Cancer and energyomics
The wedlock of genomics and metabolomics

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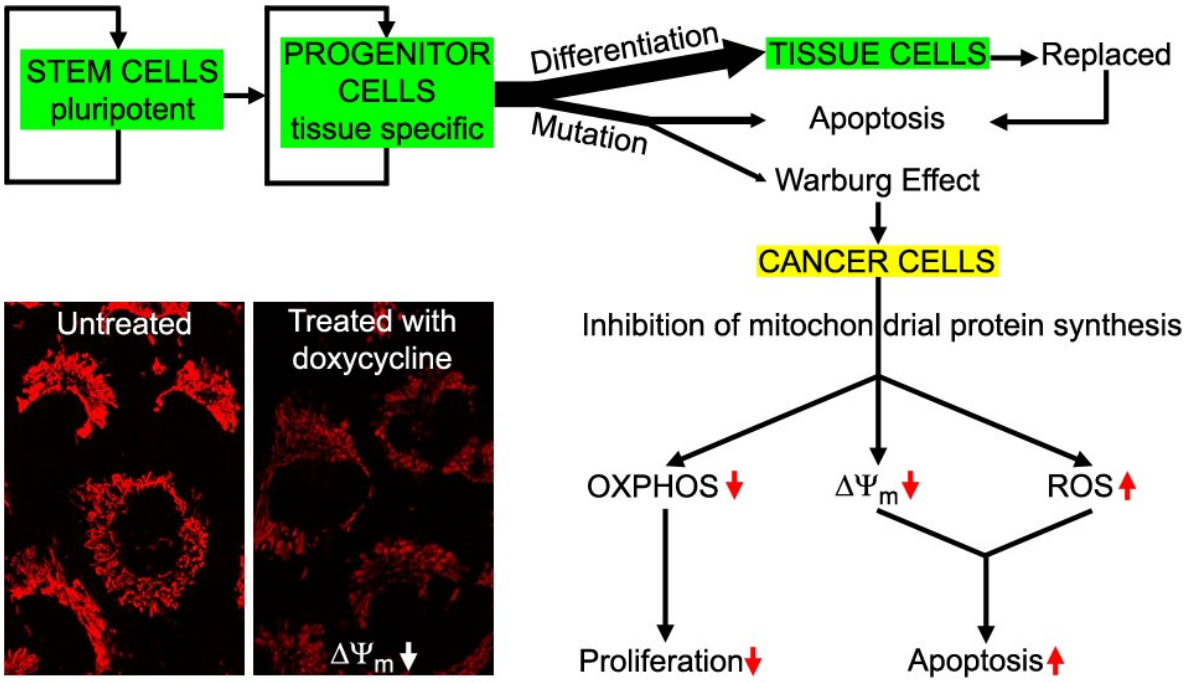
Abstract

Personalised medication of cancer, based on genomic analysis, predominates the fields of science and healthcare. Much attention is also paid to metabolic changes related to the disturbance of glucose metabolism in cancer known as the Warburg Effect: the excessive uptake of glucose and its conversion to lactate even in the presence of oxygen. We hypothesise that the unwanted clonal proliferation of cancer cells can best be prevented by lowering their energy generating capacity without hampering this process in normal tissues. Cancer is caused by overexpression of, or mutations in oncogenes. Their products are practically all dependent on ATP for their function, but drugs interfering with their expression may affect comparable processes in healthy cells. This lack of specificity applies also to current metabolic and immunotherapeutic approaches to fight cancer. For this reason, both strategies are prone to side effects. In our opinion, carcinogenesis, caused by millions of different oncogene mutations, should be treated with a generalised rather than a personalised method. Carcinogenesis relies on mitochondrial oxidative phosphorylation for energy generation. Limitation of this capacity during clonal expansion of cancer cells by the antibiotic doxycycline offers an attractive therapeutic strategy. Early detection with a general tumour marker, is desirable.

Graphical abstract



Keywords: Apoptosis; carcinogenicity; clonal expansion; doxycycline; glycolysis; mitochondria; oncogenes; oxidative phosphorylation; PKM2; reactive oxygen species; Warburg Effect.

Introduction

In the 1920s, Otto Warburg and his co-workers presented experiments showing that tumours, in contrast to healthy tissues, consume large amounts of glucose and excrete large amounts of lactic acid irrespective of the presence of sufficient oxygen to allow mitochondrial respiration [1,2]. This process is now known as aerobic glycolysis or fermentation and called the Warburg Effect. The reason for this divergent behaviour was not understood. One of the possibilities considered was that cancer cell mitochondria suffer from uncoupled or inhibited respiratory activity. Warburg himself was apparently not convinced of this explanation. He noted: "In order to kill tumour cells in living animals through want of energy it is necessary, as in experiments *in vitro*, to stop respiration as well as fermentation" [2]. Nonetheless, the assumption that the Warburg Effect is caused by the lack of sufficient energy generating capacity of mitochondria is still described as the Warburg Hypothesis, credited to him posthumously. Warburg struggled his entire life with this conundrum [3,4]. Finally, he concluded that cancer has countless secondary causes, but that the prime cause is the replacement "in great part" of respiration by fermentation of sugar [5]. How great was not mentioned but is certainly worth considering.

It is unlikely that cancer cells can survive and proliferate if the cells are unable to produce energy by mitochondrial oxidative phosphorylation (OXPHOS), which yields much more ATP per glucose molecule oxidised than glycolysis [6]. In Warburg's experiments with Jensen sarcoma [2], 66% of the glucose was fermented to lactate; hence, 34% of the pyruvate produced by glycolysis remained available for entering the mitochondria and usage by the Krebs cycle and OXPHOS. Because the stoichiometry of the ATP production by OXPHOS was unknown at the time, Warburg was unable to calculate the contribution of OXPHOS to ATP production. Using the ratio of fermentation to respiration determined by Warburg, it can now be calculated that the contribution of OXPHOS to ATP production corresponds to more than half of the total cellular ATP production [7]. Thus, despite the widespread view that cancer cells synthesise ATP mainly through aerobic glycolysis [8], the calculation clearly indicates that this opinion is untenable.

Recent studies confirmed that proliferating cancer cells generate a large part of their ATP through glucose- and glutamine-driven OXPHOS under normoxic as well as hypoxic conditions [9]. Surviving cancer cells appear to be particularly reliant on OXPHOS for their ATP production [10–12]. Therefore, there is ample reason to upgrade the neglected role of OXPHOS as an opportunity to combat cancer [13,14]. We think that cancer cells use fermentation for their survival [15] but depend on OXPHOS for their proliferation. The Warburg Effect allows the escape of cells from the intrinsic process of apoptosis by the gain of energy from fermentation [16] Here, we will clarify our opinion, taking into account the eight defining hallmarks of cancer as proposed by Hanahan and Weinberg [17] (Table 1), and the two mainstream therapeutic strategies to treat cancer as a genomic or a metabolic disease, respectively.

ATP-dependent phosphorylation of proteins is an important mechanism of signal transduction. For more than a decade, the phosphorylation of tyrosine residues has been considered to play a critical role in the development of cancer [18,19]. The links between genomic, transcriptomic and phosphoproteomic properties represent a strong signalling network,

which can be used to prioritise targets for chemotherapy. We feel that targeting the common need of all players, i.e. energy contained in ATP, deserves priority. Our opinion is based on two considerations. The first is that the majority of protein products of mutated genes that drive cancer cell formation rely on ATP-dependent phosphorylation for their function. The second is the changed balance in the energy generating capacity of the cytoplasm and mitochondria, which increases the resistance of cancer cells to apoptosis.

Table 1. The eight hallmarks of cancer[1].

1	Sustaining proliferative signalling
2	Evading growth suppressors
3	Resisting apoptosis (primary consequence)
4	Enabling replicative immortality
5	Inducing angiogenesis
6	Activating angiogenesis and metastasis
7	Reprogramming of energy metabolism (primary cause)
8	Evading immune destruction

[1] Adapted from Hanahan and Weinberg [17].

How cancer cells are defined and the inconsistency in the terms used for these cells frustrates cancer research [20]. We think that the majority of tumours develop from derailed progenitor cells at an intermediate stage of stem cell differentiation to post-mitotic, tissue-specific cells (Fig. 1).

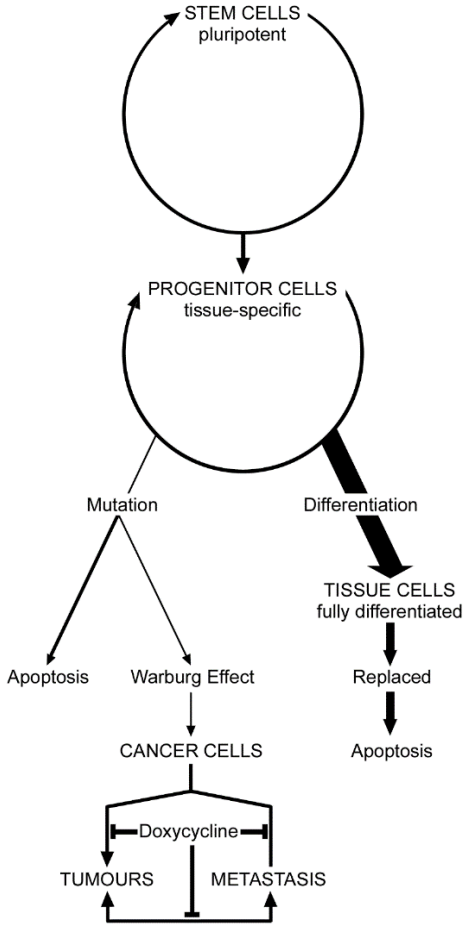


Figure 1. The developmental stages of human cells. Inhibition of doxycycline on cellular proliferation is indicated.

Hypothesis

We hypothesise that it is more effective to focus cancer treatment on the Warburg Effect as common feature of cancer than to target the millions of different causative genetic mutations. We do, however, not suggest to tackle the excessive glycolysis but, instead, propose to target OXPHOS as most important energy generating system of the cell. We think that this can best be achieved by impeding the synthesis of 13 essential subunits of the OXPHOS enzyme complexes rather than direct inhibition of the enzymatic reactions with small molecules. The genes of these 13 subunits are encoded on the mitochondrial DNA (mtDNA). We have shown that their translation can be inhibited *in vivo* with tetracyclines in clonally proliferating cancer cells without obstructing OXPHOS function in slowly proliferating or post-mitotic healthy cells.

Genomics

The cancer genome

Cancer is a disease of the genome, caused by a cell's acquisition of somatic mutations in key genes [21]. Identification of the cancer driver genes is considered essential to provide a blueprint for prospective therapeutic endeavours [22,23]. The realisation that these so-called oncogenes form the basis of cancer has a long history [24,25]. Already in the 1950s, it was recognised that tumours arise from clonal expansion of a single mutated progenitor cell that may express certain tissue-specific markers [24]. Cancer-related mutations may be inherited, induced by environmental factors or result from replication errors [26]. Oncogenes carry gain-of-function mutations. They often act in concert with tumour suppressor genes carrying loss-of-function mutations.

In the last few years, cancer is seen as a collection of many specific diseases. The apparent genomic diversity of tumours has inspired the idea of personalised medication, tailored for specific carcinogenic mutations [27,28]. Whole-genome cancer sequencing projects have established that mutations in a multitude of genes are involved in carcinogenesis [21,29,30]. Broad-based genomic testing is used as a tool to stratify patients for genetically matched clinical trials [31]. Analysis of over five million mutations in more than seven thousand cancers has yielded over 20 distinct mutational signatures that might improve cancer diagnostics and treatment [32].

Kinases, small molecule inhibitors and monoclonal antibodies

Many oncogenes code for ATP-dependent protein kinases. Their activity is linked to the energy generating capacity of cancer cells. The 'kinome' of the human genome represents more than 500 genes [33], equivalent to ~2% of the total number of protein-coding genes. The different ATP-dependent kinases are involved in the phosphorylation of a large number of specific targets responsible for the proliferation of cancer cells. Numerous small molecules and monoclonal antibodies have been developed to offset the aberrant actions of the mutated kinases. The targets may be slightly different from organ to organ; however, they all share their ATP dependence for phosphorylation to become functionally active. Unfortunately, improvement of the quality of life and overall survival gained with these chemotherapeutics has been dis-

appointing [34–38]. A seemingly attractive approach to fight certain cancers is related to mutations in genes coding for growth factor receptors [39]. Various mutation-specific monoclonal antibodies and tyrosine kinase inhibitors that block the function of these receptors are presently available. Sadly, the kinase inhibitors may lose their therapeutic activity because a mutation in another genes reduces the efficacy. One of the counteracting genes is the proto-oncogene *KRAS*. Interestingly, the mutated KRAS protein contributes to the Warburg Effect [40–42].

Recent advances in cancer immunotherapy have resulted in FDA-approved drugs with durable responses in patients with metastatic diseases. However, in many patients, immunotherapies do not help and it is difficult to predict who will benefit. Because of the often limited efficacy of chemotherapeutics, the need for combination therapy and neoadjuvant treatments in the genotype-matched personalised approach is regarded essential [43,44]. Hopefully, the development of neoantigen vaccines will improve this therapeutic strategy. Personalised protein mutanome [45] and RNA mutanome [46] vaccines are presently clinically tested (NCT02928224) [47]; however, it remains questionable whether this undoubtedly scientifically interesting research will result in a cure or at least a significant improvement of the quality of life and overall survival.

Metabolomics

The Warburg Effect

The opinion that genotype-based therapeutic agents deserve priority in the war against cancer is not generally accepted [48–50]. Many oncologists adhere to the conviction that the primary cause of cancer is metabolic. The group of Alberghina strongly promotes this view [51–54] and it forms the crux of the monograph by Seyfried [55]. Those who view cancer as a metabolic disease see the Warburg Effect as the basis of carcinogenesis. They favour cancer treatment by intervention at the level of glucose metabolism, based on the assumptions that cancer cells suffer primarily from: (1) intermittent hypoxia in pre-malignant lesions [56,57], (2) deficiencies of OXPHOS [58–62], (3) mtDNA depletion [63,64], or (4) defects of other mitochondrial functions [65,66]. Seoane *et al.* [67] support the notion that cancer is linked to mitochondrial dysfunction but, like Seyfried's group [55], did not find evidence that mtDNA mutations are directly responsible for tumour initiation, maintenance or aggressiveness. In fact, Seoane *et al.* [67] postulates that the intact mitochondrial genome has a critical function in the cellular adaptive survival response during tumour development. We do not believe that the Warburg Effect arises as a consequence of the absence of oxygen or respiratory insufficiency. We think that it is the consequence of genetic mutations and that mitochondrial energy metabolism remains adequate in cancer cells.

The energy generating capacity of cells is based on the interplay between glycolysis in the cytosol and OXPHOS in the mitochondria. Their contribution to the total energy production varies depending on the uptake of oxygen. To regulate the contribution of glycolysis and OXPHOS, cells have a high energy checkpoint and a low energy checkpoint. The former is represented by the hypoxia-inducible factor 1α (HIF-1α). Under normoxic conditions, the subdued expression of this transcription factor will keep glucose uptake as low as possible [68].

The latter is represented by AMP-activated protein kinase (AMPK). AMPK stimulates ATP generating catabolic pathways and suppresses ATP consuming anabolic pathways during metabolic stress under hypoxic conditions. AMPK stimulates glucose and fatty acid uptake and oxidation when cellular energy is low. Phosphorylation of AMPK is required for its activation. Mutations in (proto)oncogenes and tumour suppressor genes may disturb the control of these checkpoints [69]. Mutations that result, directly or indirectly, in a massive uptake of glucose will lead to cancer. The glucose transporter of the plasma membrane plays a critical role in glucose import. Inhibition of the transporter has been shown to counteract the Warburg Effect *in vitro* [70].

Metabolic treatment options

Dietary approaches

The increased mutation rate and disturbed DNA repair during senescent deterioration contribute to the rise of cancer with age [71]. Diets that restrict total caloric intake result in reduced blood glucose levels [72] and have been shown to increase the lifespan of laboratory rodents through a reduction of DNA damage [71]. *In vivo* experiments with dietary and caloric restriction measures in mice have revealed a significant induction of proapoptotic and antiangiogenic effects [56]. These processes are likely to contribute to a lower cancer frequency and aggressiveness. However, while caloric restriction is probably beneficial for cancer patients, it may compromise the patient's general physical condition [73].

Restriction of the available glucose can be particularly damaging for the brain. Representing only 2% of the body weight, the brain consumes 20% of the total glucose intake [74]. It is essential for energy but also as a precursor for neurotransmitters. When there is limited supply of nutrients, the human body will maintain the blood glucose level at the required lower limit for as long as possible by gluconeogenesis from amino acids and, eventually, even by digesting bodily proteins (cachexia). In addition, the brain can adapt its energy generation in part by the oxidation of ketone bodies produced from fatty acids by the liver during periods of starvation [55]. Various studies have focused on the possible improvement of cancer treatment by ketogenic diets, rich in fat but low in carbohydrates [55,58,75–79]; however, evidence that dietary modification on its own can effectively treat cancer patients is lacking. Dietary intervention is at best a secondary approach alongside other treatments.

Pharmacological approaches

The excessive absorption of glucose from the bloodstream by cancer cells cannot be easily tackled by dietary measures, such as substitution of glucose with ketone bodies [78, 79] of mannose [80]. Application of competitive or direct small molecule inhibitors of any of the enzymes involved in glycolysis at effective doses to kill cancer cells may be life threatening because of concurrent inhibition of glycolysis in normal cells. Inhibition will be less effective in cancer cells than in healthy cells as long as the cellular uptake and use of glucose by the cancer cells is unrestricted. Nevertheless, there have been numerous proposals to control cancer with small molecule inhibitors that act at the metabolic level, including inhibition of enzymes involved in glycolysis [81–89], the pentose phosphate pathway [90–92], pyruvate dehydrogenase [93–99], succinate dehydrogenase [83,86,87] and phosphoinositide 3-kinase [100–102].

Energyomics

The mitochondrial energy generating capacity as target of choice

We believe that mutations in genes that switch glucose metabolism from OXPHOS to glycolysis, leading to fermentation, will cause cancer, but only in progenitor cells that have retained or regained their capacity for proliferation (Fig. 1). This change was first identified by Warburg and is regarded one of the hallmarks of cancer (Table 1). As outlined in our hypothesis, we consider the energy metabolism a better target to combat cancer than the millions of genetic mutations causing the disease. When viewed in the light of energy metabolism, cancer can be seen as a singular disease with increased glycolytic activity but still adequate respiratory capacity. Although this capacity may be limited in comparison with fully differentiated cells, it is sufficient for cancer cells to proliferate because of the increased glycolysis in the presence of oxygen.

Ward and Thompson [103] presented the view that cancer is based on the oncogene-directed metabolic reprogramming to synthesise building blocks required for growth. The greater demand for building blocks derived from glycolytic intermediates may indeed explain a greater dependency of tumours on glucose [104]. However, the conclusion that the major function of fermentation in cancer cells is to maintain high levels of glycolytic intermediates for anabolic reactions contradicts the main observation of Warburg: the overproduction of lactate, which is indicative of the energy generating function of glycolysis. Aerobic glycolysis is not exploited for the synthesis of building blocks but forced upon the cells by mutations in oncogenes coding for various kinases [105,106] and this allows the cells to evade apoptosis.

Normally, the combined cytoplasmic and mitochondrial energy generating capacity of cells that are destined to die will be too low to sustain cellular physiology and, consequently, induce apoptosis with conservation of the building blocks for recycling. We believe that, while the enzymes of the glycolytic pathway are still functional and present at normal levels in these cells, glucose uptake is a limiting factor. Thus, mutations that result in increased glucose uptake will cause fermentation and power the cytosolic energy generating capacity. Consequently, cytosolic ATP levels are kept high by the raised glycolysis, reducing the need for ATP produced in mitochondria. The decreased ADP-ATP exchange across the mitochondrial inner membrane results in a high ATP/ADP ratio within the mitochondrial matrix and a high mitochondrial inner membrane potential ($\Delta\Psi_m$). Jointly, these enhance the cancer cell's resistance to apoptosis [15]. In other words, fermentation enables cancer cells to stay alive in spite of a restrained but still coupled OXPHOS. The Warburg Effect transforms cells that are on the pathway of programmed cell death to cancer cells (Fig. 1).

It is clear that the energy generating capacity of cancer cells is sufficient for proliferation. The choice between glycolysis and OXPHOS has been called a tumour's dilemma [107]; however, we do not consider the fermentation in cancer cells a metabolic choice but a fundamental property responsible for survival [15]. Respiration is not replaced by fermentation but remains essential for proliferation. Although promoting apoptosis is not considered the prevailing mechanism of tumour therapy, it is the route for the 10^9 cells per hour that are replaced in the human body [108]. Recently, it was emphasised that effective therapeutic solutions for cancer might lie outside the cabinet of the cutting-edge medicines [109]. We think that the

main goal of cancer therapy should be to bring the cancer cells back to the programmed death pathway of apoptosis. The earlier cancer is diagnosed, the more likely this approach will be successful.

There are thousands of potentially unique mutations that may serve as targets for personalised medication. As long as a single cancer cell remains able to divide, new mutations may arise that promote fermentation. For that reason. we propose to concentrate on the energy generating capacity of the mitochondria. This is already declining in cells on their way to apoptosis because of diminishing OXPHOS function. Thus, rather than to fight against the unwanted fermentation, we propose to focus on further curtailment of the mitochondrial energy generating capacity. We do, however, not recommend direct inhibition of OXPHOS, as this would also affect healthy organs, but advocate restriction of OXPHOS capacity in cancer cells without significant limitation of this capacity in healthy cells. We have shown that this can be achieved by partial inhibition of mitochondrial protein synthesis in rapidly expanding cancer cells [110]. Treatment with the tetracycline antibiotic doxycycline will partially inhibit synthesis of the 13 essential subunits of the OXPHOS enzymes encoded on the mtDNA and result in a partial OXPHOS defect. Slowly proliferation and post-mitotic healthy cells will keep sufficient reserve mitochondrial energy generating capacity to remain functionally active during treatment with doxycycline at doses routinely used for Lyme disease and Q fever (100 mg twice daily) because the mitochondrial protein synthesis is not completely blocked. In contrast, the mitochondrial energy generating capacity in cancer cells will be critically affected by doxycycline treatment since the higher proliferation rate leads to a faster and more profound depletion of the mtDNA-encoded OXPHOS enzyme subunits through cell divisions [110].

In a survival study of patients with tumours of the nasopharynx and larynx, pre-treatment with tetracyclines resulted a significant increase of overall survival as compared to treatment with erythromycin, which does not inhibit mitochondrial protein synthesis [111]. We showed that treatment with doxycycline reduced the mitotic index of tumour tissue [112] and that doxycycline alone or in combination with a variety of common chemotherapeutics blocked proliferation and even cured cancer in animal model systems [110,113–115].

Augmentation of oxidative stress as anticancer strategy to induce apoptosis

Oxidative stress is considered a cause of cancer [116]. Because of metabolic and signalling changes, cancer cells are inherently under increased oxidative stress [117]. Reactive oxygen species (ROS) may promote cancer cell initiation, maintenance and proliferation [118]. Oxidative stress may also be caused by genetic mutations. For instance, Ishikawa et al. [119] found that mtDNA mutations in the gene coding for a subunit of complex I of the respiratory chain (*MTND6*) were associated with overproduction of ROS and led to tumour progression in an experimental mouse model.

It is thought that increased oxidative stress makes cancer cells more prone to free radical-induced apoptosis when challenged by further oxidative stress. This idea has led to the proposition to treat cancer with pro-oxidants to exacerbate oxidative stress [117,120-122]. For example, high doses of vitamin C have been used to enhance oxidative stress in cancer cells. While vitamin C at daily need and recommended oral dose acts as an antioxidant, intravenous application of the maximal daily dose exerts pro-oxidant action and leads to a rise of

ROS in cancer cells [123,124]. However, a 12-week phase II clinical trial (NCT010800352) with intravenous administration of vitamin C of up to 60 g weekly did not result in remission and caused many adverse effects [125].

When administered orally or intravenously, pro-oxidants will affect all cells and tissues. Therefore, it seems wiser to boost ROS production through manipulation of respiratory function in rapidly growing cancer cells with normal or increased supply of oxygen. The respiratory chain is the main source of ROS in cells and disruption of this pathway is known to cause an increase of ROS formation [126]. Augmentation of ROS production by selective targeting of the respiratory chain has been proposed as a possible therapeutic approach as such or in combination with other therapeutic measures [127,128]. We have shown that treatment of cancer cells with doxycycline results in a respiratory deficiency, a decrease of $\Delta\Psi_m$ and an increase of ROS production [115]. Moreover, the treatment lowered the apoptotic threshold for the anticancer drug gemcitabine [115]. Thus, doxycycline may serve as a double-edged sword, decreasing the mitochondrial energy generating capacity and increasing oxidative stress.

Concluding remarks

Reconsidering the hallmarks of cancer as formulated in Table 1, we feel that the resistance towards programmed cell death (hallmark 3) is actually the only real point worth fighting. It is the physiological consequence of the reprogramming of energy metabolism (hallmark 7). The other six hallmarks are the result of the fact that cancer cells originate from mutated progenitor cells that through gain of oncogenic changes are able to evade the Hayflick limit and continue to divide [129–131].

Therapeutic measures should be directed towards the creation of conditions that lower the apoptotic threshold of cancer cells but not of healthy cells. As outlined, one approach for lowering of this threshold is to decrease mitochondrial ATP synthesis and $\Delta\Psi_m$ and increase ROS production. This can be achieved by inhibition of mitochondrial protein synthesis with doxycycline in exponentially expanding cancer cells [115]. In addition to lowering of the apoptotic threshold, doxycycline might have further advantages. Cancer cells frequently develop resistance to anti-cancer drugs by over-expression of ATP-driven efflux pumps that clear the drug before it affects cellular physiology. A doxycycline-induced OXPHOS deficiency will reduce the ATP supply, which might limit pump function. Targeting the mitochondrial protein synthesis in clonally expanding cancer cells is a potential strategy for any combination therapy, except if clonal expansion of T-cells is part of the treatment strategy [132]. Thus, doxycycline treatment may provide a radical change of the collective cancer ecosystem to achieve better outcomes for society [37].

Early diagnosis of cancer remains a major challenge [133]. We recommend early diagnosis by a common cancer marker and inhibition of mitochondrial protein synthesis in cancer cells as routine starting point for the fight against cancer in primary care. In this respect, pyruvate kinase may potentially serve as a universal biomarker for early cancer detection. Pyruvate kinase catalyses the conversion of phosphoenolpyruvate to pyruvate in the final reaction of the glycolysis. PKM2 is the dominant isoform of the enzyme in malignant tumours [134]. PKM2 can switch from a dimeric, inactive to a tetrameric, active form promoting aerobic glycolysis. The mechanism is initiated by phosphorylation of tyrosine kinase signalling proteins

[135] and is, thus, ATP-dependent. The PKM2 activity of cancer cells correlates with the level of glucose and oxygen utilisation [107,136]. The expression of PKM2 in cancer cells is reminiscent of its expression during embryogenesis [135]. PKM2 is an important marker for cancer *in vivo* [135]. Knockdown of PKM2 expression can reverse oxaliplatin-resistance in colorectal cancer cells [137] and downregulation of PKM2 expression by metformin increases the cisplatin-sensitivity of osteosarcoma cells, reversing chemoresistance [138]. Considering the importance of PKM2 in the cancer cell biology, we think that it is sensible to develop a mass spectrophotometry-based proteomic test for circulating PKM2, released from tumours in the blood, as general cancer biomarker for diagnostic purposes in primary care.

Declaration of Competing Interests

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Warburg, O., Posener, K., Negelein, N. Über den Stoffwecksel der Carcinomzelle. Z. physiol. Chem. 1924, 152, 309–344.
2. Warburg, O., Wind, F., Negelein, E. The Metabolism of Tumors in the Body. J. Gen. Physiol. 1927, 8, 519–530.
3. Warburg, O. On the Origin of Cancer Cells. Science 1956, 123, 309–314, doi:10.1126/science.123.3191.309.
4. Warburg, O. Partielle Anaerobiose der Krebszellen und Wirkung der Röntgenstrahlen auf Krebszellen. Naturwissenschaften 1959, 46, 25–29.
5. Warburg, O. The Prime Cause and Prevention of Cancer. In: Proceedings of the revised lecture at the meeting of the Nobel-laureates on 30 June 1966, at Lindau, Lake Constance, Germany; Konrad Triltsch: Wurzburg, 1969; pp. 6–16.
6. Mookerjee, S.A.; Gerencser, A.A.; Nicholls, D.G.; Brand, M.D. Quantifying Intracellular Rates of Glycolytic and Oxidative ATP Production and Consumption Using Extracellular Flux Measurements. J. Biol. Chem. 2017, 292, 7189–7207, doi:10.1074/jbc.M116.774471.
7. Koppenol, W.H.; Bounds, P.L.; Dang, C. V. Otto Warburg's Contributions to Current Concepts of Cancer Metabolism. Nat. Rev. Cancer 2011, 11, 325–337, doi:10.1038/nrc3038.
8. Lu, J.; Sharma, L.K.; Bai, Y. Implications of Mitochondrial DNA Mutations and Mitochondrial Dysfunction in Tumorigenesis. Cell Res. 2009, 19, 802–815, doi:10.1038/cr.2009.69.
9. Fan, J.; Kamphorst, J.J.; Mathew, R.; et al. Glutamine-Driven Oxidative Phosphorylation Is a Major ATP Source in Transformed Mammalian Cells in Both Normoxia and Hypoxia. Mol. Syst. Biol. 2013, 9, 712, doi:10.1038/msb.2013.65.
10. Viale, A.; Pettazzoni, P.; Lyssiotis, C.A.; et al. Oncogene Ablation-Resistant Pancreatic Cancer Cells Depend on Mitochondrial Function. Nature 2014, 514, 628–632, doi:10.1038/nature13611.
11. Lamb, R.; Harrison, H.; Hulit, J.; Smith, D.L.; Lisanti, M.P.; Sotgia, F. Mitochondria as New Therapeutic Targets for Eradicating Cancer Stem Cells: Quantitative Proteomics and Functional Validation via MCT1/2 Inhibition. Oncotarget 2014, 5, 11029–11037, doi:10.18632/oncotarget.2789.
12. Sancho, P.; Burgos-Ramos, E.; Tavera, A.; et al. MYC/PGC-1α Balance Determines the Metabolic Phenotype and Plasticity of Pancreatic Cancer Stem Cells. Cell Metab. 2015, 22, 590–605, doi:10.1016/j.cmet.2015.08.015.
13. Viale, A.; Corti, D.; Draetta, G.F. Tumors and Mitochondrial Respiration: A Neglected Connection. Cancer Res. 2015, 75, 3687–3691, doi:10.1158/0008-5472.CAN-15-0491.

14. Ashton, T.M.; Gillies McKenna, W.; Kunz-Schughart, L.A.; Higgins, G.S. Oxidative Phosphorylation as an Emerging Target in Cancer Therapy. Clin. Cancer Res. 2018, 24, 2482–2490, doi:10.1158/1078-0432.CCR-17-3070.
15. Kroon, A.M.; Taanman, J.W. Mitochondria and Cancer: The Warburg Fact. Chemotherapy: Open Access 2014, 3, 3, doi:10.4172/2167-7700.1000134.
16. Huber, H.J.; Dussmann, H.; Kilbride, S.M.; Rehm, M.; Prehn, J.H.M. Glucose Metabolism Determines Resistance of Cancer Cells to Bioenergetic Crisis after Cytochrome-c Release. Mol. Syst. Biol. 2011, 7, 470, doi:10.1038/msb.2011.2.
17. Hanahan, D.; Weinberg, R.A. Hallmarks of Cancer: The next Generation. Cell 2011, 144, 646–674, doi:10.1016/j.cell.2011.02.013.
18. Hunter, T. Tyrosine Phosphorylation: Thirty Years and Counting. Curr. Opin. Cell Biol. 2009, 140–146, doi:10.1016/j.ceb.2009.01.028.
19. Stratton, M.R.; Campbell, P.J.; Futreal, P.A. The Cancer Genome. Nature 2009, 458, 719–724, doi:10.1038/nature07943.
20. Valent, P.; Bonnet, D.; de Maria, R.; et al. Cancer Stem Cell Definitions and Terminology: The Devil Is in the Details. Nat. Rev. Cancer 2012, 12, 767–775, doi:10.1038/nrc3368.
21. Reyna, M.A.; Haan, D.; Paczkowska, M.; et al. Pathway and Network Analysis of More than 2500 Whole Cancer Genomes. Nat. Commun. 2020, 11, 729, doi:10.1038/s41467-020-14367-0.
22. Ding, L.; Bailey, M.H.; Porta-Pardo, E.; et al. Perspective on Oncogenic Processes at the End of the Beginning of Cancer Genomics. Cell 2018, 173, 305–320, doi:10.1016/j.cell.2018.03.033.
23. Bailey, M.H.; Tokheim, C.; Porta-Pardo, E.; et al. Comprehensive Characterization of Cancer Driver Genes and Mutations. Cell 2018, 173, 371–385, doi:10.1016/j.cell.2018.02.060.
24. Makino, S. Further Evidence Favoring the Concept of Stem Cell in Ascitis Tumors of Rats. Ann. NY Acad. Sci. 1956, 63, 818–830, doi:10.1111/j.1749-6632.1956.tb50894.x.
25. Weinberg, R.A. Oncogenes and the Molecular Basis of Cancer. Harvey Lect. 1985, 80, 129–130.
26. Tomasetti, C.; Li, L.; Vogelstein, B. Stem Cell Divisions, Somatic Mutations, Cancer Etiology, and Cancer Prevention. Science 2017, 355, 1330–1334, doi:10.1126/science.aaf9011.
27. Nowell, P.C. The Clonal Evolution of Tumor Cell Populations. Science 1976, 194, 23–28, doi:10.1126/science.959840.
28. Hayden, E.C. Personalized Cancer Therapy Gets Closer. Nature 2009, 458, 131–132, doi:10.1038/458131a.
29. Weinstein, J.N.; Collisson, E.A.; Mills, G.B.; et al. The Cancer Genome Atlas Pan-Cancer Analysis Project. Nat. Genet. 2013, 45, 1113–1120, doi:10.1038/ng.2764.
30. Campbell, P.J.; Getz, G.; Korbel, J.O.; et al. Pan-Cancer Analysis of Whole Genomes. Nature 2020, 578, 82–93, doi:10.1038/s41586-020-1969-6.
31. Meric-Bernstam, F.; Brusco, L.; Shaw, K.; et al. Feasibility of Large-Scale Genomic Testing to Facilitate Enrollment onto Genomically Matched Clinical Trials. J. Clin. Oncol. 2015, 33, 2753–2762, doi:10.1200/JCO.2014.60.4165.
32. Alexandrov, L.B.; Nik-Zainal, S.; Wedge, D.C.; et al. Signatures of Mutational Processes in Human Cancer. Nature 2013, 415421, doi:10.1038/nature12477.
33. Manning, G.; Whyte, D.B.; Martinez, R.; Hunter, T.; Sudarsanam, S. The Protein Kinase Complement of the Human Genome. Science 2002, 298, 1912–1934, doi:10.1126/science.1075762.
34. Fojo, T.; Mailankody, S.; Lo, A. Unintended Consequences of Expensive Cancer Therapeutics - The Pursuit of Marginal Indications and a Me-Too Mentality That Stifles Innovation and Creativity: The John Conley Lecture. JAMA Otolaryngol. Head Neck Surg. 2014, 140, 1225–1236, doi:10.1001/jamaoto.2014.1570.
35. Kim, C.; Prasad, V. Cancer Drugs Approved on the Basis of a Surrogate End Point and Subsequent Overall Survival: An Analysis of 5 Years of Us Food and Drug Administration Approvals. JAMA Intern. Med. 2015, 175, 1992–1994, doi:10.1001/jamainternmed.2015.5868.
36. Rupp, T.; Zuckerman, D. Quality of Life, Overall Survival, and Costs of Cancer Drugs Approved Based on Surrogate Endpoints. JAMA Intern. Med. 2017, 172, 276–277, doi:10.1001/jamainternmed.2016.7761.

37. Salas-Vega, S.; Iliopoulos, O.; Mossialos, E. Assessment of Overall Survival, Quality of Life, and Safety Benefits Associated with New Cancer Medicines. JAMA Oncol. 2017, 3, 382–390, doi:10.1001/jamaoncol.2016.4166.
38. Salas-Vega S., Mossialos, E. Overestimating the Benefit of Cancer Drugs-Reply. JAMA Oncol. 2017, 3, 1738–1739
39. Politi, K.; Zakowski, M.F.; Fan, P.D.; Schonfeld, E.A.; Pao, W.; Varmus, H.E. Lung Adenocarcinomas Induced in Mice by Mutant EGF Receptors Found in Human Lung Cancers Respond to a Tyrosine Kinase Inhibitor or to Down-Regulation of the Receptors. Genes Develop. 2006, 20, 1496–1510, doi:10.1101/gad.1417406.
40. Yun, J.; Rago, C.; Cheong, I.; et al. Glucose Deprivation Contributes to the Development of KRAS Pathway Mutations in Tumor Cells. Science 2009, 325, 1555–1559, doi:10.1126/science.1174229.
41. Ying, H.; Kimmelman, A.C.; Lyssiotis, C.A.; et al. Oncogenic Kras Maintains Pancreatic Tumors through Regulation of Anabolic Glucose Metabolism. Cell 2012, 149, 656–670, doi:10.1016/j.cell.2012.01.058.
42. Amendola, C.R.; Mahaffey, J.P.; Parker, S.J.; et al. KRAS4A Directly Regulates Hexokinase 1. Nature 2019, 576, 482–486, doi:10.1038/s41586-019-1832-9.
43. Ledford, H. Cocktails for Cancer with a Measure of Immunotherapy. Nature 2016, 532, 162–164, doi:10.1038/532162a.
44. Willyard, C. Cancer: An Evolving Thread. Nature 2016, 532, 166–168, doi:10.1038/nm1112-1594.
45. Ott, P.A.; Hu, Z.; Keskin, D.B.; et al. An Immunogenic Personal Neoantigen Vaccine for Patients with Melanoma. Nature 2017, 547, 217–221, doi:10.1038/nature22991.
46. Sahin, U.; Derhovanessian, E.; Miller, M.; et al. Personalized RNA Mutanome Vaccines Mobilize Poly-Specific Therapeutic Immunity against Cancer. Nature 2017, 381, 222–226, doi:10.1038/nature23003.
47. Kopetz, S.; Grothey, A.; Yaeger, R.; et al. Encorafenib, Binimetinib, and Cetuximab in BRAF V600E–Mutated Colorectal Cancer. N. Engl. J. Med. 2019, 381, 1632–1643, doi:10.1056/nejmoa1908075.
48. Wise, P.H. Cancer Drugs, Survival, and Ethics. BMJ (Online) 2016, 355, i5792, doi:10.1136/bmj.i5792.
49. Dangoor, A.; Joffe, J.; Januszewski, A.; Mansi, J.; Cunningham, D.; Selby, P. The Association of Cancer Physicians Responds to “Cancer Drugs, Survival, and Ethics.” BMJ (Online) 2016, 355, i6487, doi:10.1136/bmj.i6487.
50. Wise, P.H. Author’s Reply to Dangoor and Colleagues. BMJ (Online) 2016, 355, i6508, doi:10.1136/bmj.i6508.
51. Alberghina, L.; Gaglio, D.; Gelfi, C.; et al. Cancer Cell Growth and Survival as a System-Level Property Sustained by Enhanced Glycolysis and Mitochondrial Metabolic Remodeling. Front. Physiol. 2012, 3, 362, doi:10.3389/fphys.2012.00362.
52. Alberghina, L.; Gaglio, D. Redox Control of Glutamine Utilization in Cancer. Cell Death Dis. 2014, 5, e1561, doi:10.1038/cddis.2014.513.
53. Alberghina, L.; Gaglio, D.; Moresco, R.; Gilardi, M.; Messa, C.; Vanoni, M. A Systems Biology Road Map for the Discovery of Drugs Targeting Cancer Cell Metabolism. Curr. Pharm. Des. 2015, 20, 2648–2666, doi:10.2174/13816128113199990490.
54. Alberghina, L. Towards an Understanding of the Molecular Complexity of Cancer. Pontif. Acad. Sci. - Acta 2015, 22, 1–7.
55. Seyfried, T.N. Cancer as a Metabolic Disease: On the Origin, Management, and Prevention of Cancer; John Wiley & Sons: Hoboken, New Jersey, 2012; pp. 1–421.
56. Gatenby, R.A.; Gillies, R.J. Why Do Cancers Have High Aerobic Glycolysis? Nat. Rev. Cancer 2004, 4, 891–899, doi:10.1038/nrc1478.
57. Favier, J.; Brière, J.J.; Burnichon, N.; et al. The Warburg Effect Is Genetically Determined in Inherited Pheochromocytomas. PLoS ONE 2009, 4, e7094, doi:10.1371/journal.pone.0007094.

58. Seyfried, T.N.; Flores, R.E.; Poff, A.M.; D'Agostino, D.P. Cancer as a Metabolic Disease: Implications for Novel Therapeutics. Carcinogenesis 2014, 35, 515–527, doi:10.1093/carcin/bgt480.
59. Herrmann, P.C.; Herrmann, E.C. Oxygen Metabolism and a Potential Role for Cytochrome c Oxidase in the Warburg Effect. J. Bioenerg, Biomembr. 2007, 39, 247–250, doi:10.1007/s10863-007-9084-z.
60. Fosslien, E. Review: Cancer Morphogenesis: Role of Mitochondrial Failure. Ann. Clin. Lab. Sci. 2008, 38, 307–329.
61. Samudio, I.; Fiegl, M.; Andreeff, M. Mitochondrial Uncoupling and the Warburg Effect: Molecular Basis for the Reprogramming of Cancer Cell Metabolism. Cancer Res. 2009, 69, 2163–2166, doi:10.1158/0008-5472.CAN-08-3722.
62. Rodríguez-Enríquez, S.; Carreño-Fuentes, L.; Gallardo-Pérez, J.C.; et al. Oxidative Phosphorylation Is Impaired by Prolonged Hypoxia in Breast and Possibly in Cervix Carcinoma. Int. J. Biochem. Cell Biol. 2010, 42, 1744–1751, doi:10.1016/j.biocel.2010.07.010.
63. Chandra, D.; Singh, K.K. Genetic Insights into OXPHOS Defect and Its Role in Cancer. Biochim. Biophys. Acta – Bioenerg. 2011, 1807, 620–625, doi:10.1016/j.bbabio.2010.10.023.
64. Reznik, E.; Miller, M.L.; Şenbabaoğlu, Y.; et al. Mitochondrial DNA Copy Number Variation across Human Cancers. eLife 2016, 5, e10769, doi:10.7554/eLife.10769.
65. Frezza, C.; Gottlieb, E. Mitochondria in Cancer: Not Just Innocent Bystanders. Sem. Cancer Biol. 2009, 19, 4–11, doi:10.1016/j.semcancer.2008.11.008.
66. Ortega, Á.D.; Sánchez-Aragó, M.; Giner-Sánchez, D.; Sánchez-Cenizo, L.; Willers, I.; Cuezva, J.M. Glucose Avidity of Carcinomas. Cancer Lett. 2009, 276, 125–135, doi:10.1016/j.canlet.2008.08.007.
67. Seoane, M.; Mosquera-Miguel, A.; Gonzalez, T.; Fraga, M.; Salas, A.; Costoya, J.A. The Mitochondrial Genome Is a "Genetic Sanctuary" during the Oncogenic Process. PLoS ONE 2011, 6, e23327, doi:10.1371/journal.pone.0023327.
68. Semenza, G.L. Hypoxia-Inducible Factor 1 (HIF-1) Pathway. Sci. STKE 2007, 407, cm8, doi:10.1126/stke.4072007cm8.
69. Shaw, R.J. Glucose Metabolism and Cancer. Curr. Opin. Cell Biol. 2006, 18, 598–608, doi:10.1016/j.ceb.2006.10.005.
70. Zhang, T.B.; Zhao, Y.; Tong, Z.X.; Guan, Y.F. Inhibition of Glucose-Transporter 1 (GLUT-1) Expression Reversed Warburg Effect in Gastric Cancer Cell MKN45. Int. J. Clin, Exp Med. 2015, 8, 2423–2428.
71. Heydari, A.R.; Unnikrishnan, A.; Lucente, L.V.; Richardson, A. Caloric Restriction and Genomic Stability. Nucleic Acids Res. 2007, 35, 7485–7496, doi:10.1093/nar/gkm860.
72. Fontana, L.; Meyer, T.E.; Klein, S.; Holloszy, J.O. Long-Term Calorie Restriction Is Highly Effective in Reducing the Risk for Atherosclerosis in Humans. Proc. Natl. Acad. Sci. USA 2004, 101, 6659–6663, doi:10.1073/pnas.0308291101.
73. Kanarek, N.; Petrova, B.; Sabatini, D.M. Dietary Modifications for Enhanced Cancer Therapy. Nature 2020, 579, 507–517, doi:10.1038/s41586-020-2124-0.
74. Mergenthaler, P.; Lindauer, U.; Dienel, G.A.; Meisel, A. Sugar for the Brain: The Role of Glucose in Physiological and Pathological Brain Function. Trends Neurosci. 2013, 36, 587–597, doi:10.1016/j.tins.2013.07.001.
75. Champ, C.E.; Palmer, J.D.; Volek, J.S.; et al. Targeting Metabolism with a Ketogenic Diet during the Treatment of Glioblastoma Multiforme. J. Neuro-Oncol. 2014, 117, 125–131, doi:10.1007/s11060-014-1362-0.
76. Ho, V.W.; Leung, K.; Hsu, A.; et al. A Low Carbohydrate, High Protein Diet Slows Tumor Growth and Prevents Cancer Initiation. Cancer Res. 2011, 71, 4484–4493, doi:10.1158/0008-5472.CAN-10-3973.
77. Erickson, N.; Boscheri, A.; Linke, B.; Huebner, J. Systematic Review: Isocaloric Ketogenic Dietary Regimes for Cancer Patients. Med. Oncol. 2017, 34, 72, doi:10.1007/s12032-017-0930-5.
78. Veech, R.L.; Chance, B.; Kashiwaya, Y.; Lardy, H.A.; Cahill, G.F. Ketone Bodies, Potential Therapeutic Uses. IUBMB Life 2001, 51, 241–247, doi:10.1080/152165401753311780.

79. Cahill, G.F.; Veech, R.L. Ketoacids? Good Medicine? Trans. Am. Clin. Climatol. Assoc. 2003, 114, 149–163.
80. Gonzalez, P.S.; O'Prey, J.; Cardaci, S.; et al. Mannose Impairs Tumour Growth and Enhances Chemotherapy. Nature 2018, 563, 719–723, doi:10.1038/s41586-018-0729-3.
81. Ko, Y.H.; Pedersen, P.L.; Geschwind, J.F. Glucose Catabolism in the Rabbit VX2 Tumor Model for Liver Cancer: Characterization and Targeting Hexokinase. Cancer Lett. 2001, 173, 83–91, doi:10.1016/S0304-3835(01)00667-X.
82. Mathupala, S.P.; Ko, Y.H.; Pedersen, P.L. Hexokinase-2 Bound to Mitochondria: Cancer's Stygian Link to the "Warburg Effect" and a Pivotal Target for Effective Therapy. Sem. Cancer Biol. 2009, 19, 17–24, doi:10.1016/j.semcancer.2008.11.006.
83. Shoshan, M.C. 3-Bromopyruvate: Targets and Outcomes. J. Bioenerg. Biomembr. 2012, 44, 4–15.
84. Wang, T.A.; Zhang, X.D.; Guo, X.Y.; Xian, S.L.; Lu, Y.F. 3-Bromopyruvate and Sodium Citrate Target Glycolysis, Suppress Survivin, and Induce Mitochondrial-Mediated Apoptosis in Gastric Cancer Cells and Inhibit Gastric Orthotopic Transplantation Tumor Growth. Oncol. Rep. 2016, 35, 1287–1296, doi:10.3892/or.2015.4511.
85. Berthe, A.; Zaffino, M.; Muller, C.; et al. Protein N-Glycosylation Alteration and Glycolysis Inhibition Both Contribute to the Antiproliferative Action of 2-Deoxyglucose in Breast Cancer Cells. Breast Cancer Res. Treat. 2018, 171, 581–591, doi:10.1007/s10549-018-4874-z.
86. Dell'Antone, P. Targets of 3-Bromopyruvate, a New, Energy Depleting, Anticancer Agent. Med. Chem. 2009, 5, 491–496, doi:10.2174/157340609790170551.
87. Periera da Silva, A.P.; El-Bacha, T.; Kyaw, N.; et al. Inhibition of Energy-Producing Pathways of HepG2 Cells by 3-Bromopyruvate. Biochem. J. 2009, 417, 717–726, doi:10.1042/BJ20080805.
88. Ganapathy-Kanniappan, S.; Geschwind, J.F.H.; Kunjithapatham, R.; et al. Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) Is Pyruvylated during 3-Bromopyruvate Mediated Cancer Cell Death. Anticancer Res. 2009, 29, 4909–4918.
89. Ganapathy-Kanniappan, S. Evolution of GAPDH as a Druggable Target of Tumor Glycolysis? Expert Opin. Therap. Targets 2018, 22, 295–298, doi:10.1080/14728222.2018.1449834.
90. Wang, C.Y.; Shui, H.A.; Chang, T.C. Dual Effects for Lovastatin in Anaplastic Thyroid Cancer: The Pivotal Effect of Transketolase (TKT) on Lovastatin and Tumor Proliferation. J. Invest. Med. 2018, 1–9, doi:10.1136/jim-2017-000634.
91. Tseng, C.W.; Kuo, W.H.; Chan, S.H.; Chan, H.L.; Chang, K.J.; Wang, L.H. Transketolase Regulates the Metabolic Switch to Control Breast Cancer Cell Metastasis via the A-Ketoglutarate Signaling Sathway. Cancer Res. 2018, 78, 2799–2812, doi:10.1158/0008-5472.CAN-17-2906.
92. Tylicki, A.; Lotowski, Z.; Siemieniuk, M.; Ratkiewicz, A. Thiamine and Selected Thiamine Antivitamins — Biological Activity and Methods of Synthesis. Biosci. Rep. 2018, 38, BSR20171148, doi:10.1042/BSR20171148.
93. Michelakis, E.D.; Webster, L.; Mackey, J.R. Dichloroacetate (DCA) as a Potential Metabolic-Targeting Therapy for Cancer. Br. J. Cancer 2008, 99, 989–994, doi:10.1038/sj.bjc.6604554.
94. Stockwin, L.H.; Yu, S.X.; Borgel, S.; et al. Sodium Dichloroacetate Selectively Targets Cells with Defects in the Mitochondrial ETC. Int. J. Cancer 2010, 127, 2510–2519, doi:10.1002/ijc.25499.
95. Madhok, B.M.; Yeluri, S.; Perry, S.L.; Hughes, T.A.; Jayne, D.G. Dichloroacetate Induces Apoptosis and Cell-Cycle Arrest in Colorectal Cancer Cells. Br. J. Cancer 2010, 1746–1752, doi:10.1038/sj.bjc.6605701.
96. Anemone, A.; Consolino, L.; Conti, L.; et al. In Vivo Evaluation of Tumour Acidosis for Assessing the Early Metabolic Response and Onset of Resistance to Dichloroacetate by Using Magnetic Resonance PH Imaging. Int. J. Oncol. 2017, 51, 498–506, doi:10.3892/ijo.2017.4029.
97. Neveu, M.A.; de Preter, G.; Joudiou, N.; et al. Multi-Modality Imaging to Assess Metabolic Response to Dichloroacetate Treatment in Tumor Models. Oncotarget 2016, 7, 81741–81749, doi:10.18632/oncotarget.13176.

98. Ma, W.; Zhao, X.; Wang, K.; Liu, J.; Huang, G. Dichloroacetic Acid (DCA) Synergizes with the SIRT2 Inhibitor Sirtinol and AGK2 to Enhance Anti-Tumor Efficacy in Non-Small Cell Lung Cancer. Cancer Biol. Ther. 2018, 19, 835–846, doi:10.1080/15384047.2018.1480281.
99. Yang, C.L.; Wu, T.T.; Qin, Y.T.; et al. Afacile Doxorubicin-Dichloroacetate Conjugate Nanomedicine with High Drug Loading for Safe Drug Delivery. Int. J. Nanomed. 2018, 13, 1281–1293, doi:10.2147/IJN.S154361.
100. Li, W.; Ma, J.; Ma, Q.; et al. Resveratrol Inhibits the Epithelial-Mesenchymal Transition of Pancreatic Cancer Cells via Suppression of the PI-3K/Akt/NF-KB Pathway. Curr. Med. Chem. 2013, 20, 4185–4194, doi:10.2174/09298673113209990251
101. Han, Y.; Jo, H.; Cho, J.H.; Dhanasekaran, D.N.; Song, Y.S. Resveratrol as a Tumor-Suppressive Nutraceutical Modulating Tumor Microenvironment and Malignant Behaviors of Cancer. Int. J. Mol. Sci. 2019, 20, 925, doi:10.3390/ijms20040925.
102. Berretta, M.; Bignucolo, A.; di Francia, R.; et al. Resveratrol in Cancer Patients: From Bench to Bedside. Int. J. Mol. Sci. 2020, 21, 2945, doi:10.3390/ijms21082945.
103. Ward, P.S.; Thompson, C.B. Metabolic Reprogramming: A Cancer Hallmark Even Warburg Did Not Anticipate. Cancer Cell 2012, 21, 297–308, doi:10.1016/j.ccr.2012.02.014.
104. Lunt, S.Y.; vander Heiden, M.G. Aerobic Glycolysis: Meeting the Metabolic Requirements of Cell Proliferation. Annu. Rev. Cell Dev. Biol. 2011, 27, 441–464, doi:10.1146/annurev-cellbio-092910-154237.
105. Raimondi, C.; Falasca, M. Targeting PDK1 in Cancer. Curr. Med. Chem. 2011, 18, 2763–2769, doi:10.2174/092986711796011238.
106. Zhang, X.H.; Yu, H.L.; Wang, F.J.; Han, Y.L.; Yang, W.L. Pim-2 Modulates Aerobic Glycolysis and Energy Production during the Development of Colorectal Tumors. Int. J. Med. Sci.2015, 12, 487–493, doi:10.7150/ijms.10982.
107. Jose, C.; Bellance, N.; Rossignol, R. Choosing between Glycolysis and Oxidative Phosphorylation: A Tumor's Dilemma? Biochim. Biophys. Acta – Bioenerg. 2011, 1807, 552–561, doi:10.1016/j.bbabio.2010.10.012.
108. Rybczynska, A.A.; Boersma, H.H.; de Jong, S.; et al. Avenues to Molecular Imaging of Dying Cells: Focus on Cancer. Med. Res. Rev. 2018, 38, 1713–1768, doi:10.1002/med.21495.
109. Prasad, V. Our Best Weapons against Cancer Are Not Magic Bullets. Nature 2020, 577, 451, doi:10.1038/d41586-020-00116-2.
110. Dijk, S.N.; Protasoni, M.; Elpidorou, M.; Kroon, A.M.; Taanman, J.W. Mitochondria as Target to Inhibit Proliferation and Induce Apoptosis of Cancer Cells: The Effects of Doxycycline and Gemcitabine. Sci. Rep. 2020, 10, 4363, doi:10.1038/s41598-020-61381-9.
111. Leezenberg, J.A.; Wesseling, H.; Kroon, A.M. Possible Cytostatic Action of Tetracyclines in the Treatment of Tumors of the Nasopharynx and Larynx. Eur. J. Clin. Pharmacol. 1979, 16, 237–241, doi:10.1007/BF00608401.
112. Leezenberg, J.; van den Bogert, C.; Kroon, A. Antiproliferative Effects of Tetracyclines: A Possible Aid in the Treatment of Cancer. In Proceedings of the Current chemotherapy and immunotherapy: Proceedings of the 12th international congress of chemotherapy; Periti, P., Grassi, G., Eds.; Am. Soc. Microbiol.: Washington DC, 1982; pp. 1562–1564.
113. van den Bogert, C.; van Kernebeek, G.; de Leij, L.; Kroon, A.M. Inhibition of Mitochondrial Protein Synthesis Leads to Proliferation Arrest in the G1-Phase of the Cell Cycle. Cancer Lett. 1986, 32, 41–51, doi:10.1016/0304-3835(86)90037-6.
114. van den Bogert, C.; Dontje, B.H.J.; Holtrop, M.; et al. Arrest of the Proliferation of Renal and Prostate Carcinomas of Human Origin by Inhibition of Mitochondrial Protein Synthesis. Cancer Res. 1986, 46, 3283–3289.
115. Kroon, A.M. Taanman, J.-W. Doxycycline: a tool to reduce the proliferation of tumor cells and T-lymphocytes in vivo. In Doxycycline: medical uses and effects; Caldwell, A., Ed.; Nova Science Publishers: New York, 2018; pp. 1–46.
116. Lopez-Lazaro, M. Role of Oxygen in Cancer: Looking beyond Hypoxia. Anticancer Agents Med. Chem. 2012, 9, 517–525, doi:10.2174/187152009788451806.

117. Pelicano, H.; Carney, D.; Huang, P. ROS Stress in Cancer Cells and Therapeutic Implications. Drug Resist. Updat. 2004, 7, 97–100, doi:10.1016/j.drup.2004.01.004.
118. Weinberg, F.; Hamanaka, R.; Wheaton, W.W.; et al. Mitochondrial Metabolism and ROS Generation Are Essential for Kras-Mediated Tumorigenicity. Proc. Natl. Acad. Sci. USA 2010, 107, 8788–8793, doi:10.1073/pnas.1003428107.
119. Ishikawa, K.; Takenaga, K.; Akimoto, M.; et al. ROS-Generating Mitochondrial DNA Mutations Can Regulate Tumor Cell Metastasis. Science 2008, 320, 661–664, doi:10.1126/science.1156906.
120. Engel, R.H.; Evens, A.M. Oxidative Stress and Apoptosis: A New Treatment Paradigm in Cancer. Front. Biosci. 2006, 11, 300–312, doi:10.2741/1798.
121. Gorrini, C.; Harris, I.S.; Mak, T.W. Modulation of Oxidative Stress as an Anticancer Strategy. Nat. Rev. Drug Discov. 2013, 12, 931–947, doi:10.1038/nrd4002.
122. Gill, J.G.; Piskounova, E.; Morrison, S.J. Cancer, Oxidative Stress, and Metastasis. Cold Spring Harb. Symp. Quant. Biol. 2016, 81, 163–175, doi:10.1101/sqb.2016.81.030791.
123. Chen, Q.; Espey, M.G.; Sun, A.Y.; et al. Ascorbate in Pharmacologic Concentrations Selectively Generates Ascorbate Radical and Hydrogen Peroxide in Extracellular Fluid in Vivo. Proc. Natl. Acad. Sci. USA 2007, 104, 8749–8754, doi:10.1073/pnas.0702854104.
124. Cieslak, J.; Cullen, J. Treatment of Pancreatic Cancer with Pharmacological Ascorbate. Curr. Pharm. Biotechnol. 2015, 16, 759–770, doi:10.2174/1389201016091507151359 21.
125. Nielsen, T.K.; Højgaard, M.; Andersen, J.T.; et al. Weekly Ascorbic Acid Infusion in Castration-Resistant Prostate Cancer Patients: A Single-Arm Phase II Trial. Transl. Androl. Urol. 2017, 6, 517–528, doi:10.21037/tau.2017.04.42.
126. Murphy, M.P. How Mitochondria Produce Reactive Oxygen Species. Biochem. J. 2009, 417, 1-13.
127. Pelicano, H.; Feng, L.; Zhou, Y.; et al. Inhibition of Mitochondrial Respiration: A Novel Strategy to Enhance Drug-Induced Apoptosis in Human Leukemia Cells by a Reactive Oxygen Species-Mediated Mechanism. J. Biol. Chem. 2003, 278, 37832–37839, doi:10.1074/jbc.M301546200.
128. Trachootham, D.; Alexandre, J.; Huang, P. Targeting Cancer Cells by ROS-Mediated Mechanisms: A Radical Therapeutic Approach? Nat. Rev. Drug Discov. 2009, 8, 579–591, doi:10.1038/nrd2803.
129. Hayflick, L. How and Why We Age. Exp. Gerontol. 1998, 33, 639–653, doi:10.1016/S0531-5565(98)00023-0.
130. Jafri, M.A., Ansari, S.A., Al Qahtani, M.H., Shay, J.W. Roles of Telomeres and Telomerase in Cancer, and Advances in Telomerase-Targeted Therapies. Genome Med. 2016, 8,69, doi:10.1186/s13073-016-0324-x.
131. Khorraminejad-Shirazi, M.; Dorvash, M.; Estedlal, A.; Hoveidaei, A.H.; Mazloomrezaei, M.; Mosaddeghi, P. Aging: A Cell Source Limiting Factor in Tissue Engineering. World J. Stem Cells 2019, 11, 787–802, doi:10.4252/wjsc.v11.i10.787.
132. Kroon, A.M.; Taanman, J.-W. Clonal Expansion of T Cells in Abdominal Aortic Aneurysm: A Role for Doxycycline as Drug of Choice? Int. J. Mol. Sci. 2015, 16, 11178–11195, doi:10.3390/ijms160511178.
133. Hiom, S.C. Diagnosing Cancer Earlier: Reviewing the Evidence for Improving Cancer Survival. Br. J. Cancer 2015, 112 (Suppl, S1–S5, doi:10.1038/bjc.2015.23.
134. Bluemlein, K.; Grüning, N.M.; Feichtinger, R.G.; Lehrach, H.; Kofler, B.; Ralser, M. No Evidence for a Shift in Pyruvate Kinase PKM1 to PKM2 Expression during Tumorigenesis. Oncotarget 2011, 2, 393–400, doi:10.18632/oncotarget.278.
135. Christofk, H.R.; vander Heiden, M.G.; Wu, N.; Asara, J.M.; Cantley, L.C. Pyruvate Kinase M2 Is a Phosphotyrosine-Binding Protein. Nature 2008, 452, 181–186, doi:10.1038/nature06667.
136. Eigenbrodt, E.; Kallinowski, F.; Ott, M.; Mazurek, S.; Vaupel, P. Pyruvate Kinase and the Interaction of Amino Acid and Carbohydrate Metabolism in Solid Tumors. Anticancer Res. 1998, 18, 3267–3274.

137. Lu, W.Q.; Hu, Y.Y.; Lin, X.P.; Fan, W. Knockdown of PKM2 and GLS1 Expression Can Significantly Reverse Oxaliplatin-Resistance in Colorectal Cancer Cells. Oncotarget 2017, 8, 44171–44185, doi:10.18632/oncotarget.17396.
138. Shang, D.; Wu, J.; Guo, L.; Xu, Y.; Liu, L.; Lu, J. Metformin Increases Sensitivity of Osteosarcoma Stem Cells to Cisplatin by Inhibiting Expression of PKM2. Int. J. Oncol. 2017, 50, 1848–1856, doi:10.3892/ijo.2017.3950.