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Metabolic hallmarks of cancer cells as targets for integrative therapies

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Introduction

In 2018, 18.1 million people around the world had cancer, and 9.6 million died from the disease. By 2040, those figures will nearly double [1]. An increasing body of evidence is suggesting that the reason for this evolution is twofold: on the one hand, environmental, nutritional and lifestyle factors play an important role in the disease etiology, while, on the other hand, most current therapies are killing tumor and healthy cells alike, instead of specifically targeting common metabolic hallmarks of cancer cells. Among the latter we could mention: the significant changes of pH and redox potential inside and outside the tumor cells, their energy generating pathways relying on aerobic glycolysis, glutaminolysis or fatty acid oxidation, the activation of the NADPH:quinone-oxidoreductases, as well as the high accumulation of transitional metals and organic pollutants in the tumor tissue accompanied by inactivation of several antioxidative and detox systems. This review concentrates on the metabolic and toxicologic markers of cancer cells and proposes a new integrative approach in this respect.

Role of metal accumulation in cancer etiology and therapy

In recent decades, the presence of transition metals such as iron, copper, nickel or chromium in connection with the production of free radicals through Fenton/Haber-Weiss reactions, autoxidation of ascorbate, peroxidation processes of fatty acids and formation of DNA strand breaks has been reported [2,3]. In order to explore the connection between environment and cancer growth in humans, we assessed the accumulation of transitional metals and aluminum in healthy and breast cancer biopsies. Our Atomic Absorption Spectrometry measurements showed for the first time a highly significant accumulation of iron, nickel, chrome, zinc, cadmium, mercury, lead and aluminum in breast tumor biopsies, when compared to the control biopsies [4,5] **(Figure 1)**. Subsequent independent studies have confirmed our findings, reporting significantly increased concentrations of iron, aluminium, chromium, nickel and zinc when compared to either healthy surrounding tissue or samples from healthy persons undergoing mammoplasty [6-9].

Proliferating cells have an increased iron requirement, which is fulfilled by overexpression of transferrin receptors (TfR1) on the cell surface. Normal lymphocytes increase the density of TfR1 by 50 times after stimulation with mitogenic factors and transformed lymphoid cells even have 1,000 times the number of TfR1 [10,11]. In accordance with our findings [4,12], clinical research results show a significantly higher transferrin receptor density and ferritin accumulation in breast cancer tissue [13]. Furthermore, iron deficiency in the culture medium leads to apoptosis of cancer cells [14]. The overexpression of zinc transport proteins (ZIP4, ZIP10, LIV-1) is also well documented in cancer cells [15-17].

Once absorbed in the cell, iron is used on the one hand for the synthesis of iron-containing enzymes, on the other hand is stored as ferritin complex.

The high intracellular concentration of transitional metals leads to high ROS production via Haber-Weiss and Fenton reactions [4] **(Figure 2)**, and may be responsible for the considerable genetic variability/heterogeneity of tumor cells, even within the same tumor, among other exogenous ROS sources [18-20].

In view of these facts, the widespread clinical prescription of iron and zinc preparations for cancer patients appears to be rather counterproductive, as the malignant cells are preferentially supplied with these metals. Actually, one should try to counteract the iron excess. Indeed, chelating iron and simultaneously inhibiting the formation of the Tf-TfR1 complex by means of monoclonal antibodies were both able to inhibit tumor growth in a mouse model, much more efficiently than any single agent [21]. Furthermore, clinical studies have also shown the benefits of iron chelators as anti-cancer therapy in neuroblastoma and leukemia cases [22-25].

Ni, Cr and Cd have been identified as mutagens and carcinogens due to their ability to inhibit the repair of damaged DNA. Besides, they are also able to increase the mutagenicity and carcinogenicity of directly acting genotoxic substances [26]. The carcinogenic effects of Ni, directly or in combination with organic compounds, have been described in the literature [27,28] and slightly elevated concentrations of Fe and Ni have been found in malignant human prostate tissue [29]. Inhalation of certain forms of hexavalent chromium causes lung cancer and, at cellular level, chromium exposure can inhibit apoptosis or induce neoplastic changes [30]. Occupational cadmium exposure can cause lung cancer and high cadmium concentrations have been found in proliferative prostate lesions [31].

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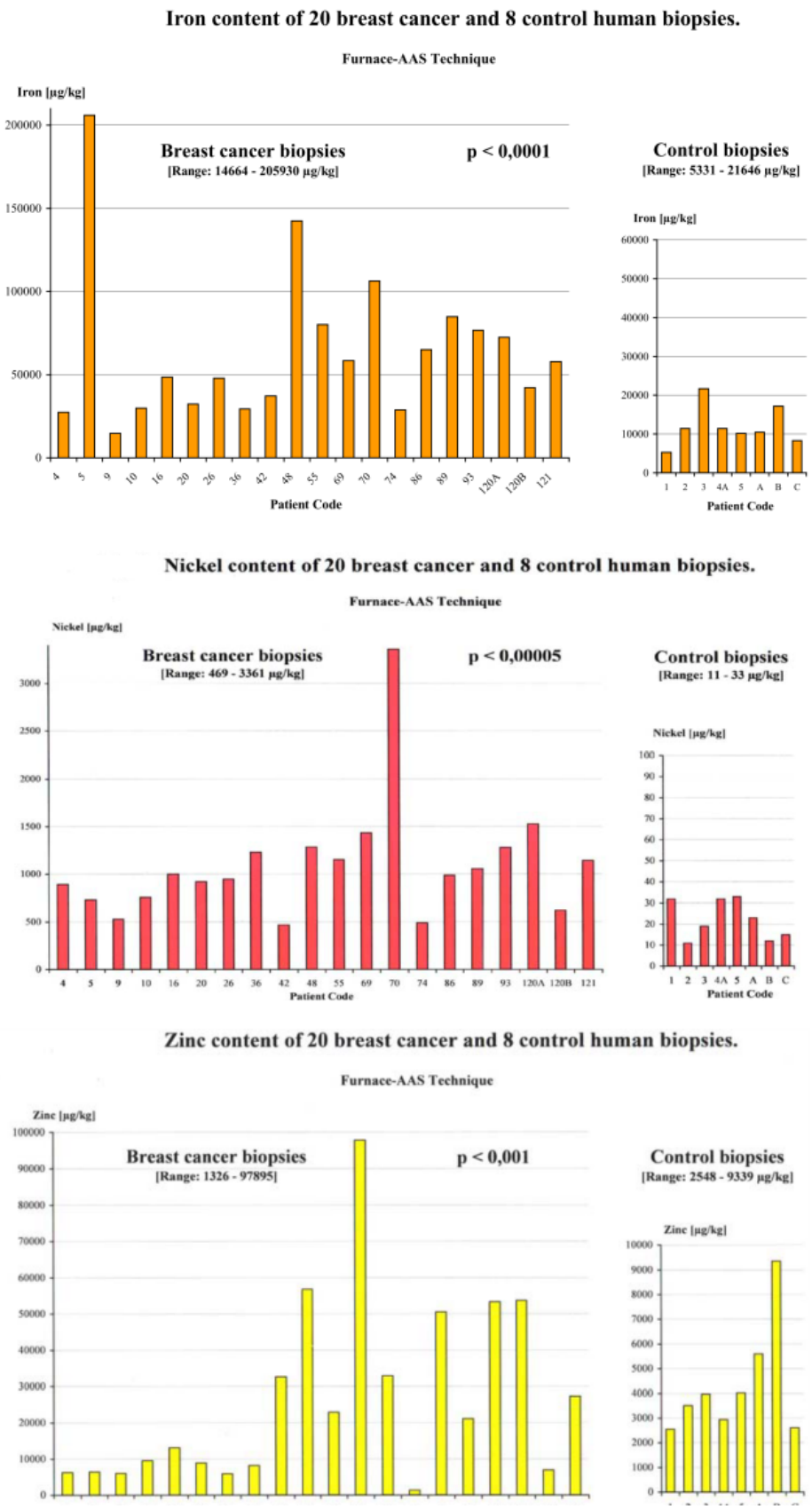

Figure 1. The iron, nickel and zinc content in the 20 breast cancer biopsies is up to 10X, 100X and 25X higher than in the control biopsies [10]

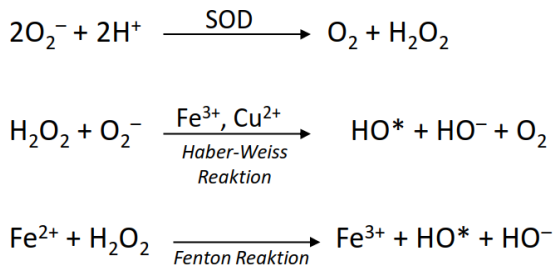

Figure 2. The production of hydroxyl radicals in H2O2- and metal- dependant Fenton and Haber-Weiss reactions

Ni, Cd and Cr activate the estrogen receptors in the absence of estradiol [32-34] thus acting as "endocrine disruptors" or "metalloestrogens". As a consequence, exposure to these metals can increase the breast cancer risk [35] and stimulate growth of ER+ breast cancer cells [32].

Metalloestrogens also trigger changes of the estrogen binding sites of genes in the cell nucleus. In mammary gland cells, this leads to an increase in cell division and more errors in DNA amplification, augmenting the risk of cancer [32].

Interestingly, it has been shown that zinc and overexpression of its transporters mediate and accelerate tumor growth as a necessary trace element [15,17], while zinc deficiency in mice and rats proved to inhibit tumor growth [36-38].

Due to the increasingly broad contact with aluminum compounds, a growing amount of data regarding the involvement of this light metal in tumor development and growth through its mitochondrial-damaging and estrogen-like effects is accumulating [39-41].

The etiology of most human breast tumors is still controversial. We have argued that environmental pollutants that cause oxidative stress and lipid peroxidation can additionally act as endocrine disruptors in the development of breast cancer [35,42].

Therefore, chronic toxic exposure to transition metals and / or organic pollutants, combined with genetic polymorphisms of detoxification phase I+II enzymes and overexpression of transport proteins or their receptors, may be responsible for this phenomenon [43].

Redox state and pH in malignant tumors

The redox and pH shifts define electron or proton transfer reactions in biological or chemical systems. They are correlated according to the rule: the lower the pH, the higher the redox potential (Eh), and the higher the pH, the lower the redox potential. Shapiro [44] defines redosis as the accumulation of non-volatile reductive equivalents (such as glutathione, NADH, cysteine, glucose), as opposed to the accumulation of oxidative substances (O_2, O_3, halogens, metals in oxidized form, environmental pollutants, etc.), which is defined as oxidosis. The measurement of the redox potentials in blood, plasma or tissue (Eh in mV) reflects the sum of all redox pairs in the sample, whereby usually the ratio between the reduced and oxidized glutathione (GSH / GSSG) and pO_2 are decisive for the cellular redox status [45]. The Eh correlates with the biological status of normal cells: -220 mV (redosis / proliferation), -200 mV (differentiation), -170 mV (oxidosis / apoptosis) [46], while proliferating cancer cells exhibit a permanent redosis (-220 mV or lower) with an increased accumulation of reduced glutathione, NADH, NADPH, cysteine, or glucose *via* GLUT transporters [47-51].

This redosis shift may be due to hypoxic states and / or a significant accumulation of electrophilic organic noxae [52-54] and transitional metals [4,42,55] in degenerated tissues after failure of the corresponding detoxification and antioxidant protection systems (GST, SOD, catalase) [56-61]. In this respect, the increased glutathione synthesis of malignant cells is regarded as an adaptive response and resistance mechanism against various pro-oxidative attacks (accumulation of heavy metals or organotoxins, chemotherapy, radiation, endogenous ROS production) and is associated with their propensity to proliferate [4,18,43,62-64].

Intracellular and extracellular pH in tumor tissues

pH measurements in healthy tissues under normal pO_2 values associate temporary growth factor-induced proliferation with an intracellular alkalization and increased aerobic glycolysis [65-67]. In accordance with the existing redosis, proliferating cancer cells exhibit permanent intracellular alkalization (pHi 7.12 - 7.65) compared to normal cells (pHi 6.99-7.20) [68-70], combined with a strong aerobic glycolysis, which was already described in the 1930's as the Warburg effect [71-73].

The permanent intracellular alkalization of proliferating cancer cells is largely due to an activation of the Na+/H+ antiporter system NHE1 [74,75], the V-ATPase proton pump [76-78] and the MCT lactate transporter, which ensure an uninterrupted discharge of protons (H+) and lactate in the extracellular space [79].

Hypoxia- and HIF1-caused hyperexpression of membrane-bound and zinc-dependent carbonic anhydrases CA2, CA9 and CA12 have already been demonstrated in many tumors [80-83] and together with anion exchangers such as Cl^-/HCO_3^- (AE1) are involved in disease progression [84,85]. Accordingly, there is a pronounced acidic extracellular environment in tumor tissue (pHe 6.2 - 6.9) compared to normal tissue (pHe 7.3 - 7.4), which clearly promotes tumor growth and metastasis [68,86] and blocks the activity of immunocompetent cells [87,88].

Activation of aerobic glycolysis (Warburg effect)

The intracellular pH increase in malignant cells can activate aerobic glycolysis at normal pO_2 concentrations [69,79,86]. Activation of the glycolysis enzymes hexokinase (HK), phosphofructokinase (PFK), pyruvate dehydrogenase kinase (PDK1) and the pentose-5-phosphate pathway (via G6PDH and transketolase-TKTL1) leads, on the one hand, to a direct inhibition of OXPHOS in the mitochondria and, on the other hand, via high pyruvate concentrations, to an increase of Hypoxia Inducible Factor (HIF1) [89,90]. The latter plays a key role in the genetic transcription of the glucose transporter (GLUT 1) and the glycolysis enzymes, as well as in the inhibition of pyruvate dehydrogenase (PDH) with reduction of pyruvate conversion to acetyl-CoA and subsequent inhibition of Krebs cycle or oxidative phosphorylation [91,92]. HIF1 also activates carbonic anhydrase CA9 in tumor cells and thus maintains the extracellular acidosis [80-82].

Cancer metabolic hallmarks as therapy targets

In view of the already mentioned genetic variability of cancer cells within a tumor and the associated resistance to therapy, the basic toxicological and molecular-biological characteristics mentioned above **(Figure 3)** are of particular importance for new complementary treatment approaches. *In vitro* and *in vivo* studies of the last years, as

well as our own therapy experiences show a significant antiproliferative and pro-apoptotic effect in tumors by means of:

- High-dose vitamin C, which has a strong pro-oxidative effect in the presence of increased cellular metal concentrations via ROS formation [4,10,93]. Accordingly, the increased heavy metal concentration in various tumors can be used for therapeutic approaches with vitamin C or polyphenols, as previously reported [94-97]. The reduction and mobilization of transition metals from their storage or transport proteins make them extremely reactive for the catalysis of free radical reactions.
- The Fenton and Haber-Weiss reactions described above generate hydroxyl radicals to a high degree, which can lead to lipid peroxidation, interruptions of DNA strands and apoptosis [3,94,98]. The autoxidation of vitamin C with production of superoxide and hydrogen peroxide in the presence of transition metals such as iron, nickel, chromium or mercury can be clearly detected in human serum using a chemiluminescence method [99]. In an acidic environment (H+ excess in extracellular space) the superoxide radical is converted into H_2O_2 and can induce apoptosis/necrosis of tumor cells.
- Due to the highly significant accumulation of heavy metals in tumor tissue, we have described for the first time the above mechanism as an explanation for the pro-oxidative, tumor-specific activity of vitamin C [4,10,43,93]. In contrast, healthy, non-metal contaminated cells are antioxidatively protected by vitamin C. Clinical studies and our own experience with pharmacologically active doses of Vitamin C administered i.v. prove that such approaches can lead to dramatic shrinkage of the tumor, an extension of the patient's life and to an increase of their quality of life [5].
- Natural polyphenols generate, in the presence of increased metal concentrations **(Figure 4)** [10,97,100] or activated NADPH : quinone oxidoreductases, superoxide and semiquinone radicals and thus exhibit a strong pro-oxidative effect. As discussed above, tumors tend to accumulate heavy metals; the activity of the microsomal NADPH : quinone oxidoreductase, has been shown to be strongly increased in various tumor types [101]. Through these two pathways, *in situ* bioactivation of phenolic and polyphenolic therapeutics occurs in a tumor-selective manner [102,103], leading to a significant production of superoxide, H_2O_2 and semiquinone radicals and thus to a selective increase of the redox potential in the tumor [101]. Curcumin combined with classical chemoterapeutics such as Cisplatin or Docetaxel led in mouse models of head and neck, respectively ovarian carcinoma to a suppression up to 96% of the tumor growth [104,105].

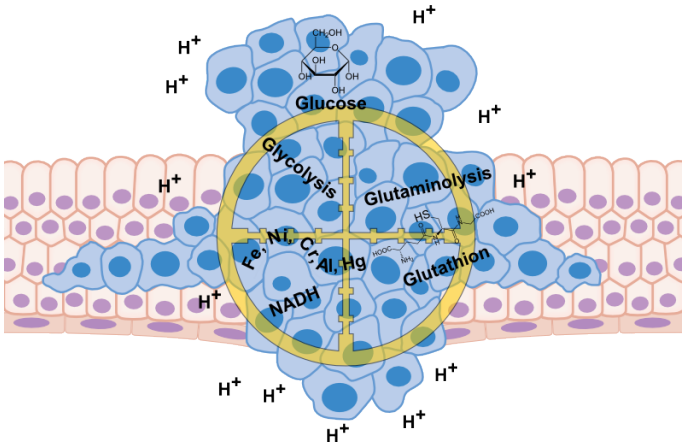

Figure 3. Metabolic targets of integrative oncology

- The elimination of intracellular redosis with the help of pro-oxidative approaches such as hyperthermia [99], short-term fasting (3-5 days) [106,107], ketogenic diet [108-110] and regular physical exercise [111].
- Studies in animal models could show that the association of a ketogenic diet with radiotherapy can lead to a complete remission of the tumor, and that the animals remained tumor-free even 200 days after withdrawal from the ketogenic diet [109]. A specially formulated nutritional formula developed for the treatment of epilepsy (KetoCal) could diminish up to 65% the growth and vascularization of gliomas implanted in mice and significantly increase their survival rate [139].
- First clincal studies on patients with advanced carcinomas show that a ketogenic diet with an insulin lowering effect can be considered safe and that the concentrations of ketone bodies correlate with the stabilization of the disease or even remission [112].
- The use of basic solutions such as Ringer's lactate or $NaHCO_3$ to buffer the extracellular acidosis with anti-inflammatory and metastasis-inhibiting effects [113-115]. Simply the use of bicarbonate perfusions leads to a selective increase of intratumoral pH concomitantly with a decrease of new metastases generation, as well as a remarkable improvement of the therapeutic efficiency of Doxorubicin in mouse models [115].
- The usage of proton pump inhibitors from the Omeprazole family [77,116], which were shown in mouse models of B-Cell lymphoma to lead to a significant slowing of the tumor growth [117] and to a significant lowering of the incidence of esophageal adenocarcinoma in patients with Barrett's Esophagus [118]. Also, V-ATPase-Inhibitors such as the macrolid antibiotics Bafilomycin A and Concanamycin A lead to a similar acidification of the tumoral millieu and apoptosis [76,119]. This finding was confirmed in clinical studies: breast cancer patients who received Esomeprazol before the chemotherapy had an almost double progression-free survival compared to the group receiving the standard chemotherapy (10.7 vs 5.8 months) [120].
- The inhibition of the Na^+/H^+ antiporter (NHE1) for the purpose of intracellular pHi reduction and apoptosis induction via 5-HMA and other amiloride derivatives in leukemia and hepatocarcinoma cells [121-123]
- The inhibition of carbonic anhydrase (CA) via acetazolamide [124], sulphonamides [80,81], coumarins, thiocoumarins or hydroxycinnamic acids [125,126].
- The inhibition of aerobic glycolysis with specific inhibitors of hexokinase (Lonidamine, 2-deoxyglucose, 3-bromo pyruvate), G6PDH (6-aminonicotinamide), the transketolase TKTL1 (oxythiamine), PDK-1 (dichloroacetate), glyceraldehyde phosphate dehydrogenase (chlorohydrin, ornidazole, arsenate) and lactate dehydrogenase A (anti-RNA) or of glucose transporters (GLUT1-3) via genistein, 5-thioglucose and mannoheptulose **(Figure 5)** [43,127-130].
- The selection of an appropriate form of nutrition with a low glycemic index not promoting tumor growth.
- Low in sugar, flour products, zinc, iron, nickel, chromium, folic acid, alcohol, glutamine, fat peroxides etc. [5].

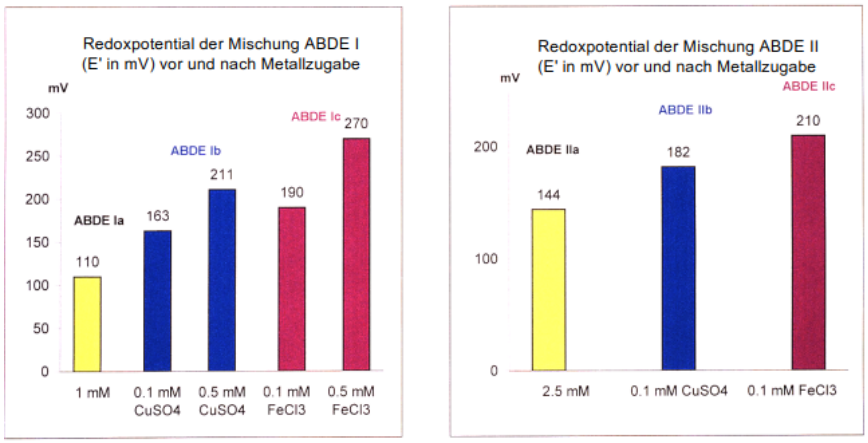

Figure 4. Substituted phenols show a pro-oxidative effect in the presence of Cu^{2+} or Fe^{3+} [10]

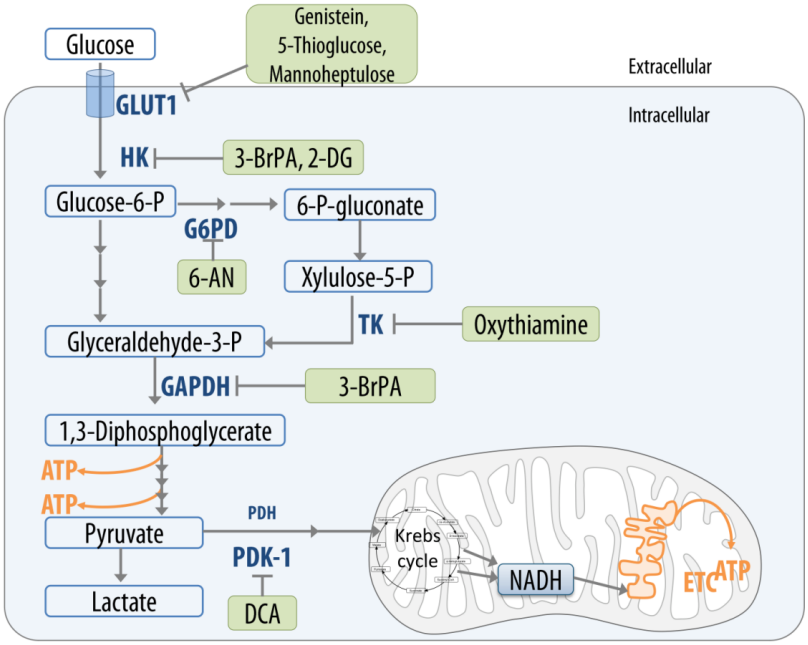

Figure 5. Inhibitors of glycolysis, of pentose-5-phosphat pathways and GLUT1 in an integrative cancer therapy model

- Rich in omega-3 fatty acids, vitamin D3, carotenoids, complex CHOs, high-dose enzyme preparations, plant proteins, mixtures of selected amino acids, sphingolipids, phytosterols, isoflavonoids, polyphenols, L+ lactic acid and pro-oxidative vegetable and fruit juices etc. [131].

Conclusion

The causal link between the increasing environmental pollution (diesel exhaust, pesticides, wood preservatives, phthalates, solvents, tobacco smoke, alcohol, heavy metals, preservatives, dyes, etc.) and the continuous raise in cancer incidence is currently well documented. Their accumulation in cancer tissue leads to oxidative stress, followed by DNA mutations and thus to an increase in the intra- and intertumoral genetic variability [19,132]. This dynamic heterogeneity is the main cause for resistance to the classic oncologic therapies.

Since the redox, pH and glycolysis shifts in tumor tissue are regarded as metabolic markers of all cancer cells, they emerge as new therapeutic targets in modern oncology.

The novel treatment approaches mentioned in this paper, *per se* or in combination with classical oncological therapies, can lead to a considerable increase in life expectancy and life quality. A detailed description of the above strategies, also addressing alternate energy-generating pathways such as glutaminolysis and fatty acid synthesis/oxidation will be published elsewhere.

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