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REVIEW

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Changing the Energy Habitat of the Cancer Cell in Order To Impact Therapeutic Resistance

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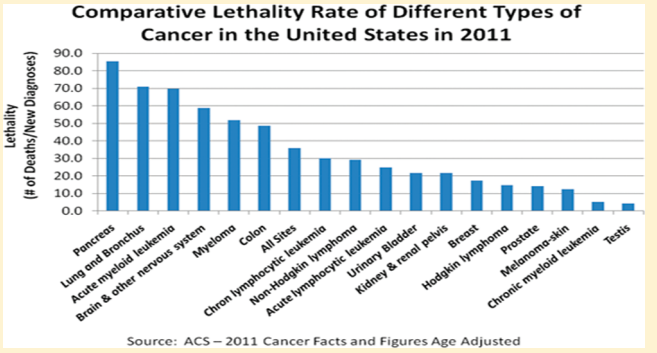
ABSTRACT: Somatic cellular evolution is becoming a popular biological explanation for the common rapid development of resistance to almost every form of cancer therapy and against almost every form of advanced human solid tumors. As a result of the historical power of evolution within nature, this common biological interpretation of the failure of cancer therapy is leading to a growing despair for many investigators and a stronger turn toward prevention through lifestyle changes. The absolute explosion of molecular scientific discoveries since 1983, in the reductionist identification of specific cancer therapeutic targets, has failed to deliver the impact in the clinic that many of us would have hoped would have resulted by this time. Personalized molecular medicine may help us reclassify appropriate therapeutic subgroups, but will it significantly impact the overall specific survival times for all of the cancers combined within the organ type for the entire population? How might we approach this therapeutic dilemma by utilizing new therapeutic insights designed on proven principles of evolution? In other words, can we fight the development of therapeutic resistance in cancer cells by turning established aspects of evolution against the survival of cancer cells within the individual patient? Here we review the concepts of changing the heat habitat and microenvironment of the cancer cell to alter the higher order organization and function of DNA. We have proposed that heat may be a major factor in determining the lasting therapeutic effect on many types of far advanced metastatic tumors.



KEYWORDS: resistance, cancer, microenvironment

MAJOR CLINICAL LIMITATIONS IN CANCER TREATMENT: THE DEVELOPMENT OF DRUG RESISTANCE AND LOSS OF DURABLE RESPONSES

Since the "War on Cancer" was declared four decades ago by President Richard Nixon on December 23, 1971, many major advances have been made in our understanding and treatment of cancer. During this 40 year window, our molecular understanding of cancer has grown exponentially. Genetic and epigenetic regulators of cellular processes involved in the development of cancer have been elucidated along with an increased understanding of the importance of the microenvironment including the vasculature, stroma and inflammation. At the same time that there have been significant advances, for the treatment of advanced, metastatic disease, one might argue that the overall therapeutic outcomes and mortality rates have almost stood still.

The principal issue is that most of these individuals have forms of their disease that are not curable using chemotherapeutic, hormonal, immunologic and radiation therapy based approaches. Many of these advanced tumors only express transient responses and soon express the evolutionary ability to become resistant to all forms of therapy. Although the concept is often debated, it now appears that many of these resistant cells came from preexisting clones that are selected for their ability to adapt to growth and/or escape death under these treatment conditions. It is this ultimate resistance that selects drugs that in principle may transiently reduce the total cell number but in only providing a delay in the continued growth of the tumors. In fact, studies have demonstrated that when these resistant tumors grow back, they do so at the same rate that the original tumor grew.[1]

A good example from the types of resistance expressed in cancer is that observed in prostate cancer. In men that have a form of prostate cancer that progresses beyond localized disease, the most frequently used therapies are hormonal androgen withdrawal based approaches. Despite the typical initial favorable response to these approaches, resistance almost always results and the tumors no longer respond to the blockade of androgens that are required for the growth and differentiation of the normal prostate. With novel anti-androgenic modalities now coming into clinical use, we may be able to delay this resistance for a longer period of time but the end result is changed very little.

In almost all types of cancer therapy, normal cells do not express that ability to develop resistance. For example, no matter

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how many treatment courses of chemotherapy are given, the toxic effects observed on normal cells continue and, if anything, worsen. This clinical ability to form resistance to therapies is a unique property of tumors. It is apparent that if we are going to truly impact cancer, we need to look at clinical and biological lessons taught to us from historical perspectives. For example, how have we been so successful in controlling some forms of cancer and so unsuccessful with others?

In evolution, in biology, we know that no species has ever become extinct by focusing only on killing off each of the individual members of the species. Species most often become extinct by altering their habitat/environment in such a way that they are no longer able to survive as a group. Therefore utilizing additional approaches that make the tumor microenvironment in the host more inhospitable for the tumor cell would seem to be required for us to make all of the tumor clones extinct. We believe this boost in clinical extinction may be at the heart of how we have successfully cured many types of some forms of very advanced cancer that have been so durable.

IMPACT OF A PHYSICAL MICROENVIRONMENT

The internal and external environments of a cell are in communication and harmony. "What a cell touches often determines what a cell does".[2] This is an old biological observation that the cellular microenvironment controls the response and function of the overall DNA information transfer within a cell. This flow of cellular information is directed from outside the cell to the central nuclear DNA and biological components by both soluble and vectoral transport systems that are interlinked networks that comprise highly dynamic polymeric structures and scaffolding with chemomechanical properties. This overall tissue matrix system[2] directly couples the cell surface receptors and pore complexes extending through the membrane to link external gradients as well as to the extracellular matrix and neighboring adherent cells. The cytoplasmic skeleton continues with similar central attachment to the nuclear DNA through a nuclear matrix of non-histone proteins and RNAs, providing higher order structure to DNA topology and folding into loops, chromosome and ultimately the chemomechanical meiotic and mitotic structures. The complex nuclear matrix is a spatial nuclear structure that is composed of self-organizing molecular complexes that orient nuclear motors and factories for the topological processing and flow and function of DNA information. This order of nuclear domains is essential for organizing the appropriately timed and developing features of tissues. This matrix system is disorganized and mistimed in cancer cells and disrupts the higher order flow of information and stability of the DNA. It is this structural disorganization of the cell that clues the pathologist to diagnose cancer. However, as a survival mechanism, stress cancer cells are known to be regulated, at least in part, by their microenvironment. There are many aspects to the microenvironment including energy, cell−cell interactions, the extracellular matrix and growth and signaling factors in the local cellular surroundings. Among these environmental components are physical factors such as thermal energy as measured as temperature. These physical components can modify pH, oxidative state, and the available food energy. The components of each of these factors have been established to play a role in normal cellular signaling as well as in cancer cells. In addition to these local cellular events, the entire body is also known to be composed of symbiotic

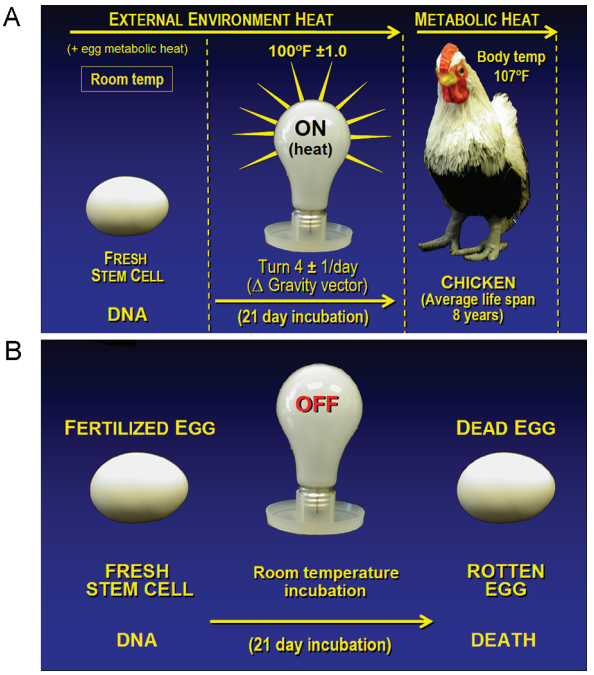

Figure 1. The influence of heat on the development of a stem cell model.

interactions between active and residual microorganisms within our bioreactor.

Of the physical microenvironmental factors, there is arguably none more important than thermal energy recorded as temperature. Optimal temperature ranges are narrow and precise and have been shown to be a central regulator of most organismal and cellular properties. At the level of the organism, for example, temperature-dependent sex selection is quite common in animal species and is most frequently found in reptiles and some birds.[3] Perhaps one of the clearest examples of the influences of heat on development is the precise and narrow temperature optimum that affects the proper incubation of bird eggs, such as a chicken egg. In many ways, the chicken egg can represent the importance of thermal energy, i.e. heat, in stem cell development. The chicken egg is a stem cell. It is a single cell with the pluripotent ability to develop into an entire chicken. The requirement for this to happen is a defined tight window of temperature (37−39 °C) for a period of 21 days. Without the necessary exact range of thermal energy, development time and egg turning, hatching will not occur (Figure 1). In many ways thermal energy is also the ultimate driver of the development process in plants (seed germination, timberline, bacteria and animals).

At the cellular level, thermal energy influences many biological components including cellular structure and higher order DNA organization. The organizational framework of the nucleus, the nuclear matrix, is considered to be one of the most heat responsive structures of the nucleus.[4−7] The nuclear matrix has been shown to be reorganized in structure and protein folding by changes in cellular temperature resulting in changes in association of complexes with this critical cellular regulatory structure.[8] Classic studies that perhaps most directly demonstrate the impact of heat on the organization of DNA are those demonstrating the chromosomal puffs associated with heat in *Drosophila melanogaster*.[9] Protein dynamics themselves are obviously modulated by thermal energy. Temperature can alter protein folding as well as the ability of proteins to interact with one

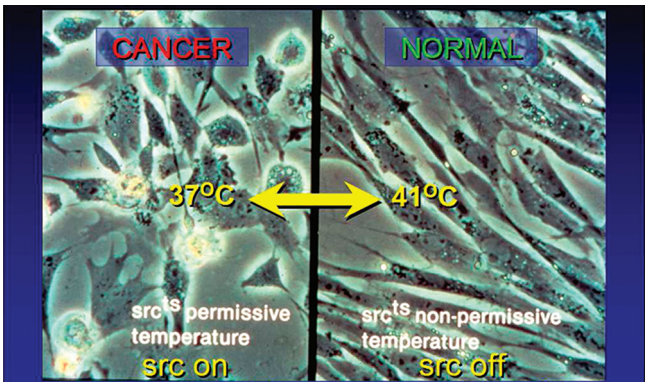

Figure 2. Cancer cell structure can be reversed by heat. Summary of discoveries by Rouse and Temins as reviewed by R. Weinberg 2006.[10]

another as well as with macromolecules such as DNA and RNA. Perhaps one of the biggest examples of how temperature impacts protein folding and function is temperature sensitive mutants. At permissive temperatures the protein carries out its cancer morphology and function, but under a sharp restrictive or nonpermissive temperature the mutant phenotype to produce the cancer cell structure is lost and the normal phenotype cell structure is reversed. A classic study utilizing the oncogene, *src*, demonstrates that, at the permissive temperature of 37 °C, the *src* gene function is on and the fibroblasts adapt a cancer structural phenotype. At a slightly higher temperature of 43 °C the function is turned off and the cells are again "normal" in structure[10] (Figure 2). This reversal can be cycled on and off with appropriate change in temperature. Protein folding and temperature are inter-related and dictate function at both the chromatin and interactome levels.

A number of important modulators of thermal and other stress responses are the heat and cold shock proteins that buffer and direct these thermal responses.[11,12] These proteins have been shown to provide cellular protection to the cancer cell. In fact, it has been proposed that lowering heat shock proteins may provide for great efficacy of chemotherapy.[13] This approach is now being evaluated in clinical trials.[14] Heat shock proteins have also been shown to be modulators of protein folding as well as chaperones that may play important roles in their function. We have been focusing on examining the other side of the equation, cold shock proteins. This family of stress response proteins are, as their name implies, turned on when the cellular environment undergoes decreases in thermal energy as measured by a decrease in temperature as well as other stress responses. We have been focusing on two members of the cold shock protein family, RBM3 and CIRBP.[15] These are both RNA binding proteins, and we are finding that they have important cellular effects in regard to how the cancer cells respond to stress and may be important targets to disrupt stress response therapies and perhaps thus make the cancer cells more vulnerable to therapies.

WHAT PRICE DO CANCER CELLS THAT DEVELOP RESISTANCE PAY IN REGARD TO FITNESS?

One question that is raised is, as the cancer cell develops resistance to therapies, what advantages or disadvantages does it generate in regard to environmental fitness? The evolution of the cancer cell to develop resistance to a therapy requires it to gather sufficient energy and resources to survive and replicate. The cellular offspring must be successful in numbers to repeat these biological events, be robust, and yet change to adapt to the changing environment and ecosystem of the tumor niche. It appears that the tumor while focusing on becoming resistant to the therapy being applied has developed an added sensitivity to other cellular stress. Development of therapeutic resistance results in an increase in the cancer cells' sensitivity to changes in their energy habitat.[16] In these studies, cells that are resistant to the chemotherapy, paclitaxel, have an increased sensitivity to heat. This increased sensitivity would appear to be an avenue in which the tumor cell environment can be manipulated in order to move them more toward to the direction of extinction.

USING THERMAL ENERGY TO ENHANCE THERAPEUTIC APPROACHES

One of the areas in cancer that has been inadequately studied is why some cancers are curable whereas others are not. An example of this is testicular cancer, where men can present with disease that has metastasized to their lungs and brain, and despite what would be a death sentence if untreated or for another cancer type, with chemotherapy, these men are almost always cured of their disease. Why? We have focused effort to attempt to understand the differences between testicular cancer and other solid tumors. One hypothesis that we termed, "the Lance Armstrong Effect",[17] describes that one potential reason for the sensitivity of metastatic testicular cancer to chemotherapy might rest in the environmental temperature differential between the site of the primary tumor and the sites of the metastases. Typically the testes are several degrees cooler than the rest of the body. This cooler temperature is required for their normal function. Barone et al.[18] reported that normal body temperature of 37 °C affects undescended, cryptorchid testes, resulting in individuals that have this condition being infertile. It has been proposed that this is a result of the induction of unstable sperm nuclear matrices that affects that higher order DNA organization. When the cancer cells that arise in the testes move into the body, they are now in a warmer environment. As stated above, it is apparent that cancer cells are more sensitive to microenvironmental stress. The metastasized testicular cancer cells are in such a stressed environment. This change may be at least one of the reasons why testicular cancer cells are so sensitive to chemotherapy.

The question would then be, if this is true, how could this be applied to other types of solid tumors? One potential solution is to warm the microenvironment of the tumor cells and, in doing so, replicate the type of conditions that the testicular cancers find themselves in. The use of heat-based therapies for cancer is not new.[19] In fact, many approaches have been applied ranging from heating the patient in their entirety to heating limbs or regions of the affected individual. Recent studies have revealed that warming tumors can reduce the interstitial pressure of the tumor, resulting in an increased effectiveness of radiation therapy.[20] As with other therapeutic approaches, the question is, how can this be done in such a way that it achieves that local microenvironmental impact without resulting in systemic toxicity? Nanotherapeutic approaches have been used to help achieve this goal. Some groups have utilized gold nanoparticles that are heated with radiofrequencies in order to heat local areas of tumor.[21] While this approach would appear to have value when the locations of the tumors are known, some questions have been raised regarding the ability of such particles to heat effectively under the described conditions.[22]

An approach that we are utilizing is called thermal enhanced metastatic therapy (TEMT). This approach utilizes iron oxide core nanoparticles to heat cancer cells, in our case, prostate cancer cells, to 43 °C in order to make them more sensitive to chemotherapy, radiation therapy and/or immunotherapy. Utilizing a multidisciplinary approach, these particles are being targeted to prostate cancer cells using prostate specific membrane antigen (PSMA) as the initial approach. This is not being developed for the treatment of localized disease but for metastatic therapy. The particles are then heated utilizing an alternating magnetic field instrument that has been specifically designed to heat these particles with minimal heat disposition in the normal surrounding tissues.[23] This approach is now being evaluated in *in vitro* and *in vivo* models. To us, this represents the kind of approach necessary to attack the tumor in its home by disrupting the local microenvironment and therefore driving it toward extinction or at least minimizing the chances for the development of resistance.

CONCLUSIONS

In order to impact cancer cell resistance to therapy we need to study what has already been shown to work for cancer successfully in the clinic. Although little focus is applied to it, we must examine the therapeutic mechanisms and biologic principles of the types of cancers that we have proven clinically that we can control in a highly effective manner with durable response without developing clinical relapses or therapeutic resistance. A target of cancers that are able to be cured may be their sensitivity to temperature. Evolution is lazy, is commonly modified and adapts existing successful modules to new species rather than designing new fundamental biological approaches. We believe that the effects of modifying energy and metabolism may explain the successful cases of therapy that interface with cancer through alteration of higher order self-organization of DNA linked to cellular structure. It is this structure that is the basis of identifying the tumor cells and is most sensitive to physical modification of the cancer cell environment in a tumor specific manner. It is clear that the price that the tumor pays as it develops resistance is sensitivity to changes in the energy habitat. It is through changes in this habitat that we may be able to develop therapeutic approaches that limit the ability of tumors to develop resistance to therapy and thereby provide patients with the much-needed impact on their diseases.

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REFERENCES

(1) Stein, W. D.; Gulley, J. L.; Schlom, J.; Madan, R. A.; Dahut, W.; Figg, W. D.; Ning, Y. M.; Arlen, P. M.; Price, D.; Bates, S. E.; Fojo, T. Tumor Regression and Growth Rates Determined in Five Intramural NCI Prostate Cancer Trials: The Growth Rate Constant as an Indicator of Therapeutic Efficacy. *Clin. Cancer Res.* **2011**, *17* (4), 907–917.

(2) Getzenberg, R. H.; Pienta, K. J.; Coffey, D. S. The Tissue Matrix: Cell Dynamics and Hormone Action. *Endocr. Rev.* **1990**, *11* (3), 399–417.

(3) Ferguson, M. W.; Joanen, T. Temperature of egg incubation determining sex in alligator mississippiensis. *Nature* **1982**, *296*, 850–853.

(4) Roti Roti, J. L.; Kampinga, H. H.; Malyapa, R. S.; Wright, W. D.; vanderWaal, R. P.; Xu, M. Nuclear matrix as a target for hyperthermic killing of cancer cells. *Cell Stress Chaperones* **1998**, *3*, 245–255.

(5) Vanderwaal, R. P.; Maggi, L. B, Jr.; Weber, J. D.; Hunt, C. R.; Roti Roti, J. L. Nucleophosmin Redistribution Following Heat Shock: A Role in Heat-Induced Radiosensitization. *Cancer Res.* **2009**, *69*, 6454–6462.

(6) Roti Roti, J. L. Heat-Induced Alterations of Nuclear Protein Associations and their Effects on DNA Repair and Replication. *Int. J. Hyperthermia* **2007**, *23*, 3–15.

(7) Falloon, E. A.; Dynlacht, J. R. Reversible Changes in the Nuclear Lamina Induced by Hyperthermia. *J. Cell. Biochem.* **2002**, *86*, 451–460.

(8) Lepock, J. R.; Frey, H. E.; Heynen, M. L.; Senisterra, G. A.; Warters, R. L. The nuclear matrix is a thermolabile cellular structure. *Cell Stress Chaperones* **2001**, *6* (2), 136–147.

(9) Lindquist, S.; McKenzie, S. H.; Meselson, M. Localization of RNA from heat-induced polysomes at puff sites in drosophila melanogaster. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72* (3), 1117–1121.

(10) Weinberg, R. A. *The Biology of Cancer Textbook*; Taylor and Francis Publishing Company: 2006.

(11) Ciocca, D. R; Fanelli, M. A; Cuello-Carrion, F. D; Castro, G. N. Heat Shock Proteins in Prostate Cancer: From Tumorigenesis to the Clinic. *Int. J. Hyperthermia* **2010**, *26*, 737–747.

(12) Phadtar, S; Severinov, K. RNA Remodeling and Gene Regulation by Cold Shock Proteins. *RNA Biol.* **2010**, *7*, 788–795.

(13) Rocchi, P.; Beraldi, E.; Ettinger, S; Fazil, L.; Vessella, R. L.; Nelson, C.; Gleave, M. Increased Hsp27 after androgen ablation facilitates androgen-independent progression in prostate cancer via signal transducers and activators of transcription 3-mediated suppression of apoptosis. *Cancer Res.* **2005**, *65* (23), 11083–11093.

(14) Matsui, Y.; Hadaschik, B. A.; Fazil, L.; Andersen, R. J.; Gleave, M. E.; So, A. I. Intravesical combination treatment with antisense oligonucleotides targeting heat shock protein-27 and HTI-286 as a novel strategy for high-grade bladder cancer. *Mol. Cancer Ther.* **2009** *8* (8), 2402–2411.

(15) Zeng, Y.; Kulkarni, P.; Inoue, T.; Getzenberg, R. H. Down-regulating cold shock protein genes impairs cancer cell survival and enhances chemosensitivity. *J. Cell. Biochem.* **2009**, *107* (1), 179–88.

(16) Li, Y.; Zeng, Y.; Mooney, S. M.; Yin, B.; Mizokami, A.; Namiki, M.; Getzenberg, R. H. Resistance to Paclitaxel Increases the Sensitivity to Other Microenvironmental Stresses in Prostate Cancer Cells. *J. Cell. Biochem.* **2011**, *1128*, 2125–2137.

(17) Coffey, D. S.; Getzenberg, R. H.; DeWeese, T. L. Hyperthermic Biology and Cancer Therapies: A Hypothesis for the "Lance Armstrong Effect. *JAMA: J. Am. Med. Assoc.* **2006**, *296* (4), 445–448.

(18) Barone, J. G,; Christiano, A. P.; Ward, W. S. DNA Organization in Patients with a History of Cryptorchidism. *Urology* **2000**, *56*, 1068–1070.

(19) Westermann, A. M.; Jones, E. L.; Schem, B. C.; et al. First results of triple-modality treatment combining radiotherapy, chemotherapy, and hyperthermia for the treatment of patients with stage 11B, 111, and IVA cervical carcinoma. *Cancer* **2005**, *104*, 763–770.

(20) Sen, A.; Capitano, M. L.; Spernyak, J. A.; et al. Mild elevation of body temperature reduces tumor interstitial fluid pressure and hypoxia and enhances the efficacy of radiotherapy in murine tumor models. *Cancer Res.* **2011**, *71* (11), 3872–3880.

(21) Curley, S. A.; Cherukuri, K.; Briggs, C. R.; Patra, M.; Upton, E.; Dolson, P.; Mukherjee, J. Noninvasive radiofrequency field-induced hyperthermic cytotoxicity in human cancer cells using cetuximab-targeted gold nanoparticles. *J. Exp. Ther. Oncol.* **2008**, *7* (4), 313–326.

(22) Li, D.; Jung, Y. S.; Tan, S.; Kim, H. K.; Chory, E.; Geller, D. A. Negligible absorption of radiofrequency radiation by colloidal gold nanoparticles. *J. Colloid Interface Sci.* **2011**, *358*, 47–53.

(23) Ivkov, R.; DeNardo, S. J.; Daum, W.; Foreman, A. R.; Goldstein, R. C.; Nemkov, V. S.; DeNardo, G. L. Application of high amplitude alternating magnetic fields for heat induction of nanoparticles localized in cancer. *Clin. Cancer Res.* **2005**, *11* (19 Part 2), 7093s–7103s.