The Natural Resistance of Cancer Cells to Natural Inhibitors of Carcinogenesis: A Philosophy Emerging from the Prokaryotic to Eukaryotic Kingdom. New Hope Above Expectation

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Abstract

Resistance of cancer cells to treatment is a common phenomenon and the reasons behind this are multiple. The evolution of resistance accumulated similarities between prokaryotic and eukaryotic cells in their mechanisms of resistance. This paper gives a historical overview of the evolution of cancer resistance to conventional therapy. We try to denote that this resistance can be overcome using the new generation of targeted therapies.

Keywords: Resistance; Cancer cells; Prokaryotic; Eukaryotic; Targeted therapy

Introduction

Historically and with the advancement of sciences, oncologists thought they can extrapolate from what they have learned in bacteriology and antibiograms of infectious diseases to discover “anti-cancer grams” for cancer patients. Moreover, testing the minimal inhibitory concentration (MIC), that is the lowest concentration of an antimicrobial that inhibits the visible growth of a microorganism after overnight incubation, is easily affordable and reproductive in routine laboratory. To incubate cancer cells from every patient and provide the oncologists with a personalized “anti-cancer gram” is obviously misleading.

The approach was probably right in its first step only. We had to understand our ancestral unicellular organisms in order to elucidate some of the characteristics and secrets of multicellular organisms. But again, the applicability of what we knew has reached a dead end and has left us with so many unanswered questions. We had to move further, which is what we have done, and we discovered that the analogy became futile as far as the gap between the species was wider. At the end, when we tried to pass from normal multicellular organisms to pathological ones, the analogy was again unreliable. And again we discovered that many secrets were still hidden inside, and that our knowledge of the “normal” is not enough to explain the “pathological”.

There has been a great progress over the past three decades on the way towards a more sustained hypothesis of what cancer is. We are inside the matrix, and the vision is becoming clearer though still not panoramic. Those unknown and dark areas over the map of the cancer world are decreasing every day. We have an overlap of discoveries, teams’ collaboration, new technologies, translational research, and all are contributing actively in the process of translating fundamental science to clinical practice. Others are working over the barriers of delivering anticancer drug to their targets and developing drug targeting systems. The advancements are quite innumerable giving us hope that we are going in the right direction.

Mechanisms of Resistance Modeling

Mechanism of drug resistance in prokaryotic cells

The four main mechanisms by which microorganisms (prokaryotes) exhibit resistance to antimicrobials are [1-3]):

1. Drug inactivation or modification: e.g. enzymatic deactivation of Penicillin G in some penicillin-resistant bacteria through the production of β-lactamases.
2. Alteration of target site: e.g. alteration of penicillin-binding patterns (PBP)-the binding target site of penicillins-in methicillin-resistant Staphylococcus aureus (MRSA) and other penicillin-resistant bacteria.
3. Alteration of metabolic pathway: e.g. some sulfonamide-resistant bacteria do not require para-aminobenzoic acid (PABA), an important precursor for the synthesis of folic acid and nucleic acids in bacteria inhibited by sulfonamides. Instead, like mammalian cells, they turn to utilizing preformed folic acid.
4. Reduced drug accumulation: by decreasing drug permeability and/or increasing active efflux (pumping out) of the drugs across the cell surface.

Mechanisms of drug resistance in eukaryotic cells and xenobiotic

The story is much simplified in prokaryotes as the only barrier between the environment (the extrinsic drug) and the unicellular organism is the cytoplasmic membrane. In eukaryotes, things are more complicated and drug delivery to a given cell is under the simple rule of the pluricellular organisms. The delivery of the drug X to a point A in a multicellular organism is affected by the normal physiological barriers and laws [4].

When we talk about multicellular organisms, it would be imperative to mention xenobiotics. A xenobiotic is a chemical found in an
organism but is not normally produced or expected to be present in that organism. Specifically, drugs such as antibiotics are xenobiotics in the human body. The body removes xenobiotics by different metabolisms including the deactivation and secretion of xenobiotics, and this mostly takes place in the liver. Secretion routes are urine, feces, breath, and sweat [5]. Hepatic enzymes are responsible for the metabolism of xenobiotics by first activating them (oxidation, reduction, hydrolysis and/or hydration of the xenobiotic), and then conjugating the active secondary metabolite with glucuronic or sulphuric acid, or glutathione, followed by excretion in bile or urine. An example of a group of enzymes involved in xenobiotic metabolism is hepatic microsomal cytochrome P450 [6].

Even more, in a multicellular organism, the circadian timing system plays a key role in the changes of toxicity of drugs by influencing their metabolisms in the liver and intestine [7]. Other physiological conditions that can affect the availability of the drug include poor blood supply in certain disease conditions, increased interstitial pressure within the tissue, and the presence of excessive extracellular matrix that may limit drug penetration into the targeted site.

**Mechanisms of drug resistance in cancer cells**

In cancer cells, both prokaryotic and eukaryotic behaviors are present to provide the cell with the capacity to intelligently overcome any drug exposition. Tumor resistance to cytotoxic drugs can occur at the start of therapy (intrinsic resistance), as early as the first treatment, or over time after an initial period of response (acquired resistance) [8-10]. The mechanisms of resistance in cancer cells can be summarized in the following:

- **Efflux pumps**: Responsible for transporting drugs out of the tumor cell, thus altering intracellular drug concentrations. Examples are P-glycoprotein and the multidrug resistance proteins, MRP 1–7 [11,12].

- **Regulation of apoptosis**: Tumor cells can evade signals that normally lead to apoptosis, conferring a survival advantage by making the cell resistant to apoptotic death. An example is the decreased cell surface expression of the Fas death receptor [13,14].

- **Drug detoxification**: Certain enzymes in the tumor cell play an important role in the cell defense against invading foreign toxins. For example, glutathione S-transferase (GST) works synergistically with the efflux pump MRPI to expel drugs from the cell [15].

- **Drug sequestration**: Drugs can be trapped in special cellular compartments, keeping them away from their site of action [16].

- **Drug target alteration**: Alterations at the drug target site may impair binding of the drug. For example, variation in microtubule composition has been associated with tumor resistance [17,18].

- **Damage repair**: Special enzymes within the tumor cell can identify and correct damage to the DNA molecules that encode its genome. For example, overexpression of the enzyme ERCC1 leads to increased DNA repair of drug-induced lesions and diminished response to apoptotic signaling [19,20].

The mechanism of resistance in cancer cells is a combination of the eukaryotes, prokaryotes, and something in between. It benefits from the protective pathways of the unicellular organisms, and relies on the multiorgan defenses (skin, liver, kidney, immune system, etc.) in the higher and most sophisticated multicellular organisms.

**Discussion**

**From genomics to proteomics**

Why wouldn't a principle of one organism apply to all other organisms? What is the difference between a prokaryotic cell and a eukaryotic cell in terms of drug resistance? By nature, all organisms would always find their ways to get rid of intruding molecules, either by effluxing them through new channels or by many other ways well established and known to contribute to the mechanisms of drug resistance. Few nuclear genes would be switched on to protect the organism. Thus, the Darwinian model is again à la mode. There is no reason to believe any behavioral differences between the primary prokaryotic cells and our eukaryotic cells.

Coming back to Charles’ soup is nothing but a proof of the evolution concept [21]; the evolution of our knowledge and sciences has brought us back to Darwin. It took us 150 years to be able to decipher the code of life. We still have some missing links in the evolution concept of organisms. We have accomplished the sequencing of human genome. But alas, sequencing the 25 thousand normal genes in our “normal cells” is nothing but the first step [22,23]. Sequencing tumor cells by microarrays technology and DNA ships and other technologies is a different affair. Cancer is by excellence a proteomic disease [10,24,25]. The first obligatory step of defining the 3 billion DNA subunits was accomplished, and now we are in front of defining the epigenetic factors influencing the genome. We are in the post-genomic delusion period, and we have entered the real era where it is the protein outcome that plays the major role in the carcinogenesis and not necessarily the gene.

**Targeted polypharmacology**

Sequencing tumor cells comes from tumor tissues and biopsies. The polyclonal challenge and the cell heterogeneity of tumors were thought to be solved by the targeted polypharmacology [26,27]. That is to find out a map of interrelated mutated proteins that can be targeted by one or more molecules to stop the signaling pathway.

Having a closer look, sequencing a soup of cells that have different levels of maturation and differentiation is quite misleading. Discovering what is wrong in one clone does not solve the problem of the other clone, and even if we were able to produce a small molecule that stops one clone. We will eventually witness the selection of a new emerging clone and the adventure will continue.

Then, some good-intention scientists would tell you: No worries, we will be able to find out all the genes “switched on” on all the clones at the same time. This is partly true, but the problem will continue unless we have the possibility to stop time. Time is the only variable that no one can introduce as constant.

The process is even more complicated. Tumors are dynamic, as they are fresh, flourishing and growing. There is always a natural tendency towards harmony, organization and filling vacancies. Even in the chaotic and anarchic primary tumor, there is finesse and “délicatesse” in the architecture of the tumor mass. The matrix holds at minima the whole. The analogy to explain the process is to look at the forests; in the primary forest, the view is possible and there are natural pathways, and there is a share of primary elements of survival (sun, rain, soil, etc.). This would assure the homeostasis of all. When it comes to the secondary forest that grows after a disaster (fire, insects, timber harvest, wind throw), the view is unclear, chaotic and unorganized and the way through is almost impossible. A long period of time would pass so that disturbances are no longer evident. The hiking inside can be of extreme
frustration, and any strategy to traverse can be misleading. But time corrects everything and going back to equilibrium is a natural process. Whereby, the survival of the fittest will take over everything.

Fortunately, even within the anarchy of tumors, there is equilibrium between well being and stress. This uncomfortable and stressful status triggers cells toward “evolution”, and activates an orchestra of trial and error.

As far as they go, due to their high proliferation rates, and their high mitotic index, their margin of survival is high. True, more errors will occur. It might be leading to fatal errors. Survival of the fittest will occur, and here we are in front of new clones with new signaling pathways. Increased secretion of vascular endothelial growth factor (VEGF) will assure neoangiogenesis [28-30], Hypoxia-inducible factor (HIF) will give the cancer cell the chance of existence even during hypoxic situations [31,32]. The increase of heterogeneity in tumors makes the puzzle more and more irresolvable as far as the music is running. The only way to stop the process is by killing the host.

Cancer is another example in nature of parasitic creatures that would sacrifice their hosts and therefore themselves for their own greed. So, genomic sequencing of a cancer cell can be of interest if we have the possibility to detect all the viable mutations inside a cancer cell within its natural history from the moment it evolved till the end of its life.

By scientific deduction, would we be able to imagine the creation by software engineering and mathematical statistical models, a complete gene mapping of every single tumor, with all possible past, present and future mutations and therefore their signaling carcinogenesis outcome? But how many inhibitory molecules would we need for each corresponding pathway within the same heterogeneous polyclonal tumor?

It is a misconception to treat one or two events. What is required is to be able to think about “the thing” or the Xenomorphic organism within its continuous and dynamic acquisition of new mutations. Would it be possible to identify every mutation in a cancer cell from the cradle to the grave? But again what does it mean grave for a cancer cell? Since those cells are non apoptotic, and therefore their possibility to detect all the viable mutations inside a cancer cell within its natural history from the moment it evolved till the end of its life.

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Cancer stem cell (CSC) story

In the 1990s, another Darwinian lesson was also learned; the single cell theory again, the cancer stem cell (CSC) story [33-35]. Those “nasty” cells remain in G0 status, and therefore do not proliferate and have no mitotic apparatus. They have some characteristics of normal stem cells and do not respond to any treatment because of their stagnant state. Only those who are asleep make no mistakes.

Conventional chemotherapies kill differentiated or differentiating cells, which form the bulk of the tumor. A population of CSCs, which gave rise to it, could remain dormant and cause relapse of the disease. The stem cells will be only watching and learning for the next round.

Cancer immunoediting

The tumor immunoediting concept is a process consisting of three phases: Elimination (cancer immunosurveillance), Equilibrium, and Escape [36].

Immunosurveillance can lead to the “Elimination” of tumor cells. If the elimination is partial, the “Equilibrium phase” may develop between the immune system and the tumor. The tumor can stay in a dormant stage, or evolve, sneaking subtly, accumulating DNA mutations and epigenetic changes. The failure of the immune system to eliminate tumor cells will contribute to the selection of tumor clones that are resistant, and so the “Escape” phase will begin. Now the immune system is no more able to control the cancer process.

Cancer evolves by a process of genetic diversification and natural selection. This tumor immunoediting process would design the chosen clone that acquires the angiogenic switch and gives it the possibility to invade neighbor tissues, activates neo-angiogenesis (secretion of VEGF), thus facilitating the passage to the metastatic stage [36,37]. The ability of the evolving tumor cell to migrating on its own and invade new tissues will need some more sophisticated power, and more arms in hand. The cell would never metastasize has it not acquired the entire autonomy for its first “exodus”. There is nothing called “kamikaze clone” in carcinogenesis.

Noah’s Ark, the kinome, and targeted therapy

God saw the extent of wickedness and decided to wipe mankind from the face of the earth. However, one righteous man among all people of that time, Noah (Genesis 6:1-9:17) found favor in God’s eyes. God told Noah to build an ark for him and his family in preparation for a catastrophic flood that would destroy every living thing on earth. God also instructed Noah to bring into the ark two of all living creatures, male and female, along with every kind of food. Noah obeyed everything God commanded him to do. This monotheistic concept for believers is only a proof of the evolutionary concept. Humans have descended from the ultimate and supreme Homo sapiens. This is only a proof of God’s intelligence. God is probably the most sophisticated evolutionist, who used the same library of molecules, the same concept in creating all living organisms on earth.

One way of proving the concept of evolution came by studying the kinome [38,39]. Gerard Manning, in Science magazine in 2002, created a catalogue of protein kinases complement of the human genome (kinome) as a first step of analyzing protein phosphorylation in normal and disease states. In his next step, he led a comparative analysis of those protein kinases in different species concluding: “Whole-genome analysis of protein kinase evolution shows that new kinase classes developed to mediate the complexity of higher organisms, and demonstrates the birth, loss, and expansion of kinase families in each fully-sequenced eukaryote” [39].

This kinome classification, opened wide the door to new targets. Some had been successfully put into practice like imatinib mesylate (Gleevec) in chronic myelogenous leukemia [40] which inhibits the BCR-ABL fusion gene product, and many new major and possible targets in cancer are the protein kinases inhibitors of cyclin-dependent kinases, phosphoinositide 3-kinases, and the Hsp90 molecular chaperone and others [41,42].

Homo sapiens at the top of the alimentary chain

We are at the top of the alimentary chain. According to the evolutionary concept, human beings came from a single cell that could have evolved from the plant kingdom to the animal kingdom.

Thus, all living forms and existing molecules are nothing but ancestral patterns and proteins of human beings. Nature hates redundancy. Cell memory is capable of coping with any natural existing product, and the cancer cell is without any exception capable of getting rid of any exposed molecules that are natural or semi-natural. Our cells are not stranger to any of those patterns of “life molecules”. Cell proliferation is a multiple pathway process. The inhibition of one single
and gallbladder cancer [46,47], colorectal cancer [48,49], and lung viruses. The medical literature is rich in association between bacteria and mitochondria are descend from specialized bacteria (probably purple germs and humans. prokaryotes and eukaryotes, and it is impossible to dissociate between cannot be seen by the eyes, which float in the air and enter the body (36 BC): “. . . and because there are bred certain minute creatures which of germ theory of disease cited by Marcus Terentius Varro (published in and call it the “germ” or the “microbe” . Some people are still in the era patients and laypeople insist that cancer is a viral or bacterial disease, and consequently we have tried to apply what we knew from molecules. From Dark Ages, Renaissance to Modern Sciences With cancer it is not Alice-in-Wonderland, where you expect to just pick up the right key placed accidentally next to a closed door. In cancer fighting, the evidence lies in the pharmacognosy and pharmacology of molecules. The problem in cancer is that, historically and back to the “Dark Ages” before the renaissance, we have considered cancer as an infectious disease, and consequently we have tried to apply what we knew from microbiology to manage this oncology problem. Still in our days, some patients and laypeople insist that cancer is a viral or bacterial disease, and call it the “germ” or the “microbe”. Some people are still in the era of germ theory of disease cited by Marcus Terentius Varro (published in 36 BC): “... and because there are bred certain minute creatures which cannot be seen by the eyes, which float in the air and enter the body through the mouth and nose and there cause serious diseases”. Briefly, there is certainly an intimate relationship between prokaryotes and eukaryotes, and it is impossible to dissociate between germs and humans. To push over the argument, some organelles inside the cytosol are nothing but a common coliving between human cells and bacteria. The endosymbiotic hypothesis for the origin of mitochondria suggests that mitochondria are descend from specialized bacteria (probably purple nonsulfur bacteria), and they were incorporated into the cytoplasm [45]. Certainly some cancers can be induced by some bacteria and viruses. The medical literature is rich in association between bacteria and gallbladder cancer [46,47], colorectal cancer [48,49], and lung cancer [50,51]. There is also abundant evidence on the link between viruses and malignancies including cervical cancer [52], hepatocellular carcinoma [53], and Burkitt’s lymphoma [54].

Conclusion
The truth lies in the new generation of targeted therapies. These new generation drugs are completely synthetic and are not related in any way to nature or to our biological legacy and heritage. It is not only because they target intelligently mutated proteins in the cancer cell signaling pathways, or its environment, but additionally because our cells are facing new patterns of molecules designed completely in the laboratory.

Hopefully, the progress in targeted therapy will cut the road in front of all impostors with their “natural products” that come up now and then pretending “miracles” and false promises. It is irrational to go back to the dark ages in treatment choice. The only way to win over the super intelligent cancer cells is to confront them with the evolution of human intelligence. It is a long and painstaking research that needs a monstrous multidisciplinary approach to win the battle. Huge financial investments are needed and should be encouraged and oriented to the field of cancer research. We are seeing resistance to some targeted therapies, but we are still in the era of penicillin, and these are only the first generation drugs, and the meant targeted therapy is only at their beginning.

Although a lot of work should be done to translate fundamental sciences into practice, we are not far from our target. We should keep on searching. We are at the end of a long dark tunnel that lasted thousands of years. In few years, our children will talk about cancer, as our grandparents have talked about plague.

References


