COMMENTARY

A Cancer Theory Kerfuffle Can Lead to New Lines of Research

Stuart G. Baker

Correspondence to: Stuart G. Baker, ScD, National Cancer Institute, 9609 Medical Center Drive, Bethesda, MD 20892-9789 (e-mail: sb16i@nih.gov).

Abstract

The standard viewpoint that cancer is a genetic disease is often stated as a fact rather than a theory. By not acknowledging that it is a theory, namely the Somatic Mutation Theory (SMT), researchers are limiting their progress. An attractive alternative to SMT is the tissue organization field theory (TOFT), which is summarized as “development gone awry.” To initiate a kerfuffle, I discuss the interpretation of various results under both TOFT and SMT, including recurrent mutations, hereditary cancers, induction of tumors in transgenic experiments, remission of tumors following the inhibition of enzymes activated by mutated genes, nongenotoxic carcinogens, denervation experiments, foreign-body carcinogenesis, transplantation experiments, and tumors with zero mutations. Thinking in terms of TOFT can spur new lines of research; examples are given related to the early detection of cancer.

Many papers and textbooks treat the standard viewpoint of cancer as a genetic disease as a fact rather than a theory. For example, a college biology textbook (1) writes, “Cancer results from genetic changes that affect cell cycle control.” An article discussing the search for driver mutations (2) starts with, “Cancer is driven by somatically acquired point mutations and chromosomal rearrangements thought to accumulate gradually over time.” An article taking an evolutionary biology view of cancer (3) says, “Cancer evolves by a reiterative process of clonal expansion, genetic diversification, and clonal selection.” None of these quotations leave any room for doubt about the genetic underpinnings of cancer.

Why is this important? When a theory is treated as a fact, it limits the possibilities for new research directions. One limitation is complacency. Peyton Rous (4), a cancer researcher awarded the Nobel Prize, wrote in 1959 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.” A second limitation is the lack of experiments that might challenge the status quo. The philosopher Paul Feyerabend (5,6) noted that, “Evidence that might refute a theory can often be unearthed only with the help of incompatible alternative.” A third limitation is lack of appreciation of paradoxes. The famous physicist Niels Bohr (7) remarked, “How wonderful that we have met with a paradox. Now we have some hope of making progress.” Once there is leap from fact to theory, new ways of thinking arise as discussed in Chamberlin’s 1897 theory of multiple hypotheses (8).

The conventional theory of carcinogenesis is called the Somatic Mutation Theory (SMT). An attractive alternative to SMT is the tissue organization field theory (TOFT) (9), which views cancer as development gone awry. Adding to a growing literature (9–20), I discuss a wide variety of experimental and observational results from the viewpoints of both SMT and TOFT. It is important not to fall into the logical trap of thinking that if a theory cannot explain everything it explains nothing. Both SMT and TOFT are not complete and each faces challenges. The key point is that a kerfuffle between SMT and TOFT can open new research directions.

Somatic Mutation Theory

SMT says that cancer starts with a mutation that gives cells a growth advantage, which leads to clonal expansion and successive mutations followed by clonal expansions. The premises of SMT are sometimes stated as: 1) cancer is derived from a single somatic cell that has accumulated multiple DNA mutations; 2) the default state of cell proliferation is quiescence; and (3)
cancer is a disease of cell proliferation caused by mutations in genes that control proliferation and the cell cycle (18). Under SMT, the adjacent tissue plays a supporting role, affecting the fitness of the clonal expansion (3) or recruiting the surrounding stromal cells to begin an interplay that enhances the neoplastic phenotype (21). Four corollaries of SMT are: 1) mutations are necessary for cancer to arise; 2) cancer takes a long time to develop (under the “classic” SMT view that mutations are rare events); 3) cancer only arises in tissue targeted by carcinogen; and 4) mutations needed for cancer do not disappear.

A brief historical background can provide perspective. SMT began in 1914 with Boveri’s theory linking chromosomal changes to cancer (22). In the 1950s researchers hypothesized that cancer involved successive mutations interspersed with clonal expansions (23–25). Based on the age-specific incidence of cancer, some researchers postulated that six or seven mutations are required for cancer development (23). Another theory was that two mutations interspersed by exponential cell growth were needed for cancer development (25). Key observational support for SMT came in 1960 with the strong association between the Philadelphia chromosome, a chromosome abnormality, and chronic myeloid leukemia (26). The oncogene theory that viral genes inserted into animal cells cause cancer (27) received a boost in 1976 with the discovery of a close similarity between genes in chickens and genes in the avian sarcoma virus (28). Additional support for SMT came in 1982 with experiments showing that introduction of DNA into normal cells could convert the normal cells to cancer cells (29–31). A standard classification of mutations thought to cause cancer was either as oncogenes, which cause a gain of function that leads to cancer, or tumor suppressor genes, which cause a loss in function that leads to cancer (32). Perhaps the high point of SMT occurred in 1990 with a genetic multistage model of colorectal tumorgenesis (33).

However, this tidy picture no longer holds. The reported number of mutations associated with tumors has increased dramatically. In various studies, investigators have reported cancers with 77 mutations per million base pairs of DNA (34), cancers with over 30 mutations (35), solid tumors averaging up to 66 mutations (36), and tumors with over 10000 mutations (37). In another study (38), only 6% of tumor mutations corresponded to six hallmarks of cancer (21), and 15% corresponded to no hallmarks of cancer. Perhaps the most widespread approach to making sense of the burgeoning number of mutations is to postulate that only a small number of driver mutations lead to cancer while the remaining passenger mutations play no causal role in carcinogenesis (34). Bioinformatics approaches try to find driver mutations focus on prioritizing the likelihood that a mutation is a driver (39); proving a mutation is a driver is much more difficult. Also, different bioinformatics methods yield different sets of driver mutations (40); yet, another complexity is that some mutations have both oncogenic and tumor suppressor roles, depending on the context. NOTCH1 is considered an oncogene in leukemia and a tumor suppressor in squamous cell cancer (41). MYC is usually considered an oncogene but sometimes has characteristics of a tumor suppressor gene (42). A further complexity is the recent discovery that some tumors are characterized by sudden catastrophic genetic changes (2), not the slow accumulation of mutations under the “classic” formulation of SMT. Noted cancer researcher Robert Weinberg (43) recently commented that, “Deep-sequencing analyses of tumors DNAs now indicate multiple, genetically distinct subpopulations whose representation seems to vary from one stage of tumor progression to another,” and concluded that, “The data we now generate overwhelm our abilities of interpretation,” and “We lack the conceptual paradigm and computational strategies for dealing with this complexity.”

Tissue Organization Field Theory (TOFT)

Tissue Organization Field Theory (TOFT) (9) says that cancer arises from the disruption of interactions with adjacent tissue, which can be mediated by intercellular chemical signals, mechanical forces, and bioelectric changes. Because these adjacent-tissue interactions are thought to play a role in embryonic development, an appropriate summary of TOFT is “development gone awry” (18). The premises of TOFT are that carcinogenesis represents a problem of tissue organization, comparable to organogenesis, and that proliferation is the default state of all cells (9,10). Three corollaries of TOFT are: 1) mutations are not needed for carcinogenesis; 2) cancer can arise in tissue where carcinogen has not been applied; and 3) genetic instability is a byproduct of carcinogenesis.

Although not strictly part of the original TOFT formulation, one additional hypothesis is that some mutations lead to cancer by disrupting morphostats (20). As discussed more later, two types of evidence support this hypothesis: 1) links between extracellular changes and mutations and 2) paradoxical results of transgenic experiments.

In contrast to the current complexity of SMT, there is an appealing simplicity to TOFT. TOFT involves only traits expressed in normal tissue under some circumstances (44). For example, rapid proliferation occurs following skin grafts (45), and invasiveness occurs in embryo implantation (46). Table 1 presents a summary of the differences between SMT and TOFT related to interpreting observational results.

Morphostats

Some investigators postulate morphostats as the chemical intercellular signal whose disruption leads to carcinogenesis (11,47–50). Morphostats are hypothesized signals that keep tissues organized despite a constantly changing environment (11). Similar to morphogens, which guide embryonic tissue development, the effect of morphostats on tissue organization is thought to depend on a concentration gradient (11). Various lines of evidence support the existence of morphostats. Some mammals regenerate tissues including deer antler (51), mouse paws (52), and the skin of African spiny mouse (53); such regeneration is likely to be under control of morphostats. Neural stem cells implanted in mammary gland stroma differentiate as mammary epithelial cells (54), a phenomenon consistent with the presence of a morphostat. Various proteins with a morphogenic role are candidate morphostats (11) because of evidence linking them with tissue maintenance. Wnt and TGF-β are involved in the regeneration of the colon crypt after injury (55). Wnt is associated with nail regeneration (56) and homeostasis of intestinal epithelium (57). Sonic hedgehog controls expression of epithelial differentiation in mouse stomach tissue and follows a gradient of expression levels in the human stomach that is consistent with a morphostat (49). Even p53, which is often discussed under SMT, is linked to both development and differentiation (58,59).

There is also evidence supporting a link between the disruption of morphostats and cancer. The proclivity of cancers to form at tissue junctions is simply and elegantly explained by intersecting morphostatic fields at these junctions, which can increase susceptibility to disruptions in any of these fields (11). Wnt is associated with brain cancer (60), colorectal cancer (61),
and breast cancer (62). TGF-β signals in fibroblasts affect carcinogenesis in adjacent epithelial tissue (63). Sonic hedgehog is associated with gastric cancer (64), and inhibition of the hedgehog pathway leads to the regression of tumors in mice (65).

Despite the suggestive evidence, the existence of morphostats has not been proven. A challenge is that morphostat proteins likely have multiple roles (50). Nevertheless, the current view of morphostats may be similar to the view of morphogens in the 1950s through the 1970s when morphogens were thought “too imprecise and simple” (66). Widespread acceptance of morphogens only came with their visualization in 1988 (66,67).

**Mechanical Forces**

Mechanical forces also play an important role in tissue development (68–73). Examples include stresses that buckle and fold tissue-forming villi in the gut (68), angular motion of cells that leads to spherical organization (69), and blood viscosity affecting the formation of blood vessels (70). There is growing evidence implicating defects in mechanical forces within the tumor (74).

**Bioelectric Changes**

Ion channels are pore-forming proteins that control the flow of ions through the cell membrane leading to a voltage difference between the cytoplasm and the extracellular environment, sometimes designated by the symbol $V_m$ (75). There is growing evidence implicating changes in $V_m$ with carcinogenesis, including associations between $V_m$ and cancer cell proliferation, migration, and differentiation, (75,76). Depolarization (less negative $V_m$) of tadpole cell membranes, regardless of the method of depolarization, led to a neoplasia-like phenotype in cells not depolarized (76,77). Further research is needed on cell membrane depolarization in mammals, long-range bioelectric effects, and spatial and time-varying $V_m$ gradients (76).

**Cancer Theory Kerfuffle**

A useful way to stimulate new types of thinking is to consider various observational or experimental results and discuss their interpretation under both SMT and TOFT.

**Recurrent Mutations**

A recurrent mutation is a mutation with a higher frequency in the tumor than expected by chance (39). The existence of recurrent mutations in tumors is certainly consistent with SMT. However, on a fundamental level, the identification of recurrent mutations only indicates association, not causation. This distinction is often not appreciated: a good example is the statement, “The high frequency of K-ras mutations and the observation that they mostly appear during early stages of tumor progression provide strong argument supporting a causative role of K-Ras in human tumorigenesis” (78). In other words, when researchers believe that oncogenes cause cancer, they are more likely to interpret association (between mutations and cancer) as evidence of causation. Under TOFT, recurrent mutations are byproducts of the disruption of intercellular communication. Support for the TOFT view comes from genetic instability in epithelial cells following alteration of the stroma by Strl (79) and genetic instability in unexposed bystander tissue adjacent to irradiated tissue (80).

**Tumor Clonality**

As a corollary of SMT, each tumor is a clone in which all cells contain the complete set of accumulated driver mutations. Contradicting this view, Nomura et al. (81) found evidence that metaplasia is polyclonal and Shah et al. (82) found multiple clonal frequencies in some tumors. An SMT proponent might argue that passenger mutations present the illusion of polyclonal tumors. Under TOFT, mutations are byproducts of carcinogenesis that could randomly cluster into multiple clones.

**Hereditary Cancer**

The existence of hereditary cancer would seem to be strong evidence for SMT. However, there are aspects that are paradoxical under SMT. For example, patients with xeroderma pigmentosa (XP), who have defects in DNA repair that greatly increase sensitivity to the sun and various mutagens, have elevated rates of skin cancer but normal rates of other cancers, despite the presence of the DNA repair defect in all cells (83). Additionally puzzling, patients with Cockayne syndrome, who have similar defects in DNA repair as XP patients and sun sensitivity, have normal rates of skin cancer (84). These results are consistent with a TOFT view that the mutations in XP patients, but not Cockayne syndrome patients, disrupt relevant morphostats or morphogens.

**Transgenic Experiments**

Transgenic experiments in which mutated genes inserted into animals lead to cancer also seem to strongly support SMT. Yet, even in this case, paradoxical results arise under SMT. For
example, Raaijmakers et al. (85) deleted the gene DICER1 in stromal bone progenitor cells and found no DICER1 deletion in the resulting acute myeologenous leukemia. This result is paradoxical under SMT, because the driver mutations should not disappear. Shachaf et al. (86) found that turning on the MYC oncogene in transgenic mice led to the development of liver cancer, but subsequently turning off the MYC oncogene led tumor cells to differentiate back to normal hepatocytes, despite the continued presence of genomic alterations. This result is paradoxical under SMT because the tumor regressed while mutations remained, although one might propose that mutations have a detrimental effect only when transcribed into protein. TOFT can readily explain these examples as instances of mutations disrupting morphostats.

Remission of Tumors Following the Inhibition of Enzymes Activated by Mutated Genes

The remission of chronic myeologenous leukemia because of Imatinib, an inhibitor of the enzyme Bcr–abl constitutively activated by the fusion gene BCR–ABL in the Philadelphia chromosome, clearly supports SMT (87). Under the SMT viewpoint, one would expect that, in cultures of marrow cells from patients with Philadelphia chromosome-positive chronic myeologenous leukemia, the Philadelphia chromosome–positive cells would clonally expand in competition with chromosomally normal cells; however, the Philadelphia chromosome–positive population rapidly disappeared while chromosomally normal cells remained (88). A possible TOFT explanation for the success of Imatinib is that the Bcr–abl enzyme disrupts a morphostat; this explanation is consistent with leukemia reemergence following discontinuation of Imatinib and the link between Bcr–abl and changes in the stroma (89,90). Also puzzling under SMT, inhibition of the BRAF enzyme, which is constitutively activated by the BCR–ABL mutation, leads to remission of melanoma tumors (91) but not to the remission colorectal cancers (92).

Foreign-Body Carcinogenesis

In the late 1930s, Turner (93) confirmed a serendipitous result that implanting a plastic disk under the skin of rats leads to the formation of a tumor. Subsequently researchers extensively investigated foreign-body carcinogenesis, noting that the shape, but not the composition, of the implanted material determined tumorgenesis (94). In a notable experiment, Karp et al. (95) subcutaneously inserted Millipore filters with various pore sizes into mice. Their results were striking (Table 2). For pore sizes equal to or smaller than 0.22 micrometers the incidence of tumors was very high, and for pore sizes equal or greater than 0.45 micrometers the incidence of tumors was zero. Karp et al. (95) postulated that tumors arose from disruption of diffusible molecules involved in cell communication. Later experiments suggested that differences in surface roughness associated with the pore size may explain these results (96,97). Both explanations fit squarely under TOFT. There is no obvious SMT explanation.

Nongenotoxic Carcinogens

A nongenotoxic carcinogen is a chemical that induces cancer without directly damaging DNA (98). Examples include chloroform and p-dichlorobenzene, which are thought to induce tumors by interfering with gap junctions (98), a phenomenon clearly related to TOFT. The existence of nongenotoxic carcinogenesis is difficult to explain under SMT.

Denervation Experiments

Various animal experiments have shown that surgical interruption of the nerve connection alters tumor growth and incidence, either as a promoter or a suppressor (99–102). An association between denervation and WNT signaling provides a possible TOFT explanation (99). There is no obvious SMT explanation.

Transplantation Experiments

In a notable experiment, Maffini et al. (103): 1) removed mammary epithelium from rats, 2) exposed the epithelium-free stromal fat pads to either no carcinogen or a carcinogen, 3) separately exposed the removed epithelium to either no carcinogen or carcinogen in culture conditions, and 4) reinserted the carcinogen-exposed epithelium into noncarcinogen-exposed stromal fat pads. They found that rats with stroma exposed to carcinogen had high tumor incidence regardless of whether or not the epithelial cells were exposed to the carcinogen. They also found that rats with stroma not exposed to carcinogen had zero tumors regardless of whether the epithelium was exposed or not to the carcinogen. See Table 3. These results are difficult to explain under SMT, which assumes cancer can only arise in tissue exposed to the carcinogen. However, TOFT offers a ready explanation in that cancer arises from the disruption of morphostats diffusing from the stroma to the epithelium.

Other transplantation experiments have also challenged SMT. Normal cells transplanted into heterologous tissues resulted in tumors (104–107). Tumor cells transplanted into normal tissue reverted to normal tissue (108–114). Human mammary cancer cells grown in a culture with albumin from unfertilized chicken eggs reverted to a normal branching process (115).

<table>
<thead>
<tr>
<th>Pore size, µm</th>
<th>0.025</th>
<th>0.05</th>
<th>0.10</th>
<th>0.22</th>
<th>0.45</th>
<th>0.80</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of tumors/No. of mice</td>
<td>11/11</td>
<td>6/10</td>
<td>8/10</td>
<td>0/9</td>
<td>0/9</td>
<td>0/9</td>
</tr>
</tbody>
</table>

Table 2. Results for Karp et al. experiment with Millipore filters (95)

<table>
<thead>
<tr>
<th>Exposure of stroma cells after removal of epithelial cells</th>
<th>Exposure of reinserted epithelial cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>No carcinogen</td>
<td>0/6</td>
</tr>
<tr>
<td>Carcinogen</td>
<td>10/13</td>
</tr>
<tr>
<td>Carcinogen</td>
<td>6/8</td>
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</tbody>
</table>

Table 3. Results of Maffini et al. experiment (103)
Spontaneous Regression

Investigators have observed spontaneous regression of tumor, namely tumors reverting to normal tissue in the absence of transplantation. Examples include stage IVS neuroblastoma that regressed without treatment to normal ganglion tissue (116), cancer-like structures induced in rabbit ears that revert to normal tissue (117), rat hepatic nodules replaced by fibrous tissue (118), and human intraductal cancer replaced by fibrous tissue (119). SMT does not allow for such spontaneous reversions, but under TOFT they can occur if morphostat communication returns to normal.

Zero Mutations

A serious and unappreciated challenge to SMT comes from recent sequencing studies in which zero mutations were reported in the DNA of some tumors. Greenman et al. (34) reported 73 out of 210 tumors with zero mutations in coding exons. In a supplemental table, Kan et al. (35) listed 64 out of 443 tumors with zero mutations based on mismatch repair detection excluding insertions and deletions. In a supplemental table, Lawrence et al. (37) listed 29 out of 3083 tumors with zero mutations in coding regions. In a supplemental table, Imler et al. (38) listed 11 out of 183 tumors with zero mutations based on massively parallel sequencing. Surprisingly, these articles either ignored or only slightly mentioned the zero mutations in some tumors. In contrast, a recent editorial prominently noted that two studies of ependymoma brain tumors found zero mutations in some subtypes (120). An SMT proponent could argue that the searches for tumor mutations were incomplete and that, with better sequencing, mutations would be found in tumors with zero reported mutations. Under TOFT, tumors can arise with zero mutations.

Markers for the Early Detection of Cancer

Under an SMT viewpoint, the future might involve collecting a tumor specimen from a patient, identifying the cancer-causing mutations, and implementing the appropriate treatment (121). Under a TOFT viewpoint, the future might involve monitoring a patient for stromal and epithelial biomarkers related to the disruption of tissue interactions and intervening when indicated to reverse the onset of carcinogenesis.

The search for biomarkers for the early detection of cancer should focus on stored specimens that are collected from individuals without symptoms of cancer who are screened by conventional modalities, often in the course of a long-term (eg, five-year) clinical trial (122). Based on considerations arising from a TOFT viewpoint, investigators should collect stored specimens from the stroma as well as from epithelium. At the completion of the trial, investigators compare the classification performance of markers in persons who developed a particular cancer and a random sample of healthy control patients. Because the incidence of a particular cancer in five years is low in asymptomatic persons, a good marker for the early detection of cancer needs good sensitivity at extremely high (almost 1) specificity (122). Often the search for an early detection marker begins with an evaluation of marker classification performance in specimens from persons with clinical cancer vs control patients without cancer (122). However, good classification performance based on specimens from persons with clinical cancers does not necessarily translate into good classification performance based on stored specimens from asymptomatic persons. For example, an SMT marker based on passenger mutations, incorrectly thought to be driver mutations, is unlikely to perform well in stored samples, even if it performs well in clinical samples. Use of a marker motivated by TOFT, as opposed to a marker motivated by SMT, may increase the likelihood of good classification performance in stored specimens following good classification performance in clinical specimens, because a TOFT marker may be detecting field changes in tissue communication pathways even before cancer arises. (On the other side of the coin, a good marker under TOFT for the early detection of cancer might perform poorly with clinical samples when the effect on early stages of cancer is no longer detectable; this should spur the initial use of stored samples for biomarker evaluation without first evaluating the biomarker in clinical samples.)

Two candidate markers for the early detection of cancer that are motivated by TOFT are desmin and voltage-gated sodium channels. The protein desmin is a marker for pericytes, which are cells that wrap around blood capillaries, communicate with endothelial cells, and contribute to the tumor neovascularature (123,124). In studying foreign body carcinogenesis, Johnson et al. (125) identified pericytes as the likely progenitors of tumors based on observations of pluripotentiality and subcellular morphology and results from a previous study (126) that ruled out a bone marrow origin. The expression of the gene for desmin contributed strongly to good discrimination between colorectal cancer and healthy tissue (127). Arentz et al. (128) found that desmin staining from clinically detected tumors and adjacent stroma cells yielded good discrimination between stage III tumors and stage I and II tumors, with (from their Figure 3) a sensitivity of 0.45 at a specificity of 1. Voltage-gated sodium channels are ion channels that control the flow of sodium through the plasma membrane. Diss et al. (129) measured the expression of voltage-gated sodium channels in prostate specimens from 17 patients with prostate cancer and five patients with benign prostatic hyperplasia. Based on their Figure 1A, the sensitivity was 0.88 at a specificity of 1. Thus, there is good motivation to study the classification performance of desmin and voltage-gated sodium channels in stored specimens.

Conclusions

The growing complexity of the cancer genomic landscape leads to a situation called paradigm instability (130). Paradigm instability is like waiting for a bus that is overdue and planning your strategy based on your view of the situation; either: 1) the bus is stuck in traffic and will arrive momentarily, so staying at the bus stop is best; or 2) the bus had a mechanical problem, so alternative travel plans should be pursued. Similarly, the complexity of cancer genomic landscape can suggest either: 1) more sequencing, bioinformatics, and systems biology research under the premise that a full SMT understanding is approaching, or 2) more investigations of the role of morphostats, mechanical forces, and bioelectric cues in carcinogenesis under the premise that a TOFT view is more relevant. However, unlike the bus example, it is possible to allocate resources to cover research under both SMT and TOFT viewpoints.

A cancer theory kerfuffle can open new lines of research by spurring critical thinking about SMT and TOFT. Importantly, a cancer theory kerfuffle should be based on the merits of the arguments without regard to prevailing opinion. As the noted twelfth century scholar Maimonides (131) wrote, “For what has been proved by a correct procedure gains nothing in truth if all scholars agree, and loses nothing if all the people on earth are of the opposite opinion.”
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