. https://doi.org/10.3402/jev.v3.24783

[20] Göransson O, McBride A, Hawley SA, Ross FA, Shpiro N, Foretz M, et al. Mechanism of action of A-769662, a valuable tool for activation of AMP-activated protein kinase. Journal of Biological Chemistry. 2007 Nov 9;282(45):32549–60. https://doi.org/10.1074/jbc.M706536200

[21] Kroll W, Loffler M, Schneider F. Energy parameters, macromolecular synthesis and cell cycle progression of in vitro grown Ehrlich ascites tumor cells after inhibition of oxydative ATP synthesis by oligomycin. Zeitschrift für Naturforschung C. 1983 Aug 1;38(7-8):604–12. https://doi.org/10.1515/znc-1983-7-818

[22] Xi H, Kurtoglu M, Lampidis TJ. The wonders of 2-deoxy-d-glucose. IUBMB Life. 2014 Feb;66(2):110–21. https://doi.org/10.1002/iub.1251

[23] Koobotse MO, Schmidt D, Holly JM, Perks CM. Glucose concentration in cell culture medium influences the BRCA1-mediated regulation of the lipogenic action of IGF-I in breast cancer cells. International Journal of Molecular Sciences. 2020 Nov 17;21(22):8674. https://doi.org/10.3390/ijms21228674

[24] Crawford J, Cohen HJ. The essential role of L-glutamine in lymphocyte differentiation in vitro. Journal of Cellular Physiology. 1985 Aug;124(2):275–82. https://doi.org/10.1002/jcp.1041240216

[25] Sun L, Meckes Jr DG. Multiplex protein profiling method for extracellular vesicle protein detection. Scientific Reports. 2021 Jun 14;11(1):12477. https://doi.org/10.1038/s41598-021-92012-6

[26] Bandyopadhyay A, Raghavan S. Defining the role of integrin αvβ6 in cancer. Current Drug Targets. 2009 Jul 1;10(7):645-52. https://doi.org/10.2174/138945009788680374

[27] Lobos-González L, Bustos R, Campos A, Silva V, Silva V, Jeldes E, et al. Exosomes released upon mitochondrial ASncmtRNA knockdown reduce tumorigenic properties of malignant breast cancer cells. Scientific Reports. 2020 Jan 15;10(1):343. https://doi.org/10.1038/s41598-019-57018-1

[28] Kubitz L, Bitsch S, Zhao X, Schmitt K, Deweid L, Roehrig A, et al. Engineering of ultraID, a compact and hyperactive enzyme for proximity-dependent biotinylation in living cells. Communications Biology. 2022 Jul 4;5(1):657. https://doi.org/10.1038/s42003-022-03604-5

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