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CELL STRUCTURE AND FUNCTION 43: 171–176 (2018) ***Mini-review and Review***

An Anti-tumorigenic Role of the Warburg Effect at Emergence of Transformed Cells

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ABSTRACT. **The Warburg effect is one of the hallmarks of cancer cells, characterized by enhanced aerobic glycolysis. Despite intense research efforts, its functional relevance or biological significance to facilitate tumor progression is still debatable. Hence the question persists when and how the Warburg effect contributes to carcinogenesis. Especially, the role of metabolic changes at a very early stage of tumorigenesis has received relatively little attention, and how aerobic glycolysis impacts tumor incidence remains largely unknown. Here we discuss a novel paradigm for the effect of the Warburg effect that provides a suppressive role in oncogenesis.**

Key words: Warburg effect, aerobic glycolysis, cell competition, EDAC

The Warburg effect is a typical metabolic status exhibited by most of cancer cells

Dr. Otto Warburg, a German physiologist, reported a monumental study that cancer cells prefer the aerobic breakdown of glucose even when they are in the presence of abundant oxygen (Warburg, 1956). Neoplastically transformed cells rewire their metabolism to satisfy demands of growth and proliferation. This metabolic reprogramming is widely recognized as the Warburg effect (Burns and Manda, 2017; Koppenol *et al.*, 2011; Vander Heiden *et al.*, 2009). The Warburg effect is a robust metabolic hallmark of most tumors, thereby leading to clinical applications such as tumor imaging (Engelman *et al.*, 2008; Gatenby and Gillies, 2007; Higashi *et al.*, 2000). Although enhanced aerobic glycolysis is a common trait of tumors, its role in cancer development remains a subject to debate. Hence ever since its discovery, the Warburg effect is still an unresolved puzzle; Are metabolic changes drivers of cancer progression or do they just come along for the ride (Devic, 2016; Liberti and Locasale, 2016)? It is generally conceived that the Warburg effect promotes both of cell viability and metastatic potential of malignant tumors (Fantin *et al.*, 2006; Shim *et al.*, 1998). Despite its intense interest, much less is known about how the metabolic alteration impacts cancer development in the context of different tumor stages. To understand cancer as a metabolic disease (Seyfried *et al.*, 2014; Seyfried and Shelton, 2010), it is necessary to uncover how the metabolic reprogramming occurs at the initial stage of carcinogenesis, in other words, at emergence of the first transformed cells. In this review, we mainly summarize recent findings on the biological consequence of the Warburg effect-like metabolic modulation in the onset of cancer incidence.

The Warburg effect-like metabolic changes cause elimination of transformed cells by cell competition

The cellular environment plays a preventive role in cancer progression. In light of this concept, it is becoming increasingly apparent that normal epithelial cells can recognize the newly emerging suboptimal cells and compare their fitness levels, leading to elimination of unfit cells. This biological phenomenon is called cell competition and is conceived to be one of essential functions to ensure tissue homeostasis (Amoyel and Bach, 2014; Claveria and Torres, 2016; Di Gregorio *et al.*, 2016; Kajita and Fujita, 2015; Kon, 2018). Our group recently reported that the Warburg effect-like metabolic changes are induced in H-RasG12V (hereafter

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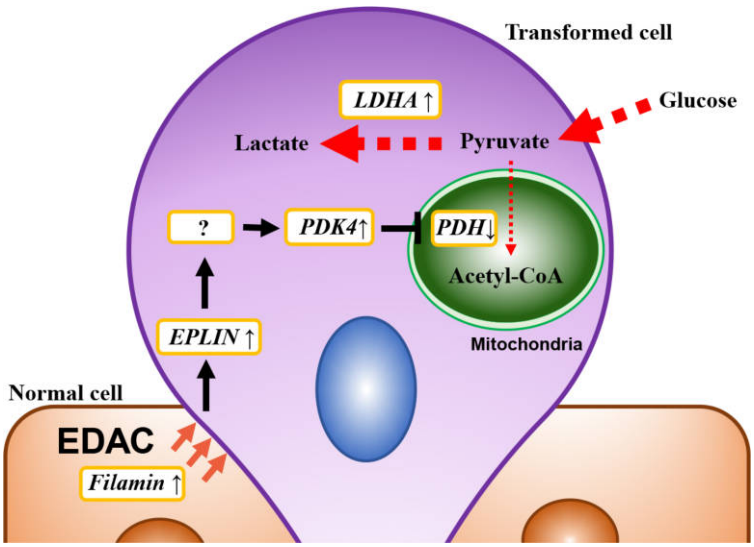

Fig. 1. The molecular pathways that cause the Warburg effect-like metabolic alterations in transformed cells surrounded by normal cells. EDAC from normal cells causes accumulation of EPLIN in the neighboring transformed cells, which turns to upregulate PDK4 and lead to mitochondrial dysfunction. The molecules that serve as a bridge between EPLIN and PDK4 are currently unidentified.

abbreviated as RasV12)-transformed epithelial cells when they are surrounded by normal cells and play a pivotal role in apical elimination of transformed cells as ineligible ones (Kon *et al.*, 2017). When RasV12-transformed Madin-Darby canine kidney (MDCK) cells are co-cultured with normal MDCK cells, RasV12 cells exhibit a reduction in mitochondrial membrane potential as evidenced by decreased incorporation of tetramethylrhodamine methyl ester (TMRM) relative to RasV12 cells cultured alone. In addition, the uptake of glucose is promoted in RasV12 cells, and lactic acid fermentation, a readout of the glycolytic pathway, is enhanced through upregulation of lactate dehydrogenase A (LDHA). These observations indicate that the Warburg effect-like metabolic changes are induced in RasV12-transformed cells when they emerge in an epithelial sheet. On the molecular basis, pyruvate dehydrogenase kinase 4 (PDK4), one of four PDK isoenzymes, is non-cell autonomously upregulated in RasV12 cells and inactivates pyruvate dehydrogenase (PDH) complex by phosphorylating its E1α subunit. PDH is a gatekeeper for mitochondrial glucose oxidation by catalyzing irreversible decarboxylation of pyruvate into acetyl-CoA, thereby inhibition of PDH activity results in declined mitochondrial activity (Roche and Hiromasa, 2007; Saunier *et al.*, 2016). The above described anti-tumorigenic function exerted by normal epithelial cells is termed as epithelial defense against cancer (EDAC) and involves a mechanical force generated by the accumulation of Filamin A, a crosslinker protein of actin filament, in normal cells adjacent to transformed cells (Kajita *et al.*, 2014). It has also been uncovered that epithelial protein lost in neoplasm (EPLIN) is a crucial regulator for the EDAC process via transducing downstream signals such as myosin-II and protein kinase A (PKA) in transformed cells, and its localization is regulated in a spatiotemporal manner (Ohoka *et al.*, 2015). Importantly, EDAC elevates the expression of PDK4, and EDAC-deficient cells (Filamin A- or EPLIN-knockdown) do not cause the cell competition-induced metabolic reprogramming. In addition, PDK4-knockout RasV12 cells are not eliminated and remained within the epithelia when co-cultured with normal cells. The fact that PDK4 inhibition diminishes the Warburg effect-like metabolic phenotype underscores that PDK4-mediated mitochondrial dysfunction markedly potentiates the glycolytic pathway. Taken together, these findings indicate that EDAC induces the Warburg effect-like metabolic shift, which is required for RasV12 cells to be eradicated (Fig. 1). Of particular note, PDK4 is downregulated in a wide variety of human tumors, implying that PDK4 could function as a tumor suppressor (Grassian *et al.*, 2011). One of essential questions is whether the aberrant mitochondrial activity itself accounts for the loser status in cell competition. In the manuscript, it is demonstrated that PDH-knockdown, thereby inhibiting the entry of pyruvate into the tricarboxylic acid (TCA) cycle does not prime cells as losers (Kon *et al.*, 2017). This result suggests that downregulated mitochondrial activity per se is not a trigger for competitive interaction.

The functional significance of PDK4-mediated mitochondrial deregulation in the elimination of RasV12-transformed cells is also substantiated in intestinal epithelial tissues of mice. The novel mouse model in which bicistronic expression of H-RasG12V and eGFP is under the control of a floxed STOP transcriptional cassette has been established. RasV12 remains transcriptionally silent

until the STOP cassette is removed by a Cre recombinase. Because the efficiency of Cre-mediated recombination in Cre-ERT2 mice is dependent on the amount of tamoxifen, injection of a low dose of tamoxifen results in the generation of genetic mosaics where only a fraction of cells undergoes a recombination event. Thus, this model not only offers the advantage of controlled expression of RasV12 mutation but also provides a platform applicable to the analysis of cell competition between normal and RasV12-transformed cells *in vivo*. Using this mouse model, it was observed that the vast majority of RasV12 cells were apically extruded in the intestinal lumen. Furthermore, mitochondrial membrane potential is decreased in RasV12 cells when surrounded by normal cells, and restoration of mitochondrial activity by antagonizing PDK4 compromises apical elimination of RasV12 cells (Kon *et al.*, 2017).

The difference between EDAC-induced Warburg effect and conventional Warburg effect

As described above, newly emerging transformed cells are apically eliminated by the surrounding normal cells through the PDK4-mediated Warburg effect-like metabolic shift. This suggests a novel mechanism for the inception of the Warburg effect-induced tumor suppressive process, and is in stark contrast to the historical view in which the Warburg effect plays a positive role in cancer progression (hereafter referred as conventional Warburg effect). However, the EDAC-induced metabolic changes share many aspects with the conventional Warburg effect. For instance, cells display an increased uptake of glucose and higher production of lactate. The aerobic glycolysis was originally viewed as a compensatory mechanism for dysfunctional respiration (Warburg, 1956), and reduced mitochondrial activity is observed in the EDAC-induced metabolic reprogramming (Kon *et al.*, 2017). Nevertheless, it should be noted that several accumulating studies have argued that mitochondria normally function in substantial cases of cancer (Crabtree, 1929; Fantin *et al.*, 2006; Koppenol *et al.*, 2011; Maldonado and Lemasters, 2014; Moreno-Sanchez *et al.*, 2007; Weinhouse, 1956, 1976), suggesting that mitochondrial impairment is not always associated with the Warburg effect phenotype. The conventional Warburg effect can be provoked through activation centered on hypoxia-inducible factor 1α (HIF-1α)-related genes such as glucose transporters (GLUTs), hexokinase 1/2 (HK1/2), pyruvate kinase M2 (PKM2), PDK1/3 and LDHA (Kim *et al.*, 2006; Luo *et al.*, 2011; Pouyssegur *et al.*, 2006; Prigione *et al.*, 2014; Semenza, 2010). Given the intimate link between HIF-1α and aerobic glycolysis, our group thoroughly investigated the involvement of HIF-1α activity in cell competition. However, there was no obvious evidence that HIF-1α activity is promoted in transformed cells when co-cultured with normal cells. Instead of PDK1/3, PDK4 was identified as one of the prime molecules to enhance aerobic glycolysis during the process of EDAC (Fig. 2). Best documented to upregulate the expression of PDK4 is peroxisome proliferator-activated receptor (PPAR) family (Abbot *et al.*, 2005; Muoio *et al.*, 2002; Wende *et al.*, 2005; Zhang *et al.*, 2006). It is very intriguing that PPARγ expression is very susceptible to mechanical stimuli as compressive forces regulate the PPARγ expression (Li *et al.*, 2013; Tanabe *et al.*, 2004). In addition, EPLIN has been proposed to function as a mechanosensor by sensing actomyosin fibers at adherens junctions (Taguchi *et al.*, 2011), suggesting that mechanical forces exerted by normal cells against transformed cells would underlie the induction of metabolic reprogramming. Whether the PPAR transcriptional complex is involved in the EDAC process remains unclear at present, which should be addressed in future studies. Interestingly, upon detachment from extracellular matrix (ECM), cells show enhanced expression of PDK4, leading to a metabolic impairment (Grassian *et al.*, 2011; Kamarajugadda *et al.*, 2012). This implies that PDK4 activation is closely associated with a dissociation phenotype irrespective of the oncogenic status of cells. Taken together, these findings indicate that the PDK4-modulated mitochondrial activity generally influences the biological behavior of cells dissociated from an epithelial layer.

What is the benefit of EDAC-induced Warburg effect-like metabolic changes for elimination of transformed cells?

Tumor cells are subjected to a remarkable array of pressures in a harsh condition such as hypoxia and scarce nutrients. The Warburg effect was initially considered as an adaptation to such environments. It has been postulated that the Warburg effect confers neoplastic cells with many biological advantages to sustain the uncontrolled proliferation. First, the enhanced glucose consumption serves to produce cellular building blocks (e.g., nucleotides, amino acids and lipids) to meet the requirement of rapidly proliferating cancer cells (Cairns *et al.*, 2011; DeBerardinis *et al.*, 2008; Levine and Puzio-Kuter, 2010; Lunt and Vander Heiden, 2011). The increase in glycolytic flux allows glycolytic intermediates such as glucose-6-phosphate or fructose-6-phosphate which can be used for nucleotide synthesis, whereas 3-phosphoglycerate and pyruvate are key precursors in the biogenesis of several amino acids. Under the condition of increased aerobic glycolysis, citrate converted from acetyl-CoA is exported from mitochondria. In the cytosol, citrate is delivered as acetyl-CoA for the synthesis of fatty acids where β-nicotinamide adenine dinucleotide 2'-phosphate, reduced (NADPH) is required for this step (Vander Heiden *et al.*, 2009; Ward and Thompson, 2012). It is currently unknown how the production rate of these macromolecules is altered and whether it accounts for the bio-

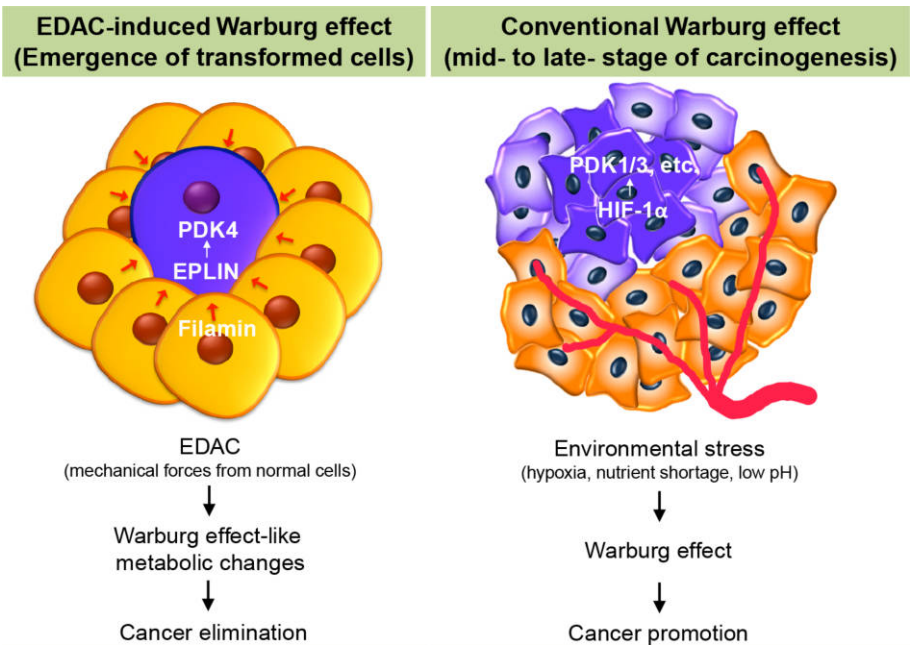

Fig. 2. A schematic representation of the difference between EDAC-induced Warburg effect and conventional Warburg effect. In the EDAC-induced Warburg effect, a mechanical force generated from surrounding normal cells causes the Warburg effect-like metabolic changes in transformed cells via the Filamin-EPLIN-PDK4 pathway. This metabolic shift results in the elimination of transformed cells. In contrast, at the mid– to late- stage of carcinogenesis environmental stresses such as hypoxia, scarce nutrients and low pH induce the Warburg effect in cancer cells, resulting in a selective advantage for survival, invasion and metastasis.

logical consequence of the EDAC-induced Warburg effect.

In addition to these biosynthetic functions, the rewiring of metabolic status also affects the generation of reactive oxygen species (ROS). There is enormous evidence that large amounts of glycolytic intermediates are diverted to the pentose phosphate pathway (PPP) to produce reducing equivalents in the form of NADPH. NADPH is a major cellular antioxidant which maintains glutathione in a reduced state to secure the redox balance. The electron transport chain (ETC) is a major source of ROS production as leaky electrons react with oxygen to produce superoxide across the respiratory chain. Given that transformed cells are inherently under increased oxidative stress as a result of a higher rate of proliferation, downregulated mitochondrial activity as an antioxidant mechanism has been proposed to function to lower oxidative burden, which potentiates cell viability (Brand and Hermfisse, 1997; Fantin and Leder, 2006; Lee and Yoon, 2015). In this regard, it is tempting to assume that the PDK4-mediated decrease in mitochondrial membrane potential is beneficial for cells to negate oxidative stress induced by EDAC. However, the opposite model of mitochondrial function in that it counteracts ROS through NADPH production by isocitrate dehydrogenase 2 (IDH2), a TCA cycle enzyme, has been recently put forward (Hawk *et al.*, 2018; Jiang *et al.*, 2016). In addition, it has been implicated that ROS is a vital regulator for cellular activity, as not only does ROS production cause DNA damage, but also function as a signaling molecule (D'Autreaux and Toledano, 2007; Liberti and Locasale, 2016). For instance, ROS inactivates phosphatases such as phosphatase and tensin homolog (PTEN) and protein tyrosine phosphatases. From this perspective, EDAC-induced ROS might act as a mediator to transduce downstream signal(s). Future studies aimed at delineating how the redox status is changed in transformed cells during cell competition are necessary.

In mitochondrial oxidative phosphorylation (OXPHOS), oxidation of one glucose generates 36 molecules of adenosine 5'-triphosphate (ATP), whereas glycolysis in cytosol produces a net gain of 2 ATP. Thus, aerobic glycolysis appears at first glance to be an inefficient means to generate ATP as the mitochondrial oxidative phosphorylation can maximize ATP production (Burns and Manda, 2017; Locasale and Cantley, 2011). However, ATP production by glycolysis is up to 100 times faster than that of OXPHOS (Pfeiffer *et al.*, 2001; Shestov *et al.*, 2014). Hence if extracellular glucose is abundant, the metabolic shift to glycolysis would allow cells to meet acute energy demand (DeBerardinis *et al.*, 2008; Guppy *et al.*, 1993; Liberti and Locasale, 2016; Locasale and Cantley, 2011). In line with this concept, our group found that the intracellular ATP level is profoundly higher in RasV12 cells surrounded by normal cells compared to RasV12 cells cultured alone based on the analysis of FRET-based ATP imaging (Kon *et*

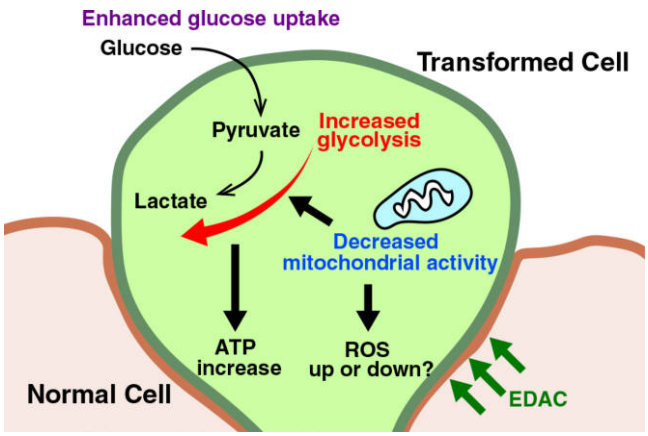

Fig. 3. A proposed model for the biological consequence of the Warburg effect-like metabolic changes at emergence of transformed cells. EDAC causes a reduction in mitochondrial membrane potential in the neighboring transformed cell, coupling with increased glycolysis. Higher production of intracellular ATP generated by the metabolic shift could be utilized for various biological reactions to leave away from an epithelial sheet.

al., 2017). The previous findings that myosin-II-driven contraction, PKA activation and enhanced endocytosis in transformed cells are required to force them out of epithelia (Anton *et al.*, 2014; Hogan *et al.*, 2009; Saitoh *et al.*, 2017) suggest that rapid ATP production might provide free energy for transformed cells to sustain those biological reactions (Fig. 3).

Future perspectives

In contrast to known pro-tumorigenic effects of the Warburg effect, the EDAC-induced Warburg effect-like metabolic shift acts in a tumor-suppressive capacity. Molecularly non-overlapped yet enhanced aerobic glycolysis establishes the diverse role of the Warburg effect in carcinogenesis; the fate of transformed cells is different depending on tumor stages. The tumor stage-specific key regulators of the Warburg effect should be identified and would present inviting target(s) for cancer treatment or prevention. Given that tumor is kind of the top of a mountain consisting of a multitude of transformed cells, a key question is whether the metabolic signature which is tagged in the evolutionary origin of cancer can be traced or vanished. Future works will help to fully understand when and how the metabolic signature is switched in cancer evolution.

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References

Abbot, E.L., McCormack, J.G., Reynet, C., Hassall, D.G., Buchan, K.W., and Yeaman, S.J. 2005. Diverging regulation of pyruvate dehydrogenase kinase isoform gene expression in cultured human muscle cells. *FEBS J.*, **272**: 3004–3014.

Amoyel, M. and Bach, E.A. 2014. Cell competition: how to eliminate your neighbours. *Development*, **141**: 988–1000.

Anton, K.A., Sinclair, J., Ohoka, A., Kajita, M., Ishikawa, S., Benz, P.M., Renne, T., Balda, M., Jorgensen, C., Matter, K., *et al.* 2014. PKA-regulated VASP phosphorylation promotes extrusion of transformed cells from the epithelium. *J. Cell Sci.*, **127**: 3425–3433.

Brand, K.A. and Hermfisse, U. 1997. Aerobic glycolysis by proliferating cells: a protective strategy against reactive oxygen species. *FASEB J.: official publication of the Federation of American Societies for Experimental Biology*, **11**: 388–395.

Burns, J.S. and Manda, G. 2017. Metabolic Pathways of the Warburg Effect in Health and Disease: Perspectives of Choice, Chain or Chance. *Int. J. Mol. Sci.*, **18**.

Cairns, R.A., Harris, I.S., and Mak, T.W. 2011. Regulation of cancer cell metabolism. *Nat. Rev. Cancer*, **11**: 85–95.

Claveria, C. and Torres, M. 2016. Cell Competition: Mechanisms and Physiological Roles. *Annu. Rev. Cell Dev. Biol.*, **32**: 411–439.

Crabtree, H.G. 1929. Observations on the carbohydrate metabolism of tumours. *Biochem. J.*, **23**: 536–545.

D'Autreaux, B. and Toledano, M.B. 2007. ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. *Nat. Rev. Mol. Cell Biol.*, **8**: 813–824.

DeBerardinis, R.J., Lum, J.J., Hatzivassiliou, G., and Thompson, C.B. 2008. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell Metab.*, **7**: 11–20.

Devic, S. 2016. Warburg Effect—a Consequence or the Cause of Carcinogenesis? *J. Cancer*, **7**: 817–822.

Di Gregorio, A., Bowling, S., and Rodriguez, T.A. 2016. Cell Competition and Its Role in the Regulation of Cell Fitness from Development to Cancer. *Dev. Cell*, **38**: 621–634.

Engelman, J.A., Chen, L., Tan, X., Crosby, K., Guimaraes, A.R., Upadhyay, R., Maira, M., McNamara, K., Perera, S.A., Song, Y., *et al.* 2008. Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. *Nat. Med.*, **14**: 1351–1356.

Fantin, V.R. and Leder, P. 2006. Mitochondriotoxic compounds for cancer therapy. *Oncogene*, **25**: 4787–4797.

Fantin, V.R., St-Pierre, J., and Leder, P. 2006. Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. *Cancer Cell*, **9**: 425–434.

Gatenby, R.A. and Gillies, R.J. 2007. Glycolysis in cancer: a potential target for therapy. *Int. J. Biochem. Cell Biol.*, **39**: 1358–1366.

Grassian, A.R., Metallo, C.M., Coloff, J.L., Stephanopoulos, G., and Brugge, J.S. 2011. Erk regulation of pyruvate dehydrogenase flux through PDK4 modulates cell proliferation. *Genes Dev.*, **25**: 1716–1733.

Guppy, M., Greiner, E., and Brand, K. 1993. The role of the Crabtree effect and an endogenous fuel in the energy metabolism of resting and proliferating thymocytes. *Eur. J. Biochem.*, **212**: 95–99.

Hawk, M.A., Gorsuch, C.L., Fagan, P., Lee, C., Kim, S.E., Hamann, J.C., Mason, J.A., Weigel, K.J., Tsegaye, M.A., Shen, L., *et al.* 2018. RIPK1-mediated induction of mitophagy compromises the viability of extracellular-matrix-detached cells. *Nat. Cell Biol.*, **20**: 272–284.

Higashi, K., Ueda, Y., Sakurai, A., Mingwang, X., Xu, L., Murakami, M., Seki, H., Oguchi, M., Taki, S., Nambu, Y., *et al.* 2000. Correlation of Glut-1 glucose transporter expression with [(18)F]FDG uptake in non-small cell lung cancer. *Eur. J. Nucl. Med.*, **27**: 1778–1785.

Hogan, C., Dupre-Crochet, S., Norman, M., Kajita, M., Zimmermann, C., Pelling, A.E., Piddini, E., Baena-Lopez, L.A., Vincent, J.P., Itoh, Y., *et al.* 2009. Characterization of the interface between normal and transformed epithelial cells. *Nat. Cell Biol.*, **11**: 460–467.

Jiang, L., Shestov, A.A., Swain, P., Yang, C., Parker, S.J., Wang, Q.A., Terada, L.S., Adams, N.D., McCabe, M.T., Pietrak, B., *et al.* 2016.

Reductive carboxylation supports redox homeostasis during anchorage-independent growth. *Nature*, **532**: 255–258.

Kajita, M., Sugimura, K., Ohoka, A., Burden, J., Suganuma, H., Ikegawa, M., Shimada, T., Kitamura, T., Shindoh, M., Ishikawa, S., *et al.* 2014. Filamin acts as a key regulator in epithelial defence against transformed cells. *Nat. Commun.*, **5**: 4428.

Kajita, M. and Fujita, Y. 2015. EDAC: Epithelial defence against cancer-cell competition between normal and transformed epithelial cells in mammals. *J. Biochem.*, **158**: 15–23.

Kamarajugadda, S., Stemboroski, L., Cai, Q., Simpson, N.E., Nayak, S., Tan, M., and Lu, J. 2012. Glucose oxidation modulates anoikis and tumor metastasis. *Mol. Cell Biol.*, **32**: 1893–1907.

Kim, J.W., Tchernyshyov, I., Semenza, G.L., and Dang, C.V. 2006. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab.*, **3**: 177–185.

Kon, S. 2018. Physiological and pathological relevance of cell competition in fly to mammals. *Dev. Growth Differ.*, **60**: 14–20.

Kon, S., Ishibashi, K., Katoh, H., Kitamoto, S., Shirai, T., Tanaka, S., Kajita, M., Ishikawa, S., Yamauchi, H., Yako, Y., *et al.* 2017. Cell competition with normal epithelial cells promotes apical extrusion of transformed cells through metabolic changes. *Nat. Cell Biol.*, **19**: 530–541.

Koppenol, W.H., Bounds, P.L., and Dang, C.V. 2011. Otto Warburg's contributions to current concepts of cancer metabolism. *Nat. Rev. Cancer*, **11**: 325–337.

Lee, M. and Yoon, J.H. 2015. Metabolic interplay between glycolysis and mitochondrial oxidation: The reverse Warburg effect and its therapeutic implication. *World J. Biol. Chem.*, **6**: 148–161.

Levine, A.J. and Puzio-Kuter, A.M. 2010. The control of the metabolic switch in cancers by oncogenes and tumor suppressor genes. *Science*, **330**: 1340–1344.

Li, G., Fu, N., Yang, X., Li, M., Ba, K., Wei, X., Fu, Y., Yao, Y., Cai, X., and Lin, Y. 2013. Mechanical compressive force inhibits adipogenesis of adipose stem cells. *Cell Prolif.*, **46**: 586–594.

Liberti, M.V. and Locasale, J.W. 2016. The Warburg Effect: How Does it Benefit Cancer Cells? *Trends Biochem. Sci.*, **41**: 211–218.

Locasale, J.W. and Cantley, L.C. 2011. Metabolic flux and the regulation of mammalian cell growth. *Cell Metab.*, **14**: 443–451.

Lunt, S.Y. and Vander Heiden, M.G. 2011. Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. *Annu. Rev. Cell Dev. Biol.*, **27**: 441–464.

Luo, W., Hu, H., Chang, R., Zhong, J., Knabel, M., O'Meally, R., Cole, R.N., Pandey, A., and Semenza, G.L. 2011. Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. *Cell*, **145**: 732–744.

Maldonado, E.N. and Lemasters, J.J. 2014. ATP/ADP ratio, the missed connection between mitochondria and the Warburg effect. *Mitochondrion*, **19** Pt A: 78–84.

Moreno-Sanchez, R., Rodriguez-Enriquez, S., Marin-Hernandez, A., and Saavedra, E. 2007. Energy metabolism in tumor cells. *FEBS J.*, **274**: 1393–1418.

Muoio, D.M., MacLean, P.S., Lang, D.B., Li, S., Houmard, J.A., Way, J.M., Winegar, D.A., Corton, J.C., Dohm, G.L., and Kraus, W.E. 2002. Fatty acid homeostasis and induction of lipid regulatory genes in skeletal muscles of peroxisome proliferator-activated receptor (PPAR) alpha knock-out mice. Evidence for compensatory regulation by PPAR delta. *J. Biol. Chem.*, **277**: 26089–26097.

Ohoka, A., Kajita, M., Ikenouchi, J., Yako, Y., Kitamoto, S., Kon, S., Ikegawa, M., Shimada, T., Ishikawa, S., and Fujita, Y. 2015. EPLIN is a crucial regulator for extrusion of RasV12-transformed cells. *J. Cell Sci.*, **128**: 781–789.

Pfeiffer, T., Schuster, S., and Bonhoeffer, S. 2001. Cooperation and competition in the evolution of ATP-producing pathways. *Science*, **292**: 504–507.

Pouyssegur, J., Dayan, F., and Mazure, N.M. 2006. Hypoxia signalling in cancer and approaches to enforce tumour regression. *Nature*, **441**: 437–443.

Prigione, A., Rohwer, N., Hoffmann, S., Mlody, B., Drews, K., Bukowiecki, R., Blumlein, K., Wanker, E.E., Ralser, M., Cramer, T., *et al.* 2014. HIF1alpha modulates cell fate reprogramming through early glycolytic shift and upregulation of PDK1-3 and PKM2. *Stem Cells*, **32**: 364–376.

Roche, T.E. and Hiromasa, Y. 2007. Pyruvate dehydrogenase kinase regulatory mechanisms and inhibition in treating diabetes, heart ischemia, and cancer. *Cell. Mol. Life Sci.*, **64**: 830–849.

Saitoh, S., Maruyama, T., Yako, Y., Kajita, M., Fujioka, Y., Ohba, Y., Kasai, N., Sugama, N., Kon, S., Ishikawa, S., *et al.* 2017. Rab5-regulated endocytosis plays a crucial role in apical extrusion of transformed cells. *Proc. Natl. Acad. Sci. USA*, **114**: E2327–E2336.

Saunier, E., Benelli, C., and Bortoli, S. 2016. The pyruvate dehydrogenase complex in cancer: An old metabolic gatekeeper regulated by new pathways and pharmacological agents. *Int. J. Cancer*, **138**: 809–817.

Semenza, G.L. 2010. HIF-1: upstream and downstream of cancer metabolism. *Curr. Opin. Genet. Dev.*, **20**: 51–56.

Seyfried, T.N. and Shelton, L.M. 2010. Cancer as a metabolic disease. *Nutr. Metab.*, **7**: 7.

Seyfried, T.N., Flores, R.E., Poff, A.M., and D'Agostino, D.P. 2014. Cancer as a metabolic disease: implications for novel therapeutics. *Carcinogenesis*, **35**: 515–527.

Shestov, A.A., Liu, X., Ser, Z., Cluntun, A.A., Hung, Y.P., Huang, L., Kim, D., Le, A., Yellen, G., Albeck, J.G., *et al.* 2014. Quantitative determinants of aerobic glycolysis identify flux through the enzyme GAPDH as a limiting step. *Elife*, **3**.

Shim, H., Chun, Y.S., Lewis, B.C., and Dang, C.V. 1998. A unique glucose-dependent apoptotic pathway induced by c-Myc. *Proc. Natl. Acad. Sci. USA*, **95**: 1511–1516.

Taguchi, K., Ishiuchi, T., and Takeichi, M. 2011. Mechanosensitive EPLIN-dependent remodeling of adherens junctions regulates epithelial reshaping. *J. Cell Biol.*, **194**: 643–656.

Tanabe, Y., Koga, M., Saito, M., Matsunaga, Y., and Nakayama, K. 2004. Inhibition of adipocyte differentiation by mechanical stretching through ERK-mediated downregulation of PPARgamma2. *J. Cell Sci.*, **117**: 3605–3614.

Vander Heiden, M.G., Cantley, L.C., and Thompson, C.B. 2009. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science*, **324**: 1029–1033.

Warburg, O. 1956. On the origin of cancer cells. *Science*, **123**: 309–314.

Ward, P.S. and Thompson, C.B. 2012. Metabolic reprogramming: a cancer hallmark even warburg did not anticipate. *Cancer Cell*, **21**: 297–308.

Weinhouse, S. 1956. On respiratory impairment in cancer cells. *Science*, **124**: 267–269.

Weinhouse, S. 1976. The Warburg hypothesis fifty years later. *Zeitschrift fur Krebsforschung und klinische Onkologie Cancer research and clinical oncology*, **87**: 115–126.

Wende, A.R., Huss, J.M., Schaeffer, P.J., Giguere, V., and Kelly, D.P. 2005. PGC-1alpha coactivates PDK4 gene expression via the orphan nuclear receptor ERRalpha: a mechanism for transcriptional control of muscle glucose metabolism. *Mol. Cell. Biol.*, **25**: 10684–10694.

Zhang, Y., Ma, K., Sadana, P., Chowdhury, F., Gaillard, S., Wang, F., McDonnell, D.P., Unterman, T.G., Elam, M.B., and Park, E.A. 2006. Estrogen-related receptors stimulate pyruvate dehydrogenase kinase isoform 4 gene expression. *J. Biol. Chem.*, **281**: 39897–39906.

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