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The tissue organization field theory of cancer: A testable replacement for the somatic mutation theory

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The somatic mutation theory (SMT) of cancer has been and remains the prevalent theory attempting to explain how neoplasms arise and progress. This theory proposes that cancer is a clonal, cell-based disease, and implicitly assumes that *quiescence* is the default state of cells in multicellular organisms. The SMT has not been rigorously tested, and several lines of evidence raise questions that are not addressed by this theory. Herein, we propose experimental strategies that may validate the SMT. We also call attention to an alternative theory of carcinogenesis, the tissue organization field theory (TOFT), which posits that cancer is a tissue-based disease and that *proliferation* is the default state of all cells. Based on epistemological and experimental evidence, we argue that the TOFT compellingly explains carcinogenesis, while placing it within an evolutionarily relevant context.

Keywords:
cancer theories; carcinogenesis; mutations; proliferation

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Most serious of all the results of the somatic mutation hypothesis has been its effects on research workers. It acts like a tranquilizer on those who believe in it, and this at a time when every worker should feel goaded now and again by his ignorance of what cancer is. (Peyton Rous. Surmise and fact on the nature of cancer. Nature 183: 1357–1361, 1959.)

An important test for any new theory is whether it is able to explain what was not explained before, and whether it is able to establish new connections between theories... (Lev Ginzburg & Mark Colyvan. Ecological Orbits. Oxford University Press. 2004, p. 10)

For the last half century, the majority view about the origin of cancer, i.e. carcinogenesis, has centered almost exclusively on only one theory, the somatic mutation theory (SMT). This theory, first enunciated in 1914 by Theodor Boveri in his book entitled *The Origin of Malignant Tumors* [1] claimed that "the problem of tumors is a cell problem" and that cancer was due to "a certain permanent change in the chromatin complex" which, "without necessitating an external stimulus, forces the cell, as soon as it is mature, to divide again." Ever since, cancer has become increasingly considered as a problem of cell proliferation due to permanent changes in the "chromatin", a term that in Boveri's time was already known to contain the heritable material.

This inference gained credibility because of the increasingly prominent role acquired by genetic research through the 20th century. This was due to the identification of DNA as the genetic material by Avery et al. [2] and subsequently, to the description of the chemical structure of the DNA molecule in a series of three articles by Watson and Crick [3], Wilkins et al. [4], and Franklin and Gosling [5]. In the light of these discoveries, the vague "chromatin changes" proposed by Boveri in 1914 morphed into the view that cancer is due to DNA mutations, a widespread notion that has dominated cancer research in the last 50 years.

The SMT has evolved over the past century in different directions. After 1914, variants to the SMT have been proposed to resolve incompatibilities

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between the collected data and the expectations anticipated by novel theoretical alternatives [6, 7]. At its core, however, supporters of the SMT implicitly or explicitly accepted the premise that cancer is a cell-based disease. In hindsight, Boveri's original version of the SMT was in fact a significant departure from the conclusions reached during the second half of the 19th century by German pathologists who considered that cancer was a tissue-based disease [8]. This latter interpretation, as well as the competing SMT, has faced many challenges over the years due to the lack of an agreed comprehensive explanation of how cancer fits within biology at large. Faced with the complexity of human cancer, some have suggested that "...a new idea seems to be needed... to start to clarify what is going on during carcinogenesis" [9]. Based on this suggestion, an evaluation of the usefulness of the SMT and of alternative approaches to it is due.

Before exploring the subject in full, it ought to be acknowledged that there is a consensus that a distinction should be made about the types of cancers that appear in the clinic; there are "sporadic" cancers and hereditary ones. "Sporadic" cancers represent over 95% of the cancers in humans. On the other hand, inherited cancers (less than 5% of total cancers) are a discrete subclass, mediated by germline mutations that have a distinct natural history, mostly appearing in early childhood and/or young adults; they have been called *inherited inborn errors of development* [10]. While the DNA mutations in this latter type of cancers are present in all cells of the organism, tumors mostly appear in one or a few organs (retina, kidney, breast, skin, neural tissue, etc.). In this paper, we deal only with the "sporadic" cancers.

As in other instances in science, the *zeitgeist* has played a significant role in accepting untested claims of the SMT without major objections. In an exceptional, critical and cogent paper entitled "An attack on cytologism" by D. W. Smithers, published in The Lancet in 1962, the main objections to the cell-based view of carcinogenesis remain as relevant today as they were then [11]. Briefly, while concluding that cancer ought to be studied as a problem of organismal disorganization, Smithers remarked that "Progress in understanding, involving a change in belief, usually becomes possible only when current opinions have been exposed for so long in an untenable position that even a new outlook is a welcome relief." However, instead of the resolution envisaged by Smithers almost 50 years ago, the field is mending these very shortcomings by adding "supracellular", tissue-based components to a cell-based theory [12–15]. These additions without any rejection of theoretical baggage may lead to a premature synthesis whereby no room will be left for hypothesis testing, an endeavor centered on the ability to reject hypotheses.[1] Hence, we pose the question: are the competing current theories of carcinogenesis testable, and if so, how?

A cell-based or a tissue-based perspective in developmental and cancer biology?

According to the philosopher D. C. Dennett, "There is no such thing as philosophy-free science; there is only science whose philosophical baggage is taken on board without examination" [16]. Therefore, ignoring the philosophical underpinnings of the postulates adopted by researchers when designing experiments is bound to hinder the interpretation of the data collected. For this reason we address the difference between the "cell-based" and the "tissue-based" stances.

For those who favor a reductionist view in biology, the cell is the "unit" of the organism, and hence, explanations of observations gathered at the tissue level of organization should necessarily be found at the cellular level. However, the vast majority of phenomena observed during embryonic development are seldom explained when focusing efforts solely at the cell level. Take for example the development of the kidney; interactions between the ureteric bud and the metanephrogenic mesenchyme results in the reciprocal induction of the collecting system, derived from the ureteric bud, and of the nephron, derived from the metanephrogenic mesenchyme. A single cell isolated from either one of these tissues, in the absence of the other tissue, fails to originate the tissues that would result from their reciprocal interactions. Moreover, the shape of the nephron, like that of other anatomical structures, requires biomechanical forces, which are generated in and by tissues [17–19]. From this evidence, we conclude that interactions among different components of a tissue cannot be reduced to cellular events. The above-mentioned mechanical forces are emergent phenomena and exert downward causation (from the tissue level to the cell level). Thus, these tissue-based phenomena are inextricably linked to the three dimensions of space (topology) and by their developmental history (time) [20].

Premises of the somatic mutation and tissue organization field theories of cancer

The SMT explicitly assumes that molecular changes in the DNA of a founder cell will make this cell unable to control its proliferation and this, in turn, will result in the formation of a tumor [21, 22]. In part, this view of cancer evolved from the experimental dependence on homogeneous cell populations living in 2D cultures, which were considered as the model *par excellence* for the study of carcinogenesis (cell transformation). Molecular changes have been reported to vary from a single one to multiple mutations in the genome of the aforementioned original tumor cell [23–25] that would affect the control of cell

[1] The objectives of science as described by Ayala are, "(i) science seeks to organize knowledge in a systematic way, endeavoring patterns of relationship between phenomena and processes; (ii) science strives to provide explanations for the occurrence of events; and finally, (iii) science proposes explanatory hypotheses that must be testable, i.e. accessible to the possibility of rejection or falsification." Ayala FJ. 1968. Biology as an autonomous science. Am Sci 56: 207–221.

proliferation, be it directly, or indirectly through cell cycle effectors or through impaired cell differentiation [26–28]. Implicitly, the SMT also adopts the premise that, unlike in unicellular organisms, *quiescence* is the default state of cells in metazoa [29, 30]. In effect, such claims ignore the fundamental fact that cancer can only arise in metazoa in the context of complex and highly differentiated tissue structures.

Over a decade ago, we proposed a competing theory of carcinogenesis that adopts alternative premises to those of the SMT [31]. The first premise of the tissue organization field theory (TOFT) states that carcinogenesis takes place at the tissue level of biological organization, as does normal morphogenesis (see example above referring to kidney development). Chronic abnormal interactions between the mesenchyme/stroma and the parenchyma of a given morphogenic field[2] would be responsible for the appearance of a tumor (Fig. 1) [32, 33]. The second premise explicitly states that the default state of all cells is *proliferation* [32]. A corollary of the TOFT is that carcinogenesis is a reversible process, whereby normal tissues (or their components) in contact with neoplastic tissues may normalize the latter [34]. From this perspective, the only adequate experimental models for the study of carcinogenesis are multicellular organisms and 3D culture models containing parenchyma and stroma that resemble the natural architecture of tissues and organs.

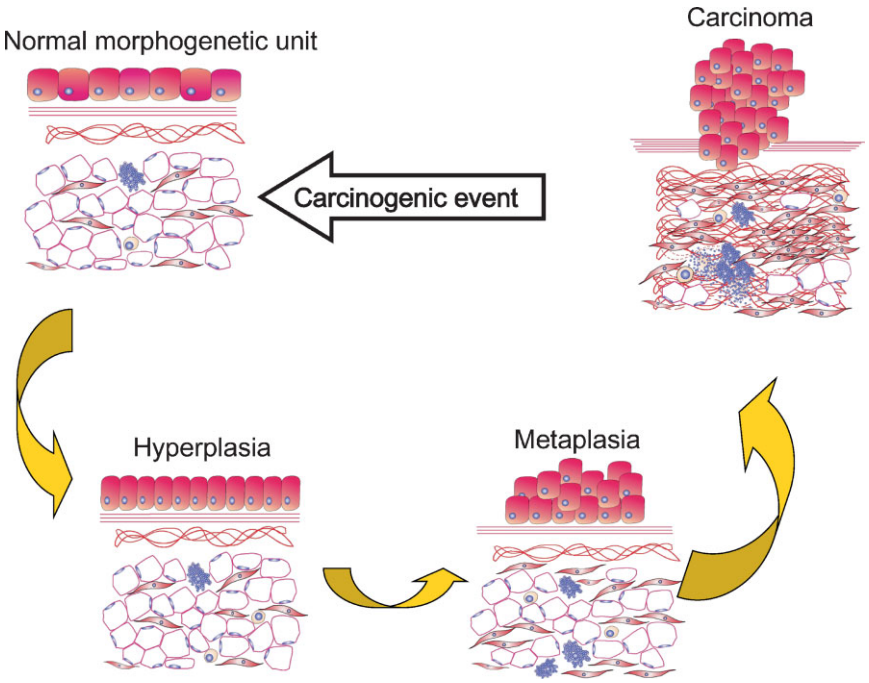

Figure 1. Carcinogenesis according to the TOFT. A single or multiple carcinogenic exposure acts disturbing the reciprocal biophysical and biomechanical communication between the parenchyma and the mesenchyme/stroma in a given morphogenic field. This results in miscues that manifest morphologically in both the stroma and the epithelium. The proliferation and motility restraints imposed by normal tissue architecture loosen and as a consequence, hyperplasia of the epithelium may occur. Further alteration of the reciprocal interactions between tissue compartments will induce metaplasia, dysplasia, and carcinoma. The stroma also may show alterations (desmoplasia, inflammatory cells).

Failings of the SMT

The current supporters of the SMT acknowledge that course corrections are necessary to accommodate conflicting data gathered "from within the SMT" with results generated from a tissue-based perspective showing that other cellular and extracellular components (e.g. matrix) are co-determinants of the neoplastic phenotype. In other words, the "renegade" mutated cancer cell is no longer sufficient to generate a neoplasm. One of those course corrections aimed at defending the SMT adduces that "...important new inroads will come from regarding tumors as complex tissues in which mutant cancer cells have conscripted and subverted normal cell types to serve as active collaborators in their neoplastic agenda..." [21]. Thus, the underlying premise of the SMT stating that cancer is a cell-based disease caused by DNA mutations remains at the core of the theory as referred to in current textbooks [22, 35] and in recently updated review articles on carcinogenesis [22, 36–40]. Additional changes were proposed, such as altered patterns of DNA methylation in one or more genes [41], or that cancer can be initiated by retrotransposon activation through changes in the transcriptional regulation of nearby genes [42]. The most extreme response has been the suggested relocation of mutations from the alleged epithelial founder cell to stromal cells [43]; this possibility has been rigorously tested and empirically ruled out [44, 45]. Because the SMT relies on DNA mutations in the founder cell that would become cancerous, in carcinomas this cell is an epithelial one[3]. Thus, while the core narrative of the SMT has remained intact, increasingly pointed criticisms due to the difficulties in fitting data within the premises of the theory are addressed by ad hoc additions or become labeled as "mysterious steps" [9]. More specifically, Brash and Cairns state: "The prime mystery in carcinogenesis remains the very first step, because it is hard to imagine how the numerous genetic changes found in cancer cells could have been produced in any cell as the result of a single exposure to a DNA-damaging agent, or why months or

[2] A morphogenic field is the collection of cells by whose interactions a particular organ or structure forms in the embryo (Gilbert, SF, "The re-discovery of the morphogenic fields, Developmental Biology On Line, http://8e.devbio.com/article.php?id=18).

[3] Carcinomas or their variants represent the overwhelming majority of clinical cancers (over 90%).

years should have to elapse before the effect of these changes is observed''. And they additionally remark: ''...the picture that emerges from the classical studies of the epidemiology of human cancers and of experimental carcinogenesis in animals is hard to reconcile with what has been learnt about mutagenesis in simple systems such as the bacteria. Initiation seems to be far too efficient to be simply mutagenesis of certain oncogenes and suppressor genes, and the subsequent time-dependent steps are even more obscure.''

In the classical version of the SMT, the ''renegade'' cancer cell (the ''seed'') is endowed with the ability to invade and form metastases in specific sites (fertile ''soil''). This conceptualization of metastases is challenged by the observation of multicelled clusters containing stromal cells from the primary tumor, stromal cells that significantly enhance the ability of the epithelial cells to metastasize. While no reference is made to the theory of carcinogenesis (SMT or TOFT?) being favored or rejected when interpreting the data collected [46], the evidence clearly points to the importance of the tissue structure in the efficiency of metastatic potential. Once again, cancers and their metastases belong to the tissue level of biological organization, which fits with the claims of the TOFT.

Efforts to identify cancer genes with the truly sophisticated technological advances made in the last few years are still fraught with uncertainties. For instance, the collected DNA sequence data comes from DNA extracted from tumors, i.e. a conglomerate of diverse, heterogeneous (epithelial, stroma, vascular) cells clearly not derived only from *the* single cancer cell that theoretically, according to the SMT, generated the tumor [22, 39]. Such DNA is then submitted to ''massively parallel'' sequencing. As mutations are identified, it would be necessary to ascertain which of them ''caused'' the cancer, an event that in humans is believed to have occurred one to several decades prior to the clinical diagnosis. This becomes a difficult, if not impossible task where there is no objective way to distinguish experimentally which mutations may have been causal (driver mutations) and which ones may have been irrelevant (passenger mutations), since the cancer has already developed [47]. An additional uncertainty is represented by the fact that the temporal sequence at which the driver mutations might have occurred in the founder cell is unknowable. Thus, the methodology used to distinguish between these two mutation types is based upon a series of unverifiable inferences.

The strategy of identifying mutations by large-scale DNA sequencing has been implemented in several studies. One, involving colon and breast cancer, estimated the presence of about 13 driver mutations per tumor. However, the mutated genes were different from tumor to tumor within a specific class (i.e. the breast) [48]. In another recently published study, the authors concluded that 49 founder mutations were present in the human pancreatic epithelial cell that originated the tumor of the patients studied [49]. It should be remembered that the SMT originally proposed a few causal oncogenes that were supposed to have ''dominant'' properties. The ad hoc alternative hypothesis aimed at resolving this lack of fit proposed that mutations might have affected different genes belonging to a common pathway. Another study aimed at searching for mutations in a likely target set of genes, namely protein kinase genes, sequenced the coding exons of 518 protein kinase genes in 210 diverse human cancers [47]. However, the distribution of mutations among tumors was quite uneven: i.e. 137 tumors showed DNA mutations, while 73 had none, and 20 of the 137 tumors with mutations had between 10 and 75 mutations each. Among them, 120 had been categorized as driver mutations. This number was much higher than expected. This ''lack of fit'' led the authors of the study to propose an even more massive sequencing effort because their finding ''implicate(d) a larger repertoire of cancer genes than previously anticipated.'' A critical evaluation of the ''state of the art'' technologies and their success in identifying causal cancer genes has been recently published [50]. These ad hoc changes to the core content of the SMT aimed at maintaining its currency have not ceased since its inception. As a corollary of all these studies, it is plausible to ask how many mutations are necessary to enable a normal cell to acquire the capability to proliferate autonomously?

An additional difference between the SMT and the TOFT is that the former claims that there are qualitative differences between cancer and normal individual cells, while the latter argues against this claim on pragmatic and theoretical grounds. Pragmatically, the vast research enterprise aimed at finding those differences can be summarized by stating that, so far, no qualitative differences have been described between a normal and a neoplastic cell. This assertion is now extended to the presence of somatic mutations [51] and aneuploidy in normal cells in vivo [52]. Theoretically, as already discussed, according to the TOFT, cancer is not a cell-based, but a tissue-based, phenomenon. Indeed, the lack of qualitative differences between cancer and normal cells was a central tenet of Virchow on cancer pathology. Given that the end-product of cell proliferation is similar in normal and neoplastic tissues (two daughter cells from a mother cell), Virchow declared that ''...there is no other kind of heterology in morbid structures than the abnormal manner in which they arise, and that this abnormity consists either in the production of a structure at a point where it has no business, or at a time when it ought not be produced, or to an extent which is at variance with the typical formation of the body. So then, to speak with greater precision, there is either a Heterotopia, an aberratio loci, or an aberratio temporis, a Heterochronia, or lastly, a mere variation in quantity, Heterometria'' [53, 54].

Altogether, the previous paragraphs make clear that the SMT has prevailed for a century without solid experimental data that would validate its currency. Given the protracted nature of the process of vindicating it, and from the perspective of a hardcore experimentalist, it is time to finally ''test the hypotheses''. How could this be done?

Testing the SMT

Based on the premise that mutations in specialized ''cancer genes'' (oncogenes and/or suppressor genes) in one somatic cell are at the root cause of cancer, a rigorous way to test the SMT is depicted in Fig. 2. The first step in this test should be to ascertain that the cell chosen as a

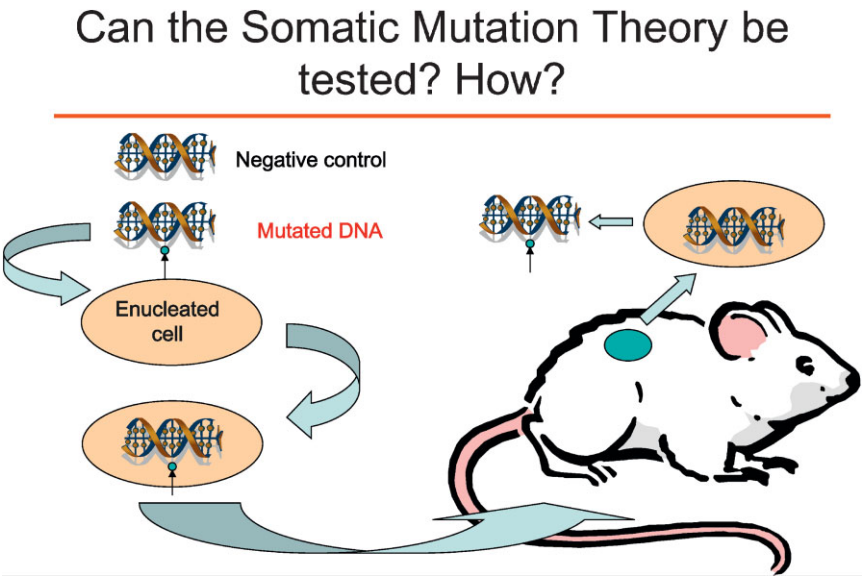

Figure 2. Testing the SMT. The DNA of one single cell needs to be extracted and its sequence checked to verify that no mutations are present. Next, the DNA should be mutated at specific sites, sequenced again to verify that only the intended mutations are present, and then introduced into an enucleated cell. After verification that the DNA organizes properly into chromatin, the cell should be transplanted into an immuno-tolerant mouse. This mouse should be checked to determine whether an organ-specific tumor arises and that all the cells in such a tumor carry the mutations originally introduced into the original founder cell's DNA.

founder cell originally carried the "wild-type" DNA. In order to verify this, the DNA of a "normal cell" should be purified and sequenced. Despite astonishing technological advances in molecular biology, pragmatically, this task cannot be performed without destroying the cell from which this molecule will be extracted. Nevertheless, assuming that this crucial goal could be successfully achieved now or in the future, the normal DNA of the selected cell should next be mutated at (a) precise site(s) in the candidate gene(s) claimed to cause cancer. Once these steps are done and the mutated locus (loci) is (are) identified, this DNA should be properly re-packed to regenerate chromatin in the native configuration of the donor cell, which then should be incorporated into an enucleated cell that would, hopefully, regenerate a nucleus that contains the mutated DNA. Again, the technical difficulties involved when following the proposed protocol, offer substantial obstacles at present. Additionally, the origin of the enucleated target cell would need to be unambiguously defined. This cytoplast should belong to an epithelial cell, say, e.g. a mammary ductal cell, or a hepatocyte or an enterocyte, etc. The next step in this effort should be to inject this putative cancer cell in the midst of any "normal" anatomical location/tissue of a syngeneic host that the proponents of the SMT would consider suitable. In this way, a cellular immunological reaction could be discarded as a cause of rejection, which would consequently prevent such a cell from proliferating and thus, forming a tumor mass. Next, the hoped-for tumor grown from the injected cell should show a phenotype traceable back to the tissue from which the cell was selected (i.e. hepatocellular carcinoma if the founder cell was a hepatocyte, mammary carcinoma in the case of a mammary epithelial ductal cell, and so forth). Finally, the protocol would require that all epithelial cells from the tumor carry the DNA mutation(s) induced in the cell originally selected for the test. This latter requirement would finally vindicate the notion that the hypothetically designated founder cell was the sole originator of the neoplasm.

Probably due to the technical challenges encountered in the stringent proposal outlined above, attempts have been made to test the SMT using genetically modified mice. In one of these models, all cells in the embryo carry the putative cancer-causing mutations, thus circumventing the obstacle of destroying the single/unique founder cell. An additional option would be the use of powerful molecular engineering techniques like conditional transgenesis and conditional knockouts that target specific tissues. In fact, in either case, the genetically modified cells would not be isolated among "wild-type" cells, but would be surrounded by equally genetically modified cells, which would configure a "field", the unit purported to be the target of carcinogens according to the TOFT. This possibility precludes testing the SMT because for over three decades it has been reproducibly shown that, through field effects, a putative cancer cell can be "normalized" by putting it in contact with "normal" tissues [55–58].

Testing the TOFT

Disturbed stroma/epithelium interactions are at the core of the TOFT. To our knowledge, the first report proposing the stroma as the target of a carcinogen based on experimental evidence was published by Orr [59]. Further, Orr concluded that the outcome of his experiments invalidated the SMT. Prodded by our analysis of his data we further explored this possibility.

When testing the TOFT, researchers are supposed to verify whether or not neoplasms having the characteristics of carcinomas emerge when disturbing the reciprocal interactions between the stroma and the epithelium. First, Barcellos-Hoff and Ravani irradiated the mammary gland stroma of mice to affect their extracellular matrix composition, cytokine production and receptors involved in cell-to-cell interactions [60]; next, they inoculated immortalized but non-tumorigenic mammary epithelial Comma-D cells into the cleared-fat pads of those mice. The inoculated Comma-D cells originated tumors mostly in the irradiated mice when compared with the non-irradiated mice[4].

[4] Comma-D cells contain two mutations in the p53 gene. If these mutations make these cells prone to neoplastic development as suggested by the SMT, it behooves to their proponents to interpret how mutated p53 induces carcinogenesis only when these epithelial cells are placed into an irradiated stoma.

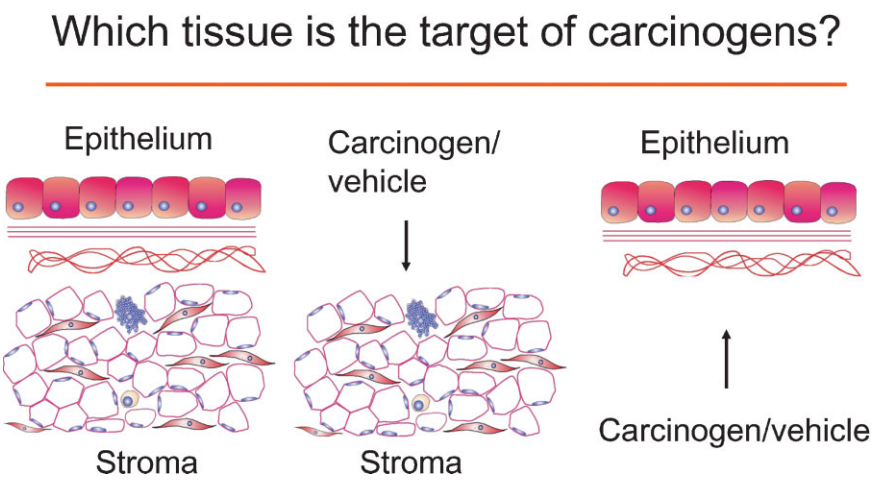

Figure 3. Testing the TOFT. The epithelium and stroma are detached from each other and separately exposed to a short-lived chemical carcinogen. After the carcinogen is thoroughly washed out, the epithelium and stroma are recombined in a 2 by 2 protocol (vehicle-exposed epithelium with vehicle-exposed stroma, vehicle exposed epithelium with carcinogen-exposed stroma, carcinogen-exposed epithelium with vehicle-exposed stroma, and carcinogen-exposed epithelium with carcinogen-exposed stroma).

Another way to explore this hypothesis is by exclusively exposing the stroma to the tissue-disrupting effects of carcinogens and observing whether carcinomas develop upon recombination with unexposed normal epithelial cells. Maffini et al. used the rapidly metabolized chemical carcinogen nitrosomethylurea (NMU) instead of ionizing radiation on rats. In these experiments, the recombination of exposed stroma with normal non-exposed epithelial cells resulted in carcinomas. The reverse combination did not (for further details see [61]). Comparable results were obtained when recombining human prostate stroma and a non-tumorigenic, epithelial cell line derived from tissue from a benign prostatic hyperplasia [62]. Subsequently, Maffini et al. reported the normalization of epithelial tumor cells isolated from NMU-induced carcinomas that reverted to form normal mammary gland ducts when injected into normal mammary gland stroma [58] (Fig. 3).

Sorting out outcomes

On the one hand, substantial technical obstacles hinder, so far, the testing of the SMT. On the other, objections have been made to conclusions drawn from experiments aimed at testing the TOFT. Both the Barcellos-Hoff/Ravani and the Maffini et al. protocols admittedly leave open the possibility that the respective carcinogens induced mutations in cells of the stroma. However, the TOFT is neutral about the role of mutations in stroma cells, while the SMT claims that the mutation(s) affecting genes that control cell proliferation occur in a single founder *epithelial* cell. Thus, there is no incompatibility between these considerations about mutations and the premises of the TOFT given that this theory considers cancer as a tissue-based and not a cell-based disease.

Of course, it would be difficult to rule out that mutations in the epithelial cells might have occurred either *before* or *after* carcinogenesis was initiated. In the first case, it is known that while normal cells already carry mutations [51], they do not end up becoming cancer cells. In the second, it might be argued that such mutations were a consequence and not a cause of the process, an eventuality already contemplated by Prehn over a decade ago [63]. From this perspective, these mutations would be irrelevant as suggested by the repeated instances where cells from diverse cancer types were normalized when placed within a normal field. Teratocarcinoma cells injected into blastocysts, hepatocellular carcinoma cells injected into normal livers, melanoma cells injected into zebra fish embryos, mammary gland carcinoma cells injected into normal mammary stroma, are just a few of the instances where the normalization of cancer cells was verified [57, 58, 64, 65] (See Box 1).

How do all these attempts to test the competing theories advance our knowledge of carcinogenesis and of its place in biology at large? The increasing need for adding qualitative changes (''course corrections'') to a theory characterizes what Lakatos described as a ''regressive'' research program, and contrasts with a ''progressive'' one whereby empirical data strengthens the framework of the theory [66]. In other words, to protect the core of the SMT (i.e. cancer as a cellular disease of the control of cell proliferation) new ad hoc components are added. This regressive quality is also evidenced by the relentless incorporation of elements of the competing, tissue-based theory into the SMT [33, 34, 67, 68]. No comparable problems have to date been found regarding the TOFT, which instead incorporates cancer within the realm of developmental biology while bringing along a paradigm change.

In 1962, Kuhn gave the word ''paradigm'' its contemporary meaning in the Preface of his influential book, *The Structure of Scientific Revolutions* [69], and defined it as ''a universally recognized scientific achievement(s) that for a time provide model problems and solutions to a community of practitioners.'' Switching premises regarding the default state of cells from *quiescence*, as adopted by the proponents of the SMT, to *proliferation*, as stated by the TOFT, qualifies as a paradigmatic change in both a narrow (limited to the field of carcinogenesis) and a broad sense because it proposes incorporating a novel evolutionary perspective into the field of carcinogenesis and in its relationship with that of biology at large.

Conclusions

We have explored theoretical and pragmatic possibilities to decide which of the two theories might be the most promising one regarding its power to articulate a coherent explanation of carcinogenesis. Direct validation of the SMT is problematic due to the technical diffi-

Box 1

Data that do not fit the somatic mutation theory of carcinogenesis

Spontaneous regression of neuroblastoma: This childhood neoplasm is probably the one with the highest documented rate of spontaneous regression [71]. The regressions of these cancers include even those classified as stage 4S, which metastasize in the liver, skin, and/or bone marrow. Cell and tissue differentiation and apoptosis are central to the regression process, which end up showing mature ganglion cells and Schwann cells [72]. This outcome contradicts the notion that ''malignant'' neoplasms are impervious to apoptotic signals and that unrestrained proliferation is a crucial, intrinsic feature embedded in the mutated DNA of cancerous cells. The changes in tissue architecture observed during regression of this tumor type are compatible with the TOFT.

Regression of hormone-sensitive tumors and their metastases: Breast and prostate tumors are not autonomous given that these tumors regress after gonadectomy or chemical hormonal ablation. Hormone antagonists (and in some case agonists such as diethylstilbestrol in the case of breast carcinomas) induce apoptosis and regression as well [73–75]. These features raise doubts about the premises of the SMT, whereby mutations in oncogenes and suppressor genes result in unrestrained cell proliferation.

Normalization by regulation of tissue architecture: Teratocarcinoma cells injected into blastocysts [55], embryonal carcinoma cells injected into the mammary gland [76], highly malignant melanoma cells injected into Zebra fish embryos [57], mammary carcinoma cells recombined with normal mammary gland stroma [58], hepatocarcinomas injected into normal liver [56], etc. are examples where cancerous cells reverse their ''malignant'' properties when placed among normal tissues.

Foreign body carcinogenesis: A number of inert substances (asbestos, plastics, etc.) embedded in tissues of susceptible animal hosts generate tumors locally [77]. These substances do not release genotoxic compounds that might mutate candidate genes (oncogenes, suppressor genes), which in turn may be somehow responsible for a tumor phenotype. What is observed, instead, are changes on tissue architecture, a result consistent with the TOFT.

Neoplastic induction by ectopic tissue transplantation: When early embryos are ectopically placed under the testicular capsule of adult animals they generate teratocarcinomas at the site of inoculation [78]. One of these teratocarcinomas was transplanted intraperitoneally for 8 years before used as a source of the embryonal carcinoma cells that when injected into normal blastocysts underwent normal differentiation in multiple tissues (see above).

Neoplastic induction of epithelial cells by altered stroma: Using a theory-neutral experimental strategy, Maffini et al. separately exposed mammary stroma and normal mammary epithelial cells to a rapidly metabolized chemical carcinogen and to vehicle, and recombined these tissues to test which combination resulted in mammary carcinomas. The recombination of carcinoma-exposed stroma with vehicle-exposed epithelium resulted in neoplasms [61]. The reverse combination did not. This observation suggests that the stroma, rather than individual cells in the epithelium, is the target of the carcinogen. These results are compatible with the TOFT and cannot be explained by the SMT. Similar outcomes were obtained recombining a quasi-normal, non-tumorigenic mammary epithelial cell line and irradiated stroma [60], and a non-tumorigenic prostate cell line and prostate cancer derived fibroblasts [62].

culties that must be overcome when one or more mutations are to be introduced experimentally into the genome of a single normal epithelial cell, while making sure that no additional alterations are inadvertently made. On the other hand, the plausibility of the TOFT has already been put to a test, and the data collected strongly supports the claim that whole tissues are the targets of carcinogens. This claim is further strengthened, though indirectly, by results obtained while modeling carcinogenesis in silico [70]. The ''normalization'' of tumor cells, a phenomenon consistently observed in various experimental models, is readily explained by the TOFT, but remains unexplained by the SMT. Thus, the need for a new outlook on carcinogenesis, predicated by Smithers [11] almost 50 years ago and recently called for by Brash and Cairns [9], is now sufficiently justified on both theoretical and pragmatic grounds. Thus, returning to the epigrams alluded to above, the TOFT and the empirical evidence in its favor collected so far ''. . .is (now) able to explain what was not explained before'', and ''it is (also) able to establish new connections between'' it and the premises derived from the Darwinian evolutionary theory and Waddington's developmental theory.

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