Why Mitochondria are Excellent Targets for Cancer Therapy

Prečo sú mitochondrie vhodné ciele pre liečbu rakoviny

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Summary

New insights into cancer cells – specific biological pathways are urgently needed to promote development of exactly targeted therapeutics. The role of oncoproteins and tumor suppressor proteins in proliferative signaling, cell cycle regulation and altered adhesion is well established. Chemicals, viruses and radiation are also generally accepted as agents that commonly induce mutations in genes encoding these cancer-inducing proteins, thereby giving rise to cancer. More recent evidence indicates the importance of two additional key factors imposed on proliferating cells – hypoxia and/or lack of glucose. These two additional triggers can initiate and promote the process of malignant transformation, when a low percentage of cells escape cellular senescence. Disregulated cell proliferation leads to formation of cellular masses that extend beyond the resting vasculature, resulting in oxygen and nutrient deprivation. Resulting hypoxia triggers a number of critical adaptations that enable cancer cell survival. The process of apoptosis is suppressed and glucose metabolism is altered. Recent investigations suggest that oxygen depletion stimulates mitochondria to compensate increased reactive oxygen species (ROS). It activates signaling pathways, such as hypoxia-inducible factor 1, that promote cancer cell survival and tumor growth. During the last decade, mitochondria have become key organelles involved in chemotherapy-induced apoptosis. Therefore, the relationship between mitochondria, ROS signaling and activation of survival pathways under hypoxic conditions has been the subject of increased study. Insights into mechanisms involved in ROS signaling may offer novel ways to facilitate discovery of cancer-specific therapies.

Key words

mitochondria – cell death – energy metabolism – cell transformation

Súhrn

Nové trendy v liečbe rakoviny sa spájajú s rozvojom presne cielených terapeutík, s účinkom na rakovinové bunky a zameraním na špecifické biologické dráhy. Úloha onkoproteínov a tumor-supresorových proteínov v proliferačnej signalizácii, regulácii bunkového cyklu a pozmenenej adhézii je už dobre preskúmaná. Chemické látky, vírusy a žiarenie sú tiež všeobecne prijímanými faktormi, ktoré vyvolávajú mutácie v génoch kódujúcich proteíny súvisiace s tvorbou rakoviny. Nedávne experimenty ukázali, že existujú dva nové kľúčové faktory pôsobiace na proliferujúce bunky – hypoxia a nedostatok glukózy. Tieto môžu iniciovať a podporovať proces malígnej transformácie v malom množstve buniek, ktorým sa podarilo uniknúť bunkovému starnutiu. Neregulovaná bunková proliferácia vedie k tvorbe bunkovej masy presahujúcej svoje rezervy, čo znižuje množstvo kyslíka a živín. Vzniknutý stav hypoxie iniciuje ďalšie kľúčové úpravy, ktoré umožňujú prežitie nádorových buniek. Proces apoptózy je potlačený a metabolizmus glukózy pozmenený. Nedávne experimenty naznačili, že vyčerpanie zásob kyslíka stimuluje mitochondrie, aby spracovávali väčšie množstvá reaktívnych foriem kyslíka (ROS). Aktivujú sa tak signálne dráhy, ako je hypoxiu-indukujúci faktor 1, ktoré podporujú prežívanie nádorových buniek a rast nádorov. Mitochondrie sú čoraz častejšie považované za kľúčové organely podieľajúce sa na chemoterapii, a preto je dôležité nájsť spôsob ako aktivovať apoptózu v mitochondriách za podmienok hypoxie, určiť vzťah medzi mitochondriami, ROS signalizáciou a procesmi aktivujúcimi prežívanie buniek. Každé nové zistenie môže otvoriť cestu pre pochopenie a odhalenie podstaty rakoviny a následné vytvorenie na mieru šitej terapie.

Kľúčové slová

mitochondria – bunková smrť – energetický metabolizmus – bunková transformácia

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Introduction

Cells in the body that retain normal growth control will eventually undergo the process of cellular senescence leading to cellular turnover by their death and replacement. By contrast, cells undergoing the process of oncogenic transformation continue to survive as immortalized cells, leading to uncontrolled proliferation that is associated with tumor formation. More recently, a relationship between changes in mitochondrial function, associated ROS production and its involvement in the process of cellular senescence have become increasingly clear [1]. Interplay between mitochondrial ROS production and the role of oncoproteins and tumor suppressors in modulating mitochondrial function to promote malignant cell transformation and avoid senescence has also become apparent [2]. Numerous studies focused their attention to the physiological process of cell aging [3,4], which is accompanied by elevated ROS production. On the other side, ROS production is increased in malignant cells in part as result of oncogene signaling via the NADPH oxidase complex and by hypoxia-related mitochondrial ROS. Increased oxidant levels contribute to enhanced cell proliferation and apoptosis suppression. Two independent therapeutic strategies targeting these pathways are possible. One target of attack would be to increase ROS scavenging, thereby dampening hydrogen peroxide signaling and depressing tumor growth. An opposite approach would be to treat cells with agents that interfere with ROS scavenging, resulting in excess reactive products that would trigger apoptosis [5,6].

It has become evident that telomere and telomerase are main components of the stem cell ignition mechanism, providing a way to restrain cancer [7]. With aging, oxidative stress accelerates vascular endothelial cell telomere shortening [8]. This process is referred to as accelerated replicative senescence, when mild repeated oxidative stress induces telomeric DNA damage [9]. On the other hand, senescence can be telomere-independent, the so-called stress induced senescence, when it is triggered prematurely by sublethal oxidative stress inducing non-telomeric DNA damages and growth arrest signals [10].

Tumor Cells and Glycolysis

Tumor cells and normal cells metabolize oxygen differentially. Because the activation of glycolysis in tumor cells is essential to prevent cell death induced by ATP depletion and H_2O_2 accumulation, the attenuation of glycolysis in tumor cells can induce their death. Normal cells would be less affected by this because they do not have increased glycolytic rates to ensure their survival [11,12]. Tumor cells exhibit profound genetic, biochemical and histological differences with respect to the original, nontransformed cellular types. In early studies on energy metabolism of tumor cells, it was proposed that enhanced glycolysis was induced by decreased oxidative phosphorylation. Since then, it has been indiscriminately applied to all types of tumor cells without an appropriate experimental evaluation and different findings [13]. The most notorious and well-known energy metabolism alteration in tumor cells is increased glycolytic capacity, even in the presence of high oxygen concentration [14]. It has been proposed that this increase in the glycolytic flux is a metabolic strategy of tumor cells to ensure survival and growth in environments with low oxygen concentrations [15]. The main mechanism responsible for the constant glycolytic flux is enhanced transcription of genes of several or all pathway enzymes and transporters, which is accompanied by an enhanced protein synthesis [16]. Experimental data have shown that in comparison to normal rat hepatocytes, all glycolytic enzymes are over-expressed by two- to four-fold while pyruvate kinase is over-expressed by ten-fold, hexokinase and phosphofructokinase type 1 are over-expressed up to 17- to 300-fold [17]. For human cervix HeLa cells, all enzymes are over-expressed by two- to seven-fold, with the exception of lactate dehydrogenase, which is expressed at a level sevenfold lower than in rat hepatocytes. However, for this last case, a more rigorous comparison should be made with normal uterine cervix epithelial cells [17]. One of the alternative approaches, in addition to building up a complex set of DNA changes, evidence suggests that the development of any cancer requires an alteration in oxygen metabolism of tumor cells. Interestingly, this alteration in oxygen metabolism can make cancer cells vulnerable to therapeutic intervention. Their increased hydrogen peroxide level and higher dependence on glycolysis for their survival make tumor cells more susceptible than normal cells to treatment with prooxidant agents or glycolysis inhibitors [12]. Several mechanisms for the enhanced glycolysis in human and rodent fast-growing tumor cells have been advanced and documented [13]. It is:

- increase in the isoform expression of glycolytic enzymes and glucose transporters,
- decreased expression of mitochondrial oxidative enzymes and transporters,
- lowering in the amount of mitochondria per cell,
- inhibition of oxidative phosphorylation by glycolysis activation (Crabtree effect),
- increased amount in the natural inhibitor protein of the mitochondrial ATP synthase,
- higher sensitivity of mitochondrial DNA to oxidative stress.

Perhaps the prime driving mechanism for the enhanced glycolysis is activation, via the hypoxia inducible factor 1 (HIF-1), of the transcription and translation of glycolytic genes in tumor cells. HIF-1 is a transcription factor constituted by two subunits, HIF-1α and HIF-1β. Factor stability mostly depends on HIF-1α. Under aerobic conditions, an active process of HIF-1α degradation is promoted, whereas, in anaerobic conditions, this subunit becomes highly stable [18]. In addition to hypoxia, HIF-1α may be induced under aerobic conditions by cytokines, growth factors, reactive oxygen species and nitric oxide; or by the energy metabolism intermediates: pyruvate, lactate and oxaloacetate [19]. HIF-1α might be detected only in malignant tumors but not in normal, healthy tissues and benign tumors. The reason for this hypothesis is change on the level of the von Hippel-Lindau protein, a tumor suppres-

sor. Von Hippel-Lindau protein binds to HIF-1α and induces its degradation, but in some aggressive tumors it is mutated, thus becoming ineffective in promoting HIF-1α degradation [20,21]. Regardless of the oxygen level, metastatic tumor cell lines (breast MDA, DU145 prostate, renal RCC4) show high levels of HIF-1α, over-expression of glycolytic enzymes and high glycolysis rate, whereas non-metastatic tumor cells (breast MCF-7, BX-PC3 prostate, A549 lung) increase HIF-1α over-expression and glycolysis only under hypoxia [21]. Simon [22] has shown direct association between HIF-1α and increasing generation of lactate from pyruvate due to pyruvate dehydrogenase complex inhibition (phosphorylation by pyruvate dehydrogenase kinase). Further association of HIF-1α with expression of other mitochondrial proteins has not yet been found.

Tab. 1. Oxidative phosphorylation/Glycolysis – dependent energy metabolism in different tumor cell types (modified from [14]).

Tissue of origin	Tumor cell type
Oxidative phosphorylation	
liver	reuber H-35, Morris and AS-30D hepatomas
lung	lung carcinoma
mammary gland	breast cancer
skin	melanoma
uterine cervix	HeLa, ovarian and uterus carcinomas
Glycolysis	
brain	glioma C6, meningioma, medulloblastoma, oligodendroglioma
colon	CT-26, Novikoff and LoVo colon adenocarcinoma
Glycolysis and oxidative phosphorylation	
brain	glioblastoma multiforme, astrocytoma C6
liver	ehrlich, Walker-256, Duning-LC18 hepatomas, MCF-7 carcinoma
mammary gland	MCF 7

Tumor Cells Energy Metabolism – Glycolysis or Oxidative Phosphorylation

Pioneering studies with solid and ascites tumor cells led to a proposal of universal mechanism that all tumor cell types were energetically dependent, mainly or only, on glycolysis. In particular, glycolysis seems to be the main energy pathway in slow-growing solid tumors, such as mammary adenocarcinoma, human melanomas [23] and rat rhabdomyosarcomas [24], as oxidative phosphorylation is apparently limited by the low oxygen availability inside the tumor [25]. It should also be considered that, in addition to lower oxygen availability in solid tumors, especially in the initial and avascular developmental stages under which a poor vascularization occurs, glucose supply can be similarly affected, thus inducing a severe decrease in the generation of glycolytic ATP [26]. Some authors [26,27] have determined a normal oxygen concentration (8–57 μM depends on tissue) in the center of glioma, carcinoma and in the hypoxic regions of human tumors. If the oxygen concentration surrounding mitochondria does not fall below 1 μM, mitochondria will work normally. Therefore, tumor mitochondrial metabolism would not be affected by hypoxia level found in tumors, unless there was prolonged exposure to weeks or months. Simultaneously, in a hypoxic microenvironment, the expression of mitochondrial enzymes alters somehow, perhaps through a p53-mediated mechanism [28]. It is assumed, but not experimentally determined, that oxidative phosphorylation in mitochondria is negligible under hypoxic conditions. Enhanced glycolysis of tumor cells is usually considered to be a sufficiently good reason for proposing that ATP supply only or mainly depends on glycolysis [19,28–30] but the quantitative contribution of each energy supply has rarely been determined. It also remains to be analyzed whether the accelerated glycolysis under hypoxia indeed serves only for ATP supply or its role is the supply of intermediates for biosynthesis of polysaccharides, precursors for lipids, amino and nucleic acids [25], which are required for active angiogenesis in solid tumors.

Initial studies proposed that a high glycolytic rate in tumor cells was the result of a damaged respiratory chain. It was shown later [30] that respiration of tumor mitochondria was as efficient as that of normal mitochondria and diminished oxidative phosphorylation observed in tumor cells was the result of a lower proportion of mitochondria (20–50%). However, significant respiratory deficiencies have been identified in some respiratory chain components – cytochrome C oxidase, iron-sulphur center, cytochrome C reductase, but an increase in activity of cytochrome C reductase has also been determined in the same brain tumors [31,32]. In contrast, no differences in oxidative enzyme activities with normal cells have been detected for Morris and Novikoff hepatomas. It is important to emphasize that a decrease of one enzyme or transporter does not automatically lead to diminution in the pathway flux or metabolite concentration. Unfortunately, detection of protein levels by Western blot, or of gene expression by Northern blot, provides information with little functional meaning, unless these measurements are accompanied by determination of enzyme activity and pathway flux.

It is intriguing that despite accelerated glycolysis in many fast-growing tumor cells, the total contribution to the ATP supply only reaches 10% [33] (Tab. 1). In contrast to other fast growing tumor cell lines, glycolysis indeed covers 50–70% of the ATP demand. Some human and rodent gliomas exhibit high or moderate susceptibility to respiratory chain inhibitors, indicating the presence of fully functional mitochondria and dependency on oxidative phosphorylation [34]. On the contrary, many brain tumors in comparison with other tissues have lower succinyl-CoA acetoacetyl transferase activity than normal neurons

or glia, and are unable to metabolize ketone bodies. Then, as fatty acids do not pass the blood-brain barrier, brain tumors seem to be dependent on glucose and glycolysis for ATP supply [35]. Therefore, the generalized statement that glycolysis predominates over oxidative phosphorylation for ATP supply in tumor cells should be experimentally determined for each particular types of tumor cell. Thus, the main thermodynamic reason for increased glycolysis in tumor cells (associated with damaged or unaltered oxidative phosphorylation) might rather be energy deficiency induced by highly ATP-dependent processes, such as an accelerated cell proliferation or stimulated nucleic acid, protein and cholesterol synthesis.

Mitochondria as Excellent Targets for Cancer Therapy

On the one hand, mitochondria are important for normal functioning and cell survival, but on the other hand, they are key regulators of intrinsic apoptotic cascade. The major nuclear encoded oncogenic proteins (MYC, p53, STAT-3, RAS) act either alone or in an integrated fashion to modulate gene expression involved in mitochondrial function, regulate the expression of gene encoding mitochondrial proteins by directly altering mitochondrial function inside cancer cells to promote cancer development [2]. Recent research established that mitochondrial associated gene expression is significantly different in cancer cells in comparison to normal cells. Most of the common features of cancer cells [36–38], can be found (either in a direct or indirect fashion) in mitochondria. Since mitochondria occupy a strategic position between bioenergetic/biosynthetic metabolism and cell death regulation, they are emerging as privileged targets for the development of novel chemotherapeutic agents [39]. During the last decade, numerous approaches that selectively target cancer cells by virtue of their mitochondrial defects have been shown to exert antitumor effects [40]. These include:

- mitochondriotoxic agents that preferentially accumulate in cancer cells due to mitochondrial hyperpolarization (e.g., F16) [41],
- pharmacological modulators of the Bcl-2 protein family (e.g., ABT-737) [42],
- compounds that bind to putative permeability transition pore complex (PTPC) subunits (e.g., PK11195) [43],
- redox active agents that trigger cell death by provoking futile redox cycles in mitochondria (e.g., arsenic trioxide, phenethyl isothiocyanate) [44],
- retinoid-related molecules that induce mitochondrial permeability transition independently of retinoid receptors (e.g., CD437, ST1926) [45].

One of the most extensively studied redox active agents is dietary phenethyl isothiocyanate (PEITC). PEITC can induce glutathione S-transferase and quinone reductase that inactivate carcinogens and promote their excretion. More recently, it has been found that PEITC could induce cell cycle arrest and apoptosis [46], and [47] have shown that PEITC treatment of the human MCF7 breast cancer cells produced significant alterations in some genes involved in tumor suppression and cellular apoptosis. Phenethyl isothiocyanate is also an effective inhibitor of HIF-1. The ability of PEITC to inhibit its activity was independent of the activity of the von Hippel-Landau protein and the proteasome, which are required for the normal turnover of HIF-1α in normoxia. Decreased expression of HIF-1α in PEITC treated human MCF7 breast cancer cells was not associated with HIF-1α RNA levels suggesting that PEITC may inhibit HIF activity by decreasing translation of the HIF-1α RNA. These results may contribute to the anti-angiogenic and anti-cancer effects of PEITC [48]. Currently, there is an ongoing phase II clinical trial for preventing lung cancer with phenethyl isothiocyanate in a group of heavy smokers [49].

Hypothetically, the most efficient mitochondrial therapies would be those that affect processes in mitochondria linked to several features of the neoplastic phenotype. As an example, compounds that disrupt the interaction between hexokinase and the voltage-dependent anion channel might display a consis-

Tab. 2. Examples of compounds targeting energy metabolism in fast-growing tumor cells.

Metabolic drug	Tumor cell type	Concentration	% of growth size reduction	Inhibition site
casiopeina II-gly	AS-30D hepatomas HeLa cells	10 μM 1 μM	> 95%	KGDH, SDH
clofazimine	bronchial carcinoma WIL	10 μM	40–50%	Mitoch. uncoupler
F16	mammary tumor and human breast cancer	3 μM	> 90%	H^+–ATPase
rhodamine 123 gossypol	human MCF-7 breast and cervical KB-3-1 carcinoma	3.4–3.8 μM 0.8–4.3 μM	50–60%	OxPhos uncoupler GAPDH and LDH
rhodamine 123 2-deoxyglucose	human MCF-7 breast	1.3 μM 300 μM	100%	OxPhos uncoupler HK II and HPI
rhodamine 123 2-deoxyglucose	human osteosarcoma	5 μM 500 μM	65–80%	OxPhos uncoupler HK II and HPI

KGDH – a-ketoglutarate dehydrogenase, SDH – succinate dehydrogenase, OxPhos – oxidative phosphorylation, GAPDH – glyceraldehyde-3-phosphate dehydrogenase, LDH – lactate dehydrogenase, HK II – hexokinase, HPI – hexose-6-phosphate isomerase.

tent dual antitumor effect: uncoupling of aerobic glycolysis from residual ATP synthesis in mitochondria and sensitization to PTPC-dependent cell death [50].

The biochemical strategy – simultaneous blockage of both ATP generating pathways for suppressing the accelerated tumor proliferation was originally proposed by [51]. For example, blockage of glycolysis (NAD+ dependent enzymes) by the drug gossypol (AT-101) in diverse fast-growth tumor cells, together with oxidative phosphorylation inhibitor rhodamine 123 (Tab. 2), decreased tumor cell proliferation by 60% [52]. Gossypol alone simultaneously inhibits anti-apoptotic proteins of Bcl-2 family [Bcl-2, Bcl-XL, Bcl-W) and demonstrates clinical activity in a phase I trial against prostate cancer [53]. Treatment of several human and rodent tumors by rhodamine 123 and 2-deoxyglucose together induced almost full blockage of growth [54], but 2-deoxyglucose alone significantly increased the cytotoxicity of cisplatin in neck and head tumor cells [55]. There are ongoing phase I/II clinical trials in patients with advanced solid tumors or prostate cancer.

In the search for the drugs that are more specific for tumor cells, some authors have used the typical mitochondrial inhibitors, rotenone and oligomycin, for blocking tumor cell proliferation. Oligomycin at low doses (0.06–0.7 μM) does not affect normal cells but it stops cell cycle progression from G1 to S phase in human leukemia cells (HL-60) [56]. At a higher concentration (3–6 μM), oligomycin has arrested over 50% of HL-60 cells in the G2/M phase, but this concentration has an impact on normal cells. Rotenon, typical inhibitor of respiratory complex I, arrests the cell cycle in G2/M phase with strong inhibition (50–90%) of cell proliferation in human lymphoma WP and 134B osteosarcoma at concentration (0.1–1 μM) [57]. This effect is related to a severe diminution of the proton gradient across the inner mitochondrial membrane, but also to an increase in the membrane fluidity and activation of apoptosis [58]. Still, there are a lot of drugs with side effects to normal cells. Therefore, therapies that are able to specifically target the respiratory chain to further elevate ROS production in cancer cells should selectively precipitate these cells into apoptosis. An example of such agents targeting mitochondria as anticancer drugs (mitocans, like vitamin E succinate or vitamin K3) have been recently reviewed [59–61]. For example, [62] have shown specific mitochondrial inhibitors of succinate-quinone reductase/complex II, which regulate production of reactive oxygen species in mitochondria and protect normal cells from ischemic damage but induce specific cancer cell death.

Conclusion

Cancer kills more than six million people worldwide every year [63]. The small decrease in some types of cancer is not attributed only to better therapies but also to the implementation of prevention and early detection campaigns. Despite these campaigns, the low efficiency of chemotherapy in patients with advanced cancers is reflected in the low, five year survival rates observed in these patients [64]. A novel therapeutic approach has emerged during the last decade. This approach seeks to attack the tumor cells selectively and is based on understanding differences between tumor cells and nonmalignant cells, particularly the intracellular organelles, such as mitochondria.

Damage to the mitochondria is at the crossroad between normal metabolism and the regulation of cell death, which is a promising direction for the development of new therapies. Molecules that can reverse malignant cells from hyperglycolytic state and, simultaneously, increase their sensitivity to induction of apoptosis appear to be very effective anticancer agents. Recent analyses of human cancers have revealed, however, that the genetic defects of tumor cells are much more numerous and unstable than expected. It varies by type of cancer of the patient, which means that two people with the same type of cancer do not have the same changes in the level of genes. Despite the complexity of the cancer genome, much effort is devoted to characterize the genetic profile of tumors with the aim of rationalization and personalization of cancer therapy.

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