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Editorial

"The new pH-centric anticancer paradigm in Oncology and Medicine"; SCB, 2017

1. Introduction

Looked at from the genetic point-of-view cancer represents a daunting and, frankly, confusing multiplicity of diseases (more than 100) that require an equally large variety of therapeutic strategies and substances designed to treat each particular tumor. However, when analyzed phenotypically cancer is a relatively uniform disease of very conserved 'hallmark' behaviors across the entire spectrum of tissue and genetic differences. This suggests that all tumors share common molecular, biochemical and physiological characteristics that are independent of the varied genetic backgrounds. Indeed, it becomes evident that, in general terms, there may be a common integrated mechanism underlying neoplastic transformation, growth, progression and metastasis. The challenge of modern oncology is to integrate all the diverse experimental data to create a new physiological/metabolic/biochemical/molecular and energetic paradigm that can unite our thinking in-order-to better understand how both neoplastic progression and therapies function.

In the present issue, we present the growing evidence, obtained from a great number of studies, for a fundamental, integrated mechanism involved not only in the initiation of the neoplastic process but in the evolution of all stages of the malignant process as-well-as in new approaches to cancer therapeutics derived from this knowledge. This new paradigm is based on a pivotal characteristic, the aberrant regulation of the pH (hydrogen ion)-dynamics of cancer, which we have defined as "the pH-centric cancer paradigm". Here, we introduce a series of reviews dealing with the growing body of evidence that shows that all cancer cells of all human tumors, have an acid-base disturbance that is completely different, and even opposite, than that observed in normal tissues: an interstitial acidic microenvironment linked to an intracellular alkalosis (the cancer-specific proton reversal). Importantly, both intracellular alkalinity and extracellular acidity are not merely the consequences of altered tumor metabolism but can be important biological signals that, in turn, have significant effects on the key processes determining tumor natural history, progression and therapeutic strategies.

Seen in this light, different fields of cancer research can now be integrated into a hierarchical one ranging from etiology to etiopathogenesis, from prevention to carcinogenesis, from cancer cell metabolism and neovascularization to drug resistance, and from cancer immunity and the spontaneous regression of cancer to some key aspects of chemotherapy and selective apoptosis.

This new and integral approach to the different stages and the many faces and manifestations of cancer opens new avenues to both increasing our understanding of cancer as a single disease process and to improve the treatment of malignant disease. This integral approach seems to follow the old Hippocratic aphorism that reads : *"Now of all diseases the fashion is the same, but the seat varies. So while diseases are thought to be entirely unlike one another, owing to the difference in their seat, in reality all have one essence and cause. What this cause is I shall try to declare in the discourse that follows"* (Hippocrates, in: "Breaths").

This issue is dedicated to those efforts and hopes.

2. Summaries and section of the different contributions

2.1. Basic and different cancer types/pH regulatory systems

Pedersen and Stock ("Roles of pH and the Na^+/H^+ exchanger NHE1 in cancer: From cell biology and animal models to an emerging translational perspective?") deal with the roles of the pH and the Na^+/H^+ exchanger (NHE1) in cancer, going from basic molecular and biological research to animal studies to propose an emerging translational effort in order to suggest new avenues of treatment. Their review covers a vast amount of data produced in many laboratories, including their own, in a very systematic way to arrive at animal models and human tissues. Importantly, the authors not only present results suggesting the probable important role of the NHE1 in cancer progression and its use as a target for therapy, but also the possible problems that could occur when translating all these pre-clinical data into clinical therapy and bedside oncology. They finally consider the role of the NHE1 in driving the dynamic interaction between microglia and glioma in the progression of these brain tumors and how pharmacological blocking of the NHE1 could have an important therapeutic effect by blocking this vicious cycle.

Granja et al. ("Value of pH regulators in the diagnosis, prognosis and treatment of cancer") have reviewed the latest evidence on which proton pumps and transporters are expressed and/or overexpressed in a series of different tumors. This serves to start considering the urgent need of stimulating further research in order to complete a molecular mapping that would allow to know with the highest possible selectivity which proton transport inhibitors should be used in each particular tumor. This subject now appears

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as a highly important one within the new pH-centric paradigm in translational oncology.

Amith and Fliegel ("Na^+/H^+ exchanger-mediated hydrogen ion extrusion as a carcinogenic signal in triple negative breast cancer etiopathogenesis and prospects for its inhibition in therapeutics") offer an original and breaking-through interpretation of the pH-related etiological and etiopathogenic mechanism responsible for the onset and dissemination of, mainly, the most malignant subtype of breast cancer. This contribution presents the role, mechanisms, regulation and, finally, the potential of inhibition of the NHE1 as a therapeutic strategy at least for triple negative breast cancer. In these situations the basal elevated NHE1 activity increases intracellular pH and decreases extracellular pH. Hydrogen ion (H^+) extrusion by itself appears as the fundamental carcinogenic insult and signal for cell transformation, at least in this subtype of breast cancer. From there on, NHE1 tends to become redistributed to the leading edge of the already malignant migrating cells and to invadopodia of invading cells, creating a localized acidic microenvironment at these sites and, in so doing, facilitates extracellular matrix (ECM) digestion, thus promoting cell movement and contributing to local growth and the metastatic process of breast cancer, all phenomena integrated within one and only dynamic process. Inhibition of NHE1, by reversing the intracellular to extracellular gradient, reduces these effects resulting in decreased metastasis. This contribution, as well as others in this issue, proposes the utilization of NHE1 inhibitors in cancer, both in prevention and treatment. It also offers new therapeutic possibilities, mainly in the triple-negative subtype of breast cancer, the subtype with the worst prognosis among the different types of malignant breast tumors.

Omran et al. ("Physical and biological characteristics of multidrug resistance (MDR): An integral approach considering pH and drug resistance in cancer") explores and discusses the controversies existing in the relationship between P-glycoprotein (Pgp) and multi-drug resistance and propose that the Pgp mediated MDR has its most important mechanism in the pH aberrant changes of the tumor microenvironment, triggered by the Warburg effect. The authors emphasize the importance of the results obtained with proton pump inhibitors in cancer in both reverting multidrug resistance and reducing the side effects of chemotherapy. This original contribution is based upon the biophysics and dynamics of drug-membrane interactions. Furthermore, while MDR has been described as a phenomenon restricted to tumor cells of tumors that initially respond to a therapy and then become unresponsive to many drugs, the majority of cancers, mostly solid tumors, do not respond from the very beginning to a variety of single and/or combined treatments.

All in all, cancers are "refractory" rather than resistant to drugs, suggesting that extracellular acidity is a highly efficient mechanism for tumors to resist to anticancer drugs. This is a leading-edge work describing in detail a new way of interpreting the field of MDR in cancer research and treatment. It also represents a new and integral approach to the understanding of MDR in cancer that, apart from integrating within it the previous Pgp model, considers the problem of MDR from the point of view of physics and mathematics in order to propose a more outreaching and comprehensive paradigm of MDR that takes into account the proton (H^+) dynamics of cancer and the selective abnormalities of cancer cells metabolism. The authors also underline how the "traditional" cancer research missed to incorporate knowledge from other scientific areas to better understand MDR because of "the hyper-specialization" of sciences. Then they point out that the Pgp transporter defies the law of enzyme specificity and that drugs only need to stay long enough in the cell membrane to be expelled by P-gp. Finally, they explain how the Warburg effect changes the membrane properties such that drugs stay longer in the membrane, a phenomenon that leads to P-gp being more efficient in expelling anticancer drugs.

2.2. Special conditions

Hardonnière et al. ("Environmental carcinogenesis and pH homeostasis: not only a matter of dysregulated metabolism") advance a brand new approach towards an integral understanding of chemical carcinogenesis, from single carcinogens to possible universal mechanisms. New ways are opened to understand several key aspects of environmental carcinogenesis, both DNA-related and pH-related. This work also brings together, perhaps for the first time, data from many laboratories, including the authors', covering essential and, so far, largely disregarded aspects on the intimate mechanisms of how environmental carcinogens can transform normal cells into cancer cells and drive local growth and metastatic progression. The physiopathological mechanisms underlying these processes and particularly how dysregulated pHi and pHe through NHE1 and cellular metabolism are involved, combining original data and theoretical modeling to support this perspective.

Parks and Poüyssegur (**"**Targeting pH regulating proteins for cancer therapy – Progress and Limitations**"**) explore the complex interactions of pH regulating transporters/enzymes with extracellular buffering and how hypoxia modifies these processes. It also points to future directions and possible applications for the treatment of cancer of the concepts and processes reviewed. The fundamental role of tumor hypoxia in providing a significant advantage for cancer cells during acidosis is considered here in depth, as well as the growing role of hypoxia-regulated proteins. The authors focus on the most recent developments in the understanding of pH regulating factors, this providing an exciting potential for novel therapeutic developments. They finally consider how targeting of the abnormal pH regulation in malignant tumors can become a synergistic strategy with other areas of oncology including immunotherapy and cancer stem cell disruption.

Huber and Rivoltini ("Role of pH microenvironmental abnormalities in malignant tumors and their relationhip to cancer immunity") present a review that is likely to be the very first one in thoroughly describing the association between tumor environmental and interstitial acidosis in hindering the organisms antitumor immune response. They review the emerging topic of the role of the cancer metabolism on anti-tumoral immune responses. This topic is particularly interesting, since it has been demonstrated that the acidity of the tumor microenvironment could negatively interfere with distinct immune surveillance mechanisms thereby leading to immune evasion and progression of tumors. The article also covers a very broad range of biological issues starting with an introduction to the field of immunotherapy that also analyzes the effects of altered tumor metabolism and its secondary extracellular acidosis as an essential factor contributing to immune escape. The authors also have hierarchically integrated the present day knowledge on this subject, reaching an integral synthesis from different fields of investigation to create a most original and valuable contribution about the importance of the environmental and interstitial acidosis (and hypoxia) of malignant tumors in escaping immune control. Still, the role of extracellular acidity on different immune cell sub-populations is not fully understood and requires further investigation. However, low pHe due to altered tumor metabolism results in the dysfunction of effector cells, such as T lymphocytes and natural killer cells as-well-as dendritic cells, while immune suppressive cells, such as myeloid-derived suppressor cells and regulatory T cells, are recruited to the tumor and/or their activity is enhanced, which blocks immune recognition. Furthermore, the effect of low pHe on antibody activity is discussed, which is an important issue due to the current implementation of antibodies for the therapy of cancer patients. The authors conclude with the

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therapeutic potential of directly targeting tumor acidosis in order to restore and/or reinforce anticancer immune response.

Dumas et al. ("Metabolic reprogramming in cancer cells, consequences on pH and tumour progression: Integrated therapeutic perspectives with dietary lipids as adjuvant to anticancer treatment") have contributed with a very comprehensive review with the main aim to cover how metabolic alterations in cancer cells may control tumor aggressiveness through effects on pHi, pHe, EMT, invasiveness, autophagy and treatment resistance. A second aim is to couple this to a discussion of the therapeutic relevance of dietary n-3 PUFAs. The manuscript spans a very wide range of topics and many very interesting points regarding both etiopathogenesis and treatment are made throughout the review. It also deals with the metabolic reprogramming in cancer cells and its consequences on pH homeostasis and tumor progression. Importantly, the authors finally propose dietary lipids (namely omega-3s) as an adjuvant therapy to other anticancer treatments.

2.3. Novel approaches to therapy

Spugnini and Fais ("Proton pump inhibition and cancer therapeutics Is it a specific tumor targeting or a phenomenon secondary to a systemic buffering?") offer a thorough review of the utilization of proton pump inhibition (PPI) in cancer therapeutics based upon the fact that proton pumps and/or exchangers activity and/or hyperactivity are the best allies of malignant tumors since they represent the key factors that allow malignant cells to survive in a metabolically hostile most acidic milieu. They do so by not allowing intracellular acidification through a continuous extrusion of H^+ either outside the cells or elimination within the internal vacuoles. If the excess of protons produced by tumor metabolism were to remain inside the cell, the consequent acid stress would drive cancer cells towards apoptosis, a feature that at the same time indicates a rational and most promising therapeutic pathway to be followed. This article summarizes the translational process that over the last few years has led to the preclinical demonstration that a class of proton pump inhibitors are indeed powerful chemosensitizers and can also improve anticancer immunity by alkalinizing the extracellular/interstitial/intratumoral environment of malignant tumors. The authors rightly conclude that PPI may have a significant role in the new pH-related anti-cancer strategies and should be included in a large variety of them.

Kolosenko et al. ("Therapeutic implications of tumor interstitial acidification") have summarized a great deal of recently published data on the effects of tumor acidosis in tumor pathogenesis with a specific focus on its therapeutic implications. In their work they have updated this field of the oncological pH-centric paradigm with novel approaches while also discussing the chemical properties of small molecules and acidity-related metabolic features as important factors in the design of potentially effective therapeutic strategies. This review discusses the general pro-tumorigenic effects of acidosis, its role in controlling and/or collapsing therapeutic efficacy (MDR) as well as strategies that can be considered for overcoming pH-dependent therapeutic resistance.

Like other groups in the field, they stress the fact that interstitial acidification is a hallmark of solid tumor tissues resulting from the combination of different factors, including cellular buffering systems, defective tissue perfusion and high rates of fermentative cellular metabolism. Besides contributing to tumor pathogenesis and progression, tumor acidosis constitutes an important mechanism in drug resistance. The acidic tumor environment is believed to create a chemical barrier that limits the effects and activity of many anticancer drugs.

Schwartz et al. ("Out of Warburg Effect: effective cancer treatment targeting the metabolism and/or the pH") base their pH-centered therapeutic approach on the Warburg effect and the still controversial Warburg issue of damaged respiration. The intention is to manipulate tumor metabolism and the dysregulated pH dynamics as specific biological targets counteracting tumor growth and dissemination when combined with conventional chemotherapy. They introduce the necessity of a concerted inhibition of the cancer-specific metabolic pathways together with the reduction of the selective reversed pH gradient of tumor cells, while at the same time trying to both reduce glucose utilization while stimulating oxidative phosphorylation. They propose a treatment with a combination of α-lipoic acid and hydroxycitrate that has been shown to be effective in animal models. Furthermore, this approach proposes this treatment in combination with conventional chemotherapy, a mixture that has yielded encouraging results in glioblastoma, brain metastasis and lung cancer. Concomitant correction of the intracellular alkalosis of cancer cells may be a very effective additional and important treatment measure to be considered.

Koltai ("Triple-edged therapy targeting intracellular alkalosis and extracellular acidosis in cancer") introduces a substantial perspective outlining the reasoning for a novel therapeutic strategy based on the dysregulated pH dynamics characteristic of all types of cancer. This represents a logical progression from less integrated perspectives, which finishes in the proposed "triple-edged strategy" of attacking simultaneously both the intracellular and extracellular pH dynamics. From the three well-established and selective pH-related hallmarks of cancer, namely, that the intracellular tumor milieu is slightly or strongly alkaline, the extracellular microenvironment of a malignant tumor is acidic ("proton reversal"), and, finally, that normal cells show exactly the opposite acid-base characteristics, the author concludes that by modifying the pH characteristics of cancer cells it has to be possible to decrease proliferation and invasion. During the last few years these important concepts have opened the way to a whole new branch of research based on the paradigm of counteracting the abnormal hydrogen ion dynamics that cancer cells require for their growth, survival, evolution and finally killing neo-strategy. The aim of this review is to describe a method that may be able to modify the pH dynamics of malignant tumors with repurposed drugs based on the attack of the specific hydrogen ion dynamics of cancer from three different but coordinated angles (triple-edged approach).

Gdovin et al. ("Focal photodynamic intracellular acidification as a cancer therapeutic method") describe a recently 'newborn' method to induce intracellular acidification as a cancer treatment that uses a focal photodynamic procedure. Its scientific contents and principles on which is based are most important and promising and well within the main subject of this Issue. Cancer cells utilize an array of proton transporters to regulate intra- and extracellular pH to even thrive in the most hostile hypoxic conditions, so increasing tumor growth and metastasis. In this vein, efforts to target many of the transporters involved in cancer cell pH regulation have yielded initial promising results. Following a review of the status of photodynamic cancer therapy, a novel light-activated process is presented here. This new method creates localized, rapid, selective and significant decreases exclusively in intracellular pH (pHi), leading to cell death. The light-activation of the H^+ carrier, nitrobenzaldehyde (NDA), has shown to be effective in initiating pH-induced apoptosis in numerous cancerous cell lines *in vitro*, including breast, prostate, and pancreatic cancers. In addition, this intracellular acidification technique causes significant reductions in tumor growth rate and enhanced survival in mice bearing triple-negative breast cancer. The efficacy of an NBA-converting nanoparticle methodology to kill breast cancer cells *in vitro* is described, as well as a discussion of the potential intracellular mechanisms underlying the photodynamically-induced pHi-driven apoptosis.

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2.4. Wrap-up review

Finally, **Harguindey et al.,** introduce a comprehensive review of the new pH-centric paradigm in biomedical research ("Cellular acidification as a new approach to cancer treatment and to the understanding and therapeutics of human neurodegenerative diseases"). The main areas and features covered by the pH-centric paradigm in cancer, from basic research to clinical oncology ("translational research") are here extended horizontally ("transversal research") to embrace human neurodegenerative diseases (HNDDs) under the same pH-centric paradigm. This transversal view creates a newly integrated model that aims to synthesize under a single and unitarian perspective the common grounds of the etiopathogenesis of these pathologies while suggesting new possibilities of treatment of either human malignant tumors and neurodegenerative processes.

The abnormalities of intracellular alkalinization along with extracellular acidification of all types of solid tumors and leukemic cells have never been described in any other disease and now are accepted as a new and specific hallmark of malignancy. The increasing attempts to induce cellular acidification using proton transport inhibitors (PTIs) and other intracellular acidifiers of different origins, considered in depth here, are becoming a new therapeutic concept and selective target of cancer treatment, either as metabolic mediator of apoptosis, in the overcoming of multiple drug resistance (MDR) and/or in restoring immune function. Finally, we discuss the extension of this new pH-centric oncological paradigm into the opposite metabolic and homeostatic acid-base situation found in Human Neurodegenerative Diseases (HNDDs). In this way, novel concepts in the prevention and treatment of HNDDs are advanced from this associated perspective to propose the utilization of a cohort of neural and non-neural derived hormones and growth factors in HNNDs.

Note: Parts of the text of this Introduction belong to the different referees that have generously reviewed the original submissions of the Reviews published in this Issue of Seminar in Cancer Biology.

Addendum:

Order of Topics and subjects in the Issue

1. *Basic and different cancer types/pH regulatory systems:*
Roles of pH and the NHE1 in cancer: **Pederson**
Diagnostic, prognostic and treatment use of pH regulators: **Granja**
Etiological and therapeutic role of the NHE1 in Triple Negative Breast Cancer: Amith
Role of pH in determining multidrug resistance (MDR): **Omran**
2. *Special conditions:*
Environmental carcinogens: **Hardonnière**
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3. *Novel approaches to therapy*
Use of V-ATPase inhibitors: **Spugnini**
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Metabolic targeting: **Schwartz**
Triple-thronged strategy: **Koltai**
Photodynamic therapy to induce intracellular acidification: **Gdovin**
3. *Wrap-up Review*
Cancer, pH and Neurodegenerative Diseases: **Harguindey.**

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