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Review

Triple-edged therapy targeting intracellular alkalosis and extracellular acidosis in cancer

Tomas Koltai

Obra Social del Personal de la Industria de la Alimentación, Departamento de Oncología Estados Unidos 1532, Buenos Aires, C1101ABF, Argentina

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ABSTRACT

Extracellular acidity and intracellular alkalinity are two of the characteristics hallmarks of malignant cells and their environment. This involves an inversion of the extracellular/intracellular pH gradient when compared with normal cells and it gives malignant cells proliferative and invasive advantages. Thus, the reversal of the pH gradient is a legitimate objective in the treatment of cancer and may be accomplished with drugs already used for other purposes and/or with specific new drugs that are currently being studied.

The aim of this review is to describe a triple approach for reversing this gradient inversion using the concerted utilization of proton extrusion inhibitors, mitochondrial poisons and lysosomal poisons that should act synergistically through different mechanisms. The scheme presented here is compatible with almost all the chemotherapeutic protocols currently being used.

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1. Introduction

There are certain established facts regarding tumor pH:

1) The extracellular microenvironment of a malignant tumor is acidic.
2) The intracellular tumor milieu is slightly or strongly alkaline.
3) Normal cells show exactly the opposite picture (this difference between normal and malignant cells is known as inversion of pH gradient) [1]. It is also known as proton reversal [2].

These findings represent a hallmark of cancer, even if they are not included as a hallmark in the seminal description of cancer by Hanahan and Weinberg [3]. Furthermore, this alteration of the acid-base balance becomes more pronounced as the tumor advances. These cancer pH characteristics represent a necessary and advantageous feature for mitosis and proliferation (intracellular alkalosis) on one side, and for migration, invasion and metastasis on the

E-mail address: tkoltai@hotmail.com

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other (extracellular acidosis). By modifying the pH characteristics of cancer cells, it is possible to decrease proliferation and invasion, and this important finding has given rise to a whole new branch of research based on the paradigm of counteracting the abnormal hydrogen ion dynamics [2] that cancer cells require for their growth and evolution. The aim of this review is to describe a method that may be able to modify a tumor's pH with repurposed drugs based on the attack of cancer's pH peculiarities from three different angles (triple-edged approach). The origin of tumoral extracellular acidity is an excess of protons produced by the cancer cells' metabolism, that are generously extruded to the cancer's extracellular environment. Hypoxia also collaborates in the creation of this acid environment, but since the elegant demonstration by Schornack and Gillies [4] in 2003, we know that it does not represent the main cause, as it was previously maintained by some authors. The excess of protons in the cancer cell is due to a highly-increased production of lactic acid, because malignant cells metabolize 10–17 times more glucose than their normal counterparts. A large proportion of the increased glucose metabolism takes place in the aerobic glycolytic pathway (Warburg effect) instead of following the oxidative phosphorylation pathway (OXPHOS). Schwartz et al. maintain that the peculiarities of cancer metabolism and acid-base balance are mainly a consequence of the Warburg effect [5]. Therefore, cancer cells produce high amounts of lactic acid even under normal oxygen levels. If this excess of protons were to remain inside the cell, the consequent acid stress would drive it towards apoptosis. This does not happen because malignant cells develop redundant and overexpressed extruding mechanisms.

Intracellular alkalinity is probably related to an over-functioning of the proton extruder systems, but there are still certain issues to be further clarified. For example, during the cell cycle of a normal cell pHi (intracellular pH) shows an oscillatory mechanism that is absent in malignant cells, where oscillations are reduced and established at an alkaline value. Reduced oxidative phosphorylation and lower CO2 production in malignant cells may lead to less cytoplasmic acidification. Cancer cells require this alkaline cytosol because, as McBrian [6] demonstrated, alkaline pHi leads to histone acetylation which permits an increased transcription process and increased protein synthesis. But proton extruder systems are still the main gate for intracellular alkalization. The main proton extruders are the Vacuolar-ATPase proton pump (V-ATPase), Sodium-hydrogen exchanger-1 (mainly NHE-1), voltage gated sodium channels (VGSCs), monocarboxylate transporters 1 and 4 (MCT1 and MCT4), and carbonic anhydrase (CA). There are others too, but most of the proton trafficking in the cell is carried out by these transporters.

1.1. The fundamentals behind the triple-edged approach

The triple-edged approach is based on the idea of producing acid stress in the malignant cell by three different but synergistic mechanisms:

1) Increasing intracellular lactic acid production.
2) Inhibiting or decreasing proton extruder activity.
3) Increasing intracellular acidity by altering lysosomal membrane permeability.

All three effects can be achieved with repurposed FDA (Food and Drug Administration) approved, non-toxic drugs. Here we propose that a rational, and well-designed association of repurposed drugs that produce any of the above-mentioned effects, could work synergistically to achieve acid stress and apoptosis or at least decrease the proliferation rate and invasion. (See Fig. 1).

Since normal cells produce much less lactic acid than malignant ones, they should not be affected or only minimally so, by this type of approach. Thus, for the triple-edged scheme we propose the simultaneous use of three different groups of drugs

1) Proton extruder inhibitors
2) Mitochondrial "poisons".
3) Lysosomal "poisons".

So, nature and evolution have built a highly redundant and specialized system for proton extrusion. It is not enough to target only one of these systems, because another (or others) will take its place or compensate for its inhibition. Another reason why we try to downregulate as many proton transportation systems as possible, is because tumor cells do not always overexpress the same proton transporters. Unless a "proton transportome", which would allow specific inhibition, is developed in the future, we must use a galenic type of massive attack on almost all the proton transportation systems. On the other hand, the pharmacological build down of all the proton extrusion systems is not possible without high toxicity for the normal cells, but partial inhibition is feasible. Now, if at the same time, we increase proton production in the malignant cells and we also achieve proton release from lysosomes in the same cells, we should be able to alter the pH balance in a significant way and thus create an intracellular proton overload that may drive the malignant cells into apoptosis.

1.1.1. Proton extrusion inhibitors

1.1.1.1. V-ATPase inhibitors. The V-ATPase proton pump is in the membrane of many organelles, including lysosomes, but it is also present on the plasmatic membrane in all eukaryote cells. Its main function is to pump protons out of the cytoplasm to the extracellular matrix or into organelles like lysosomes in which case this enzyme is responsible for lysosomal acidity. Perona et al. [7] demonstrated the relationship between V-ATPase, cytoplasmic alkalosis and cancer in a simple but very meaningful experiment: they transfected mouse fibroblasts with a yeast V-ATPase gene. The fibroblast's cytoplasm became alkaline and induced a tumorigenic phenotype. They proposed the gene of V-ATPase as an oncogenic gene. Cotter et al. found that subunit isoform a3 of the vacuolar ATPase is highly expressed in invasive human breast tumors and it is particularly localized at the level of the invadopodia plasmatic membrane in migrating cells but it is absent in normal epithelial cells of the breast. Furthermore, its knockdown reduces migration [8]. Also, omeprazole and other proton pump inhibitors (PPIs) like pantoprazole, lansoprazole, esomeprazole, rabeprazole have shown interesting anti-cancer effects. There are at least four ongoing clinical trials with the objective of determining the role of these drugs in the treatment of cancer (NCTs: 02595372, 01748500, 01069081, 01163903) [9].

Using esomeprazole on cervical cancer cells Song et al. found that this drug decreased pHi and enhanced chemosensitivity to paclitaxel [10]. This is only one in more than 20 recent publications, all of which point in the same direction. Falcone et al. published three cases of advanced refractory gastrointestinal cancer treated with high doses of rabeprazole and metronomic chemotherapy with good results [11]. The possible benefit of PPIs in chemotherapy refractory malignancies comes from the fact that the altered pH gradient at the plasma membrane of many tumors is an important mechanism of drug resistance [12].

1.1.1.2. NHE-1 inhibitors. NHE-1 extrudes protons interchanging them with the sodium that it imports, this increases intracellular pH. It is probably one of the main proton extruders in cancer cells because it is highly concentrated in the invadopodia, which is responsible for the migration and invasion processes.

In 1981 Moolenaar et al. [13] experimenting with ion flows in mouse neuroblastoma cells observed that when Na^+ was added to

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