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Mini-Review: The Influence of Respiratory and pH Imbalance in Cancer Development

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Author's contribution

This whole work was carried out by the author GD.

Mini-review Article

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ABSTRACT

Aims: In this review, we bring further evidence in support of the hypothesis on the so-called respiratory and pH imbalance (RpHI) as a cause of long standing hypoxia within the whole organism, characteristic to the preneoplastic stage.
Background: Carcinogenesis is a process by which normal cells are transformed into cancer cells. Cancer is a multifactorial disease with contributions from environmental, genetic and lifestyle factors. Cancer prevention is a global priority, yet the proximate causes of most cancers are still little understood. However, under hypoxic conditions, the overbusy cells, getting less oxygen than needed, turn into anaerobic adenosine triphosphate (ATP) production, excessive multiplication and finally, tumor development. Since hypoxia depends on the intensity and duration of action of the stress agent, human and animal organisms may compensate hypoxia only if the causal agent stops acting continuously. Cancer prevention might be associated with an increase in oxygen delivery to overstressed cells, carbon dioxide removing, as well as pH and glucose concentration balancing. The role of RpHI in chemical carcinogenesis and cancer incidence is also discussed.
Conclusion: Evidence from the literature data is brought to show that cancers develop at the physiological level, while the molecular changes in cancer cells are the consequence and not the main cause of malign processes. A link between the Warburg effect and the Macovschi's biostructural theory was suggested.

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1. INTRODUCTION

Ancient Asian medicine was mostly a preventive medicine, while current western medicine is mainly a curative one. However, the query still remains: should the medical act balance the body or repair the already occurred damage? Imagine that we want to prevent cancer by administering the anti-cancer drug, cyclophosphamide. In a few years we will have become ill from cancer! Presently, the gap between the two medical approaches seems to be even more evident.

Cancer prevention and etiology are medical priorities, but history study suggests that the journey towards achieving this goal is difficult and full of detours and roadblocks [1]. The proximate causes of most cancers are still less understood even though this is what we want to learn how to avoid [2]. For instance, it appears difficult to understand why it has to be such a long time interval between the exposure of a certain cell to a mutagen and the expression of the resulting mutations. It is hard to imagine how the numerous genetic changes found in cancer cells could have been produced in any cell as the result of a single exposure to a DNA-damaging agent, or why months or years have to elapse before the effect of these changes are observed. Malignant tumors exhibit increased dependence on glycolysis, resulting in abundant export of lactic acid, a hypothesized key step in carcinogenesis [3,4]. It is also well-known that the development of cancer has been associated with microbial infection, injury, inflammation, and tissue repair [5-7]. Chronic inflammation conditions, caused by exposure to environmental factors can increase the risk of cancer [8-10]. Most cancer cases are mainly attributable to long-term exposure to environmental factors, via a multi-year, multi-step, and multi-path process of tumor genesis [11,12]. They may involve cumulative genetic and epigenetic alterations in the chronic carcinogenesis of body cells from a non-cancerous stage to precancerous and cancerous stages [13]. Chemopreventive effects of dietary phytochemicals used at the precancerous stage can hardly be explained in light of current theories [14]. Several dietary phytochemicals such as β-carotene, curcumin, resveratrol, gallic acid, chlorogenic acid, caffeic acid, carnosol, capsaicin, 6-shogaol, 6-gingerol and their derivatives have been largely investigated against cancer invasion and metastasis [15,16]. Since carcinogenesis is a multistep process, these bioactive compounds might act on a variety of stages of the precancerous process to prevent inflammation, enzyme activities, or lactic production [17,18]. Evidence for cancer-related anti-inflammatory activity of natural and synthetic phenols was provided by Aspirin, which inhibits colorectal carcinogenesis [19].

The role of metabolic reprogramming in carcinogenesis is being increasingly recognized [20]. The identification of metabolic processes of cancer cells has been used to create strategies for treating cancer. Warburg stated that there are fundamental differences in the central metabolic pathways operating in tumor genesis [21-25]. In recent years, the metabolism of malignant tissue has once again become one of the most intense areas of research in cancer biology [26-29]. Considering all these issues, new ideas are needed to clarify what happens during carcinogenesis. In spite of the fact that Otto Warburg studied aerobic glycolysis long ago, the molecular mechanisms how increased glycolysis is regulated by oncogenic and/or tumor suppressive signaling pathways are unclear [30]. Starting with the observation that the cellular structure is partially broken down during hypoxia, a so-called respiratory and pH imbalance (RpHI) was supposed to be involved in cancer etiology [31-33].

Therefore, this work aims at presenting evidence for the role of RpHI during the precancerous stage, as well as some concluding remarks that could be useful to design new preventive strategies.

2. HYPOTHESIS AND EVIDENCE

2.1 RpHI hypothesis

Normally, various stress agents, such as physical and chemical stressors, viruses, other infectious agents, hormones in excess, but also long lasting anxiety, emotions, conflict states, etc., are able to affect the body as a whole [34-37]. In order to preserve the identity and relative independence of living organisms confronted with the external environment, some co-ordinate physiological processes are involved in keeping their internal states of equilibrium [38-40]. The living bodies react against aggressions using metabolic energy obtained by the oxidation of organic substances, including organic acids in the mitochondria [41,42]. Under normal conditions, when the supply of oxygen is sufficient, cells can carry out aerobic respiration [43,44]. Hence, when busy, cells produce the energy they need from the food stores, including the organic acids they possess. During the Krebs cycle, carbon dioxide, of a weaker acid type, is released, normally out [45,46]. Since stronger organic acids such as succinic, malic, 2-ketoglutaric and oxalylacetic acids are replaced by carbon dioxide, the cell milieu tends to be more alkaline, just blood and urine [47]. The tendency of blood alkalization entails retaining of carbon dioxide, so that the pH of blood should alter as little as possible. The retaining of carbon dioxide in blood does not allow the oxygen shift at a normal rate in lung [48,49]. As a consequence, oxygen pressure in blood decreases albeit it stays normal all around. However, oxygen may reach quite a low level without affecting cell breathing in any drastic way. Unfortunately, the busiest cells in the body need more oxygen. Oxygen partial pressure getting lower, these cells receive less oxygen than they need and fermentative processes develop alongside with breathing.

In principle, the RpHI, which can be regarded as a physiological reaction against any stress agents, may cause an oxygen crisis within the body and consequently, the busiest cells divide faster and faster producing first preneoplastic cells and in time, malignant tumors. The tumors thus acquired, as well as their fermentation products, may disturb the normal functions of most cells and tissues in the body [50].

RpHI was discovered by putting together the main experimental data in cancer with the most important findings in physiology, biochemistry, cybernetics, etc. and treating them as a whole; it represents the mechanism that could create a long standing intracellular hypoxia state, which, over time, will generate the conditions for malignant transformation [51,52]. Nevertheless, it is hard to believe that a tumor is generated starting from a physiological imbalance, prior to the first real malign cell, while the modern biology is looking for precise molecular mechanisms involved in carcinogenesis. However, the impairment of cellular breathing, altered intracellular and extracellular pH, as well as hypoxic conditions might mostly be related to a precancerous pathology.

2.2 Cancer - related Physiological Changes

Since the oncogene revolution pushed tumor metabolism to the margins of cancer research [23,53], interest in the fundamental differences of central metabolic pathways operating in malignant tissue has only been renewed in the recent years. The potential causes of

deranged metabolism in cancer should be discussed with emphasis on changes in energy metabolism of glucose, fat and protein [53].

However, hypoxia is widely considered as the natural environment in which life appears and proliferates and DNA auto-replication and transcription takes place *in vivo* in all eukaryotes [54-57]. Nuclear division unfolds anaerobically using the energy produced by glycolysis [58-60]. Consequently, the cells forced to manifest themselves in rather anaerobic conditions will divide more intensely [61]. Aerobic breathing, which provides cells with a great amount of energy, creates the necessary conditions for the existence of fine structures of the cells, the specific functions running unimpaired [62]. Lack of oxygen, even partial, causes rupture of these structures, hence the gradual disappearance of specific functions of cells, as well as contact inhibition, while it entails cell-division to a greater extent than necessary for the tissue in question [63]. It follows the first stage of the RpHI, which affects the cell respiration and division. Nevertheless, the organism possesses buffer systems, lung ventilation and the kidney mechanisms to control the concentration of hydrogen ions within the cellular milieu (Fig. 1) [64]. Alveolar ventilation is responsible for carbon dioxide elimination [65]. A slight respiratory acidosis results primarily when alveolar ventilation is decreased or if carbon dioxide production is increased [65]. However, the organism has several compensatory systems to minimize a decrease in pH. For example, deoxygenated hemoglobin readily accepts hydrogen ions to prevent substantial changes in pH. Normally, carbon dioxide, a respiratory stimulant, induces an increase in minute ventilation to normalize the pH by eliminating increased quantities of CO_2. Unfortunately, this effect is attenuated if the carbon dioxide concentrations remain elevated for more than several hours. The kidneys are also capable to control both the blood pH and some other blood parameters; however, this process is slow and lasts for several hours or days. In fact, renal compensation begins in 6-12 hours, but maximal compensation occurs in 3-5 days. The kidneys increase excretion of hydrogen ions predominantly in the form of ammonium. If the stress agents act continuously, the blood will become slightly more alkaline than usual and the blood oxygen concentration will be lower than normal. The overstrained cells, getting less oxygen than needed, will lead to anaerobic fermentation and also to a lower content of $NADH+H^+$ and $NADPH+H^+$ and a higher one of NAD^+ and $NADP^+$. Therefore, a decrease of the oxidation-reduction potential will occur as well. The quantity of sulphydryl groups in the blood and tissues also decreases. Besides, marked decrease in succinic dehydrogenase and slight increase in cytochrome oxidase levels could be found, suggesting the alteration of Krebs cycle. For this reason, the cell with an excessive fermentation will not reach an upper energetic state, if the neighbor cells and blood cannot interfere with its metabolism.

The second stage of the RpHI is reached when lactic acid is produced due to hypoxic conditions. This time, the CO_2 concentration may decrease; however, part of the produced carbon dioxide is not removed, because the cell content may remain slightly alkaline in spite of the lactate production. Again, the kidneys should control the hydrogen concentration in blood, releasing acidic species, such as ammonium ions or phosphates, into urine. The process is complicated by the existence of lactic acid in blood which decreases blood pH, while intracellular pH of overstrained cells is increased.

However, if the stress agents act continuously, the neighbor cells involved in curing or rebalancing the overbusy cells will work hard, also being overstrained and deprived of oxygen. There follows a third stage of the RpHI in which a real state of illness (infections, viruses) occurs. A very special balance between the two types of cell (attacked cells and neighbor ones) is established. As a result, the pH value of the blood is little altered. If the stress agent is very strong, it can be lethal to the organism and this one does no longer

reach the cancer stage. It is the case of microorganism- or virus-induced diseases, which, if untreated, have a bad prognostic. Contrary, a long-standing, still mild action of the stress agents may cause a slow shift of this imbalance, resulting in a stepwise decrease of blood pH value. Long standing infectious pathologies may thus result in a RpHI, which create the conditions for either the transformation of normal cells into preneoplastic cells followed by preneoplastic to neoplastic pathway or for the multiplication of malignant cells. It is well-known that only the bodies with a significant amount of morbidity may become cancerous. Most cells become glycolytic while fewer remain normal, but overstressed. This is the pathway to reach the fourth stage of the RpHI, which is that of tumor formation.

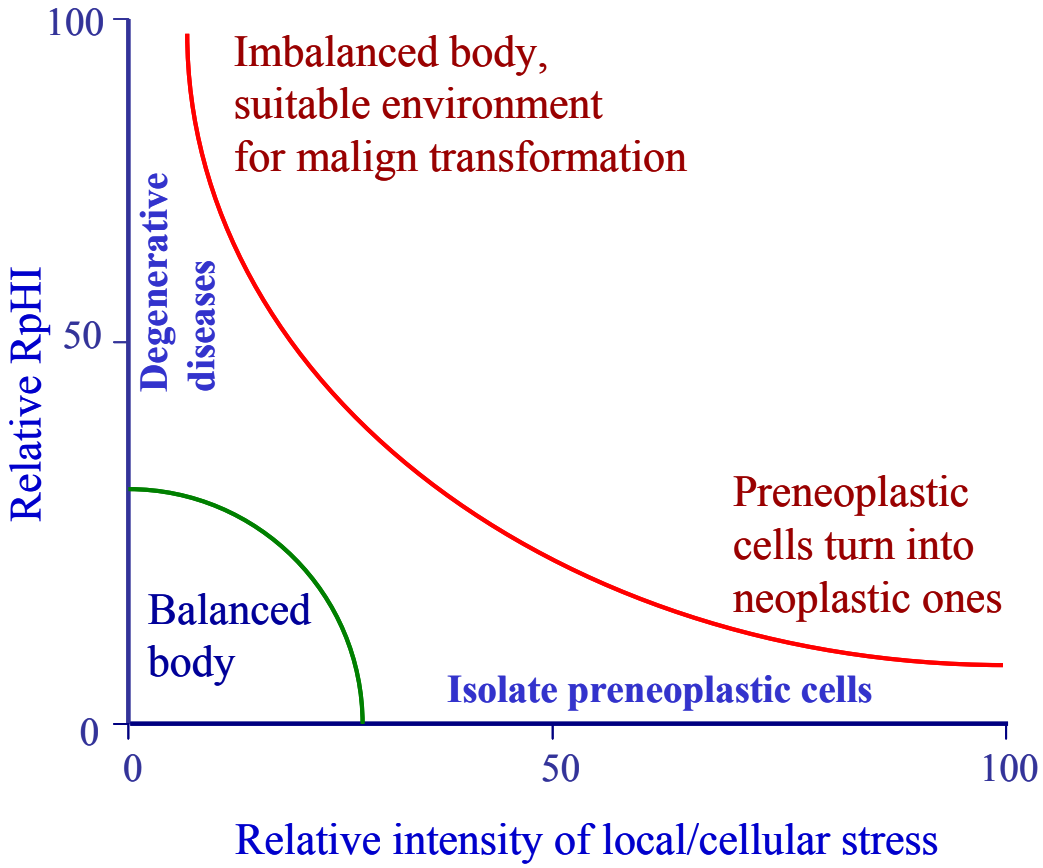

Fig. 1. Suggestive scheme for the role of the Respiratory and pH Imbalance (RpHI) in cancer development. Relative RpHI means both the relative intensity and duration of respiratory and pH imbalance within the body, whereas local/cellular stress may have different intensities or is variably long standing

The RpHI causes an oxygen crisis and the busiest cells divide faster and faster under anaerobic conditions. The preneoplastic cells thus appeared may become neoplastic, which, in their turn, will generate tumors, if the imbalance persists; these ones and their fermentation products can kill the body, if they are not surgically or by other means removed. The RpHI cannot be easily measured, since the affected individuals seem to be normal and look like they are in good health. Otherwise, if stress agents are more powerful, patients might die during the first stages of body interaction with the environment stressors, without

reaching the cancer stage. At this stage, the neoplastic cells and tumors may be found in the body. To reach cancer, the RpHI should be less strong, yet long-standing in order to get a chronic lack of oxygen in the blood and cells. Cancer could be considered an impairment of the whole body, while malignant cells are quite normal cells that, although are overstrained, get less oxygen than needed and turn into anaerobic fermentation, being divided to a greater extent than what the body requires.

The local/cellular stress means the reaction of the individual cell against the external environmental agents. There is a reciprocal relationship between the RpHI, which affects the entire body and the local solicitation, which creates a local (in a target cell or tissue) RpHI. Thus, an imbalanced body, found in apparently good health, will die when confronted to a less important local solicitation (imbalanced body, suitable environment for malign transformation). On the other hand, as in the case of experimental chemically produced tumors, a long-standing local stress induced by the carcinogen may cause a general imbalance, which could also promote cancer (Fig. 1). There are many intermediary states between these extreme situations, giving cancer its characteristic complexity. The RpHI caused by the difficulty of the lung-kidney system to remove the excess of CO_2 and alkaline ions produced during the stress reaction means/determines a general weakness of the entire body. At the same time, such degree of weakness may be associated either with cancer or other diseases.

The living body can be considered a relatively independent entity, which possesses the physiological mechanisms to preserve its specific structure in order to adapt to the environmental conditions. The RpHI is the expression of exceeding the adaptive capability of the body. Generally, the body fights against the alien disturbing agents through a well-known phenomenon, involving the humoral immunity and the capacity of immune response mediated by cells. At the same time, the organism possesses metabolizing and transformation systems, which transform alien and complex substances into simpler ones to be released.

The body must also release its own excessive cells because they are no longer necessary for the organism structure and functions. This process is more clearly understood if one considers wound healing. Thus, the injury puts the inner cells and the external environmental agents together. To react against the changes of environmental conditions, these cells use energy, alkalize the surrounding cell environment, get less oxygen and pass to an excessive fermentative (glycolysis) state. Indeed, because of the fundamental role of oxygen in metabolism, a drop in tissue oxygen level to the point where oxygen demand exceeds supply leads rapidly to metabolic crisis [43].

They divide excessively even after the healing of the wound, but not for too long. Then several cells, which have multiplied in excess for that tissue and are being no longer necessary, are released; thus, the body's own structure is restored. This process takes place in balanced bodies only. Otherwise, the useless cells divide continuously, becoming cancerous. Their neighbors cannot destroy them or help them integrate into the body.

In order for maintaining its specific structure, the body tends to remove the existing malignant cells, too, even if they are its own cells. This process seems to be dependent on the size of the RpHI. The surgical removing off the tumor cells causes a decrease in this imbalance. As a result, both the immunity and the capacity of restoration of the body's own structure, will increase because the tumors are strong stress agents, as well.

Cancer cells can thus be considered as normal; however, they behave abnormally under hypoxic conditions. Consequently, carcinogenesis only develops in exhausted organisms, hence where a RpHI occurs. A carcinogenic promoter acts in a similar way as those agents that provoke a wound, but lasts longer; a local RpHI causing cellular hypoxia develops.

2.3 Chemical Carcinogenicity and the Abnormal Metabolism of Carcinogens

The carcinogens may cause a RpHI, which causes in its turn a slight intracellular hypoxia; this one provides the normal specific conditions for expressing the ancestral gene existing in every cell. It follows that a carcinogen entering a living tissue must be released at any price, because the affected living cell will become exhausted in its effort to destroy and release that compound and its metabolites. Besides, this effort is subsequently assumed by the neighbor cells and a RpHI at the level of the whole organism takes place. The RpHI will cause in its turn the cellular hypoxia that represents the specific medium for malignant transformation.

The degradation pathway of carcinogens is based on oxidation reactions, particularly those classified as microsomal monooxygenation reactions. These reactions occur with oxygen as the oxidizing agent, generating a long standing hypoxia if carcinogen degradation is a multi-step process. Many carcinogens are polyaromatic hydrocarbons that required activation to electrophiles to form covalent adducts with cellular macromolecules. Conney and coworkers identified microsomal enzymes (P450s) that activate many drugs and chemical carcinogens [66].

Initiation involves a brief and irreversible interaction between a carcinogen and the target tissue, and implies a local cellular stress/solicitation. Gradually, the stress caused by carcinogen removal may promote an overstraining of the neighbor cells and then of the whole body, causing a general imbalance. The two stages of cancer occurrence provoked by carcinogenic substances can easily be explained by the existence of the RpHI. In the two-stage skin carcinogenesis, the initiation phase is carried out by the application of a sub-carcinogenic dose of a carcinogen [67].

Although all cells are able to degrade chemicals, this capacity differs from one tissue to another and depends on age, sex, health status, etc. Cell will degrade the foreign compound through a series of eight consecutive chemical reactions within the microsomal mixed function oxidase system [68]. Cytochromes P450, existing in each cell, provide the principal, initial source of biotransformation of xenobiotics that cell comes in contact with. The role of intestinal P450 in detoxifying specific carcinogens like 3-methylcholanthrene has clearly been demonstrated [69]. Moreover, elevated intestinal P450 levels have been indirectly linked to gastrointestinal cancer. Intestinal metabolism of 2,2,2-trifluoroethanol, which is not an carcinogen, produces intestinal lesions with consequent systemic bacterial infection. The activity of these enzymes requires nicotinamide adenine dinucleotide phosphate (NADPH) and molecular oxygen; in a typical reaction, one molecule of oxygen is consumed (reduced) per substrate molecule, with one oxygen atom appearing in the product and the other in the form of water. However, noxious reactive oxygen species (ROS) and H_2O_2 may appear. Some other degradation reactions may also be possible, depending on the type of cell. To form NADH from NAD^+, glucose, fatty acids, amino acids, citric acid, malic acid, etc., are consumed. During this process, cell content will become more alkaline and if oxygen supply is not enough for removing the carcinogen, fermentation will develop alongside with respiration. Glucose from blood will help the fermentative processes, because it forms acetyl-CoA, both in the presence and absence of oxygen. Acetyl-CoA cannot be used in the

absence of oxygen; therefore, glucose will give only pyruvate, which, in turn, is reduced by NADH to lactate.

Whenever a cell is damaged, its neighbors undergo inflammation by which this cell can be destroyed. The interesting dependence between chemical carcinogenesis and inflammation was reviewed by Loeb and Harris [66]. In this process, the need of oxygen of the neighbor cells is increased. They undergo an inflammatory process, during which they become slightly alkaline and retain carbon dioxide. A hypoxia state may occur in the surrounding tissue. Paradoxically, blood will provide a cell with less oxygen if it acts against a carcinogen than if it is in the neighborhood. If the carcinogen does not react directly with the cell chemical components, so as to be degraded and removed, the cell will continue to act against it. The degradation process, in which the carcinogen is transformed into metabolites may last long enough. Whereas a long standing hypoxia state is created, the fine cellular structure is damaged by ROS and the lactic acid is released from the contaminated cell, being then reconverted into glucose with ATP consumption as energy source. The neighbor cells support the carcinogen-contaminated cell, undergoing an inflammatory reaction. Both the degradation and the inflammatory reaction may damage the involved tissue. Omega-3 polyunsaturated fatty acids have been recognized as having anticancer activity [69], probably as a consequence of their anti-inflammatory effect.

Logically, both the carcinogen and the poisoned cell should be removed. If not, more oxygen is needed to help cells destroying that carcinogen.

2.4 Chromosomal Instability During Precancerous Stage

Epidemiologic studies and clinical evidence clearly indicate that specific genetic, environmental and behavioral factors are associated with an increased risk for cancer development [1,70]. The stepwise progression from preneoplastic lesions to cancer seems to be associated with gradually increases in genomic instability [71]. Nevertheless, at each step of carcinogenesis, there are natural mechanisms protecting the body against cancer development [72]. If DNA damage occurs, it is repaired in most of the cases.

Changes in DNA methylation consisting of either DNA hypomethylation or hypermethylation are commonly seen in cancer cells [73]. Nevertheless, hypermethylation of tumor-suppressor genes in cancer could merely be a secondary event to help maintain gene inactivity which has been induced by some other mechanism. DNA methylation changes in cancer cells have been shown not to be the by-products of malignant transformation, but can play an instrumental role in the cancer process [74]. Cancer cells usually exhibit important changes in DNA methylation and histone modification [75]. Hence, targeting epigenome-modifying enzymes could be an attractive therapeutic strategy. The cancers with poor prognosis demonstrate evidence of a metabolic shift, involving down regulation of genes involved in the TCA cycle, increased glycolysis, decreased AMPK and PTEN protein levels, glutamine-dependent lipogenesis, fumarate hydratase, upregulation of the pentose phosphate pathway and the glutamine transporter genes, increased acetyl-CoA carboxylase protein and altered promoter methylation of miR-21 [76]. Nevetheless, epigenetic changes such as DNA methylation act to regulate gene expression in normal mammalian development [77]. In fact, DNA methylation helps to maintain transcriptional silence in noncoding or nonexpressed regions of the genome. Such findings suggest that gene silencing by hypermethylation is relatively common both in human cancer and many other human diseases [78]; interindividual epigenetic variations in normal tissues due to aging, environmental factors, or innate susceptibility are also characterized. Besides, the variability

of gene expression is related to epigenomic states that vary across individuals. For instance, methylation profile classes are significantly associated with age, being significant predictors of tissue origin as well [78]. Nevertheless, renal carcinogenesis is associated with neither chronic inflammation nor persistent viral infection and any histological change is hardly evident [79]. However, regional DNA hypermethylation on C-type CpG islands has already accumulated in such non-cancerous renal tissues, suggesting that, from the viewpoint of altered DNA methylation, the presence of precancerous conditions can be recognized even in the kidney. Since DNA methylation of genes is age-dependent, being related to the tissue origin, it cannot be a hallmark of cancer as some authors claim [80], but a phenomenon associated with excessive cell multiplication in a specific tissue.

Malign process is preceded by the genetic deficiencies, yet, only 0.3 per cent of the recessive-lethal mutations give neoplasm in *Drosophila melanogaster* [81,82]. Moreover, tumor cells of this species become easily reversible because of the fact that these mutations do not seem to be the cause of cancer. In addition, even if cancer occurrence is directly related to the recessive-lethal mutations, the resulted abnormal proteins are normally hydrolyzed to amino acids.

The accumulation of unfolded proteins in the endoplasmic reticulum represents a cellular stress induced by multiple stimuli and pathological conditions. These include hypoxia, oxidative injury, high-fat diet, hypoglycemia, protein inclusion bodies and viral infection [83].
Eugen Macovschi stated that the biostructure is partially broken down during hypoxia [84]. Since the biostructured matter is found under an altered, abnormal state in cancer cell [85,86], the RpHI hypothesis was advanced to explain first the transformation of a normal cell into a preneoplastic one and second, the transition from preneoplastic cells to malignant ones [31,32,50]. The RpHI hypothesis is concordant with Macovschi's statement, because it considers the role of hypoxia in the malignant transformation of cell biostructure. Genomic instability may also be associated with hypoxia, which might be the causal phenomenon. However, on using metabolic inhibitors like dinitrophenol and sodium azide, Macovschi demonstrated partial and reversible biostructure breakdown [84]. He concluded that these compounds inhibit oxidative phosphorylation and consequently, the ATP content of cells decreases. Since ATP is needed to support the biostructure, then any carcinogenic agent alter the state of living matter by decreasing the ATP pool. Cancerogenesis is therefore accompanied by the partial breakdown of the biostructure, leading to alteration of cell behavior. Nevertheless, further research showed that cellular ATP concentrations only slightly decrease by switching from aerobic production of ATP to glycolysis. Under such circumstances, RpHI hypothesis admits that carcinogens are chemicals difficult to be degraded and eliminated from the body and not very reactive compounds. Metabolic processes of carcinogens occur in cells in organisms [87]. The most important degradation pathways are oxidation reactions, particularly those classified as microsomal monooxygenation reactions. Monooxidations occur with oxygen as the oxidizing agent, generating a long standing hypoxia if carcinogen degradation is a multi-step process, this one is not too toxic to kill the cell and its degradation products are also difficult to be eliminated. Dinitrophenols also induce hypoxia by inhibiting respiration, without affecting too much the ATP concentration in poisoned cell. Under hypoxic conditions, ATP production is stepwise shifted from respiration to glycolysis, resulting in extracellular pH decrease, enhanced proteolysis [33] etc. In fact, these biochemical processes as well as some others like the hydrolysis of proteins involved in oxidative phosphorylation, cytochrome c or K+ leaking affect the biostructure integrity. RpHI hypothesis has thus a contribution to rationale understanding of biostructure breakdown in cancer, since it refers to the cause for long standing hypoxia, which creates the conditions for biostructure breaking down.

2.5 Increased Glucose Metabolism of Cancers

In solid regions of tumors the most consistent finding is: a higher glucose utilization rate, a higher lactate production, and a lower pH than in the surrounding tissues [88]. The acidic extracellular pH that is lower than pH in surrounding tissues is a key feature of solid malignant tumors. Tumor ATP was found to be slightly higher and glucose slightly lower than in the neighbor tissues. Most measurements of regional glucose and oxygen consumption by positron emission tomography have confirmed that oxygen extraction is lower than in normal tissue and that the oxygen/glucose uptake ratio corresponds to a glycolytic rate of more than 65% [89]. Since ATP production by glycolysis is less than 6% of that of oxidative phosphorylation, restriction of glucose delivery to the tumor by the blood was supposed to be more important than acidosis for inducing energy failure and subsequent tissue necrosis [88].

It was suggested that glycolysis is elevated because it produces acid, which provides an evolutionary advantage to cancer cells vis-à-vis normal parenchyma which they invade [90]. The RpHI hypothesis states that the slight hypoxia in the precancerous stage will induce an increased consumption of glucose as required by cells to produce the same amount of ATP as that obtained by normal respiration.

However, hypoxia occurs in physiological situations such as during embryonic development, as well as in pathological conditions such as ischemia, wound healing and cancer [35].

The link between hypoxia and the regulation of angiogenesis is an area of intense research [91] Moreover, molecular insights into these processes are rapidly being generated [92]. A primary effector of the adaptive response to hypoxia in mammals is the hypoxia-inducible family factor of transcription regulators. These proteins activate the expression of a broad range of genes that mediate many of the responses to the decreased oxygen concentration, including enhanced glucose uptake, increased red blood cell production and the formation of new blood vessels via angiogenesis [91].

The limiting role of glucose - and not of oxygen - for maintenance of energy state is also reflected by the relationship between ATP and glucose content under conditions of critical blood perfusion. Diabetics treated with an insulin-lowering drug, known as metformin, have less chances of cancer than those who receive insulin as treatment [93]. Indeed, metformin may lower cancer risk in diabetics and improve outcomes of many cancers [94]. Metformin may act by impacting mitochondrial respiration leading to the activation of the AMP-activated protein kinase, which controls energy homeostasis in cells. This drug also seems to inhibit cancer cell growth in vitro and delays the onset of carcinogen-induced cancer in mice. According to RpHI hypothesis, the drug metformin may lower the glucose concentration in cells, reducing thus oxygen consumption and glycolysis.

The tumors are slightly more alkaline than normal brain tissue, although lactate was consistently increased [95]. Thus, in animals with pronounced peritumoral brain edema, the latter is distinctly more alkaline than the healthy tissue. Moreover, high CO_2 levels decrease O_2 consumption and ATP production and impair cell proliferation independently of acidosis and hypoxia [96]. Quantification of tissue pH in solid regions of glioma reveals an average value of 7.26. This value is higher than in the cortex of the opposite hemisphere and lower than that in adjacent edematous cortex.

In tumors, ATP content generally decreases when glucose begins to fall, whereas in the normal brain, ATP declines before glucose is reduced [97]. Although these considerations and the observed lactate increase are in support of a preferential glycolytic pathway for ATP production, tumor pH was slightly more alkaline than in normal brain tissue [98-100. Nevertheless, at an extracellular pH of 7.25, the oxygen consumption rate of cells increases by nearly a factor of 2 as the glucose concentration decreases from 5.5 mM to 0.4 mM [101]. This effect of glucose concentration on the oxygen consumption rate, however, was slight at an extracellular pH of 6.95 and disappeared completely at an extracellular pH of 6.60. Hence, low glucose concentrations, associated with a normal extracellular pH will increase oxygen utilization, slowdown glycolysis, increase respiration and aerobic ATP production. An ill animal will consume less food and will avoid effort. This behavior could be a lesson for human beings to reduce blood glucose concentration according to physiological requirements.

Inhibition of proton pumps in cancer and changes in pH are associated with the mechanism by which tumor cells avoid intracellular accumulation of toxic substances [102].

2.6 Respiration and glycolysis

It is well known that respiration and fermentation are two independent biochemical processes, which may occur simultaneously in the living cell. Given an oxygen concentration of 10 per cent or more in the surrounding atmosphere, respiration occurs in the living cell. Given a concentration below 3% of oxygen, fermentation (glycolysis) will occur. Both processes occur in the range from 3% to 10% oxygen (Fig. 2). The growth and lifespan of human diploid cell strains at oxygen concentrations of less than 20% is enhanced and an extension of about 25% of the lifespan of both cell types was carried out by long term cultivation below 10% oxygen [103].

Whenever a cell within the metabolic system (muscle, liver, kidney, lung etc.) uses oxygen at a faster rate than it can be supplied by the circulatory system, the cell begins functioning anaerobically, reducing the pyruvate to lactate instead of further oxidizing it, as it would occur if oxygen supplies were adequate. Lactate thus accumulates in that cell, diffuses out through the bloodstream and eventually finds its way to the liver, where it is reoxidized to pyruvate and converted back to glucose by the gluconeogenic pathway operative there.

Mitochondria play an important role in the functional responses of eukaryotic cells to changes in O_2 concentration, being involved in the adaptation to hypoxia. Previous investigations showed that when eukaryotic cells are exposed to hypoxia, glycolysis is stimulated and it can compensate the low ATP production within the oxidative phosphorylation process. Moreover, Otto Warburg showed that normal cells would become irreversibly cancerous if the environment they rested in had their oxygen levels lowered by 35% for two days. Besides, long-term cell cultures may become malignant without any other influences.

The glucose consumption rate of cancer cells increases much when the oxygen concentration is reduced [101]; however, severe conditions are required to stop cell growth (8.2 μM oxygen and an extracellular pH of 6.6).

Hypoxia inducible factor 1, a marker of the Warburg effect and altered tumor metabolism, is associated with worse overall and recurrence free survival in peripheral tumor regions [104]. Meanwhile, central tumor expression of HIF-1α has no significant correlation with outcome.

These findings highlight the importance of assessing metabolic markers in the context of tumor microenvironment and oxygen tension.

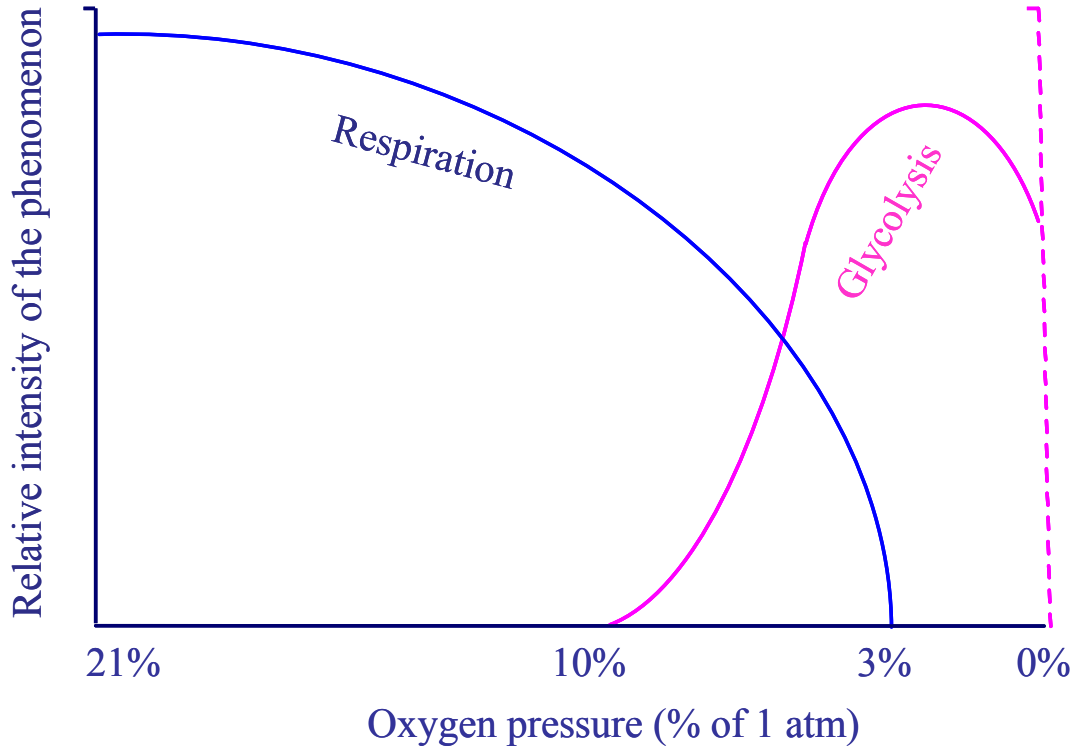

Fig. 2. Schematic presentation of the relative intensities of cellular respiration and glycolysis as a function of oxygen partial pressure

Indeed, hypoxia and the hypoxia-inducible factors (HIFs) step in the metabolic crosstalk between cancer cells and their microenvironment [105]. Nevertheless, tumor cells are able to maintain a high glycolytic rate even if enough oxygen is supplied. HIFs were suspected to control the response of malignant cells to this specific microenvironment by shifting energy production from oxidative phosphorylation towards glycolysis. According to RpHI hypothesis, although enough oxigen may be present in the microenvironment, the cells cannot exchange it with carbon dioxide, which is kept by these cells. Furthermore, the mechanisms by which hypoxia and HIFs regulate tumor metabolism should be reconsidered. Possibly, RpHI may induce hypoxia, which in its turn, creates the necessary conditions for HIF production, a marker of hypoxic state.

Hypoxia prevents normal ATP production by oxidative phosphorylation [106]. Hypoxic cells swell, whereas the swollen mitochondria produce less energy. Chronic inflammation begins following continuous exposure to noxious exogenous influences [106]. Most cancers are mainly attributable to long-term exposure to environmental factors, via a multi-year, multi-step and multi-path process of tumor genesis [11]. Despite ATP synthesis rate is reduced when oxygen availability decreases, the exposure of cells to low oxygen level do not significantly affect the total ATP content, which is quite the same level of normoxia [107]. These data suggest that cells respond to lowered oxygen tension by increasing the activity

of glycolytic enzymes and glucose transporters via the hypoxia-inducible factor-1 (HIF) [107]. HIF up regulates pyruvate dehydrogenase kinase 1, which inhibits the pyruvate dehydrogenase decreasing TCA cycle and consequently oxidative phosphorylation in mitochondria. HIF, which mediates the transcriptional response to hypoxia, is a strong promoter of tumor growth and invasion [108,109].

2.7 Stressed Cells and Neighbor Cells

As the metabolic control mechanism which regulates the transition from aerobic to anaerobic metabolism seems to be of key importance in the development of pathological changes, several studies were initiated to correlate the effects of anoxia on normal and aged mitochondria to the various biochemical and functional parameters of these organelles.

In isolated mitochondria inhibition of respiration and energy production have been demonstrated to be important factors in cell injury especially when nitrogen-induced anaerobiosis was investigated [110]. It was observed that aging, especially under nitrogen, leads to a decrease of the affinity constants of all mitochondria. The effect of anaerobiosis is increased by the combination with decreased pH; it was demonstrated that decreased pH exerts an effect on the redox state of cytochrome *a* in anaerobic, ATP-treated intact mitochondria [111].

Cytochrome *c* oxidase (EC 1.9.3.1.) acts as a final electron acceptor in the mitochondrial respiratory chain and catalyzes the molecular oxygen reduction to water. The fact that the radical intermediates of oxygen reduction have a carcinogenic potential calls to a special consideration of the cytochrome *c* oxidase pathway [112].

The cytochrome *c* oxidase activity is the highest in all forms of host liver mitochondrion; aging of mitochondria was associated with a decrease in enzyme activity, which was more intense in host liver mitochondria than in the tumor ones. As depicted in Fig. 3, ageing and anaerobiosis provoke a partial breakdown of the mitochondrial biostructure; the cytochrome c oxidase activity mirrors this process [103]. The biostructure of ascitic mitochondria is also broken-down; anaerobiosis and carcinogenic factors induce a similar breaking down of mitochondria. Synthesis of mitochondrial-encoded subunits of cytochrome c oxidase in *Saccharomyces cerevisiae* is also suppressed in response to oxygen deprivation both *in vivo* and *in vitro* [113]. Cytochrome c oxidase activity among other respiratory enzymes monitored an imbalance, which characterizes both a precancerous stage and cancer. While cancer cell is hypoxic, the surrounding cells are overstrained.

Angiogenesis is a necessary component of all healing wounds. Tissue hypoxia is a well-known stimulus to angiogenesis [114,115]. Malignant, ischemic, inflammatory, infectious and immune disorders are characterized by dysregulated formation of new blood vessels [92]. There are, however, inconsistent data and widely differing hypotheses concerning how much (what pressure or concentration) is required to support cellular metabolism and how the oxygen levels in cells within tissues are regulated [116]. There is a wide disparity in the published data and in the hypotheses used to interpret the cell and tissue responses to changes in oxygen pressure [117,118].

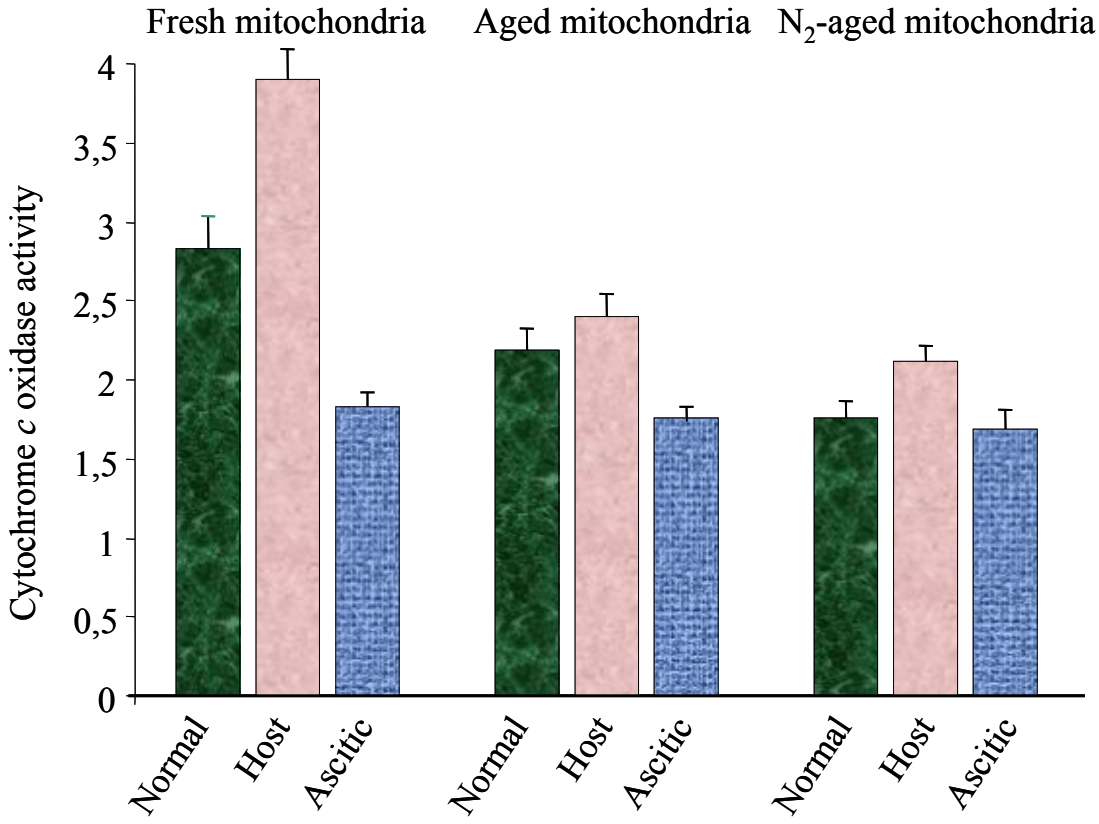

Fig. 3. Scheme showing decreased enzyme activities in tumor cells as compared with those of normal cells, whereas increased enzyme activities are measured around the tumor

Cytochrome c oxidase activity (expressed as micromoles oxidized cytochrome c/mg protein/min) of normal mitochondria, host liver mitochondria and ascitic mitochondria is different under various conditions (Fresh and aged mitochondria for 15 min at 22 °C in air or nitrogen-based anaerobiosis), which brings convincing evidence for RpHI hypothesis. Please, see the papers by C. Mihai for experimental details [110]

2.8 Life Style and Cancer

The American Cancer Society has recently published the Nutrition and Physical Activity Guidelines, which are developed by a national panel of experts in cancer research, prevention, epidemiology, public health and policy. They reflect the current scientific evidence related to dietary and activity patterns and cancer risk [119]. Moderate to vigorous levels of physical activity lower the risk of developing several cancers. Although the lung is a well oxygenated environment, hypoxia has been demonstrated in the center of an invasive lung tumor [104]. The molecular markers of hypoxia like HIF-1α and CA9 suggest that carbon dioxide removal rather than oxygen supply is a benefit of exercise. However, we may suspect the uncoupling activity of some carcinogens in lung cancer such as tar from smoking, which may inhibit oxidative phosphorylation even in the presence of oxygen. Recent studies referred to the mitochondrial uncoupling in leukemia cells, with energy dissipation and low ATP synthesis, as a potential mechanism of impairment of mitochondria

in Warburg phenomenon [120,121]. Moreover, uncoupling proteins are over-expressed in breast cancer, supporting the link between mitochondrial uncoupling and the Warburg effect [26,122]. Besides, overexpression of *E2f1* and *c-Myc* was identified at an early dysplastic stage of hepatocarcinogenesis [123]; the combined activity of *c-Myc* and *E2f1* enhanced the expression of genes involved in mitochondrial metabolism, which results in increased ATP synthesis by the respiratory chain. Overweight and obesity are clearly associated with an increased risk of developing many cancers. Overeating seems to be the main avoidable cause of cancer in nonsmokers [124]. The excess body weight is associated with risk of cancer at several organ sites, including colon, breast, endometrium, oesophagus and kidney [125]. Maintaining a healthy weight, staying physically active and consuming a healthy diet can substantially reduce the risk of developing, or dying from cancer. These same behaviors are also associated with a decreased risk of developing cardiovascular disease and diabetes. All these recommendations are concordant with the RpHI hypothesis. Moreover, anxiety, rather than depression, seems to be a problem at long-term cancer survivors and their spouses, compared with healthy controls [126]. Cancer and depression commonly co-occur, while chronic and severe depression may be associated with elevated cancer risk [127]. Some evidence shows that providing psychosocial support to reduce depression, anxiety, and pain may increase survival time with cancer. Depression and emotional disorders may reduce treatment success in cancer [128]. A bidirectional relationship between cancer and depression was thus found. Women suffering from depression were reported to be more vulnerable to breast cancer [129]. The complex relationship between the psyche and the nervous, immune and endocrine systems seems to be implicated in both cancer risk and survival [130].

It was suggested that cancer cells have dysfunctional mitochondria, which prevent them to use the citric acid or other compounds in the Krebs cycle [45,131]. The mitochondrial function seems to be altered in the precancerous stage. However, if cell content becomes more alkaline due to the continuous work under stress conditions, it will retain carbon dioxide and will get less oxygen regardless of its concentration. The RpHI approach is in full compliance with the findings on cancer occurrence reported by other authors [132]. A more comprehensive theory on cancer etiology to highlight not only the molecular aspects but also the biostructural ones and possibly psychic-related processes as Macovschi stated is expected from a more integrative life science [133].

2.9 Inflammation and Cancer

Chronic inflammatory conditions predispose susceptible cells to neoplastic transformation [134]. In general, the longer the inflammation persists, the higher the risk of cancer. Inflammatory processes have been implicated in the etiology of inflammation-associated cancers [135]; they may induce DNA mutations in cells *via* oxidative/nitrosative stress. However, inflammatory cells and cancer cells themselves produce free radicals and soluble mediators. Reactive intermediates of oxygen and nitrogen may directly oxidize DNA, or may interfere with mechanisms of DNA repair. Free radicals may also rapidly react with proteins, carbohydrates and lipids and the derivative products may induce a perturbation in the intracellular homeostasis, until DNA mutation. The effectors are represented by an imbalance between pro-oxidant and antioxidant enzyme activities [134].

Viral infection-induced chronic inflammation significantly increases the likelihood of cancer development [136]. Many viruses have already confirmed their role in cancer development and progression. Proteins involved in ROS generation are upregulated during chronic inflammation and cancer [135]. Gastrointestinal cancers are strongly linked with chronic

inflammation [137]. Some authors have demonstrated a higher risk of hepatocellular carcinoma in obese individuals. Since obesity is a low-grade inflammatory disease, a direct role of visceral adipose tissue in carcinogenesis was investigated [138]. Cytokines such as IL-6 and TNF-α secreted from the adipose tissue are able to induce a chronic inflammatory condition predisposing the liver to a preneoplastic stage. Chronic inflammation promotes also prostate cancer formation [139]. Cellular glycolytic and oxidative metabolism is significantly influenced by low-level inflammation in prostate epithelial cells. Thus, the micro-environmental inflammatory cytokine TNFα induces aerobic glycolysis while inhibiting oxidative metabolism (Warburg effect). Nevertheless, although the inflammatory cytokine TNF-α can directly induce metabolic changes, similar to those produced by oncogene expression, this effect can simply be blocked by the natural dietary compound curcumin [140].

The Warburg effect is an anomalous characteristic of glucose metabolism in cancer cells [141]. Cancer cells are characterized by increased aerobic glycolysis. Moreover, the pro-inflammatory cytokines promote glycolysis in breast cancer cells, where the inflammation-induced miR-155 function is an important mediator. The miR-155/miR-143/HK2 cascade may be involved in the mechanism linking inflammation to the metabolism of cancer cells [141]. The Warburg phenotype may serve as a main causative mechanism for cellular hyperproliferation and resistance to apoptosis [142]. Indeed, hypoxia and inflammation alter cellular metabolism.

According to Warburg, cell respiration is damaged or insufficient in cancer cells. However, oxygen consumption and carbon dioxide production seem to be high in many cancerous tissues and cells, suggesting that their respiration is not irreversible damaged [24]. The Pasteur effect also appears to be normal in many cancerous tissues since oxygen inhibits lactate production. Many tumor cells show high rates of lactate production in the presence of oxygen, a phenomenon known as the Warburg effect, which has diagnostic and possibly therapeutic implications. The basal lactate production is 3-5 fold higher in T98G glioma cells than in normal astrocytes [143]. On the contrary, the rate of lactate accumulation in response to mitochondrial inhibition with sodium azide is 10 times lower in glioma than in astrocytes, consistent with defective tumor metabolism. The biostructure of these cells seems to be severely broken down. Indeed, RpHI hypothesis clarifies this paradox showing that cancer cells are in fact normal cells, which apparently behave differently due to hypoxic conditions. If a cell is in trouble, neighboring cells will help or destroy it. Such phenomenon can not take place in cell culture and cancer cells thus multiply incessantly. The biostructure breaking down is a gradual process, which may have many stages such as the adaptive one, followed by others like the preneoplastic, neoplastic, or apoptotic stages. The efficacy of cancer therapies, especially for late-stage disease, remains poor overall [144]. Focusing on cancer prevention requires novel targeted therapies. During the process of identifying preventive agents, the task now is to understand how these agents interact with their target molecular compounds or biostructure components [145].

A detailed understanding of preneoplastic phenomena and cellular mechanisms of tumor formation can lead to new therapeutic approaches for improvement of clinical outcome.

3. CONCLUDING REMARKS

Literature findings reviewed here support the hypothesis on the RpHI, which may explain the long interval between exposure of a certain cell to a mutagen and the expression of the resulting mutations, as well as carcinogenesis without any apparent genetic alteration.

Mutations of certain genes have been identified as risk factors for development of certain cases of cancer. However, gradual progress in understanding the origins of neoplasms should also be considered. The hypothesis discussed here takes into consideration the long standing hypoxia within the whole organism, characteristic to the precancerous stage. Under hypoxic conditions, the overstressed cells, getting less oxygen than needed, will lead to anaerobic ATP production, excessive multiplication and finally, tumor development. Such assertion is consistent with most literature data. Consequently, new strategies of cancer prevention should be drafted, based on a physiological approach of precancerous stage. Cancer prevention may be associated with carbon dioxide removal from blood in order to stimulate blood and cell oxygenation and oxygen exchange in cells. Besides, pH and glucose concentration control is important to prevent cachexia. Natural mechanisms protecting against cancer should be taken into consideration. Future medicine can also be seen as caring for physiological balances and not only as an emergency surgery.

Further studies are required to highlight the essential mechanisms characteristic to the preneoplastic stage hidden in the extremely complex oncology. At least, our theoretical approach suggests that hypoxia is a key parameter that positively influences cancer development in overstressed tissues.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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