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REVIEW ARTICLE

A nonmainstream approach against cancer

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Abstract

The discovery of antibiotics as specific and effective drugs against infectious agents has generated the belief that the famous Paul Erlich theory on magic bullet should be applied to cancer as well. However, after around 60 years of failures in finding a magic bullet against cancer, a question appears mandatory: does the magic bullet against cancer really exist? In trying to understand more on the issue, we propose three discoveries are coming from a nonmainstream approach against cancer. Tumor is acidic, and tumor acidity impairs drugs entering within tumor cells and isolates tumors from the rest of the body. Proton pumps are key in allowing tumor cells to live in the acidic microenvironment. A class of antiacidic drugs, proton pump inhibitors (PPIs), were shown to have a potent anti-tumor effect, through inhibition of proton pumps in tumor cells. PPIs are indeed prodrugs needing acidity to be activated into the active molecule. So they use protonation by H+ as an activating mechanism, while the vast majority of drugs are totally neutralized by protonation. An anti-tumor therapy based on PPI showed to be effective both *in vitro* and *in vivo*. Differently from normal cells, cancer cells meet their energy needs in great part by fermentation, and it appears conceivable that hypoxia and low nutrient transform tumor cells into fermenting anaerobes. This suggests that cancer cells are more similar to unicellular organisms, aimed at surviving in a continuous fighting, rather than cooperating, with other cells, as it occurs in the normal homeostasis of our body. We have shown that cancer cells take their fuel by ''cannibalizing'' other cells, either dead or alive, especially when starved and in acidic condition. This finding led to the discovery of a new oncogene TM9SF4 that human malignant cell shares with amoebas. The evidence is accumulating that almost all the cells release extracellular vehicles (EVs), from micro- to nanosize, which shuttle a variety of molecules. Tumor cells, particularly when stressed in their hostile microenvironment, release high levels of EVs, able to interact with target cells in various ways, within an organ or at a distance. They may represent both valuable tumor biomarker and shuttles for drugs with anti-tumor properties. This article wants to burst a real change in future anti-cancer strategies, based on the idea that tumors are much more common features than specific molecular targets.

Keywords

Acidity, cancer nonmainstream, cannibalism, exosome, therapy

History

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Introduction

''Drug research needs serendipity''. This was the title of an article published in The Financial Times in 2008[1]. The authors discussed the reasons of a dramatic failure in drug discovery. In their own words: ''*The molecular revolution was supposed to enable drug discovery to evolve from chance observation into rational design, yet dwindling pipelines threaten the survival of the pharmaceutical industry. What went wrong? The answer, we suggest, is the mismeasure of uncertainty, as academic researchers underestimated the fragility of their scientific knowledge while pharmaceuticals executives overestimated their ability to domesticate scientific research*.'' Of course, we have no chance to find a reason for not agreeing with this statement. There is looseness between academic science and the pharmas in biomedical research. There is something looking like ''unrealistic ambition'': we take the discoveries of scientists, and we apply to the concept of Research and Development the potentially applicable findings coming from basic research. This approach did not get to innovative and effective therapies for major diseases. The authors of the article wrote further: ''*For all the breathless headlines proclaiming breakthrough discoveries, the truth is that we still do not understand what causes most disease. Even when we can identify a responsible gene or implicate an important mutation, we have made only limited progress in turning these results into treatments*.'' Unfortunately, this is dramatically true, and I fear we have to regard seriously for this truth, to find a way to overcome this failure that is becoming a tragedy for the human beings. Again from the words of the authors ''*Medical research is particularly hampered by the scarcity of good animal models for most human disease, as well as by the tendency of academic science to focus on the ''bits and pieces'' of life – DNA, proteins,*

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cultured cells – rather than on the integrative analysis of entire organisms, which can be more difficult to study.'' In fact, from the age of the big discoveries in medicine, where medical scientists often tested their ideas on their own bodies, today biomedical research is mostly deprived of MD or physicians, while full up of basic scientists, with no medical culture and more badly with no interest in discovering ''the causes of the diseases''. From the same article ''*Nevertheless, real scientific progress has occurred, inviting the question: why do pharmaceutical companies, which spend billions of dollars each year trying to turn advances into treatments, have so little to show for their efforts? Answer: spreadsheets are easy; science is hard*.'' I fear the problem is that during the last decades ''science'' has become a sort of spreadsheet application. This is because the research projects in biomedicine where to set up in the NASA-like way. Something similar to ''we want to get to the moon'', Yes; but discovering the cause/s of the diseases, to try to cure them, does not correspond to the will to get to the moon. The unforgotten genius and 1931 Nobel Prize for Medicine Prof. Otto H. Warburg suggested to all medical scientists at the beginning of the last century: ''*We can only cure what we can understand first*.'' I think we should reset our research in aiming at the understanding of the diseases. The example of cancer is emblematic, since we still ignore the prime etiology of tumors. The result is that after more than 60 years from the introduction of chemotherapy in human beings, the gold standard anti-tumor strategies offered to cancer patients are still based on chemotherapy, surgery and radiotherapy, which physically try to destroy cancer with brutal force rather than selectively interacting with cancer cells' unique biological characteristics. Cancer represents an area with significant unmet medical need, with millions of people worldwide being diagnosed annually and in spite of the currently available therapy, millions of patients die from this disease every year[3]. There is an urgent need for safe and effective new treatments resulting in durable disease remissions and increased overall survival. This point is consistent with an article by Robert A. Gatenby[3]. This article proposed to change the strategy in the war against cancer. Gatenby began from some facts ''*The German Nobel laureate Paul Ehrlich introduced the concept of 'magic bullets' more than 100 years ago: compounds that could be engineered to selectively target and kill tumor cells or disease-causing organisms without affecting the normal cells in the body. The success of antibiotics 50 years later seemed to be a strong validation of Ehrlich's idea. Indeed, so influential and enduring was medicine's triumph over bacteria that the 'war on cancer' continues to be driven by the implicit assumption that magic bullets will one day be found for the disease*''. After so many years we are still waiting for this magic bullet against malignant tumors and, of course, this is generating the idea that something went wrong along the way. Gatenby concluded, ''*However, in battles against cancer, magic bullets may not exist and evolution dictates the rules of engagement*.'' Gatenby proposed that a reasonable approach may be to set up therapeutic strategies aimed at controlling cancer rather than trying to cure or even heal it, through very toxic drug combination that is seeming to be more destructive to the patients' body rather than cancer, by itself. All in all, these considerations suggest that we should proceed along two different but parallel paths in trying to find a way to cure the disease, but avoiding at the same time to be uselessly aggressive for the patient's body with extremely toxic and poorly effective drugs. It also seems that to adopt ''take care of tumor patients'', is more reasonable than trying to heal entirely them from the disease, probably causing more toxicity than healing. Thus, it seems highly reasonable that changing the strategy against cancer has become an urgency. The first and more important fact that should be re-discussed is the belief that cancer, but some hematologic malignancies, is a localized disease and that a surgical or a surgical-like approach is always the best strategy in order to eradicate this ''monster'' from our body. Very recently a interesting article has been published in ''Medical Hypothesis'', providing evidence that cancer surgery not only does not represent a definitive eradication of cancer, but it does not lead to a real survival benefit, except in a few immediately life-threatening situations[4]. The author concluded that this is due to the fact that surgery appears to be based on an invalid paradigm of what cancer is. In fact, cancer appears to be a systemic rather than a localized and therefore eradicable disease. Of course, he expressed the hope that the standard cancer treatments will be reassessed in the light of his demonstration. But actually, while the author clearly stated that cancer is a systemic or at least non-localized disease, he did not precisely propose how to step ahead in changing the strategy. Here, I would like to propose a series of discoveries generated by a ''nonmainstream approach'' to research on cancer. First, I would come back to a concept which in the past had a pivotal role in the identification of drugs proven effective in different diseases: ''serendipity''. Today, the word ''serendipity'', while with ancient origin, is used worldwide with different definitions ''the faculty of making happy and unexpected discoveries by accident'' or ''the faculty of finding valuable or agreeable things not sought for'' or ''an accidental discovery;'' or ''finding one thing while looking for something else''. However, serendipity is one of the pivotal factors contributing to drug discovery. Whether we want to save the definition that serendipity implies the finding of one thing while looking for something else, we have to recall the discovery of penicillin first. Fleming was studying ''*Staphylococcus influenzae*'' when one of his culture plates had become contaminated and developed a mold that created a bacteria-free circle. Then he found within the mold a substance very active against the vast majority of the bacteria infecting the human beings. Serendipity had a key role in the discovery of a wide panel of psychotropic drugs as well, including aniline purple, lysergic acid diethylamide, meprobamate, chlorpromazine and imipramine[5]. In introducing some examples obtained with a nonmainstream approach to cancer research, I would like to first recall and emphasize that to notice something that some others did not realize before you, and, therefore, to get to a serendipity-mediated discovery, you need to pay a high level of attention on what is occurring with a 360° view around you. But this is not entirelyenough, since, and properly talking about scientific discoveries, you should have your mind sufficiently unbiased from mainstream infrastructures, normally making you extremely focused on a particular endpoint, without paying attention to potential ''unexpected discoveries''. A researcher in medicine should look at the things with the curious and the innocent eyes of a child. Probably, research in medicine should come back to the age of innocence, which should cancel the age of mainstream reports, definitively not contributing to real advances in the cure of human diseases. Max Planck said ''Science progresses not because scientists change their minds, but rather because scientists attached to erroneous views die, and are replaced'' and Otto Warburg used the same words when he realized the lack of acceptance of his ideas.

Some nonmainstream examples

Example 1: tumor acidity and the Warburg's effect

Probably we should have a different look, with different eyes, to the thousands of drugs we have on the market. Probably we have to think better of the off-targeting and/or an off-label use of drugs that are commonly used in the treatment of other diseases or at lower dosages. In fact, there is an interesting approach using side effect similarities for drug target identification[6]. An example of the off-targeting approach is PPIs, that together of having some

off-targets in the central nervous system[6], have been shown to exert a potent anti-tumor effect, through inhibition of proton pumps expressed by malignant tumor cells, that are similar but not identical to the gastric proton pumps, thought to be their specific targets[7–12]. Hyperfunction of proton pumps is a hallmark of very cancer cells[9,11]. However, many other ''proton or ion exchangers'' are involved in cancer cell homeostasis, and inhibition of these molecules always leads to potent anti-tumor effects[13]. In fact, tumor cells are obliged to survive in a very hostile microenvironment characterized by low nutrient supply, hypoxia and low pH[14]. Proton pumps help the cancer cells to eliminate quickly H+, to avoid intracellular acidification[13]. The way cancer cells get to the generation of this acidic microenvironment, so hostile to normal cells that are not equipped to survive in the same conditions[15], is not entirely built. In fact, this murderous microenvironment isolates cancers from the rest of the body, and probably the most crucial factor in determinating this condition is the extracellular acidity of tumors[14]. What is appearing from the ensemble of the research of the last century is that tumor acidity is a progressive phenomenon beginning with the acquisition of a tumor-specific metabolism, also called ''Warburg's effect''. In 1924, biochemist and Nobel Laureate Otto Heinrich Warburg postulated that cancer cells differ from normal healthy cells in how they convert fuel (food, glucose) to energy. As already mentioned, most normal cells follow the Krebs cycle, which requires oxygen to convert glucose to energy and produces 36 ATP molecules per unit of fuel. Warburg presented evidence that many cancer cells make energy through glycolysis, with a lactic acid byproduct that accounts for the acidic cellular microenvironment common to cancerous tissue[16]. Glycolysis is also a much less efficient energy production pathway, yielding just two ATP molecules per unit of fuel, only one-eighteenth of Krebs cycle production. Therefore, the cancer cell has to consume much more glucose to generate enough energy to thrive[58]. Although Otto Warburg was a brilliant biochemist, most of his peers did not take his hypothesis seriously (except for a few, such as Nobelist and co-discoverer of vitamin C, Albert Szent-Gyorgyi). In fact, until recently, the Warburg Hypothesis had been forgotten. One of the issues with the Warburg hypothesis was why cancer cells would use less efficient glycolysis to produce energy even when sufficient oxygen was present, a condition normally favoring the Krebs cycle. Warburg reported that cancer cells maintain a lower pH, as low as 6.0, due to lactic acid production and elevated CO_2[16]. A take home message we want to preserve from the Warburg's hypothesis is that, while Cancer, above all other diseases, has countless secondary causes (almost anything can cause cancer), probably there is only one common prime cause, ''the replacement of the oxygen respiration (oxidation of sugar) in normal body cells with sugar fermentation'', with production and accumulation of lactic acid leading to a progressive acidification of the tumor microenvironment. The acidity of the extracellular spaces also leads to a progressive selection of cells that are equipped to survive at low pH. In fact, malignant cells survive thanks to the activity of proton pumps that avoid intracellular acidification. However, another important issue related to tumor acidity is the resistance of tumors, particularly solid tumors, to anticancer agents[17]. The way acidity impairs the effect of chemical drugs, which are dreadful poisons in principle, is that the great majority of them are weak bases. If in a H + rich milieu, they are immediately protonated and neutralized outside the tumor cells, and in few words they do not enter the target cells quickly enough[7,9] and even the few drugs that manage to enter, probably through a sort direct ''auto-buffering effect'', are internalized by the intracellular acidic vacuoles, where they are neutralized and possibly eliminated by nanovesicles outside the cells, as we have shown for cisplatin[18]. We provided a series of evidence that PPIs can render resistant cancer cells and tumors fully responsible to chemotherapeutics[7] even at sub-optimal doses[19]. The pre-clinical data led to some clinical trials in patients with different cancer histologies with very encouraging results[20] (clinicaltrial.gov, NCT01069081), also supported by clinical trials in domestic animals with spontaneous cancers[12,21]. However, the most stimulating and original hypothesis was to deprive cancer cells of crucial survival options, such proton pumps are, to induce a sort of suicide in cancer cells through intracellular acidification and consequent activation of lytic enzymes, in turn leading to a quick and inexorable cell death. The experiments performed in this direction invariably showed that PPIs, currently used worldwide as antiacidic and gastroprotector molecules (i.e. omeprazole, esomeprazole, lansoprazole, rabeprazole), were highly cytotoxic for a variety of human cancer cells and cancers, and the earliest event of this PPI-induced tumor cell death was intracellular acidification[8,10]. To note, the results obtained with PPI were echoed by inhibitors directed against almost all proton exchangers regulating the proton traffic between the intracellular and the extracellular tumor compartments[13]. This may represent an example of a nonmainstream therapeutic approach to cancer, inasmuch the current strategies against cancer are either to attack it with the most destroying forces (surgery, chemotherapy, radiotherapy) or trying to target specifically each cancer, probably each, with specific and/or personal drugs, with what was called ''the magic bullet''. This strategy was called molecular targeting approach to cancer, and, but some few cases, unfortunately, did not lead to the expected results. The anti-proton pump approach proposes the use of a class of simply anti-acidic drugs that are out of any pipeline of the Big Pharmas and are out of the minds of the vast majority of scientists involved in research on cancer. As a direct consequence, clinical oncologists fear in using PPI in the first line treatments for cancer patients, since PPI are not included in the standard protocols approved by the national and international regulatory agencies for the treatment of cancers. Thus, the only one way to have access to clinical trials was through a backdoor strategy, which was the combination of standard chemotherapy with PPI. This was possible because acidity is a major cause of the resistance of malignant tumors to a wide array of drugs[17] and PPIs induce chemosensitization by reducing extracellular acidity that is a very effective mechanism of chemoresistance[7]. The results of the first clinical trial performed in patients with osteosarcoma showed that PPI increased the effectiveness of neoadjuvant polychemotherapy, particularly in a subclass of patients that currently show a low level of clinical response[20]. These results were supported by a clinical trial performed in pets with spontaneous tumors based on the combination of high dosage PPI with standard chemotherapy; the results showed a very remarkable increase not only in the rate of clinical remissions, but also in the quality of life of the treated animals[12]. In a further study, PPIs combined with systemic alkalization markedly improved the clinical response to metronomic chemotherapy (i.e. daily treatment with suboptimal doses of chemodrugs, instead of each 2–3 weeks treatment with the standard high dosages) in a group of domestic animals with spontaneous cancers[21]. Together with the use of potent inhibitors of proton pumps also, the use of systemic alkalization has been evaluated as a potential anti-cancer approach[14]. Very recently it was clearly shown that simply through adding sodium bicarbonate to the tap water drunk by mice which spontaneously develop prostate cancer[22], it is possible to prevent the generation of cancer in almost the 100% of the animals. Of course sodium bicarbonate is not the ideal compound for some reasons including: (i) sodium bicarbonate is unpalatable which might affect the patient compliance especially when the study recommends very high dosages, as it seems the case, (ii) but also because it is a sodium

salt and a long-standing treatment may lead to both cardiovascular and kidney dysfunction. However, the results obtained in prostate cancer developing mice are amazing and cannot be ignored, at least because they represent a clear proof of concept that a simple and continuous correction of the pH balance in our body may represent the future of both prevention and therapy of cancer. Of course, it may appear overly nonmainstream to be acceptable, but it was demonstrated with solid scientific evidence[22], with a solid scientific background, and to be honest, it is a paradox that scientific evidence cannot be accepted only because it is nonmainstream. The idea of treating cancers with anti-acidic approach or inhibition of proton exchangers was the background that convinced a group of scientists to found a new society, called International Society for Proton Dynamics in Cancer[23], now International Society of Cancer Metabolism. However, the idea to pool together the proton exchanger inhibitors is becoming one of the future strategies for treating cancer patients, and Salvador Harguindey is one of the few clinical oncologist worldwide who experimented this approach in cancer patients, without other concomitant treatments[24].

Example 2: cancer cells behave as amoebas

All normal body cells meet their energy needs by respiration of oxygen, whereas cancer cells meet their energy needs in great part by sugar fermentation. All normal body cells are thus obligate aerobes, whereas all cancer cells are partial anaerobes. From the standpoint of the physics and chemistry of life, this difference between normal and cancer cells is so great that one can scarcely picture a greater difference. Oxygen gas, the donor of energy in plants and animals, is dethroned in the cancer cells and replaced by the energy yielding reaction of the lowest living forms, namely the fermentation of sugar.

In every case, during the cancer development, the oxygen respiration always falls, fermentation appears, and the highly differentiated cells are transformed into fermenting anaerobes, which have lost all their body functions and retain only the now useless property of growth and replication. Thus, within a tumor mass when respiration disappears, life does not disappear, but life relates to a cell that is more similar to unicellular organisms than the cells aimed at cooperating with other cells, in organs and compartment of our body. Now it is the time to stop and think. All these, unfortunately, neglected, features of malignant tumors suggest that while cancer has been considered a disease in which rapid proliferation and uncontrolled cell growth are the most relevant hallmarks, cancer cells are obliged ''to evolve or regress'' in order to survive in a very hostile and challenging microenvironment including hypoxia, acidosis and low nutrient supply. It is likely that these adaptations significantly contribute to the ability of cancers to metastasize to other organs and survive to the aggression of very toxic therapies, probably through extracellular acidity. A hypothesis might be that cancer cells' secret is to develop strategies, probably through a microenvironmental, Darwinian-like induced cell selection, aimed at maximizing tolerance and flexibility to unfavorable conditions of the environment where tumor cells are obliged to live, or at least survive, and grow. From these thoughts and considerations, we should rethink cancer cells not as a pool of stratifying cells with a very speed turn over due to uncontrolled growth, but as single units armed to survive in a very hostile microenvironment. The evidence is growing supporting the hypothesis that tumor cells live as individual beings. One question may be: how they do that? How is it possible that within a human body there are cells that behave and live in a totally different way from the vast majority of the cells generating from the same body? What it seems conceivable is that very active proton exchangers help a lot of tumor cells at least in facing-off microenvironmental H+ accumulation. However, how cancer cells deal with the very low nutrient supply[57]? If we consider cancer cells similar to unicellular organisms, they may use another mechanism to face up starvation or nutrients deprivation that is cannibalism of other cells, either dead or alive that is to feed on other cells, as unicellular microorganisms do[25,26]. Phagocytosis is usually thought of as a process by which professional phagocytes, such as macrophages, engulf unwanted material in order to clear/scavenge it from the body. This notion is rooted in a century of nearly exclusive focus on the role of phagocytosis in immunity and the understanding of mechanisms involved in this process. Nevertheless, initial studies on phagocytosis, including those of Metchnikoff, stemmed from investigations of amoebas ingesting and feeding upon other microorganisms. Only later was the existence of macrophages discovered in higher organisms. The fact that phagocytosis in multicellular organisms can also be used for obtaining nutrients is apparent in the observation that tumor cells feed upon neighboring cells in a process called ''tumor cell cannibalism''. The original characterization of phagocytosis was based on observations of the microbiological world, specifically free-living amoebae that ingested, killed and digested other microorganisms to feed upon them[27]. Later, mobile cells whose phagocytic activity was used for host defense were found in multicellular organisms. Those studies led to a radical change in the vision of phagocytosis: from ''eating to feed'' to ''eating to defend''. In fact, in addition to clearing microorganisms, macrophages internalize, process and present antigens to the immune system, playing a critical role in the adaptive immune response as well. However, cancer cells show a clear feeding behavior primarily oriented against neighboring cells[15]. Interestingly, such behavior was virtually undetected in cells derived from the primary tumors, suggesting that cannibalism may represent a hallmark of metastatic or very malignant/undifferentiated cells. Unlike macrophages, which engulf only dead or transformed cells, metastatic tumor cells indiscriminately internalize amorphous material as well as both apoptotic and healthy cells, including immune cells[15,25]. In fact, our recent report suggests that tumor cell cannibalism is a very important survival option of tumors by either (i) increasing nutrient levels through feeding upon other cells and (ii) allowing escape from the specific immune response by cannibalizing tumor infiltrating T lymphocytes that should represent their murderers[15]. In this scenario, upregulation of phagocytosis in human tumor cells may be a sign of regression to a simpler (i.e. ancestral) life, similar to that of amoebae, where the goal is to survive and propagate in a hostile microenvironment. In fact, human cancer cells behave very similarly to unicellular microorganisms, such as amoebas, inasmuch they live when ingesting cells while they die when engulfed by amorphic material[15]. This line of research has led to the discovery of a protein that amoebas and cancer cells share, TM9SF4[28], that is related to their ability, to cannibalize other cells[26]. More recently, the same line of research has shown that TM9SF4 represents a novel V-ATPase-associated protein involved in V-ATPase activation[29]. V-ATPase is a proton pump highly active in malignant tumors we have shown to be a target, while not fully specific, of PPI[9,11]. TM9SF4 knockdown is associated with a significant inhibition of the invasive behavior of colon cancer cells and with increased sensitivity to the effect of chemotherapeutics. These effects were also consistent with reversing tumor pH gradient with a decrease of cytosolic pH, alkalization of intracellular vesicles and a reduction of extracellular acidity[29], further supporting the importance of TM9SF4 as a potential target for future anticancer therapies. All in all this line of research, that started simply from the microscopic observation of the cells within other cells, has led to the discovery of a new oncoprotein involved in a key function

of malignant cells, that is the ability to feed on other cells, probably representing a very important butt for future anticancer therapies, whose discovery was achieved through a definitive nonmainstream way of doing research in medicine. We want to emphasize that to get to the discovery of new tumor biomarkers and/or molecular targets for new anticancer approaches, the mainstream approach is a genomic investigation, which in fact is not leading to remarkable and useful discovery for epochal changes in cancer patients' management. In fact, tumor cannibalism is included in a list of so called ''cell-in-cell phenomena'', together with entosis (overholtzer), ''emperitosis'', ''emperipolesis'' and ''suicidal emperipolesis''[30–34]. An interesting debate on this issue may be read online in ''It's a Cell-Eat-Cell World'' By Jef Akst (1 August 2011, The Scientist).

Example 3 – exosome, a natural shuttle for biomarkers and drugs

To date the way the cells interact with other cells, both in a paracrine way within an organ or at distance is not entirely understood. However, in the last decades a novel mechanism for cell-to-cell interaction is overbearingly coming out. In fact, evidence is accumulating that almost all the cells release extracellular vesicles (EVs) of various sizes, from micro to nano, that shuttle a paramount variety of molecules, including proteins, lipids and nucleic acids, and again with a nonmainstream approach we contributed at various levels in discovering a key contribution of EVs in cancer pathogenesis and as cancer biomarkers. Among EVs, exosomes have currently a central role in discoveries, showing the complexity of our body, through a series of mostly unknown mechanisms. Exosomes are nanovesicles, naturally released from almost all the cells of our body, that either in normal or disease states deliver a great deal of molecules including proteins, lipids and nucleic acids. These ''nanosized'' vesicles actually interact with target cells, within an organ or at distance, using different mechanisms. We contributed to show that the way EV interacts with cells include: ligand-to-receptor interaction[35,36] and fusion with the target cell plasmamembrane, followed by the uptake of the exosome content by the target cell[37]. Thus, exosomes appear as a vectorized signaling system operating from inside a donor cell towards either the extracellular counterparts or all the internal compartments of the target cell. These evidences place exosome in the center of the real novelties in translational science, and as the potential candidate as the self nanovectors for future strategies in NanoMedicine. The future use of exosomes for new therapeutic and diagnostic approaches not only needs serious discussion and consideration, but also continuous new and fruitful information. Exosomes are becoming the real novelty in the identification of novel biomarkers. In fact, new tests offering the possibility to contemporarily characterize and quantify exosomes in the human body fluid have been recently set up by our group[38]. This double potentiality ofexosomes suggests the use of these nanovesicles as the ideal tool in ''Theranostic''. This new area of Nanomedicine focuses on multi-disciplinary research to build new systems for various nanobiomedical applications ranging from the medical use of nanoplatform-based diagnostic agents to therapeutic agents, and even possible future applications of diagnosis + therapy, theranostics. Theranostics is the medical application of nanobiotechnology and refers to highly specific medical intervention at the nanoscale for diagnosing, treating or preventing diseases. It includes early detection of diseases, monitoring therapeutic response and targeted delivery of therapeutic agents. However, the most important task of the Theranostic strategy is the Theranostic NanoFormulations that deal with the development of new agents based on ''whole in one approach'' that should have its maximal application in the field of personalized medicine, because they allow detection as well as monitoring of an individual patient possibly at an early-stage, and with a specific delivery of the drug/s at the site of the disease. Exosome appears the ideal nanovector for theranostic, with the maximal potentiality of targeting to the disease site, with only minimal side effects. If successful, the proof-of-concept in the use of exosomes as the autologous nanovector for both diagnosis and therapy of major diseases will allow for widespread preclinical and clinical applications. As far as cancer is concerned, exosomes and the other EVs have proven to be extremely helpful in setting up new early diagnosis strategies through identification of new tumor biomarkers exclusively shuttled by these nanovesicles[39,40]. However, evidence is accumulating on the ability of EV to deliver preferentially the known tumor-related proteins (tumor biomarkers), and currently used in the clinical management of tumor patients, but very often with questionable results often leading to overdiagnosis[39,40]. In fact, the EVs, and in particular exosomes, are detectable not only in plasma or saliva or in the amnion, but in the urine and the stools as well[39,40]. This means that, while expressing ubiquitous markers, making them detectable and quantifiable[38,39], EVs not only have different routes of excretion, but also different functions and activities, including the purpose of making cells and compartments in communication within our body (e.g. plasmatic EV), and also with the commitment to free our body from toxic or unwanted material (e.g. urine and stool EVs). In the case of cancer, it is highly conceivable that the ability of exosomes to eliminate toxics is hijacked from malignant cells, that are continuously under the pressure of a very hostile microenvironment that is hypoxic, acidic and very poor of nutrients, but very rich in oxidants and free radicals[11,14]. Very recently, we have shown that exosomes participate into the framework of cancer resistance to cytotoxic drugs (i.e. cisplatin), through the elimination of the drug outside the tumor cells. Moreover, the exosomes-delivered drug appears in its native form, and therefore totally functioning, in turn possibly delivering the cytotoxic drug in non-tumor cells through fusion[18,37]. In the same study, we have also shown that cisplatin-containing exosomes may circulate in the blood, thus potentially participating to the systemic toxicity of the standard single or polychemotherapy. The tumor-released EV may also participate in the tumor escape from the immune response, since they express the ligands for the death receptors fully expressed by the lymphocytes that should be their assassins. In fact, tumor-released EV killer lymphocytes through either Fas or TRAIL-mediated apoptosis[35,36], while the same EV do not kill the tumor cells that release them, probably because either they do not express a sufficient level of death receptors or the intracellular apoptotic pathways are blocked or inhibited. Lastly, a further study showed that the microenvironmental acidity increases the release of exosomes by the tumor cells and the level of exosomes release is dramatically reduced by both buffering the culture medium and treating the tumor cell culture with PPIs[37]. The same effect was shown by treating human tumors/SCID mice xenografts with PPIs[18], being the level of circulating exosomes directly related to the tumor size[38]. Research on EV has led our group to set up an immunocapture-based method able to characterize and quantify exosomes into the plasma of tumor patients[38].

EVs deliver both viruses and prions[41,42]. Intriguingly, in the period, I studied HIV-1 infection participates in studies showing that HIV-1 virions were released by infected cells together with some cellular proteins[43], and this was related to the ability of HIV-1 virions to bud through the cell membrane just acquiring membrane proteins through this process[44]. Interestingly, a vast majority of these proteins were adhesion molecules that participate in the cell-to-cell adhesion that allow a full intercellular

communication in normal condition, but allowing a cell-to-cell spreading of HIV-1 virions without extracellular dissemination, and frequently through cell-to-cell fusion with polykarions formation as well[45]. Now we know that HIV-1 virions are released through exosomes from infected cells and that this may well represent a key mechanism of HIV-1 infection[46,47].

In a recent study in collaboration with Corrado Spadafora's group, we have also shown that tumor exosome may transfer codifying nucleic acids to the germ line *in vivo* just contributing to a somato-to-germ line transmission of genes[48], and creating some doubts on the Watson and Crick paradigm. This finding is of course of paramount importance in the light of clear evidence that EVs not only deliver nucleic acids but can transfer both mRNAs and miRNAs within target cells[49,50].

Consequences of a nonmainstream strategy in cancer research

Very recently the 2012 report of IARC, based on GLOBOCAN estimates, has shown that, about 14.1 million new cancer cases and 8.2 million deaths occurred in 2012 worldwide, and the burden has shifted to less developed countries, which currently account for about 57% of cases and 65% of cancer deaths worldwide[2]. This suggests, at least, two conceivable thoughts. First, not only that cancer incidence is increasing, but also that the deaths for cancer are increasing as well, without any evidence that the annual incidence of both is decreasing. Second, that cancer is becoming a global health problem, with a novelty, as compared to the usual problems normally managed by the global health agencies, that it includes both developed and less developed countries with comparable percentages of incidence. Thus, cancer patients'' management has to be considered a big problem for the whole humanity and unfortunately far to be solved. What this article wants to suggest is to change the strategy against cancer, in trying not only to change its social impact on one hand, but also to change the strategy of a research program that is too based on a mainstream approach. Currently, the social impact of cancer is that it is considered a sort of ''death sentence''. Recent reports suggest that a cancer diagnosis significantly increases the risks of cardiovascular events and suicides, as it occurs in prostate cancer patients[51]. Therefore, a first step in the enterprise aimed at changing the current strategy against cancer is to trigger a profound social revolution in the conception of cancer; in turn trying to make that cancer will become a disease, most conceivably a chronic disease, not a sentence to die anymore. I am sure that, before a more effective and less toxic therapy will be obtained, the best way to go ahead is to apply one of the most important concept of medicine: that patients should accept to live together with their tumors, as better as possible and as longer as possible. This can be achieved to think at a long-standing therapy; that should not be aimed at destroying cancer with very aggressive treatments, probably contributing to markedly impair the normal homeostatic balance of our body, but to control the tumor growth and spreading. This strategy is indeed in the course to be tried in cancer patients treatment[3], but I am sure that it should be matched with a well thought social campaign that, over the years, should contribute to changing the concept of cancer into the minds of people worldwide. In fact, in medicine it is a general rule that disease are not healed but mostly under a control or rendered chronic, as it occurs with for instance hypertension, diabetes, celiac disease, chronic inflammatory diseases and honestly with the vast majority of human diseases, but cancer. The only condition for which patients, relatives and the human beings worldwide ask to be healed is cancer, and probably this is a central issue, because cancer is actually a disease and not, as it is unfortunately believed, a death sentence.

However, and this is the central issue of this article, also the strategy in the research of cancer should change a lot. A paradigm of this change should be not to think of molecular targets anymore, but rather to target phenotypes; meaning that we should focus our attention, not on the factors that distinguish cancer by cancer, and more ambitiously patient by patient, looking at proteins or nucleic acids, but rather to phenomena that are in common between cancers. This article, while autoreferentially, provides three examples of what target phenotypes may mean. First, tumor acidity that may represent either a target for antiacidic therapies or a target for drugs that are specifically delivered to acidic compartments and there, through protonation, transformed into the active molecules (e.g. PPIs)[14]. This is of course important not only for setting up new therapies entirely based on anti-acidic approach, but also for combined therapies being cancers extremely resistant to virtually all chemical drugs, through acidity[52–54]. Then, the high level of exosome released by tumors, and therefore perfectly useful as tumor biomarkers; and the way tumors are so attractive for exosome, probably negatively charged, suggesting to use exosomes as a natural nanodelivery for anticancer drugs[39,40]. Lastly, tumor cannibalism that may represent both a common marker of tumor malignancy and a target for antitumor therapies aimed at depriving cancer for a very efficient way to face off low nutrient supplying[25]. I hope this article will convince the readers to sit down and think, as the human beings did in the past, where the insufficient technology left much more time to reflect upon the occurrence of our life, and probably to better figure out the future direction of the research in medicine. However, this approach has led to the use of PPIs in clinical trials with patients with tumors of various origin[20,55], showing that this approach is able at least to improve the efficacy chemotherapy in terms of either time to progression or overall survival, and this with a solid rational and a solid background. It is however conceivable that, in terms of evolutionary biology[56] the antiacidic approach through PPI may well target the whole tumor cell population, inasmuch as on one hand it may kill tumor cells expressing high levels of proton pumps, on the other hands by buffering the tumor microenvironment it may eliminate the most important factor of the selective pressure within tumors that is microenvironmental acidity. The selective pressure due to the acidic extracellular microenvironment of cancers, together with the aerobic glycolisis, hypoxia and more in general the tumor metabolism represent all a way tumor evolves in a malignant way[57,58]. The concept of malignancy should be re-thought not simply related to an uncontrolled growth or a deranged capacity to migrate or metastasize, rather to a selection of cells naturally armed to survive in very hostile conditions, that are hypoxia, acidity, low nutrient supply, aberrant accumulation of toxics. The same conditions that usually kill normal cells in a while.

Declaration of interest

The author reports no declarations of interest.

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