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COMMENTS AND CONTROVERSIES

Research on Early-Stage Carcinogenesis: Are We Approaching Paradigm Instability?

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INTRODUCTION

In a recent article in the New York Times,[1] Gina Kolata reported that a major change is occurring in the way researchers view early-stage carcinogenesis. We offer a more detailed perspective on this change and discuss implications for research funding.

The prevailing paradigm for early-stage carcinogenesis is the somatic mutation theory, which states that "cancer results from an accumulation of mutations and other heritable changes in susceptible cells."[2pp146] The somatic mutation theory has changed over time, and multiple variations have occurred in recent years. In the early 1900s, Theodor Boveri postulated that imperfect or irregular division of the chromosome leads to cancer, and Ernest Tyzzer first used the term somatic mutation in connection with cancer.[3] The discovery of DNA as the genetic material and the observation that cancerous changes are transmitted from one generation of cells to the next pointed to DNA as the critical target of carcinogens.[4] This viewpoint was so ingrained in the research community that, by 1959, future Nobel Laureate Peyton Rous wrote that "numerous workers on cancer are now content to think it [cancer] results from somatic mutations. Hence they see no other reason to seek in other directions to learn its nature."[5pp1357] Not surprisingly, in light of the comment of Peyton Rous, the seminal discovery in 1981 that DNA from human cancers introduced into mouse cells resulted in tumors[6,7] and later experiments that induced human tumors in transgenic mice carrying an oncogene[8,9] were widely viewed as supporting the somatic mutation theory, and there was little serious consideration of alternative explanations. Since then, an ever-growing list of somatic mutations has been associated with cancer; these are now frequently classified as either driver mutations that confer growth advantage or passenger mutations that do not.[10]

Recently, researchers have proposed variations on the somatic mutation theory that share the assumption that carcinogens directly alter the DNA structure or function in cells in the tissue from which cancer arises. These variations, which include epigenetic, chromosomal, and cancer stem-cell theories, differ in how the alteration occurs and in what types of cells are involved. Under the epigenetic theory, heritable changes in gene expression that are not caused by an alteration in the DNA sequence are postulated to contribute to carcinogenesis by increasing chromosomal instability, by reactivating transposons (sequences of DNA that move around the genome), or by loss of imprinting (ie, loss of a silenced genetic locus that leads to monoallelic gene expression).[11] The link between epigenetics and cancer began with the observation of hypomethylation of human tumors[12] and was followed by the identification of hypermethylated tumor-suppressor genes and inactivation of microRNA genes by DNA methylation.[11] However, the epigenetic theory cannot explain unpredictable effects,[11] such as an experiment in which hypomethylation led to fewer tumors than expected.[13] According to the chromosomal theory, a carcinogen induces random aneuploidy, which slowly leads to chromosomal variations and eventual expansion of the most adaptable cells. The chromosomal theory offers an explanation for nonmutagenic carcinogens, the strong association between aneuploidy and cancer, and long latency periods.[14] The more-or-less repeatable patterns of chromosomal changes seen particularly in hematologic cancers is consistent with this theory.[15] According to the stem-cell theory, carcinogens induce cancers by altering those cells that possess characteristics associated with normal stem cells, such as self-renewal and generation of mature cells through differentiation. The stem-cell theory explains experimental results that only a small fraction of injected leukemia cells produce spleen colonies.[16,17] Support for the stem-cell theory also comes from the apparent identification of stem cells in solid tumors;[18] however, this conclusion was later challenged by findings of substantial genetic differences between the purported stem cells and their descendents.[19] A common feature of the somatic mutation theory and the related epigenetic, chromosomal, and stem-cell theories of cancer is the underlying notion that cancer originates at the cellular level of biologic organization.

A central problem for the somatic mutation theory and its variations derives from experiments in which various observations remain unexplained.[20] For example, tumors arise when filters with small holes are inserted subcutaneously in mice but not when filters composed of the same material, but with large holes, are similarly inserted.[21] Equally paradoxical is the observation that tumors arise in epithelial cells at a much higher rate than in controls when normal rat mammary epithelial cells are transplanted adjacent to stroma that had previously been exposed to a chemical carcinogen after clearing out the local epithelial cells.[22] It has been observed that just transplanting normal cells into another (untreated but inappropriate) stromal environment (eg, testis cells to kidney capsule) is enough to induce carcinoma predictably and that, despite the abnormal phenotype of the subsequent cancer

cells, transplanting them back to their proper environment ensures that they return to normal.[23,24]

These paradoxes can be explained by an alternative paradigm, namely the tissue organization field theory, which proposes that cancer is a tissue-based disease.[25,26] Under the tissue organization field theory, cancer arises from disruption of tissue microarchitecture, driven by the collapse of morphostat gradients that maintain that integrity.[24-26] A by-product of a sufficiently long disruption of the morphostat gradient is genetic instability and clonal expansion of the most adaptable cells. Although those subscribing to the somatic mutation theory might attribute the collapse of the morphostat gradient to the effect of mutations yet to be identified, there is experimental evidence compatible with the notion that such a collapse (occurring at the tissue-level of biologic organization) can be unrelated to an effect of mutations at the cellular level. This paradigm has been additionally corroborated by a theoretical (in silico) approach, in which a computational model illustrated how a blockage in the stromal layer could disrupt the morphostat field and induce aberrant epithelial cells.[27]

In addition to explaining results that are paradoxical according to the somatic mutation theory, the tissue organizational field theory also explains results previously interpreted as supportive of the somatic mutation theory and its variations. The tissue organization field theory explains cancer initiation by oncogenes as a result of oncogene alteration of supporting tissue, which, in turn, disrupts the morphostat gradient. The tissue organization field theory is consistent with nonmutagenic carcinogens disrupting morphostatic fields and with a long latency period for this disruption before genetic instability (including chromosomal changes) and clinical manifestation of cancer.

The somatic mutation theory and tissue organization field theory are both plausible theories of early-stage carcinogenesis, so it is reasonable to ask why the vast majority of research funding related to early-stage cancer goes to investigate the dominant somatic mutation theory. This question prompted us to formulate the concept of paradigm instability, which posits that the longer the time without a breakthrough according to a dominant paradigm, then both the more likely that a breakthrough in the dominant paradigm will be seen to be imminent and the more likely that the competing paradigm is correct. To introduce these ideas, we first present two hypothetical examples, which involve the hunt for buried treasure and waiting for a bus (Fig 1).

Treasure hunting
The more you dig for treasure marked by Map A without finding definitive evidence, the closer you think you are to finding the treasure at the location on Map A and the more you think the location on Map B is correct.

Waiting for the bus
The longer you wait for the bus after the scheduled arrival time, the more you think the bus will arrive at any moment and the more you think the bus will not be coming any time soon due to unexpected problems.

Theories of early-stage carcinogenesis
The more resources expended to try to uncover the exact nature of somatic mutation theory, the more you think that you are nearing a breakthrough in understanding somatic mutation theory and the more you think that a competing theory such as tissue organization field theory is correct, especially in light of observations that are paradoxical under somatic mutation theory.

Fig 1. Examples of paradigm instability.

HUNTING FOR BURIED TREASURE

For one hypothetical example of paradigm instability, imagine you and four other treasure hunters disembark on a deserted island. You have two treasure maps, each of which marks a different location of the buried treasure on the island. The vast majority of experts believe Map A is correct, but a few experts offer cogent reasons to support the veracity of Map B. You have 5 days to dig for treasure before the boat returns to pick you up and you run out of food and water. Let us say the common wisdom is that there is a 95% chance Map A is correct and a 5% chance Map B is correct. Also, because the exact location of the treasure is imprecise, you will probably need to combine efforts to unearth the treasure. Your best initial strategy is for all five of you to start digging in the location designated by Map A. The more you dig at the location specified by Map A, the closer you believe you are to the buried treasure. You find a piece of wood that you interpret as part of the treasure chest that had broken off, but it could also be a random piece of wood. Random pieces of wood are, after all, more common than treasure chests. After 4 days of digging, you have not found the treasure; nonetheless, you think you are very close to finding the treasure and that the next shovel of dirt will unearth it. To give up when you may be so close may be foolish. However, the fact that you have not yet found the buried treasure despite your extensive efforts makes you entertain the thought that you are digging in the wrong place. At this point, you think the chances of finding treasure at the location specified by Maps A and B are approximately equal. You have reached a point of paradigm instability. Therefore, you agree that two of the treasure hunters should dig at the location specified by Map B.

WAITING FOR A BUS

As a less fanciful example of paradigm instability, imagine you and four coworkers are in a foreign city. You are each scheduled to give a 5-minute presentation related to the same project, with the first starting at 10:30 AM. The more of you that arrive in time for the presentation, the more likely your project will be well received. The five of you arrive at a bus stop at 9:55 AM. You can either walk to your destination or catch a bus scheduled to arrive at 10:00 AM. As you are not sure of the distance, walking may take anywhere from 20 to 50 minutes. The bus would take 10 minutes. Your best strategy is to take the bus, so all of you wait. At 10:10 AM, the bus has not arrived. Initially, you reason that the bus is delayed by traffic and will arrive at any moment, so that waiting longer is the best strategy. Conversely, you think the absence of the bus means that it will not come for a long time (caused perhaps by a unexpected road closure, your misunderstanding of the schedule written in a language you do not understand, or the bus having arrived and departed just before 9:55 AM), in which case you are better off walking. You have now reached a point of paradigm instability, in which waiting for the bus or walking are equally likely to be successful. To diversify your strategy, you decide that two persons should walk and the remaining three should wait for the bus.

EARLY-STAGE CARCINOGENESIS

We believe that cancer research into early-stage carcinogenesis may be approaching a period of paradigm instability. Proponents of the somatic mutation theory argue that massive DNA sequencing "will comprehensively elucidate central questions related to the nature of human cancer" because "the human genome is finite".[7pp723] That is, the more one digs (to uncover mutations), the more likely one is to strike pay dirt (a comprehensive understanding of carcinogenesis). But are the mutations just random pieces of wood, not the buried treasure chest?

Proponents of the tissue organization field theory argue that, even if one could prove that mutations may be sufficient to cause germline neoplasms and some cancers in specific experiments, such as in the seminal discovery noted in the introduction, they cannot explain all cancers, as evidenced by foreign-body and transplantation experiments. Hence, there is a gap in our knowledge of carcinogenesis that cannot be resolved simply by cataloging DNA mutations in somatic (or inherited) cancer variants. No matter how many different mutations are cataloged, they will not be able to explain these (and other) paradoxical aspects of the somatic mutation theory of carcinogenesis.

In contrast, the tissue organization field theory explains both the foreign body and transplantation experiments that are paradoxical according to the somatic mutation theory as well as experimental results that seemingly support the somatic mutation theory, such as the induction of cancer from DNA inserted into mice, which can be explained according to tissue organization field theory as the result of the collapse of the morphostat gradient. In addition, recent investigations into the role of the extracellular matrix support the view that "disrupting tissue architecture is sufficient in the long run not only to disrupt function, but also to induce tumorigenesis."[28pp173]

IMPLICATIONS FOR FUNDING

Currently, the vast majority of funding for research into early carcinogenesis supports studies involving the somatic mutation theory, such as the large genome-wide search for genetic markers of susceptibility to cancer.[7] We believe that, for research into early-stage carcinogenesis, most funding agencies think only in terms of the first part of paradigm instability, namely that, as more genetic cancer susceptibility markers are investigated without a breakthrough, there is an increasing probability that, with larger studies, a breakthrough is imminent. This viewpoint overlooks the key second part of paradigm instability that, as more genetic cancer susceptibility markers are investigated without a breakthrough, there is an increasing probability that the alternative tissue organization field theory is correct, particularly in light of the experimental evidence that supports the tissue organization field theory.

The current paradigm also tends to be reinforced, because the experimental tools used to investigate it may be increasingly excellent for investigating somatic mutations but suboptimal for investigating a new paradigm, which adds another hurdle to exploring new paradigms. Just as there is no such thing as theory-free investigation, there are no such things as theory-free tools of investigation. For example, if Map A points to sandy soil and Map B points to rocky soil, a wide shovel for digging at the location specified by Map A might be a poor tool for digging at the location specified by Map B. Similarly, if one is originally planning to take the bus, the absence of the right shoes makes walking less appealing. This reinforcement of the current paradigm by the nature of the experimental tools suggests that extra consideration needs to be given to investigating the new paradigm. Another advantage of considering a new paradigm is the opportunity, early in the investigation, to find new evidence that supports the new paradigm or that refutes the old: persons walking instead of riding the bus might either spot the bus nearby and call their coworkers to advise them to wait or might notice a road closure and call their coworkers to advise them to walk.

On the basis of our conclusion that research into early-stage carcinogenesis may be approaching a period of paradigm instability, we suggest that some of the additional funding for investigating cancer susceptibility markers be reallocated to the study of tissue organization field theory.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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