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Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	2013_IJBcRR_8617
Title of the Manuscript:	Mini-review: Respiratory and pH imbalance at the early precancerous stage
Type of the Article	Mini-review

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PART 1: Review Comments

	Reviewer's comment	Author's comment <i>(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)</i>
<u>Compulsory</u> REVISION comments	Although opinions are rational in some aspects, major revision is still needed to implement before the publication. 1. In this review, authors was sought to present some evidence to support the hypothesis that RpHI was a cause of long standing hypoxia within the organism, and was speculated as the initiating factor for carcinogenesis. Therefore, it is better to give a clear definition of RpHI at the beginning of introduction instead of explain it after redundant foreshadowing.	We would like express our gratitude to Editorial Board and the Reviewers for their pertinent suggestions made to improve the manuscript quality. Following the comments and recommendations by the experts, we made the required changes in the document. 1. Indeed, the hypothesis on a respiratory and pH imbalance which occurs under the stress agents was previously advanced to explain the complex phenomena related to almost all pathologies. It is a biochemical and a physiological process which takes place under the stress conditions, and not the first but ultimate cause of disease. The first cause may be a virus, a bacteria, a carcinogen etc., which distresses a cell, a tissue or the whole body for long. The stressor, acting for a long time, may cause a syndrome we called RpHI. The RpHI results in a long standing hypoxia, which helps the malignant transformation. However, in case of chemical carcinogens as in specific experiments, just a few cells are obliged to face the hazardous material, when, in their effort to destroy and release it, they need much more oxygen than the body can afford. In brief, RpHI means a quality imbalance, not something easily to be quantified. It is not possible to measure the intensity of RpHI, or how intense is hypoxia. There are too many variables to measure. In fact, our aim is to understand better the mechanism of respiratory and pH changes, cachexia etc., if admit such biochemical processes. Indeed, the hypothesis is based on many factors interacting in preneoplastic stage. Hypoxia may play an important role in cancer etiology, but we



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	2. Although RpHI might exist during cancer progression, the author did not provide potent evidence to demonstrate the existence of RpHI at the early precancerous stage. Just as describe in page 6, the RpHI cannot be easily measured, which makes it difficult to identify the time course when the RpHI occurred.	cannot speculate that it is the single initiating factor for carcinogenesis. However, a hypoxic environment may induce a severe prognostic. Besides, hypoxia might be associated with glycolytic processes resulting in a slight lowering of blood pH. Low pH may be associated with an increased rate of proteolysis, which results in cachexia. A better definition of RpHI was introduced to avoid confusions. Thus, after the row 65 in “Introduction” the rows 82-84 were introduced, followed by a subchapter “the RpHI definition” described in the original manuscript from rows 92-111. 2. The reviewer is right. We gave only a few examples of how anyone can demonstrate the existence of RpHI. For instance, under hypoxic conditions, mitochondria swell pH decreases, proteins are hydrolyzed etc. All these things are obvious; still we put all symptoms together and give a rational mechanism. Thus, RpHI hypothesis could explain why it is almost impossible to heal somebody with metastases. The imbalance is too long standing and the whole body too tired, exhausted. Breathing can improve the prognostic but it is not enough for a divorced woman, whose child died, which suffers from breast cancer. Therefore, it is about the quality of life and how to adapt to present date life conditions. First of all, health means quality and then quantity (blood pressure, blood sugar, etc. When measured the blood content of copper and iron ions under slightly shifted pH around 7.4, only this value was associated with the highest concentration of iron. We supposed that proteins and phosphate ions may interfere with these ions depending on pH. Additionally, a slight decrease of pH may also increase the protease activity. Indeed, this manuscript introduced only little direct evidence for RpHI, since it focused mainly on the proposed
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	3. According previous studies, tumor hypoxia induces intracellular pH decrease as a result of lactate acid accumulation. Thus, the author cannot rule out the possibility that RpHI is the consequence of hypoxia but not cause of it.	mechanisms and some confirmatory findings in literature. Other papers by our group should discuss both the new and existing data on this matter. 3. Lactic acid decreases blood pH and that of extra-cellular environment, while intracellular pH of overstrained cells is increased (Chiche et al. <i>Int J Cancer</i>, 130, 1511, 2012). Consequently, increased activity of cell will result in increased intracellular pH, retaining carbon dioxide and a state of hypoxia. These changes are related each to another, but further research should clarify how they interact.
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Minor REVISION comments	More data and discussion are required for drawing the conclusion that the RpHI hypothesis is concordant with Macovschi's statement.	We added at page 11, row 303 the following "Macovschi stated that the biostructure is partially broken down during hypoxia. Since the biostructured matter is found under an altered, abnormal state in cancer cell, RpHI is proposed to explain first the transformation of a normal cell into a preneoplastic one, and second, the transition from preneoplastic cells to malignant ones". From page 11, row 303, it was deleted: "According to Eugen Macovschi, the structure in the cancer cell is found under an altered, abnormal state; the investigation of this state is essential for the knowledge of carcinogenesis." According to Macovschi, under hypoxic conditions, mitochondrial biostructure is broken down. Besides, the cell and mitochondrial biostructure is damaged in neoplasm. The partial breakdown of the biostructure depends particularly on the activity of cellular regulatory mechanisms so that either ones or others of the DNA genes may be genetically activated. We explain the mechanism by which low hypoxia state is created, and how it contributes to the biostructure break down. We added some new data and discussed the findings in the light of the biostructural theory. For instance: "However, on using metabolic inhibitors like dinitrophenol and sodium azide, Macovschi demonstrated partial and reversible biostructure breakdown. 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 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
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		admits that carcinogens are chemicals difficult to be degraded and eliminated from the body. Metabolic processes of carcinogens occur in cells in organisms [Manahan SE. Toxicological chemistry and biochemistry. 3rd Ed., CRC Press LLC, Boca Raton London New York Washington, D.C., 2003, pp. 162-166]. 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 but 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 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. Hence, RpHI hypothesis may bring a rationale explanation for biostructure breakdown in cancer."
<u>Optional/General</u> comments		Other changes we made: The text from rows 485-497 on hypoxia was cut and put at row 408. Besides, more references from E Macovschi and other authors have been added. Please, see the text with track changes.