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Targeting mitochondria for cancer treatment

Individuazione dei mitocondri per il trattamento del cancro

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Summary

The paper is aimed to overview the published papers for analysis of the cancer treatment by restoration of normal physiological processes, in particular electromagnetic activity and apoptotic function of glycolytic phenotype cancer cells. Achievement of this target is based on estimation of the treatment based on bringing back normal mitochondrial function through inhibition of molecules (kinases) blocking the pyruvate pathway using dichloroacetate (DCA) or drugs with similar properties. This method is supported by considerable *in vitro* and *in vivo* experience by DCA treatment even in humans. Opening pyruvate pathway restores the proton space charge layer, the strong static electric field around mitochondria, ordering of water, and induction of strong non-linearity of microtubule electrically polar oscillations. Normal level of excitation of oscillations dependent on energy liberated from mitochondria and low damping keeps running physical processes regulated by electromagnetic field. DCA may act on a wide spectrum of cancers. Cancer cells might be brought back to a

Riassunto

Lo studio cerca di fornire una visione d'insieme dei lavori pubblicati per l'analisi del trattamento del cancro tramite ripristino dei normali processi fisiologici, in particolare attività elettromagnetica e funzione apoptotica del fenotipo glicolico delle cellule tumorali. Il raggiungimento di questo obiettivo si basa sulla valutazione del trattamento inteso a riportare la normale funzione mitocondriale attraverso l'inibizione delle molecole (chinasi) bloccando la via del piruvato utilizzando il dicloroacetato (DCA) o farmaci con proprietà simili. Questo metodo è supportato da un notevole numero di esperienze *in vivo* e *in vitro* mediante trattamento con DCA anche negli esseri umani. L'attivazione della via del piruvato ripristina lo stato di cariche protoniche spaziali, il campo elettrostatico forte attorno ai mitocondri, l'orientamento dell'acqua, e l'induzione di una forte nonlinearità delle oscillazioni elettricamente polari dei mitocondri. Il normale livello di eccitazione delle oscillazioni che dipendono dall' energia liberata dai mitocondri e il basso smorzamento continuanoa far funzionare i processi fisici regolati da

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normal healthy state or be subject to apoptosis by DCA or a similar drug treatment. Clinical trials should test this method, verify the results, determine effectiveness, and assess adjuvant treatment methods. Experience with DCA would accumulate experimental data for development of novel drugs for cancer treatment through restoring the mitochondrial function. Eur. J. Oncol., 17 (1), 23-36, 2012

campo elettromagnetico. Il DCA può agire su un ampio spettro di tumori. Le cellule tumorali possono essere ricondotte a uno stato normale sano o essere soggette ad apoptosi dovuta a DCA o a trattamento farmacologico simile. Gli studi clinici dovrebbero testare questo metodo, verificare i risultati, determinarne l'efficacia e valutare metodi di trattamento adiuvante. L'esperienza con DCA sarebbe in grado di accumulare dati sperimentali per lo sviluppo di nuovi farmaci per il trattamento del cancro attraverso il ripristino della funzione mitocondriale.. Eur. J. Oncol., 17 (1), 23-36, 2012

Key words: **Cancer glycolytic phenotype, mitochondrial dysfunction, dichloroacetate (DCA), physical processes in cells, apoptosis**

Parole chiave: **fenotipo glicolitico del cancro, disfunzione mitocondriale, dicloroacetato (DCA), processi fisici nelle cellule, apoptosi**

Introduction

The organization of a biological system is not only organization of the form (morphogenesis, chemical reactions, and genetic code expression), but also organization of physical forces and information transfer between its different parts. But physical mechanisms of biological activity and high capacity information transfer have been neglected. A concept of organization based on the rôle of physical fields in living matter was formulated by H. Fröhlich who postulated long-range quantum mechanical phase correlations, existence of electrically polar vibration modes excited far from thermal equilibrium, and generation of endogenous coherent electromagnetic field in biological systems (1-7). Fröhlich predicted the rôle of endogenous electromagnetic field in cancer development (8). Microtubules were assumed to form the generating structures in the eucaryotic cells (9-13). Mitochondria create fundamental conditions for generation of the cellular electromagnetic field including energy supply for excitation of oscillations (14-16). Positively charged protons transferred from the matrix space produce strong static electric field around mitochondria (17) which contributes to interfacial ordering of water (18-21). Disturbances of mitochondrial function are detrimental to biological activity and form an essential base for pathological states (14-16). O. Warburg disclosed that cancer cells display decreased oxidative metabolism (22) caused by mitochondrial dysfunction (23). Warburg effect was assumed to be a secondary side effect but this point of view was revisited in the last decade (24-27). Analysis of the rôle of mitochondrial dysfunction in cancer process and its treatment is an urgent question.

It is extremely rare and difficult to find an essential target that is unique to cancer cell and that may be utilized for development of selectively acting drugs. But another reason for a poor performance of anticancer drugs exists. Heterogeneity and adaptability of cancer cells, that make possible to escape treatment effects, are a general feature of the cancer process. For instance, recurrence after definitive radiation therapy for localized prostate cancer is a common phenomenon (28). Any reasonable analysis of the problem cannot omit the Warburg effect, i.e. suppression of oxidative mitochondrial metabolism, a distinct difference between a healthy and a cancer cell (24-27). Almost all cancer cells demonstrate enhanced uptake and utilization of glucose by increased fermentation, whereas mitochondrial activity is suppressed, including its key rôle in regulation of cell death pathways (29). Mechanism of the unique metabolic profile of a type of cancers was disclosed by Bonnet *et al.* (30). Some human cancers have high

mitochondrial membrane potential, low expression of the K^+ channel Kv1.5, and apoptotic resistance. Pyruvate dehydrogenase kinase (PDK) blocks the pyruvate pathway in mitochondria. Glycolytic phenotype forms an essential difference between healthy and a large group of cancer cells. (In the wide sense the glycolytic phenotype might include all types of mitochondrial defects. In the narrow sense the glycolytic phenotype contains only mitochondrial disturbances formed by interruption of pyruvate pathway in mitochondria. The latter mechanism seems to be generally connected with the glycolytic phenotype term. In either case the glycolysis is enhanced).

Mitochondrial dysfunction is an active contributor to the malignant process or its essential part. Pyruvate dehydrogenase (PDH) complex is regulated by PDK's (isoforms PDK-1–4) and pyruvate dehydrogenase phosphatases (PDP-1, 2). PDK-1 was identified to cause decreased glucose oxidation through PDHα phosphorylation and PDH complex inhibition (31). Different groups of pathways and oncogenes result in resistance to apoptosis and a glycolytic phenotype. The glycolytic phenotype is a widespread phenomenon in solid cancer forms. Experimental results in vitro and in vivo confirmed reversal of the glycolytic phenotype of the metastatic breast cancer cells through inhibition of PDK by dichloroacetate (DCA-sodium salt of dichloroacetic acid). Reversal of glycolytic phenotype and reduction in the number of metastatic lung tumors by DCA was observed (32). Connection of the Warburg effect with genetic code alterations should be analyzed. Mitochondrial genome (mtDNA) represents only about 1% of the cellular DNA, but its gene products are essential for normal cell function. Accumulation of mutations in mtDNA is approximately tenfold greater than that in nuclear DNA (33).

Treatment through anti-cancer drugs targeting mitochondrial defects and triggering apoptotic function offer a selective method for restoring normal function of mitochondria and cancer cells or their natural programmed killing. Published papers very often describe drugs targeted to mitochondria under a general name "mitocans". In particular, effects of various small molecules on mitochondrial function are examined. Natural compounds and their analogues that preferentially impact cancer with mitochondrial dysfunction are overviewed in Chen *et al.* (34). Some of the drugs directly target mitochondria or indirectly affect their function. A review of published papers on targeting mitochondria for cancer therapy is in Fulda *et al.* (35).

DCA is a drug that very likely attacks the widest spectrum of cancers. DCA restores the healthy state from glycolytic phenotype cells that induces apoptosis of too much damaged cells (30, 36-37). Mitochondria from glioblastoma (GBM) cells showed hyperpolarization that was reversed by DCA. DCA also increases mitochondrial reactive oxygen species and induces apoptosis in GBM cells. The target of DCA, PDK-2 is highly expressed in GBM tumor (38). Isoforms PDK-1, 2, and 4 were inhibited by DCA but PDK-3 was not (32). DCA may significantly reduce cellular proliferation and sensitize prostate cancer cells (overexpressing Bcl-2) to radiation (28). DCA may enhance cell death in combination with oncolytic adenoviruses (39). Cytotoxicity of DCA in combination with anti-cancer platinum compounds was studied in vitro. DCA significantly increased toxicity of carboplatin, satraplatin, JM118, and oxoplatin, but not cisplatin, picoplatin, and oxaliplatin (40). Significant palliative effects of DCA were observed in a patient with cancer of unknown origin with metastases to liver and neck (41).

Nevertheless, some results of DCA treatment are not positive. Effect of DCA on reduced PDHα phosphorylation was not clearly demonstrated by experimental results published in McFate *et al.* (31). The study suggested a need for developing better PDK inhibitors. Treatment by DCA may be connected with encephalopathy and polyneurophaty. Confusion, gait disturbances, impaired mental processing, aggressive behavior, hallucinations, and dysphasia were observed (42). The side effects of DCA treatment are age and dose dependent (for instance reversible peripheral neuropathy and neurotoxicity) (43). Adults are considerably more susceptible to these adverse effects than children.

The chlorinated acetic acid, in particular dichloroacetic acid (denoted here by DCAa) exists as chlorine disinfection by-products in drinking water supplies. Chronic study on male Fisher (F344) rats to 1.6 g/l DCAa in their drinking water (corresponding to the time-weighted mean daily doses of 139 mg/kg/day) displays a statistically significant increase of carcinogenicity (hepatocellular carci-

noma) (44). Six differentially expressed genes were found in mice (B6C3F1 strain) treated with 2 g/l DCAa in drinking water (corresponding to about 400 mg/kg/day). The expression pattern of four of these genes was similar in DCAa-induced hepatocellular carcinomas. Hepatocellular carcinomas were induced in male mice exposed to 3.5 g/l in drinking water (of about 700 mg/kg/day) for 93 weeks (45). At the concentration of 20 mM/l (of about 130 mg/l) and 576 days of exposure increased incidence of hepatocellular carcinoma was observed on B6C3F1 mice (46). DCA was administered to pathologic states associated with mitochondrial energy failure, for instance as a metabolic modulator or treatment drug of human hereditary mitochondrial metabolic diseases such as congenital lactic acidosis (CLA) and hyperlactatemia. Stackpoole *et al.* (47) described inhibition of PDK by DCA and restoration of PDH complex activity. Toxicity of DCA (administered at 25 mg/kg/day) was examined in healthy adults (men and women) and CLA children patients to test whether the chronic use of the chemical causes hepatocellular, peripheral nerve, ocular, or renal toxicity. The DCA appears to be relatively nontoxic when acutely administered to healthy adults or to patients with acquired lactic acidosis. There was no clinical evidence that long-term administration at low doses of DCA causes toxic effects to eyes, kidneys, or gonads or induces neoplasia in any human tissue. It was not determined whether important differences in DCA metabolism and toxicology exist in humans for its low and high doses (47).

Small molecules targeting mitochondria as anticancer agents are described in Wang *et al.* (48). For instance, altered mitochondrial transmembrane potential is targeted by Rhodamin-123, cancer mitochondrial respiration (low electron transfer activity) by arsenic trioxide and vitamin E analogues, cancer mitochondrial membrane permeability by betulinic acid, Bcl-2 inhibitors by gossypol, mtDNA by cisplatin, altered metabolism associated with mitochondrial dysfunction by DCA. Mitochondria as an intrinsic and extrinsic regulator of apoptosis are described in Mammucari *et al.* (49).

Natural vitamin E includes molecules of tocopherols and tocotrienols (both of them α-, β-, γ-, δ-). Vitamin E subcomponents or their analogues display anti-cancer effects. For instance, α-tocopherol inhibits cell proliferation. α- and γ-tocotrienol are effective against sarcoma 180 cells, Ehrlich carcinoma, and invasive mammary carcinoma cells (50). An important rôle of vitamin E is in scavenging reaction on reactive oxygen and nitrogen species (51). Vitamin E derivates are a novel group of anticancer agents targeting mitochondria. They cause mitochondrial destabilization with activation of mitochondrial mediators of apoptosis (52). Vitamin E analogues such as α-tocopheryl succinate (α-TOS) or α-tocopheryl acetyl ether (α-TEA) are selective inducers of apoptosis in cancer cells and efficient suppressors of tumors acting on mitochondrial complex II (CII). Effects of modified vitamin E succinate are analyzed in Dong *et al.* (53). Drugs possessing anti-cancer activity by destabilizing mitochondria may be modified with mitochondria targeting molecules, for example tagging them with lipophilic cations (54). Antiproliferative activity of the vitamin E analogues is described in Neuzil *et al.* (55).

Frataxin is a natural protein acting on oxidative process. Frataxin promotes antioxidant defense resulting in diminished malignant transformation in vitro (56-57). Disruption of frataxin expression impairs mitochondrial function (58).

Attempt for combination of cisplatin with two dichloroacetic acid units in one molecule (called mitaplatin) for cancer treatment is described in Dhar *et al.* (59). Fig. 1 shows the mitaplatin molecule that after entering the cell is reduced to cisplatin and dichloroacetic acid. Dichloroacetic acid inhibits PDK in mitochondria and cisplatin interacts with nuclear DNA. The cytotoxicity of mitaplatin in a variety of cancer cell lines equals or exceeds that of all known Pt(IV) compounds and is comparable to that of cisplatin. Mitaplatin alters the mitochondrial membrane potential gradient of cancer cells, promoting apoptosis by releasing cytochrome c and translocating apoptosis inducing factor from mitochondria to the nucleus. In vitro experiments were performed on cancerous NTera-2 and healthy fibroblasts cells, and human lung cancer A549 and human lung fibroblast MRC-5 cells.

Physical mechanisms in cell will be overviewed and their disturbances in cancer process analyzed. Cancer treatment in humans through mitochondria remodeling on the basis of the present knowledge will be presented too.

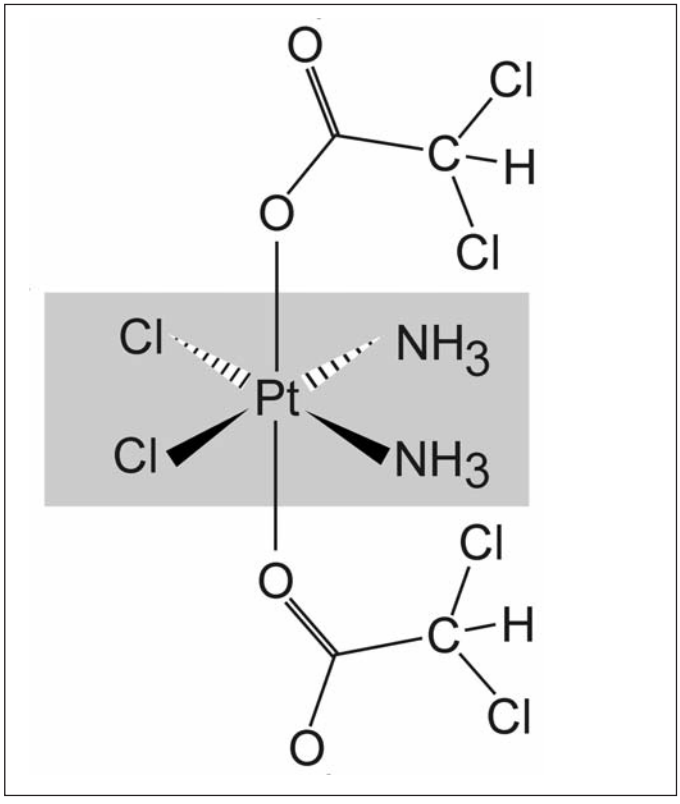

Fig. 1. Combination of cisplatin with dichloroacetic acid for cancer treatment.

Energy metabolism

Glycolysis and mitochondrial oxidative metabolism are the basic processes of energy supply. Pyruvate (produced by glycolysis) and fatty acids are transported to mitochondrial matrix, broken to acetyl that is bound to coenzyme A (CoA). In the citric acid cycle the energy of the acetyl CoA is used for pumping protons across the inner mitochondrial membrane and transformed into electrochemical proton gradient energy. A strong static electric field around mitochondrion is formed. The strong static electric field is measurable up to a distance of several μm's from a mitochondrion. Intensity of the electric field was measured by fluorescent particles of 30 nm in diameter sensitive to electric field (17). At the outer mitochondrial membrane intensity of the electric field is of about 3.5 MV/m. The mitochondria may occupy of about 22 % of the cellular volume and, therefore, the mitochondrial static electric field fills up the rest of the volume. Cytosol, cytoskeleton, and biological molecules are electrically polarized.

Production of ATP is driven by the electrochemical proton gradient across the inner mitochondrial membrane. The efficiency of ATP production greater than about 40% is measured. The rest of the energy that is nearly about 60% [often denoted as "wasted" energy (60)] is liberated from a mitochondrion as heat, photons, and remaining chemical energy (for instance of reactive oxygen species – ROS). Emission of UV photons was observed in the course of citric acid cycle (61-63).

A near zone electromagnetic field is assumed to be generated by cellular cytoskeleton that forms the oscillating structure (9-11, 14-16). Cytoskeleton is a filamentary structure organizing the cell. It participates in cell motility, ribosomal and vesical transport, mitosis, and transduction of pressure and tension. Actin filaments, intermediate filaments, microtubules, and associated proteins are the main components of the cytoskeleton. The cytoskeleton is in a state of dynamic equilibrium (tensegrity) when some filamentary structures are contracted and other filaments are under pressure (64-65). Pressure in the cell is resisted by microtubules. Elastic modulus of a cell depends on the cytoskeleton state and changes as a result of internal cellular organization, biochemical conditions, and pathological state.

The microtubule structure may be non-linear by itself but non-linear properties may depend on the static electric field generated by mitochondria too. Microtubules form an electrically polar structure with energy supply. Structure of microtubules satisfies conditions for excitation of Fröhlich's coherent electrical vibrations (9-11).

In the interphase mitochondria are aligned along microtubules, the main organizers of the cellular cytoskeleton. In the M phase distribution of mitochondria is not known. Microtubule subunits, the tubulin heterodimers, are strong electric dipoles. Microtubule mechanical vibration analysis disclosed that different modes may have frequencies from acoustic up to the GHz region (66-68). Electrodynamic oscillations were observed at about 8-9 MHz (11) and 465 MHz (69). The microtubules seem to form a main structure for Fröhlich's coherent vibrations. Conditions for non-linear oscillations may depend on the strong static electric field of mitochondria. Energy is supplied to microtubules by hydrolysis of GTP (guanosine triphosphate) to GDP

(guanosine diphosphate) in β tubulin after polymerization, by motor proteins moving along microtubules, and by non-utilized energy liberated from mitochondria. The supply of mitochondrial non-utilized energy may be dominant (14-16).

Fig. 2 shows a theoretical model of the physical links of biological activity based on cooperation of mitochondria and microtubules. Mitochondria form conditions for coherent excitation of oscillations in microtubules by energy supply, low damping, and shift of oscillations into highly non-linear region.

Water ordering

Water ordering is very likely a crucial phenomenon for biological activity of living cells. Excitation of coherent elasto-electrical oscillations depends on low damping of cytoskeleton vibration system conditioned by water ordering. A few most fundamental concepts about the cellular water will be pointed out. Ordering of the liquid phase of water depends on the static electric field. A strong static electric field is excited by surface charges of molecules and subcellular stuctures (70). For instance, the ordered water around microtubules forms cylindrical layers (clear or exclusion zones) 5-20 nm thick depending on concentration of cations (18, 71). The clear zones of layered water may cause significant reduction of microtubule vibration damping.

Ordering of the water molecules takes place in the form of layers parallel to the surface in the ideal case of a flat plane interface. The organization of water at the interface, which contains several layers, may be measured through atomic force microscope (72-73).

Organization of water by the electric field is a general feature and may be also achieved by electric field of external sources. Fuchs *et al.* (74-76) and Giuliani *et al.* (77) have shown that strong electric field (about 600-700 kV/m) organizes water and forms a floating water bridge (about 1-3 cm long) between two glass beakers.

Many interfaces are present in the cell (average distance between non-water molecules in a cell is of about 2 nm), thus all of the cellular water will be ordered to some degree. The ordered water shows different physical properties compared to bulk water including higher viscosity (78), different pH (79), decreased thermal motion of molecules (21), and different spectroscopic properties (80). Ordered layers of water exhibit separation of charge (79) and solvent exclusion (20-21). Infrared radiation promotes formation of the exclusion zones (79). Properties of ordered water are described for instance in (78, 81). Ordering of water may extend to

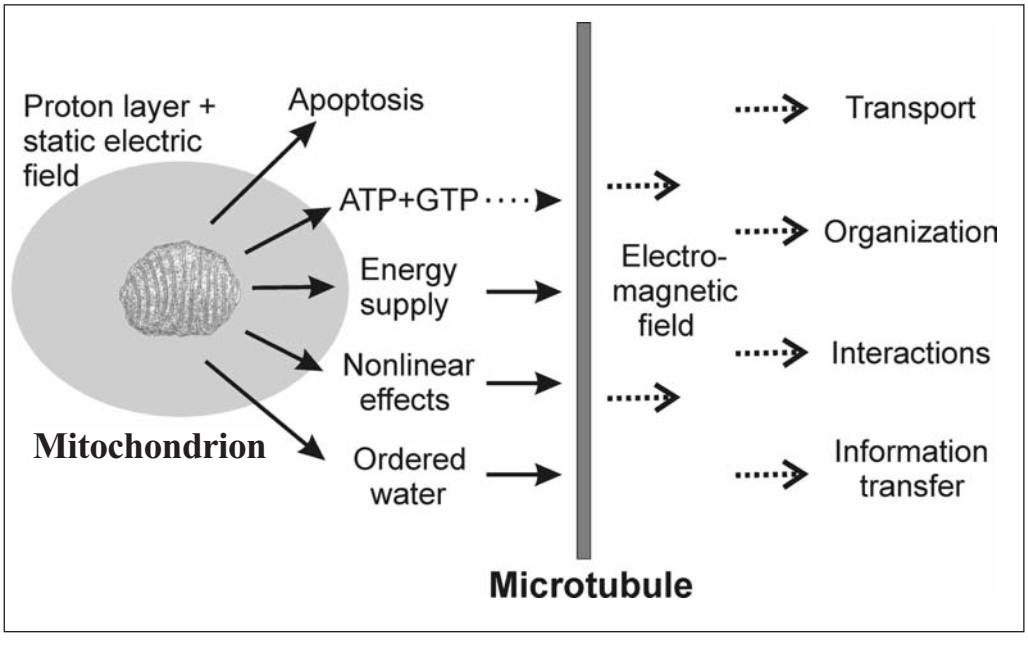

Fig. 2. Physical processes in a normal living cell based on cooperation of mitochondria and microtubules in generation of electromagnetic field. A strong static electric field causes ordering of water and shifts oscillations into a highly non-linear region. Efflux of energy non- utilized for production of ATP and GTP may supply energy to microtubules. Energy from ATP and GTP is delivered through motion of motor proteins and by hydrolysis after microtubule polymerization, respectively.

a distance of few hundreds of micrometers from the surface as was found by Zheng and Pollack and Zheng *et al.* (20, 21).

Based on the quantum electrodynamic theory Preparata (82) and Del Giudice *et al.* (83) analyzed the structure of water and proved that the "liquid" water is actually two mixed phases of water: coherent domains which form ordered water phase and gas-like water phase. The clear (or exclusion) zones observed at the interfaces are actually macroscopic manifestation of a partial separation of ordered water domains and bulk water regions. Coherent domains are a reservoir of quasi-free electrons and may have a tremendous impact on the biological activity (83-84). Theoretical treatment that does not take into consideration formation of coherent domains (85) contradicts the experimental findings (20, 74-77).

Mitochondrial water organization deserves a special attention. Mitochondria contain ordered water within their inner polar interfaces (86) and water is ordered by mitochondria also in their surroundings (87). Fully functional mitochondria are a source of a strong static electric field (17), which was measured to a distance of several μm's. Level of mitochondrial water ordering is different in healthy and cancer cells.

Mitochondrial dysfunction

Otto Warburg disclosed suppression of the oxidative mechanism of ATP and GTP production and its replacement by fermentative process in cancer cells (22). Only about a half of the ATP cell production is covered by mitochondrial supply (23). Warburg assessed essential influence of mitochondrial dysfunction on cancer cell development and properties and predicted that "The adenosine triphosphate synthesized by respiration therefore involves more structure than adenosine triphosphate synthesized by fermentation." (23). Mitochondrial dysfunction was found in many types of cancer and is also denoted by Cuezwa *et al.* as "the bioenergetic signature of cancer" (25). Function of mitochondria is suppressed, for instance, in human breast, ovarian, colorectal, and brain cancer. An overview was published by Carew and Huang (24). A different group of signaling pathways and oncogenes results in resistance to apoptosis and in a glycolytic phenotype, the majority of carcinoma has hyperpolarized mitochondria, and most solid state tumors have increased glucose uptake (36). (But only some types of glycolytic phenotype tumors were treated, in particular, non-small cell lung cancer, glioblastoma, breast, endometrial, and prostate cancer.) This suggests that drugs targeted to glycolytic phenotype, such as DCA, may be effective in a large number of different tumors. On the other hand the lack of mitochondrial hyperpolarization in certain types of cancer, including oat cell lung cancer (that belongs to the small-cell lung cancer), lymphomas, neuroblastomas and sarcomas (36), suggests another type(s) of energy production defects and apoptotic blocking.

The energy stored in the mitochondrial membrane potential and used for production of ATP is a regulator of life and death (37). Blockade of normal mitochondrial function in the glycolytic phenotype cancer cells is caused by phosphorylation of PDH enzyme family that breaks down pyruvate and binds the acetyl group to CoA (coenzyme A). Fig. 3 shows a schematic picture of a glycolytic phenotype of a cancer cell. Function of the enzymes PDH is blocked by PDK. Hyperpolarization of the mitochondrial inner membrane is accompanied by diminution or strong suppression of the layer of the static electric field, by the decrease of the non-utilized energy efflux, and by low expression of the K^+ channels.

Mitochondrial dysfunction evolves in precancerous stages along the cancer transformation pathway. Development of mitochondrial dysfunction was analyzed in cervical lesions. Immunity response of cervical cancer patients to different antigens was investigated. Antigens were prepared from cervical tumors (cervical antigen) and from blood of mice infected with the lactate dehydrogenase (LDH) virus (LDH antigen) (88). In the presence of cervical or LDH antigen T lymphocytes prepared from blood of a cervical cancer patient display decreased adherence properties to solid state surfaces. Immunity response to cervical antigen is specific with respect to cervical cancers. LDH antigen displays similar adherence defect in all cancers that were investigated. Therefore, LDH antigen contains a component that is common to cancer process. LDH virus, a

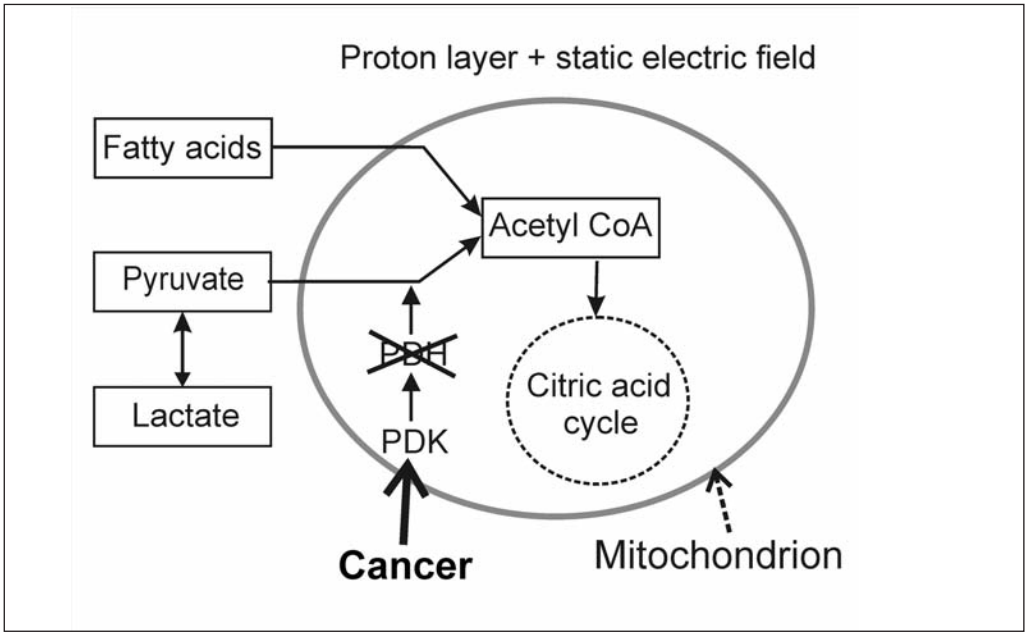

Fig. 3. Glycolytic phenotype of a cancer cell. The pyruvate pathway in the mitochondria is blocked through phosphorylation of PDH enzymes by PDK. DCA inhibits PDK and restores PDH activity and normal mitochondrial function. Another defects, for instance in the citric acid cycle, may cause diminution of the proton transfer from the mitochondrial matrix.

parasite on cellular energy system, is observed at mitochondria. Therefore, the immune response to the LDH antigen is assumed to reveal mitochondrial defects in energy production activity. In cervical lesions the observed adherence defect, i.e. mitochondrial dysfunction gradually develops in the period in which the precancerous cell transforms to cancerous one (88). Dysfunction of mitochondria triggers a key mechanism of malignity in cancer. Sensitivity of T lymphocytes to LDH virus antigen was also detected in patient with different diseases [besides cancer also in acute myocardial infarction and schizophrenia (89)]. These findings might suggest connection of different pathological states with energy parasitic effects of LDH virus.

DCA disturbs PDK and reverses mitochondria to normal function (30). Functional mitochondria may restore the normal cell function or switch on apoptosis of too aberrant cells. Nevertheless, DCA attacks PDK and not the mechanism of its pathological production.

Cytoskeleton disturbances

Cytoskeleton disturbances are induced along the pathway of cancer cell development before malignant properties are formed (90). Mechanical deformability of human epithelial breast cells may display the relationship between cancer promotion and cytoskeleton defects. For instance, deformability of human non tumorigenic epithelial breast cells (MCF-10), of not metastatic cancer (adenocarcinoma) cells (MCF-7), and increased metastatic potential cells (modMCF-7) is 10, 20, and 30%, respectively. Mechanical properties of human pancreatic cells (Panc-1) were measured by Beil *et al.* (91), Suresh *et al.* (92), and Suresh (93). After application of sphingosylphosphorylcholine (SPC) which plays a critical rôle in the metastatic invasion of gastrointestinal cancers, the keratin network is pathologically reorganized. Keratin structure shrinks around the nucleus and the distance between the nuclear membrane and the outer edge of the keratin structure is reduced to about 50% of the unperturbed value. In this case dynamic deformation experiment disclosed that the relative spring constant decreased to about 40%, and energy dissipated per one cycle of measurement increased to about 400% in comparison with the value before application of SPC. Morphological changes which are used for cytological and histological assessment of cancer evolution may result from cytoskeleton defects induced along the pathway of cancer transformation.

Mitochondrial dysfunction results in disturbances of cytoskeleton generated electromagnetic field, in particular its low power and diminished coherence. Tensegrity of the cytoskeleton is disturbed. Interac-

tion forces between cancer cells are lowered. Cancer cells are attracted by the healthy cells into healthy tissue; this may represent the mechanism of the local invasion (94). After further decrease of the power and coherence of cytoskeleton oscillations and additional defects of the cytoskeleton structure the spatial and the spectral pattern of the generated electrodynamic field might be damaged to such extent that the cancer cell may evade from interaction with its surroundings, liberate, and form metastatic nodules in distant organs. These physical mechanisms have to be closely connected with the extracellular matrix defects. It should be emphasized that cancer problem cannot be solved without understanding the electromagnetic activity of cells.

DCA cancer treatment in humans

Patients with advanced stage of cancer are treated by DCA in the Medicor Cancer Centres in Toronto (95). A wide spectrum of various tumors was treated. For instance, metastatic renal, lung, and ovarian carcinoma, mesothelioma, glioblastoma, and melanoma with brain metastasis were reported. The minimum and the maximum doses were 10 and 25-50 mg/kg/day, respectively. The courses were continual or cyclic (1-3 weeks on followed by 1 week off). Duration of the treatment should be at least one month. Doses are limited by severity of the side effects. Individual response is similar to that of conventional chemotherapy. For a dose greater than about 20 mg/kg/day response is developed within 2-4 weeks. For smaller doses the response is weaker or delayed. Positive response was experienced by 60-70% of treated patients.

The side effects are dose and age dependent. In some experimental animals dichloroacetic acid may induce a liver cancer by long-term exposure and doses 100-1000 mg/kg/day (44-46). However, in humans a short-term DCA administration appears to be relatively nontoxic. Exposure to low doses of about 25 mg/kg/day for several months did not reveal adverse effects (47). The side effects are mild and reversible in the majority of cases (95). The side effects are of neurological and gastrointestinal origin. Neurological side effects include peripheral neuropathy (which is age dependent for doses of about 50 mg/kg/day and higher), sedation, fatigue, confusion, hallucinations, memory problems, hand tremor, and gait disturbances. Gastrointestinal side effects may be manifested as heartburn, nausea, vomiting (stomach upset), and indigestion. Mechanisms of the side effects are not elucidated. (The side effects might be caused by action of DCA on body regulations.) After starting treatment some patients experienced pains at the sites of their tumor(s) within the first few days. It was only a transient effect and within a short time the pains disappeared.

The most dangerous process is the tumor lysogenic syndrome. If a large number of tumor cells are rapidly killed, a sudden release of the content of the dead cells into the blood stream may cause abnormal heart rhythms and kidney failure.

DCA has anti-tumor effects, relatively low toxicity (however, dependent on doses and period of administration) and low cost. In the positively responded patients DCA may have palliative effect but it may also transform aggressive cancer form from a fatal disease to a chronic disease with simple medications. Medicor Cancer Centres announced about 800 treated patients up to the end of the year 2011. All these patients were previously treated by standard methods, but the treatment was either ineffective or could not continue.

Discussion

Physical processes in living cells are very often not considered as a part of biological activity. It may lead to neglect of important mechanisms and misunderstanding of some links along the cancer transformation pathway. The disregard of physical processes in living cells is also based on rejection of Fröhlich's hypothesis. Ordering of water was not taken into consideration and totally omitted by some workers (96-98). They assumed highly damped oscillators in cells which contradicts the aspects of ordered water. Reimers *et al.* (97), and McKemmish *et al.* (98) in their assessment of the Fröhlich's energy condensation hypothesis (1-3, 8) analyzed non-linear relation for spectral energy transfer with constant coefficients and used a parameter roughly corresponding to SQ (where S is energy supply and Q is the quality factor). Therefore, their method of

analysis makes the *Q* factor – an essential parameter of oscillation systems – indistinguishable (in different cases with the same *SQ* values) too. The results are not applicable to living cells as the parameters of ordered water are quite different from those of bulk water (97, 98).

Physical processes are closely connected with mitochondrial function. Mitochondria play a unique role in cells. Mitochondrial function is regulated by chemical messengers. But besides release of chemical apoptotic triggers their main role is not in the chemical region. Mitochondria are the boundary entities between chemical and genetic processes on the one hand and physical ones on the other hand. Mitochondria are producers of energy in the form of ATP and GTP. But mitochondrial function in physical processes cannot be reduced to energy production. Mitochondria create a layer of the proton space charge and of a strong static electric field around them with consequences in non-linear effects and water ordering. Mitochondria are multifunctional organelles and play a critical role in cell organization and activity. Mitochondrial dysfunction degrades physical processes in cells. Cancer transformation contains mitochondrial links.

Cancer is a complex microevolution process. Cancer transformation pathway is a multistep and multibranched development of a fatal disease. Cancer utilizes and gradually alters essential life mechanisms. Genetic, chemical, and physical mechanisms are mutually coupled. Very often only quantitative differences of processes in healthy and cancer cells are observed. Due to adaptability of cancer cells any one-point treatment may be overcome by an altered cancer mechanism. The treatment should be complex, target essential links in different branches along the cancer transformation pathway, and based on combination of convenient chemical compounds.

Heterogeneity and adaptability is one of the obstacles to effective cancer treatment. Another obstacle may be caused by the character of the differences between healthy and cancer cells. The differences may be only of quantitative type. Any cancer treatment based on therapeutic strategy of killing the cancer cells may also damage healthy cells. The degree of the healthy cells impairment may depend on the relationship between quantitative levels of processes in healthy and cancer cells. Therefore, the negative side effects have to be individual and may vary from negligible to serious. The difference in increased fermentative production level of ATP in cancer cells may belong to quantitative types. The therapeutic strategy based on killing the cancer cells through inhibition of fermentative production [for instance by 2-deoxyglucose or 3-bromopyruvate (99)] may cause consequences to healthy cells.

The therapeutic strategy should be based on differences between healthy and cancer cells and methods that may render reliable results with small side effects. One of the most remarkable differences between the healthy and cancers cells is the dysfunction of mitochondria. Restoration of their function revives the physical processes in the cell and unlocks the apoptotic pathway. Decreased power, coherence, and disturbed spectrum and spatial patterns of the endogenous electromagnetic field in cancer cells are the fundamental functional differences in comparison with healthy cells. If the cell systems are damaged too much (for instance by disorganization of the cytoskeleton or the DNA structure) the cell undergoes the preprogrammed death. Targeting mitochondria very likely affects the region of the main differences between the healthy and the cancer cells and restores the physical processes. The therapeutic strategy is pointed to restoration of normal function of the cell.

DCA returns the mitochondrial membrane potential towards the normal physiological level without affecting the mitochondria of non-cancerous cells (100). DCA is an anti-tumor drug with generally mild side effects. It may be used against cancers in a wide spectrum of their types. A substantial preclinical evidence of DCA utilization in vitro and in vivo (28, 39, 40, 42, 43) and also experience in cancer treatment in humans are present (38, 41, 95, 101). Early-phase clinical trials should be started to determine the possible role of DCA in cancer treatment. Clinical trials would disclose the tumor reaction and development, patient states determined from laboratory tests and measurements, and specific symptoms after DCA application. Clinical trials with DCA may determine specific requirements for the future drug development (for instance for inhibition of the mechanism of pathological production of PDK).

Variability of cancer mechanisms is enormous. Even Warburg effect may exist in a reverse form in tumors. Fibroblasts associated with cancer cells produce pyruvate by aerobic glycolysis. Pyruvate reduced to lactate is transported to cancer cells for utilization in efficient mitochondrial production (102).

Conclusion

Mitochondria are multifunctional organelles that are important for cell organization and function. Mitochondrial dysfunction developed along cancer transformation pathway degrades physical processes and blocks apoptosis of the cell. Targeting mitochondria affects the region of significant difference between healthy and cancer cells and makes possible to restore the physical processes (in particular normal level of electromagnetic activity) in cells and the apoptotic function. Restoration of mitochondrial function cuts off a considerable part of cancer mechanisms.

The glycolytic phenotype was found in several types of cancers. DCA may be effective in restoring mitochondrial function and apoptosis by inhibiting pyruvate dehydrogenase kinases in a considerably wide spectrum of cancer types. Preclinical evidence of DCA utilization in vitro and in vivo and experience in cancer treatment may be found in literature (28, 38-43, 95, 101). Early clinical trials by DCA treatment should be started to verify the effects of the treatment method and to accumulate experience. DCA application may increase positive results in cancer treatment and contribute to better understanding of the cancer process for future synthesis of more effective drugs for mitochondrial cancer treatment, for instance for inhibiting pathological PDK production.

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