



Targeting cancer's weaknesses (not its strengths): Therapeutic strategies suggested by the atavistic model

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In the atavistic model of cancer progression, tumor cell dedifferentiation is interpreted as a reversion to phylogenetically earlier capabilities. The more recently evolved capabilities are compromised first during cancer progression. This suggests a therapeutic strategy for targeting cancer: design challenges to cancer that can *only* be met by the recently evolved capabilities no longer functional in cancer cells. We describe several examples of this target-the-weakness strategy. Our most detailed example involves the immune system. The absence of adaptive immunity in immunosuppressed tumor environments is an irreversible weakness of cancer that can be exploited by creating a challenge that only the presence of adaptive immunity can meet. This leaves tumor cells more vulnerable than healthy tissue to pathogenic attack. Such a target-the-weakness therapeutic strategy has broad applications, and contrasts with current therapies that target the main strength of cancer: cell proliferation.

Keywords:

■ adaptive immunity; cancer therapy; carcinogenesis; evolution of multicellularity

Introduction

Current cancer therapy is based on radiation, chemotherapy, and surgery. Radiation and chemotherapy target

cancer cell proliferation by damaging DNA. However, DNA damage interferes with normal cellular proliferation throughout the body and often has significant toxicity (e.g. [1]). Modern molecularly targeted therapies have, on the whole, proven less selectively toxic to cancer cells than hoped, and are unquestionably associated with a range of unusual and sometimes debilitating adverse effects; as an additional disappointment, they often exercise very temporary benefits before resistance sets in.

The effectiveness of surgery is compromised by the invisibility of micrometastases, irresectability of either the primary tumor or overt metastases, and the common reactivation of dormant secondary micrometastases [2, 3]. Simi-

lar problems apply to radiotherapy. Despite certain clear benefits of current therapies, more effective and better-tolerated approaches are needed.

The main challenge facing cancer researchers is to develop therapies that more specifically target cancer cells, while leaving normally functioning cells unscathed. However, since the capabilities of cancer cells seem to be based on accessing normal cellular functions that play important roles in embryogenesis and tissue self-renewal [4, 5], targeting these capabilities without producing side-effects on normal cells is difficult. Finding a therapeutic window between proliferating cancer cells and proliferating normal cells remains a major challenge in the design of successful cancer therapies [6].

Current therapeutic treatments attack the strengths of cancer: they predominantly target what cancer cells, and all cells, have deeply embedded in their genomes – strategies for cellular proliferation. It may seem rational to treat a proliferative disease with anti-proliferative drugs. However, after ~4 billion years of evolution (the first ~3 billion of which were characterized by the largely unregulated proliferation of unicellular organisms) cellular proliferation is probably the most protected, least vulnerable, most redundant and most entrenched capability that any cell has. The redundant and robust supports for cellular proliferation are ~2 billion years older than the many layers of recent differentiation and regulation that evolved with multicellular eukaryotes. Thus proliferation, not terminal

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differentiation, is the ancestral and default state of cells [7–9].

Since the separation of the germline and the somatic cell line (~1.5–2.0 Gya) many layers of regulation have evolved to control somatic cell proliferation and differentiation. These layers include transcription factors, epigenetic controls, chromatin remodeling, histone modification, RNAi's, apoptosis, anoikis, autophagy, necroptosis, methylation of mRNA, senescence, and the Hayflick limit [8, 10]. Despite these fairly recently evolved controls (~1 billion years ago), all somatic cells of multicellular organisms still have proliferation, the most fundamental of all capabilities, built into them as a default. Cellular proliferation remains essential to embryogenesis, growth, and tissue self-renewal. Therefore the option of rapid proliferation is retained in the genomes of complex organisms. As cancer progresses, epigenetic and genetic changes increase. In the atavistic model of sporadic cancer [11], these changes and loss of function are hypothesized to accumulate in the most recently evolved layers of control. These layers are less well protected and hence more susceptible to damage than the more deeply entrenched, older genetic pathways of cellular proliferation. Most conspicuously, recently evolved control over cellular proliferation is lost.

If the model is correct, and cancer's capabilities are based on the deeply entrenched orchestration of multiple and redundant drivers of cellular proliferation, then targeting one or even a few capabilities will not be very effective. Such redundancy allows cancer to be a moving target. This is familiar to clinicians [9, 12]. Drug cocktails to block *multiple* proliferative pathways seem to be slightly more effective [13]. However, this strategy targets multiple *strengths* of cancer and is limited by the speed with which multiple drug resistance evolves [14], and by cancer's ability to access, through mutations, the alternative redundant drivers of cellular proliferation. Despite being limited to onset-delaying clinical outcomes [15], this conventional strategy of directly confronting cancer's deeply entrenched strengths continues (Table 1 of [9]).

The rest of this paper is organized as follows. First we describe the main therapeutic idea that emerges from

our atavistic model of carcinogenesis [11], and how it can be applied to any physiological system with major features that evolved during the evolution of metazoan multicellularity ~0.5 to ~1.5 billion years ago. Then we describe some potential applications of this main idea, including a detailed application to the immune system. Finally we discuss irreversibility and target-the-absence therapies, and then summarize.

The main therapeutic implication of the atavistic model

The atavistic model

It has been periodically postulated that cancer represents some sort of reversion to a more primitive phenotype (e.g. [16]). The atavistic model asserts that this general concept can be refined into a more precise, quantitative theory with specific testable consequences and therapeutic implications. The model proceeds from the hypothesis that the distinctive hallmarks of cancer possess deep evolutionary roots that extend back to, and perhaps even precede, the dawn of multicellularity. When cancer is triggered, cells default to ancestral phenotypes and express these ancient modalities in an inappropriate setting. The genetic toolkit and functional pathways associated with the ancestral phenotypes are retained in modern organisms because of the crucial role they play in embryogenesis, tissue renewal, and wound healing; they are accessible to damaged cells and tissues, because it is easier to revert to, or co-opt, existing pathways than to evolve new ones.

Multicellularity and many of the capabilities of eukaryotes evolved in our metazoan ancestors between ~1.5 and ~0.5 Gya. Those were the formative years in which somatic cell differentiation and the regulation of proliferation evolved. Cancer's proliferative capabilities (and proliferative capabilities in general) are much older, having evolved over the preceding ~2 billion years. The near ubiquity of stem-like cells in most tissues, and their ability to form cascades of transit amplifying cells, may be

the main reason that rapid cellular proliferation has been preserved and seems so easily accessible in tumors.

A key prediction of the atavistic model is that mutational burden and epigenetic dysregulation during cancer progression will be preferentially concentrated in younger genes that are less well embedded, less protected and generally less well maintained than the core cellular proliferation pathways. This suggests that recently evolved mechanisms should be the first to manifest dysfunctionality during carcinogenesis [11], providing a new way to distinguish cancer cells from normal cells.

The altered functionality of cancer cells includes the loss of function of tumor suppressor genes and other mechanisms that have evolved over the past approximately billion years to regulate cellular proliferation in the somatic cells of multicellular organisms. The accumulating loss of the evolutionarily more recent functions associated with cellular differentiation is manifested in the gradual loss of epigenetic control of gene expression and the emergence of a de-differentiated phenotype with increasing grade of cancer. The order of the reversion to phylogenetically earlier functionalities is illustrated schematically in Fig. 1.

In addition to loss of normal functionality, cancer progression is characterized by what is called "gain of function". So-called "gain" or "acquisition" of functions by cancer cells is interpreted in the atavistic model as the "regain" or "reacquisition" or "de-repression" of functions that have been repressed [20]. This is sometimes also described as genes being "resurrected opportunistically from early embryonic genes" as cancer exploits ancient pathways [21]. The epithelial-to-mesenchymal transition (EMT) is a prominent example.

A new target-the-weaknesses strategy

A new strategy suggested by the atavistic model targets cancer's weaknesses, which are to be found among the irreversible losses of function. Lost functionality is not easily re-conjured by somatic evolution [22, 23]. The

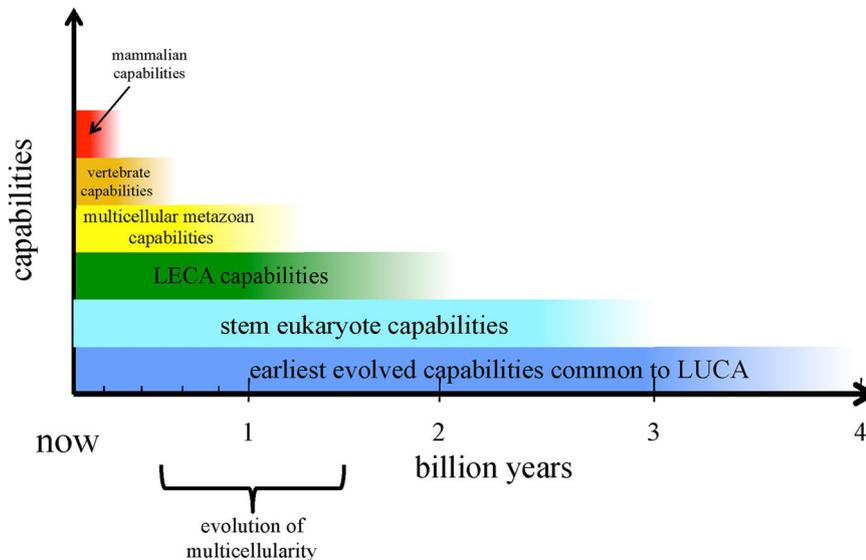


Figure 1. The physiological capabilities of human cells evolved at different times. Some capabilities are ancient and fundamental, and evolved in our bacterial ancestors (blue), while some are relatively new (few hundred million years old), and are shared exclusively with our mammalian relatives (red) [17, 18]. Functional capabilities are usually not discrete in that new capabilities are often modified and co-opted versions of previously existing capabilities. This dependence of the more recent capabilities on earlier capabilities is represented here by placing the new capabilities on top of the old in a stepping stone structure (e.g. [19]). In the atavistic model of cancer, the most recently evolved capabilities (red) are the ones most susceptible to damage and are more likely to be dysfunctional in cancer. In more advanced grades of cancer, the next most recent genes, the vertebrate genes, would be preferentially dysfunctional, leaving the more dedifferentiated cancer cells with basal multicellular metazoan capabilities (yellow). LECA, last eukaryotic common ancestor; LUCA, last universal common ancestor (of all life).

Achilles heel of cancer is the dysfunctionality of the most recently evolved genetic pathways. If cancer progression correlates with reversion to earlier phenotypes, one may appeal to the increasingly available knowledge of phylogenetic history to determine the target vulnerabilities. We hypothesize that cancer's strengths stem from capabilities that evolved more than a few hundreds of millions of years ago, and their weaknesses arise from the loss of, or damage to, capabilities acquired over the past few hundred million years. These more-recently-evolved capabilities are retained by healthy cells. If cancer is a highly capable atavism depending on old robust weapons for its survival – if cancer is the dysregulation and degeneration of recently evolved genes and the complementary up-regulation of ancient genes – then a potentially useful therapy is to apply a specific stress to the organism that is relatively easily dealt with by healthy cells using recently evolved capabilities, but is not easily dealt with by the older

capabilities available to cancer cells. Thus one can preferentially stress cancer cells while minimizing damaging side effects to the normal cells. Viewed in the context of the atavism model, cancer's niche creation can be thought of as the re-creation within the host organism of ancient environments in which ancestral physiologies are more comfortable [9]. Our therapeutic strategy is to drive the tissue micro-environment out of the comfort zone of cancer cells. In bridge one plays to the strengths of one's partner (which are the weaknesses of one's opponent). Here we play to the strengths of the more recent genes in normal cells (which are the weaknesses of cancer cells).

Applications of the target-the-weaknesses strategy

In principle our strategy can be applied to any of the many ways in which cancer cells differ from normal cells [5]. In

practice, it is necessary to focus on capabilities, the evolution of which is well enough known to distinguish the oldest (>1.5 Gya), more deeply entrenched, capabilities from the more recently evolved (<0.5 Gya) capabilities. This ~0.5 to ~1.5 billion year range spans the evolution of multicellularity and the evolution of the mechanisms that regulate and limit cellular proliferation. To proceed, one first determines the phylogenetic order of the principal genes involved in a hallmark of cancer in order to identify the most recent genes and their adaptive significance. Then one may devise a challenge that normal cells can handle. The application of differential stress in this manner may not result in the complete eradication of cancer cells, but a sustained weak response may actually be clinically more successful in the long run (in terms of the mortality and morbidity rates) than a brief strong response [24, 25].

The Warburg effect

Prior to the second great oxygenation event about ~0.8 Gya, our ancestors metabolized in either anoxic or hypoxic conditions. These conditions prevailed at the transition to multicellularity. The atavism theory therefore predicts that cancer cells will generally prefer hypoxic conditions, and use metabolic pathways appropriate to hypoxia. This seems to be the case. As cancer progresses, there is usually a shift in the balance of energy metabolism away from oxidative phosphorylation and towards aerobic glycolysis. That is, even in the presence of oxygen, cancer cells perform a less efficient form of ATP production that uses glucose. This is known as the Warburg Effect [26]. In accordance with our strategy to stress cancer cells more than healthy cells by disrupting the creation of “ancient” micro-environments, we advocate elevating the oxygen tension in the vicinity of the tumor together with systemically restricting the glucose supply. We note that hyperbaric oxygen and ketone-rich (glucose-poor) diets are finding some success, especially in combination, as cancer therapies [27]. Anti-anti-oxidants are also proving effective [12]. This success may be attributed to what is

effectively a target-the-weakness strategy: in oxygen-rich, sugar-poor environments, normal cells can use their recently evolved genes to perform oxidative phosphorylation, and digest ketones in a higher pH environment. Cancer cells have difficulty metabolizing ketones possibly because of the dysfunction of the more recently evolved capabilities of mitochondria [28–30]. In addition, Fan et al. [31] found that glutamine-driven oxidative phosphorylation is a major ATP source in transformed mammalian cells. A target-the-weakness strategy could include glucose and glutamine restriction with a heavier reliance on the newest foods that our cells have learned to metabolize. Cancer cells are also better adapted than normal cells to the acidic conditions that they produce through their glycolytic build up, and vigorous export of protons derived from lactic and other acids. Thus in the tumor environment, maintaining a higher than normal extracellular pH that would still be tolerable for normal cells, can form part of a target-the-weakness strategy [32, 33]. In contrast, inhibiting the dominant regulator of low extracellular pH in cancer cells would be a target-the-strength strategy, since there are multiple redundant ways to maintain low extracellular pH [34, 35].

Transmembrane pumps (ABC pumps)

Multi-drug resistance (MDR) efflux pumps are a major obstacle to effective delivery and efficacy of chemotherapy [36]. Cancer cells can acquire drug resistance through various ATP-binding cassettes (ABC). ABC drug transporters have been shown to protect cancer stem cells from chemotherapeutic agents (see Table 1 of [37] for details). In the atavistic model [11] we predict that cancer cells develop MDR as a *generic* up-regulation of ancient efflux pumps. A further prediction of the atavistic model is that when cancer cells are exposed to new drugs, their resistance is non-compound-specific because the ability to pump the toxins out is not based on newly evolved efflux pumps, or even substantially modified old efflux pumps, but is the result of the more easily accessed up-regulation of

pre-existing pumps that ancestors used to protect their chemical integrity. In other words, MDR is a reversion to, and up-regulation of, a more ancient, less-specific efflux pump defence system. However, this up-regulation is not exclusively “ancestral”, because normal stem cells and much of the normal gastro-intestinal tract is replete with these pumps, especially ABC-B1.

The branch lengths derived from ABC superfamily phylogenetic trees [38, 39] can be used to assign relative ages to ABC transporters. In Fig. 2, we have used short branch lengths from the root of the tree as a proxy for ancient (i.e. less different from the ancestral state), and long branch lengths as a proxy for recent (more different from the ancestral state). The absolute dates on the x-axis are schematic only. Assuming that ABC pumps evolved substantially in the range ~0.5–1.5 Gya, the relative ages of these ABC pumps should indicate very approximately the order in which they become dysfunctional during carcinogenesis.

Pumps at the bottom of Fig. 2 are deeply embedded strengths of cancer. Attempts to inhibit them have not been

very successful. A target-the-weakness therapeutic strategy requires the identification of the different varieties of substances that the more recently-evolved efflux pumps (at the top left in Fig. 2) are most efficient at pumping out of the cell. If drugs can be developed that can *only* be pumped out by the 16 pumps in the top three rows (and not by the other more ancient pumps) then advanced cancer cells with compromised versions of recent transporters (and their regulators) should be less effective than normal cells at pumping these drugs out of the cell. These drugs are then likely to prove more toxic to cancer cells than to normal cells. As far as we know, the relationship between specific ABC pump dysfunction and cancer progression has not been investigated.

DNA repair mechanisms

It has been estimated that, on average, there are 10^3 – 10^6 molecular lesions per cell per day in normal cells [40] (but see [8]), arising from a large variety of physical and chemical sources of

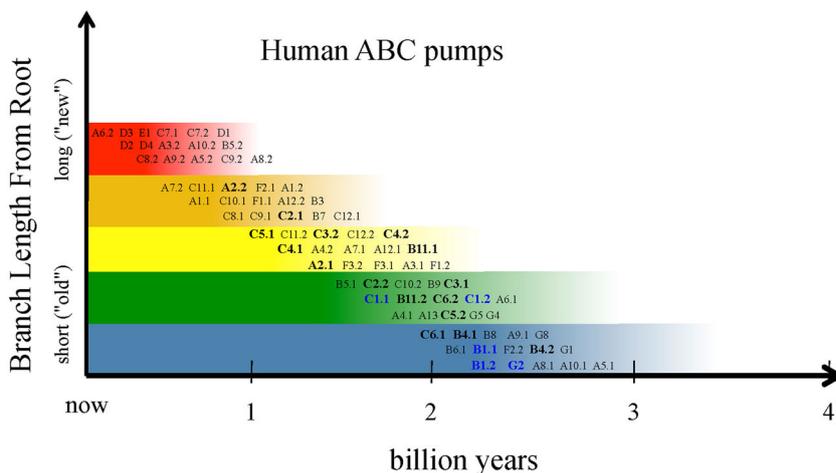


Figure 2. Same concept and color scheme as Fig. 1 but here, applied to ABC transmembrane efflux pumps. In the phylogenetic tree of 76 human ABC pumps [38] we take the branch length from the root to the extant gene for each pump, as a proxy for the age of the pump. Short branch lengths indicate genes close to the root, hence “old”. Genes with long branch lengths have evolved the most from the root, hence “new”. Bold blue labels indicate the most predominant pumps implicated in MDR (B1, C1, and G2). Other pumps found to be responsible for cancer cell resistance to chemotherapy (Table 1 of [36] and Table 1 of [37]) have bold black labels. The oldest pumps are more associated with MDR than the younger pumps. The atavistic model predicts that in advanced cancers or cancers with a heavy mutational burden (ovarian, basal breast) that the ABC transporters with the longest branch lengths in the upper left (and their regulators) are more likely to be compromised. This is a testable hypothesis with important implications for therapy. ABC transporter names are taken from [38].

damage. To deal with this, there are many DNA damage sensors and repair mechanisms [41–43], whose expression depends on cell type, age and extracellular environment. Cancer cells have differentially incapacitated DNA repair systems [42], a factor that contributes to their well-known elevated mutation rate [44]. A prediction of the atavistic model is that these incapacitated DNA repair systems will generally be the more recently evolved.

The many mechanisms for DNA repair did not all evolve at the same time. Phylogenetic dates for their origin remain sparse, although there is some evidence [45, 46] that one kind of non-homologous end joining pathway (D-NHEJ) evolved more recently than another kind (B-NHEJ) and would thus be more likely to be lost as cancer progresses. An obvious therapeutic strategy emerges from the foregoing considerations. If cells are targeted with a restricted set of DNA damaging agents, namely those for which the damage can be repaired by the D-NHEJ pathway, then normal cells will be less adversely affected than cancer cells. Conventional radiotherapy and radiomimetic drugs produce double strand breaks for which D-NHEJ is a major repair pathway; single-strand break repair, base excision repair, and homologous recombination are backups (Fig. 1 of [42]). Thus, to the extent that the

dysfunctional parts of these DNA repair mechanisms in tumors are the most recently evolved parts, some of the success of radiotherapy and conventional cytotoxic chemotherapy may be attributable to an inadvertent target-the-weakness strategy.

Target-the-weaknesses strategy applied to the immune system

Applying the target-the-weakness strategy to the immune system depends on knowing the approximate order in which various components of the human immune system evolved [47–51]. This is complicated. However, using a convenient simplification to illustrate the basic idea, we have divided the immune system into two conventional parts (Fig. 3): an adaptive immune system that evolved over the past ~500 million years (the basis of immune system memory and vaccination) and an innate immune system that evolved earlier [52–54]. The period ~1.5–0.5 billion years ago corresponds to the evolution of cellular differentiation, including the hematopoietic cell differentiation that led to our current immune system. A prediction of the atavistic model of cancer progression is that cellular dedifferentiation and reversion will compromise the effective-

ness of adaptive immunity in the tumor environment while leaving innate immunity largely intact.

The roles of immunoeediting, immunosuppression, and macrophages

Thomas [56] and Burnet [57] hypothesized a cancer surveillance role for the immune system. This has now been generalized to the concept of immunoeediting [58], involving three phases: surveillance, dormancy/equilibrium, and final escape from immune control [59–61]. Many elements of the immune system are linked to cancer progression. Tumor-associated macrophages (TAMs) aggregate in primary tumors and micrometastases, and have been implicated in their activation and proliferation [62, 63]. In ovary, melanoma and breast tumors, the density of T cells (adaptive immune system) is usually correlated with a more favorable treatment outcome. In contrast, cells of the innate immunity, especially macrophages, are often correlated with tumor progression and a less favorable outcome [64]. Tumor cells and the tumor microenvironment often exhibit an immune suppression phenotype [65]. Growing evidence suggests that this immunosuppression is mediated by TAMs and related myeloid cells of the innate immune system, and the interactions between TAMs and transformed cells (Chap. 13 of [30], see also [64, 66, 67]).

The normal immune response can be divided into an early pro-inflammatory stage and a later anti-inflammatory stage. Both are mediated by macrophages [68]. The switch from the pro-inflammatory stage to the anti-inflammatory stage corresponds to the macrophage switch from an M1 to an M2 phenotype. By performing their normal anti-inflammatory immune suppression program, M2s protect tumors from the adaptive immune system as if the tumor were a recent site of inflammation now free of infection, that no longer needs T cells, but does need the proliferation of neighboring cells. Apoptosis and remodeling complete the normal wound healing process, but in cancer these seem to be missing (“wounds that do not heal” [69]).

Our atavistic model predicts that as cancer advances, tumor cells

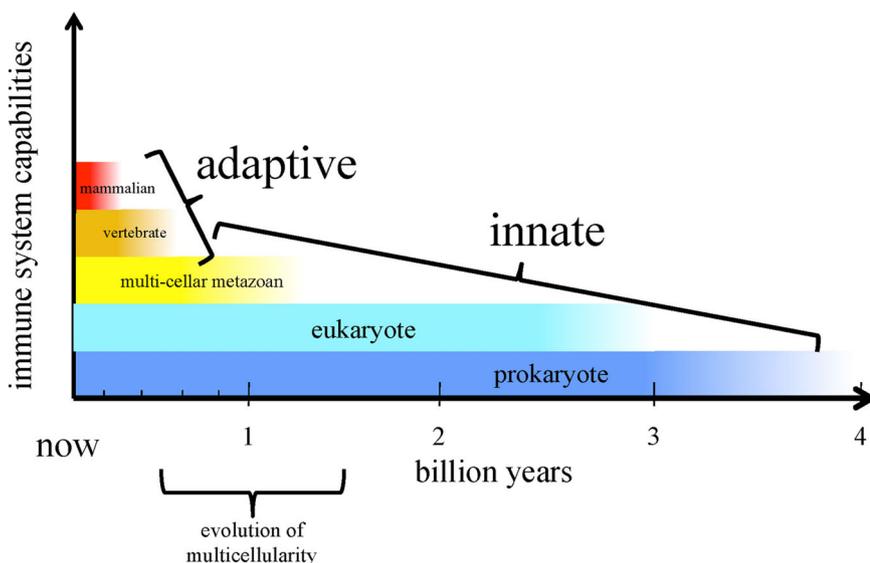


Figure 3. Evolution of the immune system. The more recently evolved adaptive immune system has a memory that is the basis for vaccination. The more ancient innate immune system does not. This stepping stone diagram represents the “layering hypothesis” [47, 55].

progressively revert to earlier phenotypes. M2 macrophages of innate immunity are doing what they did before the emergence of adaptive immunity. Immunosuppression is a generic expression of cancer cells losing contact with the more recently evolved aspects of the immune system.

Immunotherapy and existing cancer vaccines

The suppression of the immune system by TAMs is seen as an obstacle to cancer management because it interferes with the normal tumor surveillance mechanisms of the adaptive immune system (phase 1 of immunoediting). The main idea behind existing immunotherapy is to boost immunosurveillance by artificially activating the adaptive immune system against the tumor [24]. In the context of immunoediting, the main idea of this immunotherapy is to exit phases 2 and 3 (dormancy and escape) and return to the effective immunosurveillance of phase 1. In other words, cancer immunotherapy tries to reduce or reverse tumor-induced immune suppression: “manipulating the local tumor suppressive microenvironment is crucial” [65]. This approach suffers from the same problem as other forms of therapy: how to target cancer cells while avoiding healthy ones. Tumors are often not sufficiently distinct from normal tissues to be attacked by the adaptive immune system without the risk of triggering unacceptable levels of auto-immune pathology [24, 70, 71]. Our proposal, based on the atavism model, differs fundamentally from immunotherapeutic attempts to boost adaptive immunity.

How a vaccination/inoculation therapy could work

From the tumor’s point of view, immunosuppression (the absence of adaptive immunity) offers protection against attack by the adaptive immune system. As cancer progresses, this protection is strengthened as the immune system is increasingly suppressed in the vicinity of the tumor. However, the very same immunosuppression that protects the tumor can also be a weakness – most

obviously when the tumor needs the adaptive immune system to protect it from infection.

Our main therapeutic idea is to exploit this weakness as follows. In the case of a non-metastatic primary solid tumor of a particular organ (e.g. breast, liver, colon) the therapy would be:

- a. Identify a highly effective vaccine that protects the host organ (and the body in general) from a specific virus, bacterium or parasite that targets the host organ.
- b. Vaccinate the patient (or verify that the patient has been previously vaccinated).
- c. Inoculate the affected organ (specifically the tumors in the organ) with the disease-causing infectious agent at a dosage that will allow the vaccine-primed adaptive immune system to protect normal cells but, because of tumor-dependent immunosuppression, will be less able to protect tumor cells from the disease.

This therapy should be most effective in cases of strong immunosuppression. The more advanced the cancer, the more immunosuppressed the patient and the more difference there is between normal and tumor cells in terms of communication with the adaptive immune system. Thus, this therapy may complement standard cancer immunotherapies which are *least* effective in highly immunosuppressed patients. This therapy would not be plausible in patients too weakened by long illness to mount a normal immune response outside the tumor environment.

There is already some suggestive evidence in favor of our proposal. In the late 19th century William Coley noticed that rare cases of spontaneous tumor regression were often preceded by acute bacterial skin infections (erysipelas) and fevers [24, 72–74]. Coley experimented by deliberately inducing fevers and infections in cancer patients by inoculating them with live *Streptococcus*. He reported some success but the results were mixed [72]. Clinical studies in the 1950’s with live inoculants also had some successes, but again the results were mixed. Side effects were often severe [75]. The successes have

usually been interpreted as an up-regulation or “inciting” of the normal adaptive immune response (e.g. p. 715 of [76]). Under this interpretation, and in an effort to reduce infection and other side effects (while maintaining the up-regulation), attenuated bacteria and viruses, and non-human viruses, were used instead, again with mixed results [77]. We suggest that at least some (possibly most) of the reported efficacy of the live inoculant technique in these trials occurred not because of the immune system being up-regulated to kill tumor cells, but *because the tumor cells were killed by the infectious agent itself* [78]. This is plausible because the tumor cells are in an immunosuppressed region and are not as well defended as normal cells by the adaptive immune system. We propose that vaccination before inoculation with the infectious agent should increase our ability to challenge tumor cells more than normal cells. If this is the case, there could be an important difference in effectiveness between whether one used live bacteria (as Coley did) or killed/attenuated bacteria (as in BCG and most current cancer vaccines). In cases of remission, to distinguish these mechanisms, we need to be able to differentiate tumor cells that have been killed by an up-regulated adaptive immune system, from those directly killed by the infectious agent. Kim et al. [78] provide evidence that even with attenuated *Listeria* in a mouse breast tumor model (4T1), direct killing of the tumor cells may be more effective than the intended mechanism of cytotoxic T lymphocyte-mediated cytolysis in response to tumor associated antigens (e.g. Mage-b). They report: “*Listeria* bacteria in the tumor microenvironment may be protected from clearance by the immune system, but not in the normal tissues.”

Metastasis: Bacterial inoculant targets tumor-associated macrophages

Since ~90% of cancer deaths are due to metastasis, it is important to apply the target-the-weakness strategy to metastatic cancer. Cells believed to be most closely associated with micro-metastases are tumor-associated-macrophages

(TAMs). Therefore, to reach the sites of micro-metastases successfully, viruses, bacteria or parasites with a TAM-tropism can be recruited. Tumor-targeting (usually macrophage targeting) bacteria have been studied [79] in terms of their ability to carry therapeutic molecules [80, 81] or radioactivity [82] to tumors. Our proposal is to use them to directly kill tumor cells.

Quispe-Tintaya et al. [82] used attenuated *Listeria monocytogenes* laden with radioactive rhenium-188 to target macrophages in a pancreatic mouse tumor model (Panc-02). In normal tissue the immune system was able to efficiently clear the attenuated *Listeria*, but in the “heavily immune-suppressed microenvironment of metastases and primary tumor” attenuated *Listeria* could not be efficiently cleared [82]. This is a target-the-weakness strategy, challenging immunosuppressed regions with high concentrations of radiation. However, high concentrations of radioactivity as the main weapon is problematic, since clearing of normal tissues produces high doses of radiation in the liver and kidney. Another problem is that radioactivity-laden *Listeria* cannot reproduce to create more radioactivity-laden *Listeria*: the radioactivity gets diluted with time. A further possible problem occurs if there is any infection or wound where macrophages assemble to fulfil their normal function: the radiation will kill those cells too.

Based on the predictions of the atavism model, we suggest the following modification to the approach of Quispe-Tintaya et al. [82]. After vaccination against *Listeria*, an inoculation with *non-attenuated Listeria* is carried out. TAMs should be preferentially susceptible to attack from the *Listeria*, but normal macrophages at wound-healing sites will be relatively well-protected by the adaptive immune system. If non-attenuated *Listeria* is not just the carrier but also the killer, then with time, *Listeria* reproduction increases its effectiveness in immunosuppressed tumors.

Galmbacher et al. [81] used an attenuated strain of the macrophage-infecting bacterial pathogen *Shigella flexneri* (an intracellular invader) to induce apoptosis in TAMs in murine breast tissue. Side effects on non-tumoral macrophages were not dis-

cussed. Coster et al. [83] have developed a *Shigella* vaccine for humans based on attenuated *Shigella*. We suggest repeating the murine experiments of Galmbacher et al. [81] but using Coster et al.’s [83] vaccine (if effective for the invasive strain of *Shigella*, and if effective in mice), followed by an inoculant of live invasive *Shigella*.

In the case of widely disseminated metastatic cancer, one cannot make targeted inoculations directly into the tumors. Instead, the inoculant has to search the whole body. However, in a pre-vaccinated body, a general intravenous inoculant would immediately incite an adaptive response and may not be able to reach the micrometastases. The infectious agent has first to avoid systemic attack by the immune system en route. Time-delayed inducibility may be one solution. For example, if it takes 10 days for a vaccination to become effective, one could inoculate on the 8th or 9th day after the vaccination, thus giving the inoculant enough time to spread, but not enough time to do much damage to normal cells before an adaptive immune response kicks in. It is possible that at a low enough dosage, inoculated *Listeria* would act as its own vaccine, providing enough time to spread and target macrophages, but not enough time to kill macrophages in normal tissue.

A common feature of macrophage-targeted therapies is markedly suppressed metastatic tumor growth, but also the unwanted side effect of toxicity in non-tumoral macrophages. Our vaccination/inoculation therapy is designed to improve on these results by increasing the treatment’s ability to distinguish between TAMs (that one wants to attack) and non-tumoral macrophages (that one does not want to attack). An important difference between these two kinds of macrophages is the level of immunosuppression. If non-tumoral macrophages do not reside in immunosuppressed regions then they should be protected by the adaptive immune system during the vaccination/inoculation therapy.

The idea behind using live inoculant is direct killing of tumor cells. To avoid killing normal cells, dosage and degree of attenuation can be regulated. A potentially useful side effect of live inoculation is inflammation, which

may be necessary to properly “up-regulate” the immune system.

Discussion

Irreversibility

The effect of targeted drugs and cancer vaccines are usually only temporary [9]. Unattenuated oncolytic viruses [84] such as reolysin (e.g. [85]) are designed to be unable to replicate in terminally differentiated, non-dividing cells, but able to infect, replicate and cause lysis of cells with activated Ras pathways (20–25% of human cancers, but also normal dividing cells). Because a cell proliferation pathway is being targeted, this is another “target-the-strength” strategy. In some virotherapy, viruses are designed to target cells displaying tumor-specific antigens. But in a repeat of the familiar story, neoplastic mutation and selection ensures the eventual emergence of a tumor subpopulation that does not express the targeted antigens.

The way around the evolution of therapeutic resistance is to distinguish between mutable targets and targets that derive from irreversible changes in cancer cells. The atavistic model predicts which targets are mutable and the direction of their mutability: cancer tends to revert, *irreversibly*, toward phylogenetically earlier states. For example, immunosuppression is increasingly irreversible as cancer advances. The more permanent vulnerabilities of cancer are the irreversible losses of function.

Genetic profiling shows the presence of great intratumoral heterogeneity that frustrates conventional therapeutic approaches [86] and intratumoral heterogeneity of immunosuppression could limit the efficacy of the proposed therapeutic approaches. The atavistic model, however, predicts that although this heterogeneity is an inevitable consequence of cancer’s elevated mutation rate, the general direction of mutation is reversion to ancestral forms via disablement of the more recently evolved capabilities. If a major capability (such as engagement with the adaptive immune system) has been lost, there is an infinitesimally low probability that it will be rediscovered, or regained, via a reverse mutation.

“Target-the-absence” therapies

Our target-the-weakness strategy differs from *Bacillus Calmette-Guérin* (BCG) in that the infectious agent would not be attenuated. Our strategy also differs from Coley [72] and from current immune therapies in that we recognize the infectious agent as the main and direct killer of tumor cells – not the boosting of the adaptive immune system [78] or the boosting of the innate immune system [87]. Our strategy also differs from other target-the-absence suggestions [88–90], since it does not depend on identifying homozygous deletions in the face of tumor heterogeneity. The target-the-weakness strategy depends on the increasingly available knowledge of phylogenetic history and the predicted tendency for cancer cells to revert towards earlier forms. The identified weaknesses also have the advantage of being increasingly irreversible and thus less susceptible to one of the main problems of most current therapies, which is the temporary nature of their effectiveness.

Conclusions

Most current cancer therapies target cell proliferation and other deeply embedded strengths of cancer. The atavistic model of cancer [11] suggests an alternative approach: a target-the-weakness strategy in which challenges are designed that can *only* be met by recently evolved capabilities, which the atavistic model predicts will be decreasingly effective in tumor cells as cancer progresses. The atavistic model is a fertile source of target-the-weakness therapeutic ideas, and we describe examples involving the Warburg Effect, transmembrane ABC proteins, DNA repair mechanisms, and the immune system. Many more applications are possible. If the phylogenetic order of the components of a physiological system is known, and there are important identifiable changes in the time period 1.5–0.5 billion years ago that overlap with the evolution of cell differentiation, then a target-the-weakness strategy can be devised based on that information.

Our most detailed example involves the immune system. The atavistic model

suggests that as cancer progresses, cancer cells “lose contact” with the more recently evolved adaptive immune system of the host and that this immunosuppression is increasingly irreversible. The absence of adaptive immunity in immunosuppressed tumor environments can be exploited by using a vaccination/inoculation strategy, which in metastatic cancer could involve non-attenuated *Listeria* or other pathogenic agents.

For our approach to work, two important outstanding questions need to be addressed. First, how specific can the atavistic model be in predicting loss of functionality? Second, how specifically can therapeutic challenges be designed to ensure enough differential stress between healthy cells and cancer cells, such that the cancer can be slowed and managed more effectively than with current treatments? It seems likely that a combination of challenges to cancer will be necessary to accomplish this goal.

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