

Editorial

Ten unanswered questions in cancer: “If this is true, what does it imply”?

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Introduction and tribute to Donald S. Coffey, Ph.D.

Don Coffey immortalized many aphorisms for his trainees, but perhaps the most famous of these is “If this is true, what does it imply?” This simple statement guides scientific research. If what we observe is real, what does it mean? By extension, how does that truth affect or change our thinking? How does it guide the next questions we must ask?

Dr. Coffey’s thinking about what cancer was and how it evolved was driven by two fundamental observations or truths that have vast implications for the field: 1) cancer is a disease of cell structure and function and 2) life is an isothermic chemomechanical engine and therefore cells can be thought of as chemomechanical engines [1]. He was constantly trying to understand how the mutations of the genome that lead to cancer contribute to or are caused by the changes in nuclear, cytoplasmic, and tissue shape that allow the diagnosis of cancer to be made [1]. Simultaneously, he tried to understand how the disorganized structure of the cancer cell allowed it to survive, proliferate, and function despite its non-physiological aberrant state. The disorganized structure and function of cancer cells, however, only touches the surface of the myriad of unanswered questions associated with the evolution of a cancer cell to not only form a successful primary tumor, but also to genotypically and phenotypically adapt to different and changing environments

resulting in a metastatic, lethal disease (**Figure 1**).

As we seek truth, we are guided by these unanswered questions and the implications for our research, for our understanding of cancer biology, and for our patients.

Question 1. How long does it take a normal cell to acquire the appropriate type and number of mutations to become a malignant cell?

There are two current models of carcinogenesis: the stochastic model that postulates that every cell in the body has intrinsic tumorigenic potential and the cancer stem cell model that proposes that cancer can arise only from a small subset of normal stem cells. It is accepted that a malignant cell emerges over a period of years. What remains unclear is how many cell divisions are required for a normal cell to evolve into a cell that proliferates past Hayflick’s limit and acquires the other required features of malignancy historically depicted as Hallmarks of Cancer [2]. It is now evident that the time and number of mutations required to give rise to a cancer cell is dependent on the inherited genetic background of the host (i.e., patient) as well as the effects of the changing environment that host lives in. It is unknown how frequently healthy cells evolve into premalignant cells that are simply not viable and undergo apoptosis or senescence or how often the immune system clears abnormal cells before malignancy develops. It is often said that each person develops “cancer” thousands of times in their life that do

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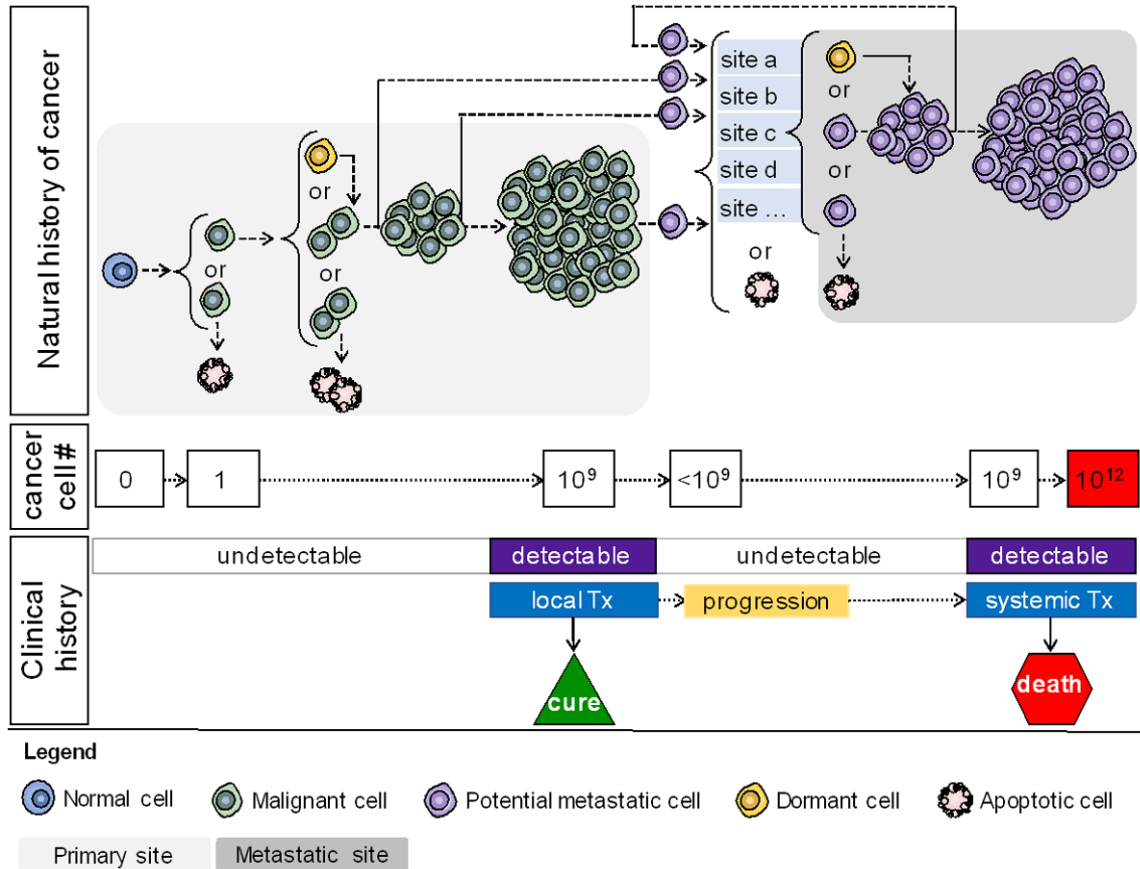


Figure 1. Unanswered questions of the journey of cancer over time.

not grow to become clinically significant, but these events still cannot be measured.

Question 2. What lessons from normal development and natural variation can we apply to understanding cancer?

There are many lessons to be learned about cancer by observing the differences between normal biology and malignant biology. Dr. Coffey was fond of asking his trainees why the human seminal vesicles never develop cancer, while the prostate and testes develop cancer relatively frequently (1 in 9 for prostate cancer and 1 in 250 for testicular cancer in the US) [3]. In contrast, there have been only 51 reported cases of cancer of the seminal vesicles in published literature [4]. What is it about the nuclear, cellular, or tissue organization and structure that allows the cells in the seminal vesicles to escape cancer? The answer may partially lie in the evolution of the gland itself, as exclusively meat-eating animals (e.g. dogs, cats, and sea lions) do not have seminal vesicles, whereas plant-eating animals or omnivores (e.g. apes,

horses, and humans) do [5]. Drs. Coffey and Getzenberg also found that the nuclear matrix proteins in prostate cells were differentially expressed relative to the seminal vesicles in castrated rats, indicating that a fundamental structural difference may partially explain the difference in malignancy in the two tissues [6]. These types of questions that seek to differentiate the normal characteristics of healthy tissues, combined with understanding the “cellular history” of both the individual cancer cell and larger architecture of a tumor are crucial to form a better understanding of how to find and treat cancer.

Question 3. Once a cancer cell emerges, how long does it take to become clinically evident?

How much time is required and what are the necessary environmental conditions for the newly emerged proliferative malignant cell to form a tumor of clinically detectable size? A clinically detectable tumor, i.e., one that can be discerned by current imaging modalities, is generally considered to be 1 cm³ in size and

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consists of approximately one billion cancer cells. How often malignant cells form clinically significant tumors depends on a number of factors including, among others, proliferation rate of the malignant cells, the permissiveness of the normal host tissue microenvironment, the activation and participation of the tumor microenvironment, the angiogenic capacity of the malignant cells, and their ability evade immune surveillance. Each of these is likely dependent on host specific factors that remain difficult to quantify or estimate. 80% of men who die at age 80 years of non-prostate cancer related causes have histologically malignant prostate cancer present in the prostate, yet these cells are not “clinically” important [7]. We still do not understand why different cancers develop at different rates in individuals.

Question 4. How, when, and where do cancer cells metastasize?

Approximately 50% of all patients diagnosed with cancer can be cured by local therapy such as surgery or radiation [8]. This truth implies that the patients who fail primary therapy already had microscopic metastatic disease at the time of primary therapy. We do not understand what enables a cell to successfully leave a primary tumor or when this emigration initiates. While it is clear that cancer cells with a subset of phenotypic characteristics may have a selective advantage as a potential metastatic seed (e.g., mesenchymal or stemness characteristics, high motility, resistance to anoikis, invasive ability), it remains unknown how or why a rare subset of cancer cells would gain these characteristics. It continues to be a “Holy Grail” of the cancer community to be able to identify patients who already have pre-clinical disseminated tumor cells (DTCs) at the time of primary therapy, and to define which of those cells will become a clinically significant metastasis.

Since Paget published his “Seed and Soil” hypothesis in 1889, the cancer metastasis field has struggled with understanding why different cancer types exhibit propensities to successfully colonize specific organs [9]. For example, nearly 100% of men who die from prostate cancer have bone metastases and we still do not know why [10]. Observed tropism implies that metastatic location is not random, but the distribution of cancer cells from the primary tumor to potential metastatic sites should be unbi-

ased: blood flow dictates that cancer cells should be distributed with equal probability to all organs [11]. This unbiased distribution would likely also apply to cells leaving one metastasis to seed another, a phenomenon that complicates efforts to detect residual micrometastatic disease. It is also unclear how metastatic spread is related to primary tumor site, the relationship to neighboring organs, or location with respect to nearby circulatory, lymphatic, and nervous systems. Given the frequency of bone metastases in prostate cancer patients, it would be logical to sample the bone marrow for evidence of residual disease at the time of primary therapy, but the bone may not actually be the first site of colonization for those cells [12]. Our knowledge of where and when cancer cells initially metastasize is limited by the sensitivity of current imaging techniques.

Question 5. Why do some disseminated tumor cells proliferate to become clinically evident metastases and some do not?

Based on data on the frequency of circulating tumor cells, it appears that the entire process of metastasis is extremely inefficient - likely on the order of one successful metastatic cell for several billion cells that leave the primary tumor [11]. We do not know, nor can we currently measure, the critical points of this process: How many cells reach each secondary organ? How many invade the secondary site? How many of those potentially metastatic seeds subsequently die or are eliminated by the immune system? Of those that survive, how many regain proliferative capacity to colonize the metastatic site? Beyond the simple quantitation of these events, we also do not know factors and selective pressures that contribute to determining whether a cell remains dormant or proliferates into a metastatic tumor. As discussed in Question 4, cells emigrating from the primary tumor are likely distributed to all organs of the body without bias. What are the characteristics of the secondary organ “soil” or the would-be metastatic “seed” that enable the cancer cell to proliferate in that particular site to generate a clinical metastasis?

Question 6. Do metastatic cancer cells undergo a period of dormancy?

The concept of cancer dormancy was first introduced in 1952 when Rupert Willis observed

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decades-long disease-free periods between elimination of the primary tumor and clinical relapse [13]. Today, the idea of cancer dormancy has expanded to refer to both individual cellular dormancy (a non-proliferative cell reversibly arrested in G0/G1) and tumor mass dormancy (a balance of proliferation and cell death resulting in zero net growth). It is unknown which of these mechanisms is responsible for long-term dormancy of malignant cells. Is it possible for the balance of cellular proliferation and death within a mass to be maintained for a decade without disruption by additional mutations or systemic influences? If individual cellular dormancy exists, what factors of the “soil” could possibly induce mitotic arrest of a “seed” that has already acquired proliferative mutations, invaded through tissue, survived the bloodstream, and successfully homed to a new site? It has also been proposed that dormancy does not exist, and that latent periods can be explained by slow cycling, similar to hematopoietic stem cells. Answers to these questions will have profound implications for understanding the natural history of metastatic cancer.

Question 7. What is the role of the normal host and tumor microenvironments in each step of the metastatic cascade?

There are a number of internal factors (e.g., mutations, inherited epigenetic marks) that contribute to cell malignancy and cancer progression. A cancer cell, however, does not exist in isolation: there are numerous external factors in the tumor microenvironment that influence disease progression. Factors such as tissue architecture, local nutrients (e.g., growth factors, lipids), and neighboring cells (e.g., immune cells, stromal cells) all contribute to malignant cell behavior and fate [11, 14-17]. As we learned from Dr. Coffey, it is critical to draw from the laws of normal development and healthy cell function to understand the role of hijacked healthy cell players in the setting of a tumor ecosystem. It remains unclear how each factor of the tumor microenvironment amalgamate to promote or inhibit establishment of the primary tumor, permit or restrict escape of potential-metastatic cells from the primary tumor, establishment of metastases at distant sites, and response to therapy. Characterizing the function of host T-cells in the tumor microenvironment formed the basis of the current immunotherapies that have demonstrated dramatic efficacy in a subset of tumors. Defining and

understanding the characteristics of the positive and negative effects of the other players in the tumor microenvironment would define other points of intervention to positively impact disease management.

Question 8. What is the role of the host (patient) in cancer?

The life-history of a tumor, from the initial emergence of a malignant cell to forming lethal metastases at secondary sites, is all set within the confines of the host patient, encompassing both inherited and acquired genetic and phenotypic characteristics. Every individual is born with a particular genetic background that influences all cells of the body, including pre-malignant cells and the cells that ultimately participate in the tumor microenvironment discussed in Question 7. There is large inherited variation between populations, highlighted by epidemiological studies that identify particular ethnic groups at higher risk of specific tumor types (e.g. African American men are at increased risk for prostate cancer than Caucasian men). Moreover, even within a population, there is a great deal of variability of physiological processes such as immune surveillance that likely influence the emergence and progression of cancer. These inherited phenotypes not only influence pre-malignant cells, but also influence the cancer-supportive or -suppressive stroma of an individual. There is evidence that when malignant epithelial cells are combined with normal stromal cells, their malignant phenotype becomes altered and in some cases reversed [18, 19]. In addition to inherited variability, an individual's environment (and his/her physiological response to it) also contributes to cancer initiation and progression. A number of environmental risk factors have been identified that induce mutation in cancer cell of origin (e.g. smoking and lung cancer, *h. pylori* infection and gastric cancer). There is not a clear understanding, however, how “every-day” environmental factors may influence tumorigenesis, either through the effects on the pre-malignant cell or to microenvironment cells (e.g. response to chronic stress, immune challenge, sleep debt, etc.).

Question 9. How and when does tumor cell heterogeneity emerge?

Cancer cells develop resistance to all known natural and synthetic drugs while normal cells do not have this capability [1]. Dr. Coffey noted

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that this resistance is a reflection of the wide diversity of functions expressed by cancer cells within a tumor and that this variation in function (pleiotropism) is accompanied by variation in structure (pleomorphism); that is, form follows function. While there are many mechanisms that contribute to tumor cell heterogeneity, how and why these mechanisms are part of the evolution of cancer remains undefined.

Cancer cell heterogeneity is most often described (and most readily interrogated) in the setting of therapeutic resistance. Resistance to a therapy may be solely cell intrinsic and therefore present in a treatment-naïve setting. Given the genetic instability inherent to cancer cells, clonal diversity may be a product of neutral evolution (genetic drift). It is also possible that the clonal heterogeneity in treatment-naïve tumors may result from the adaptive responses to the selective pressures within the tumor microenvironment, such as hypoxia, overcrowding, or acidic pH [15]. In this case, therapy resistance mechanisms may have “piggybacked” on the traits actually selected for survival in a harsh environment. Similarly, cancer cell heterogeneity can also be induced through application of external selective pressure: therapeutic treatment. There is strong evidence that a subset of cancer cells will evolve in response to therapy, allowing that clonal population to propagate and survive. In both cases, resistant clones adapt and acquire a more aggressive phenotype and are resistant to future treatments, which ultimately results in disease relapse and metastasis. How and when tumor heterogeneity arises has profound implications for the treatment and management of the disease, to inform treatment type, aggressiveness, and timing. It is critical to understand these mechanisms to create rational treatment strategies to balance reduction of tumor burden and the application of severe selective pressures.

Question 10. How does cancer kill the patient?

One of the most common questions a patient asks is: “How is this cancer going to kill me?” It is still a great mystery. Cancer rarely kills by mechanically blocking an organ - rarely does a patient die of liver failure or kidney failure because the cancer has effaced the organ. What we know is that as patients approach a total tumor burden of approximately a kilogram of

tumor (approximately one trillion cells), that this is not compatible with life. The major syndromes associated with cancer death like cachexia or embolism appear to be mediated by cytokines released from the tumor microenvironment [15, 16]. What will ultimately kill a particular cancer patient remains unpredictable and therefore untreatable. Thus, the most common and most fundamental patient question remains unanswered.

Dr. Coffey continued to try to answer these questions, and many others, literally to the day he passed away [20]. He continues to challenge us every day from the other side.

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