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Competing views on cancer

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Abstract

Despite intense research efforts that have provided enormous insight, cancer continues to be a poorly understood disease. There has been much debate over whether the cancerous state can be said to originate in a single cell or whether it is a reflection of aberrant behaviour on the part of a 'society of cells'. This article presents, in the form of a debate conducted among the authors, three views of how the problem might be addressed. We do not claim that the views exhaust all possibilities. These views are (a) the tissue organization field theory (TOFT) that is based on a breakdown of tissue organization involving many cells from different embryological layers, (b) the cancer stem cell (CSC) hypothesis that focuses on genetic and epigenetic changes that take place within single cells, and (c) the proposition that rewiring of the cell's protein interaction networks mediated by intrinsically disordered proteins (IDPs) drives the tumorigenic process. The views are based on different philosophical approaches. In detail, they differ on some points and agree on others. It is left to the reader to decide whether one approach to understanding cancer appears more promising than the other.

Keywords

Cancer; histogenesis; intrinsically disordered proteins; morphogenesis; protein interaction network; stroma-epithelium interaction; tissue organization field theory

1. Introduction

Many factors have contributed to cancer's status in the public mind as 'the emperor of all maladies' (Mukherjee 2010). Central to them is the supposedly inexorable nature of the disease and, often, the absence of a generally agreed pathogenesis behind its appearance. After a century of research on cancer, one would expect that much would have been learned given the generous funding earmarked to the study this disease. Instead, cancer continues to be a poorly understood disease to most cancer researchers, biologists and the public at large. As a result, all participants in the cancer debate have been increasingly concerned with the

possibility that something fundamentally important is being missed by the cancer research community when applying accepted notions to their research programs. Still, regardless of the epistemological value of its premises, research on cancer has generated knowledge in a host of related fields. Enormous insight on how cells proliferate, how cells regulate gene expression, how organelles and cellular structures operate in an integrated manner within cells, etc., has been acquired through studies of cancer, always under the premise that cancer is a cell-based disease. However, despite the incessant avalanche of data based on this perception, the cancer puzzle remains unsolved.

At the level of basic science too, there is often a sense of perplexity, even uneasiness, regarding how cancer fits within biology. One reason for this is the perceived mismatch between the single word description of the condition and the plethora of explanations that have been put forward for its existence. Among the latter, a distinction is commonly drawn between causal elements that are internal to the organism (e.g. spontaneous somatic mutations, stochastic fluctuations in metabolic fluxes, cell cycle regulation, etc.) and those that are external to it (e.g. carcinogens), analogous to the familiar nature/nurture distinction. Irrespective of which of the two is more important in a particular type of cancer, there has been much debate over whether the cancerous state can be said to originate in a single cell (or one or more genes or proteins within a cell) or whether it is a reflection of aberrant behaviour on the part of a 'society of cells' taken as a whole. Note that the alternative referred to in the previous sentence is *not* between 'gene' (or 'protein') and 'system' (or 'network'); it is, rather, between views that are 'cell-based' and 'tissue-based'. As one might guess from this, semantic issues have plagued discussions about cancer.

The maintenance or spread of a cancerous state is not the same as the origin of cancer (carcinogenesis), which is a distinct phenomenon. It is important that the terminology reflects this distinction. By referring to all three as 'cancer', one risks conflating issues whose bases are partly or wholly different. Also, it is not often appreciated that with regard to the origin of cancer it may be difficult to make an operational distinction between external and internal causes.

The merits of these ideas were discussed by Carlos Sonnenschein (Tissue Organization Field Theory), Annapoorni Rangarajan (Somatic Mutation Theory) and Prakash Kulkarni (Intrinsically Disordered Proteins Theory) at a meeting held in May 2012. The theories present rival hypotheses for the origin of cancer. The somatic mutation theory (SMT) posits a mutation in a single somatic cell as the first step. The tissue organization field theory (TOFT) is based on a breakdown of tissue organization involving many cells from different embryological layers (epithelium, mesenchyme). The intrinsically disordered proteins theory (IDPT) focuses on instability of the normal network of protein interactions, either spontaneous or triggered externally, and, to begin with, occurring in a single somatic cell. Following the initial trigger, all these theories postulate a cascade that progresses to full-blown cancer.

What follows portrays arguments that, in turn, favour or challenge each theory. Epistemological arguments as well as pragmatic, experimental evidence either favouring or rejecting the discrete theories are currently proposed. By doing so, the contributors to this

debate commit themselves to defend or attack the premises adopted by the competing options (which may be quite different). The debaters put before the reader testable hypotheses that can be used to clarify the issue further. As might be expected from their adopting different premises, they do not reach the same conclusions. The authors highlight the strength of their own case and raise questions regarding the tenability of others. It is precisely this disagreement that constructively informs the readership about which arguments carry more weight and may serve to reach closure to what in fact has been a century of unproductive exchanges without apparent resolution. The sooner a consensus is reached – and the consensus may well be that the phenomena do not lend themselves to a unitary explanation – the sooner the scientific and clinical cancer establishment may concentrate on what matters most to the societal community we all serve. We should celebrate the willingness of the debaters for sharing their competing views in the same venue.

In the spirit of the meeting that provided the motivation for bringing out this special issue of *Journal of Biosciences*, the reader is offered divergent viewpoints. In effect, it is left to the reader to judge the outcome. The conclusion highlights the salient features of the preceding three sections, each of which comes with an abstract or brief background in order to make it somewhat self-contained. It should be stressed that the sections that follow reflect the independent, and at times divergent, views of CS and AMS (section 2), AR (section 3) and PK (section 4).

2. Paradigmatic changes in carcinogenesis: What to keep and what to drop

Although study of the molecular biology of cancer has not yet laid bare the causes of most cancers or produced a cure, it has enormously increased our understanding of the molecular biology of the mammalian cells.

– John Cairns, *Matters of LIFE and DEATH*, 1997

The somatic mutation theory (SMT) of carcinogenesis has dictated the research agenda in this topic for over half a century. An alternative theory on the same subject, the tissue organization field theory (TOFT), was proposed in 1999. While the former is centered at the cellular level of biological organization, whereby cancer would arise from a single cell that underwent mutations in genes that control its proliferation, the latter posits that cancer is a problem of tissue organization akin to development gone awry. These theories also sharply differ on their premises and on epistemological grounds: while the SMT considers that proliferative quiescence is the default state of cells in metazoa, the TOFT acknowledges that proliferation and motility are the default state of *all* cells. Additionally, the SMT adheres to a structure of biological determination based on the concept of information, a search of causality at the molecular level, and to bottom-up reductionism. This way of thinking has hindered the study of biological organization. The TOFT, instead, adheres to an organicist view whereby there is bottom-up, top-down and reciprocal causality. Accordingly, biological objects, endowed with agency and autonomy, are already full of ‘causes’, and thus, molecules do not play a privileged causal role as proposed by a reductionist agenda. Molecules, including nucleic acids, would then represent just one of the many constraints, as do physical constraints that jointly determine biological organization. The lack of fit

between the theoretical core of SMT and experimental results showing the central role of tissue organization in carcinogenesis is being addressed by SMT followers with *ad hoc* explanations aimed at amalgamating these irreconcilable theories. Acceptance of TOFT and its premises will have profound consequences in biology and society.

2.1 Background

Over the last century and a half, dozens of theories of carcinogenesis have been proposed. They fall into two main categories, namely, (a) cell-based and (b) tissue-based theories. The former consider that cancers originate in a cell that has irreversibly changed (mutated) usually at the genomic level but that other cell components have also been proposed to have changed. These components include somatic DNA mutations, mitochondria, the plasma membrane, metabolic pathways, cell cycle pathways, cyto-architecture, cyto-'differentiation', etc.; changes in one of these cell components or processes did not rule out that more than one component was involved in carcinogenesis. As it is mentioned below, none of these theories is concerned with the default proliferative state of the original mutated cell. Because of lack of success in explaining carcinogenesis by these cell-based theories, some researchers have proposed to merge them with a tissue-based theory in what can be considered as a 'theory of compromise' or a 'hybridized theory of carcinogenesis'. The only tissue-based theory of carcinogenesis is the TOFT that in addition to claiming that cancer is a tissue-based disease posits that proliferation and motility are the default state of all cells (figure 1). We expand on these topics in the text below.

The text of the epigram that appears at the beginning of our article can be considered a truism. It is therefore relevant to ask, why is it that the resolution of the many cell-centered and molecular phenomena that were expected to explain carcinogenesis have fallen short in resolving the cancer puzzle? In itself, this widely acknowledged lack of explanatory success represents a clear indictment on the notion that cancer is a cell-based, molecular disease. Moreover, the increasingly sophisticated understanding of intracellular processes deepens the puzzlement over the lack of resolution of this topic. Notwithstanding, for the last half a century and even today, most efforts dedicated to explain carcinogenesis have been based on the SMT or its cell-based variants (see sections 3 and 4).

The acknowledged originator of the SMT was the famed German biologist Theodor Boveri, a zoologist who authored a book in 1914 entitled *Zur Frage der Entstehung Maligner Tumoren*; this book was originally translated into English in 1929, by his wife Marcella, under the title *On the Origin of Malignant Tumors*. In the initial pages of his book, Boveri acknowledged that he himself had not conducted experiments in the area of carcinogenesis (Boveri 1929). Instead, he based his narrative on bibliographic searches and experiments he conducted in the area of cell division during early development in sea urchins. Another English translation and annotated version of Boveri's book was authored by Sir Henry Harris, Regius Professor of Medicine Emeritus at Oxford University, under the title *Concerning the Origin of Malignant Tumors* (Harris 2007); in it, Sir Henry dismisses the notion that cancer may be considered a tissue-based disease.

In the original version of SMT, Boveri proposed that a normal cell would become a cancerous one as a result of rearrangements taking place in its nuclear chromatin. During the

first half of the 20th century, this cell-based interpretation of carcinogenesis competed on an equal footing with others which, instead, posited that cancer was a tissue-based phenomenon (Sonnenschein and Soto 2013). This competition persisted until the momentous descriptions in 1953 by Franklin and Gosling (1953), Watson and Crick (1953), and Wilkins *et al.* (1953) of the structure of the DNA molecule, which brought about the molecular biology revolution. Almost two decades later, SMT was unambiguously adopted as the paradigm of record in cancer research; despite attempts to challenge its hegemony, it still holds this status today.

Toward the end of last century, a series of developments in the fields of control of cell proliferation and in developmental biology exposed some of the shortcomings of SMT and highlighted novel evolutionarily relevant premises that helped explain some aspects of carcinogenesis not addressed by this theory. Indeed, in *The Society of Cells*, we exposed both epistemological arguments and experimental data that offered a plausible alternative explanation for the process of carcinogenesis (Taylor and Francis, London, UK, 1999). Such explanation is based on two fundamental premises. The first one posits that *proliferation* and *motility* are the default state of *all* cells and the second, that *tissues* are the target of carcinogens.

The first premise of SMT explicitly claims that cancer is a *cell-based* disease, whereby an alleged normal cell over time accumulates mutations that affect the control of its proliferation, thereby becoming the original cancer cell. The second premise of the SMT considers that the default state of cells in metazoans is *quiescence* (Varmus and Weinberg 1993; Alberts *et al.* 1994; Alberts 2010); this premise is rarely referred to, if ever, in articles and reviews by those who side with the SMT. Nonetheless, it is carried implicitly in methodological protocols that have been designed to explore the worthiness of this theory (Hanahan and Weinberg 2000, 2011; Jiao *et al.* 2012).

In order to remedy the shortcomings of SMT, a number of *ad hoc* variants of this theory have been offered while retaining unaltered the notion that cancer is a cell-based disease (Nowell 1976). Among these variants, the cancer stem cell (CSC) one has recently received considerable attention (Esteller 2008; Kalari and Pfeifer 2010; Portela and Esteller 2010; Marusyk and Polyak 2013; Timp and Feinberg 2013); this has been so despite arguments that claim that the definition of such cells as stem cells is based on mostly operational basis. In the parlance of Conrad Waddington, the biologist who originally introduced this term, epigenesis was meant as the temporal and spatial control of embryonic development that is not directly due to changes in the DNA sequence of cells involved in this process. Now, in the context of SMT, epigenetic alterations are, so far, limited to DNA methylation and histone modifications. Implicitly, the supporting argument of this SMT variant claims that the plasticity of the tumorigenic process would justify the conversion of non-stem cancer cells into cancer stem cells and vice versa (see section 3).

Still another cell-based variant of the SMT postulates that tumorigenic events are driven by intrinsically disordered proteins (IDPs) (see section 4). Since most IDPs exhibit considerable conformational and stochastic flexibility through numerous network iterations, the system would select outputs that impact increased 'fitness'. Thus, by exploring the network search

space, IDPs would rewire protein interaction networks to activate previously masked options. The resulting output would drive ‘cellular transformation’ and enable the cancer cell to ‘assimilate’ unmasked options facilitating it to adapt to perturbed environments while guiding its evolution. Given that several oncogenes, epigenetic modifiers and chromatin remodelers are IDPs, this variant theory would propose an increased expression of IDPs in cancer as an add-on to the SMT.

2.2 What's wrong, or right, about merging theories?

An undesirable consequence of the merging of theories of carcinogenesis involving the SMT and the incessant generation of its *ad hoc* variants has been the inability of scientists to fulfil one of the crucial explanatory purposes of theories; that is, as described by the geneticist Francisco Ayala, scientific theories should provide opportunities to be tested and, if falsified, to be discarded (Ayala 1968). More specifically, the merging of the SMT as the theory of record plus the extemporaneous add-ons (epigenesis, stroma-epithelial interactions, CSCs, gene overexpression, etc.) preclude ruling out, or in, which component of the merged theory ought to be discarded. As a result, the co-existence of contradictory components in a theory preclude weeding out which is the one that is half-right or half-wrong.

In 1914, Boveri was concerned with whether his theory of cancer was testable. Up to the present the SMT has remained untested by those who abide by it; despite aggressive, generously funded efforts to definitively vindicate the SMT since the advent of the molecular biology revolution, they have fallen short of the target. The record shows, instead, that evaluation of its merit is based on correlations and inferences (Bizzarri *et al.* 2011; Soto and Sonnenschein 2011; Baker 2012; Sonnenschein and Soto 2013). Doubtless, exploration of the SMT under the aegis of the molecular biology revolution has had an enormous impact on biology in general and genetics in particular; this validates John Cairns insightful comment quoted in the epigram of this article. For a more detailed account of historical aspects of cancer theories and their impact in biology see Rather (1978) Sonnenschein and Soto (1999), Pitot and Loeb (2002) and Stuart Baker (2012).

2.3 Alternatives to SMT and its premises

The biological, epistemological and heuristic importance of the notion that *proliferation* and *motility* are the default state of all cells has been overlooked or just ignored by biologists and cancer researchers in particular (Hanahan and Weinberg 2000; Hanahan and Weinberg 2011; Vaux 2011), including the current contributors in this piece (see below). *Proliferation* is unambiguously acknowledged to be the default state of prokaryotes, unicellular eukaryotes and plants; from an evolutionary perspective, it stands to reason to extend this same status to metazoa (Luria 1975; Steward *et al.* 1958). Moreover, to the best of our bibliographic search, we found no empirical data that would, instead, buttress the claim that *quiescence* is the default state of cells in metazoa (Sonnenschein and Soto 1999; Soto and Sonnenschein 2011; Deves and Bourrat 2012; Parr 2012). A closer analysis of the proposition that the default state of cells in metazoa is *quiescence* would suggest that (a) the accepted default state of *proliferation* in unicellular organisms mysteriously changed with the advent of multicellularity. No epistemological or empirical precedent to justify such a

switch has been proposed in the literature consulted. Nevertheless, biology textbooks at all levels of instruction implicitly or explicitly claim that cells in metazoa should be directly stimulated in order to proliferate (Alberts *et al.* 2002, 2008; Biggs 2003). Additionally, (b) if the *cell* from which life evolved indeed needed a nudge from something (a ‘growth factor’?), or someone, in order to enter the cell cycle, it would come perilously close to acknowledging a form of creationism (intelligent design?). We wonder whether researchers who side with the SMT or its variants would associate themselves with this implication.

The *ad hoc* course-corrections proposed for the SMT are predicated on several at times separable, and/or overlapping, notions. The most popular are that (a) epithelial mutated cancer cells recruit their neighbouring stroma for the initiation and progression of the tumour (Hanahan and Weinberg 2000, 2011), and/or (b) metabolic pathways (Griffin and Kauppinen 2007; Hanahan and Weinberg 2011), (c) mutations in fibroblasts that become ‘activated’ and synergistically affect epithelial cells that become neoplastic (Bhowmick *et al.* 2004) and (d) immunological events (Hanahan and Weinberg 2011; Coussens *et al.* 2013). According to their proponents these are crucial participants in the multifactorial processes of cancer initiation and progression.

2.4 Can the options to explain carcinogenesis be sorted out?

We devised an experimental protocol that would simultaneously test the TOFT and the SMT while using a theory-neutral approach. Thus, we separately exposed stroma and parenchyma to a carcinogen or to vehicle prior to their recombination. These experiments revealed that the stroma was the target of the carcinogen because tumours developed in tissue recombinants where only the stroma was exposed (for details, see Maffini *et al.* 2004). Using a different rationale and methodology, others reached a comparable conclusion (Barcellos-Hoff and Ravani 2000; Nguyen *et al.* 2011). Thus, we and others concluded that TOFT more closely explains the cancer phenotype when compared with SMT (Maffini *et al.* 2004; Soto and Sonnenschein 2011; Bizzarri *et al.* 2013; Sonnenschein and Soto 2013). Additionally, cells isolated from a cancer may become ‘normalized’ when placed in the midst of the normal tissue of origin, an outcome compatible with the TOFT and incompatible with the SMT (Mintz and Illmensee 1975; Maffini *et al.* 2005; Baker and Kramer 2007; Hendrix *et al.* 2007; Baker *et al.* 2009; Bussard *et al.* 2010; Booth *et al.* 2011), or within a morphogenetic field (Bizzarri *et al.* 2011).

And, what about the somatic mutations present in cells belonging to tumours? When Rich Prehn wrote ‘Cancers beget mutations *versus* mutations beget cancers’ (Prehn 1994), he meant to offer to his readership a choice on whether mutations played either a causal role in carcinogenesis (‘mutations beget cancer’) or that they were merely a consequence of carcinogenesis (‘cancer begets mutations’). In our view, the latter alternative is an accurate reflection of the relevance of somatic mutations in carcinogenesis.

At the request of an anonymous reviewer of our current submission, we will now address Drs Medina and Kittrell’s comments on the conclusions Barcellos-Hoff and Ravani (Barcellos-Hoff and Ravani 2000) and we (Maffini *et al.* 2004) draw on the role of the stroma in carcinogenesis (Medina and Kittrell 2005). Differences regarding our respective rodent models, experimental designs and the carcinogen used (DMBA vs radiation vs NMU)

may have been responsible for the contrasting results. Their alternative explanation of carcinogenesis when resulting from recombining carcinogen-exposed stroma with vehicle-exposed epithelial cells is that the latter cells could have mutated. This eventuality could be ruled out in our experiments because we also tested a recombinant of NMU-exposed epithelial cells with vehicle-treated stroma, and here found no neoplastic development. While we used normal rat epithelial cells, Medina and Kittrell acknowledged using ‘transplantable immortalized, preneoplastic mammary outgrowth line TM10’ epithelial cells that, when implanted into a naïve mammary stroma, developed tumours; hence, they were testing whether a particular experimental condition significantly shortened the mean latency period. Additionally, the effect of carcinogen exposure exclusively on the epithelial cells was not tested, nor was addressed as the key issue of whether epithelial cells are necessary targets of the carcinogen. Also, when epithelial cells were jointly exposed to carcinogen while in contact with their stroma and were transplanted into naïve fat pads, tumour latency was not statistically different from that of naïve controls. Moreover, Medina and Kittrell’s experiments applied DMBA to the whole tissue (epithelium plus stroma); this only accelerated the appearance of tumours; thus, their claim that carcinogen treatment of the epithelium is essential for carcinogenesis appears as unwarranted. In addition, Medina and Kittrell found no effect of the stroma on carcinogenesis, a result clearly in contrast to that by Barcellos-Hoff and Ravani. Medina and Kittrell attributed this discrepancy to experimental differences (epithelial cell lines, carcinogenic agent), as they did regarding ours. Recently, Dr Medina co-signed a paper with Dr Barcellos-Hoff and others (Nguyen *et al.* 2011) that refined the methodology and analysis described in the 2000 Barcellos-Hoff and Ravani paper which concluded that the stroma is a crucial target of carcinogens. Still, Nguyen *et al.* did not rule out that genomic mutations may play a role in rodent mammary carcinogenesis.

2.5 The cancer wars: Results and hopes

A criticism levelled at the TOFT has been that it does not provide a ‘molecular understanding’ of the disease, an argument that reflects a reductionist approach to biology adopted by these critics (see section 3). Indeed, the TOFT aims at explaining carcinogenesis and metastases at the tissue level of biological organization, i.e. the level at which these events take place, where cancers are diagnosed and ‘cures’ may be ascertained. On the other hand, the point has been made abundantly clear even by those favouring the SMT that they themselves have not fared much better at providing such a long-sought mechanistic understanding (Hanahan and Weinberg 2000, 2011; Vogelstein *et al.* 2013). Moreover, studies using the latest powerful technology have concluded that the different genomic profiles in cancer cells do not correlate well with specific clinicopathological behaviour (Kreso *et al.* 2013; Marusyk and Polyak 2013). Parenthetically, an integrated genomic, transcriptomic and proteomic characterization of 373 endometrial carcinomas using array- and sequencing-based technologies revealed that endometrioid tumours belonging to hypermutated and ultramutated groups correlated positively with groups that had a rather better prognosis (Cancer Genome Atlas Research Network *et al.* 2013). Most disturbing for the stated goal of reaching a ‘mechanistic (molecular) understanding of cancer’, a novel sophisticated data-mining model has revealed that the alleged ‘driver mutations’ described so far appear to be unreliable representatives of the mutations that, according to the SMT, are responsible for human cancer phenotypes in primary and metastatic tumours (Lawrence

et al. 2013). Additionally, the disconnect between the linear, reductionist approach of the SMT and its lack of clinical-biological relevance is exemplified by the phenotype of the human RAS/MAKP syndromes, involving Costello's, Noonan's, LEOPARD's and CSF syndromes, which represent complex phenotypic alterations that are seldom linked to neoplasia (Aoki *et al.* 2008). Comparable inconsistent conclusions have been drawn from efforts to link somatic mutations to diverse cancer phenotypes of the sporadic variety of cancers and also in Down's syndrome patients (Satgé 2013). As several reports have pointed out, the return on investment for these concentrated efforts explored under a reductionist strategy has been characterized by committed supporters of the SMT as either modest (Hahn and Weinberg 2002; Vogelstein and Kinzler 2012; Institute of Medicine 2013; Fisher *et al.* 2013) or meagre (Quaranta and Tyson 2013).

And, if the success of a theory is measured by the eventual benefits derived from its acceptance (for instance, the adoption of novel, effective therapeutic approaches) and for the application of its principles to illuminate related issues, the SMT can be summarily qualified as mostly a failure. The consensus being slowly reached in this regard would justify considering going back to the metaphoric 'drawing board'. Instead, in a recurrent denial attitude, it is now reported that it is not the faulty rationale used by the scientific community for a century to interpret the genesis of tumours (SMT and variants) but 'the redundancy and remarkable complexity of cellular signaling pathways' which should be blamed for the failure of oncogene-targeted drugs. Thus, according to this view, the solution is not to radically revise the theoretical and epistemological reasons for the failure but 'to improve therapeutic strategies and outcomes in cancer patients by using systems analysis and computational modelling approaches' (Quaranta and Tyson 2013). While acknowledging past failures in predicting future success in this area of research, we favour the thought that this type of analysis and modelling approaches may have a much better chance to be effective in improving treatments when based on evolutionarily relevant premises like the ones adopted by the TOFT (Soto *et al.* 2011; Soto and Sonnenschein 2012).

Again, our severe judgement on the SMT does not invalidate claims that much has been learned in the process of exploring those cell-based mechanisms that carcinogens were supposed to affect in order to finally produce a neoplasm. The 'collateral benefits' stemming from expanded knowledge of intracellular phenomena (DNA replication, transcription, translation, organelle function, signal transduction, etc.) and the spectacular technological advances generated by genetic engineering are indeed welcome. Remarkably, however, the single-minded concentration on the intracellular level as dictated by the SMT has been rewarded with neither the highly touted 'mechanistic understanding' of the disease nor the translational benefits of 'cures'.

2.6 Carcinogenesis in the midst of science

Science has a rather long record of accomplishments and failures. Historians of science have explored this record and drawn conclusions that have been debated extensively (Fleck 1981; Kuhn 2012). As in most endeavours where research funds are distributed to explore any given subject, evaluators of applications usually reward applications that abide by the 'consensus'. Alternative solutions to a puzzle are usually either ignored or plainly rejected,

especially when they represent a serious challenge to the ‘law of the land’. This critical assessment remains valid despite genuine intentions by funding agencies claiming that they are willing and ready to fund ‘out-of-the-box’ approaches to explain, and eventually ‘cure’, cancer. These intentions are systematically neutralized in the process of choosing experts to participate in review panels, since those ‘most qualified’ are for the most part supporters of the *status quo* (Huang 2013; Soto and Sonnenschein 2013).

2.7 Conclusions

On what grounds is a switch from SMT to TOFT plausible and desirable? A radical paradigmatic switch of the magnitude we are proposing will necessarily apply to all levels of biological organization, which includes the cellular, the organismic and even the societal levels. This interpretation resonates with Charles Darwin’s view on the exponential nature of reproduction as when he remarked, ‘...There is no exception to the rule that every organic being naturally increases at so high a rate, that, if not destroyed, the earth would soon be covered by the progeny of a single pair’ (Darwin 1909). It is worth recalling that this passage reflects Darwin’s concern with Malthus’ remarks on human overpopulation. A final resolution of the controversy regarding the default state of *all* cells may not be imminent but when achieved, it may represent a true paradigmatic change of the magnitude contemplated by Thomas Kuhn in his highly influential 1962 book *The Structure of Scientific Revolutions* (Kuhn 2012).

Finally, on the basis of epistemological and theoretical arguments and of pragmatic experimental evidence, promoting the merits of the SMT is becoming unsustainable. To the contrary, for over a decade now, comparable arguments and evidence make the TOFT not only a plausible alternative to explain carcinogenesis but the bases for a radical reinterpretation of biological phenomena at all levels of biological organization (Soto *et al.* 2008; Saetzler *et al.* 2011; Longo *et al.* 2012).

3. Stem cells and cancer

Increased understanding of stem cell biology over the past two decades has uncovered several similarities between cancer cells and normal stem cells, leading to the notion that cancers may arise due to the accumulation of mutations within normal, tissue-resident stem cells. Irrespective of the origin of cancer, a fundamental question that has remained unaddressed in cancer biology is that once manifested, are all cells of a cancer functionally equivalent? Interestingly, recent research has identified a subset of cells within several cancers, termed as cancer stem cells (CSCs), which appear to selectively possess tumour-initiating properties. In addition, the CSCs have been found to be inherently drug resistant, and hence predicted to contribute to cancer aggressiveness and relapse. Thus, unlike current chemotherapy that targets the bulk of the cancer leading to reduction in tumour size, but perhaps leaves behind the CSCs unscathed, the CSC hypothesis posits that targeting CSCs is likely to offer a better and more durable treatment for cancer.

3.1 Background

Before embarking on discussing the role of stem cells in the process of carcinogenesis, I will present a more neutral viewpoint to the hotly debated theories that explain cancer initiation. The previous section criticizes the SMT model in several places and questions the relevance of the experimental evidences that supports it as mere inferences. In spite of that, it is difficult to gauge from it the main divide between the SMT and the TOFT models of cancer. In simple terms, the SMT posits that external cancer-causing agents (environment, chemicals, radiations, carcinogens) enter the cell and damage the cellular DNA, leading to the generation of mutations, which, through subsequent rounds of cell proliferation and clonal selection, drives the process of carcinogenesis (figure 2a). Thus, gene mutation is the primary cause of cancer. Indeed, several cancer-associated gene mutations have been identified, some grouped as drivers that are directly involved with cancer initiation and others as passengers, which may be bystanders –perhaps a consequence of the process of cancer progression itself (Stratton *et al.* 2009). Importantly, mice engineered to harbour several oncogenic driver mutations develop cancer, lending further support to the mutation-driven cancer model. These observations have led to the generation of several transgenic mouse models of cancer harbouring cancer-associated mutations (both gain of function and loss of function) (Van Dyke and Jacks 2002) with the aim to understand cancer initiation and progression. Nevertheless, narrowing the differences between the biology of mice and humans has remained a challenge (Rangarajan and Weinberg 2003). More recently, efforts are underway to sequence the entire genome of cancer patients (Vogelstein *et al.* 2013), with the aim and hope to obtain a better understanding of the contribution of various gene mutations to the process of carcinogenesis.

The TOFT model, on the other hand, posits that cancer-causing agents affect the underlying mesenchyme/stroma, leading to the disruption of cell–cell and cell–extracellular matrix communications. These altered organizations at the tissue level are predicted to lead to cancers in the parenchyma (the epithelium) (figure 2b). Further, under the TOFT model, mutations may be a result of the altered cellular communications, which may not contribute to the development of cancer. Thus, mutations may be a consequence of the process of carcinogenesis; although it is accepted within the TOFT that some mutations, such as inherited mutations associated with familial cancers, may contribute to carcinogenesis by disrupting cell–cell communications. So, the focus in the TOFT, right from the beginning, has been on the disruption of tissue architecture as a whole. However, the molecular events that lead to alterations in the stroma, the nature of these alterations, and why alterations that affect the stroma predominantly lead to epithelial, and not stromal, cancers remains unexplained.

Although the SMT does not altogether neglect the importance of the surrounding stroma to the process of carcinogenesis, it predicts that the growing tumour alters its neighbouring normal stroma (figure 2a), thus leading to a reactive stroma, which results in the remodelling of the tissue architecture surrounding the tumour (Bissell and Radisky 2001). Thus, the emphasis in the SMT is on tumour-mediated alterations to the normal neighbouring stroma, while the TOFT predicts that alterations in the stroma precede cancer initiation in the epithelium.

In one study undertaken to directly address whether carcinogens affect the epithelia or the stroma to initiate carcinogenesis, the proponents of the TOFT model performed rat mammary gland transplantation experiments (Maffini *et al.* 2004). Rats with cleared fat pads (containing mammary stroma devoid of mammary epithelium) and *in vitro* cultured mammary epithelial cells were independently exposed to the carcinogen NMU (figure 3). Either carcinogen- or vehicle-treated mammary epithelial cells were transplanted into the cleared fat pads of rats exposed to carcinogen or vehicle. These experiments led them to uncover that mammary epithelial cells (carcinogen-treated or not) generated tumours only when the stroma was exposed to carcinogen. In contrast, carcinogen-treated mammary epithelial cells failed to initiate tumours in the background of an intact stroma (Maffini *et al.* 2004). However, a very similar set of mammary gland transplantation experiments performed in mice failed to reproduce these results (Medina and Kittrell 2005). In this latter study, only when the mammary epithelial cells were exposed to carcinogen (DMBU) did tumours arise, but not when the stroma alone was exposed to carcinogen, thus supporting the SMT model. These opposing results could be due to differences between the biology of rat and mice, or between the nature of carcinogens used, or due to pre-existing alterations present in the epithelial cells prior to transplantation into the host; it suffices to point out that the TOFT model may be applicable, albeit, to a subset of tumours, and cannot replace the SMT. Further, even though several experiments have highlighted the role of altered stromal microenvironment in tumour formation when starting with ‘pre-initiated’ epithelial cells (cells already harbouring pro-tumorigenic genetic mutations) (Olumi *et al.* 1999; Barcellos-Hoff and Ravani 2000; Mueller and Fusenig 2004; Nguyen *et al.* 2011), conclusive experiments to demonstrate that altered stroma suffices to *initiate* cancer in *normal* epithelial cells are still lacking. Thus, although based on limited experimental evidences, the TOFT is emerging to challenge the widely accepted SMT model.

In addition to gene mutations that have remained the mainstay of the SMT, recent studies have highlighted a role for non-genetic or ‘epigenetic’ alteration of gene expression in tumour initiation and progression. Promoter methylation, histone modifications, miRNAs, chromatin remodelling complex proteins, and the more recently identified intrinsically disordered proteins (IDPs) (see the following section) can all lead to alterations in protein levels without genetic mutations and contribute to tumorigenesis. Indeed, the importance of these mechanisms in the generation of intra-tumoral heterogeneity, in switching cell-fates during the process of epithelial-mesenchymal transitions associated with metastasis, in generating epithelial cell plasticity, and in determining the states of drug resistance and stemness, are increasingly being recognized within the SMT framework (Baylin 2011; Scheel and Weinberg 2011; Munoz *et al.* 2012; Suva *et al.* 2013; Tam and Weinberg 2013). In contrast, the proponents of the TOFT fail altogether to provide molecular insights into cancer progression, and leave it, by and large, to the imagination of the readers.

So far as the rift concerning the default state of the mitotic potential of metazoan cells is concerned, it is difficult to understand the logic extended by the TOFT and the criticism(s) hurled against the SMT. The proliferation of most normal cells *in vivo* is ‘limited’ owing to the confines of the tissue environment; when removed from such confines, and placed in tissue culture plates, most normal metazoan cells restore their proliferation. Within the tissue

culture environment when cells reach their boundaries and become confluent, they once again stop proliferation, which can be rekindled yet again by sub-culturing the cells in 1:3 or 1:4 ratio. This process can continue for several generations until the cells reach a proliferative block termed as replicative senescence (or the Hayflick limit) owing to the erosion of telomeric ends and shortening of chromosomal ends with each cell division (Harley *et al.* 1994). On the other hand, cancer-causing mutations result in ‘unlimited’ proliferation of cells. Thus, in the tissue culture environment, such mutation-bearing cells often divide even when they reach confluence, without due respect to the confines or boundaries. So the apparent quiescence of normal cells is re-kindled when the cell is provided with appropriate environments, but within limits; however, cancer-causing mutations lead to proliferation without limits.

It is well recognized that cancer is not a single disease, but a group of diseases, and given that two cancers, even within the same patient, can look and behave differently, it is possible that both these theories are valid in their own rights and may apply to different contexts. Similarly, the aneuploidy theory of cancer or the speciation theory of cancer (Duesberg *et al.* 2006), which predicts that cancers arise as a result of changes in the number of chromosomes (aneuploidy), may be applicable to certain contexts. Thus, instead of a ‘one size fits all’ approach, a ‘multiple roads lead to Rome’ approach may be better suited in understanding this complex disease. Perhaps the debate on how cancer arises is useful to the academician, but to the clinician and to the cancer patients, what matters is that once cancer is manifested (which is when most cancers are detected), how does one treat it? Importantly, how does one tackle the problems of drug resistance and cancer relapse? The emerging concept of CSCs attempts to bridge this gap. Dubbed as a variant or modern ‘avatar’ of the SMT, the stem cell theory of cancer highlights the importance of functional heterogeneity within cancers, and challenges the current chemotherapeutic practice of shrinking the tumour as the yardstick to test the efficacy of an anticancer agent.

3.2 Stem cell hypothesis of cancer

Recent advents in the field of stem cell biology (Reya *et al.* 2001; Pardal *et al.* 2003; Cho and Clarke 2008) have led to the emergence of the ‘stem cell hypothesis of cancer’. The hypothesis comprises two features: (i) the possibility of normal stem cells as the ‘cell-of-origin of cancer’, and (ii) the pre-existence of a rare and distinct subpopulation of cells within cancers (termed as the CSCs) that fuels cancer growth, and is likely responsible for drug resistance and cancer relapse.

3.2.1 Stem cell origin of cancer—Several tissues in our body present a hierarchical organization of cells (Marshman *et al.* 2002; Chao *et al.* 2008; Makarem *et al.* 2013) – the tissue-resident *adult stem cells* divide and generate more of their own kind (via self-renewal), and also generate committed cells (*progenitors*) that after few rounds of proliferation undergo terminal differentiation to give rise to the *differentiated* cells of the tissue (figure 4a). Of these various cells within a tissue, which could be the cell-of-origin of cancer that ends up accumulating the various genetic alterations proposed to be required to culminate in a successful tumour?

Differentiated cells of the epithelial tissues lack proliferative potential, and hence it is hard to envisage the accumulation and propagation of mutations within these cells. Further, the turnover time of the differentiated epithelial cells within the tissue is short, ranging from few days to weeks, and these cells get quickly replenished, and thus do not allow enough time for the accumulation of 4–8 genetic perturbations that are predicted to generate a full-blown cancer. Progenitors are the committed cells within a tissue that proliferate rapidly, but briefly, before undergoing terminal differentiation and losing their proliferative potential. Thus, the narrow time window in which the progenitors proliferate may not be sufficient yet again to accumulate multiple mutations. In contrast, tissue-resident stem cells stay within the epithelial compartments for a long time, and have long-term proliferative potential, and thus present as attractive candidates in which mutations can accumulate and propagate. In addition, in recent years, several similarities have been identified between normal stem cells and cancer cells (Pardal *et al.* 2003), including extensive proliferative potential, telomerase activity, similar expression of cell surface markers, increased expression of ABC family of transporters, etc. Together, these have led to the suggestion that cancers may arise in tissue-resident stem cells (Martinez-Climent *et al.* 2006). Indeed some studies have indicated a stem cell origin for haematological malignancies like AML (Bonnet and Dick 1997) and CML (Pasternak *et al.* 1998; Perez-Losada *et al.* 2001). More recently, elegant genetic-tracking studies in mice have revealed a stem cell origin for gut tumours (Schepers *et al.* 2012), thus further supporting the notion of stem cell origin for cancers. However, this topic still remains hotly contested in the context of human solid cancers.

If indeed normal stem cells possess several properties hitherto associated with cancer cells, then is it possible that they require fewer genetic alterations to becoming a successful tumour than their differentiated counterparts. Indeed, work from our laboratory has shown that *in vitro* transformation of normal human mammary epithelial cells (HMECs) growing in suspension as mammospheres (that are enriched in stem/progenitor cells) required fewer genetic elements for transformation (Paranjape *et al.* 2012) compared to those growing in adherent conditions (Elenbaas *et al.* 2001) that favour differentiation.

Together, these arguments and studies strongly propose a stem cell origin for cancers. Does this mean cancers cannot arise in other cell types? Mutations and/or epigenetic alterations that can nudge the progenitors/differentiated cells back in lineage, enabling them to re-acquire properties of self-renewal and long-term proliferation, can surely contribute to tumorigenesis. Thus, the nature of cells within a tissue in which the cancer originates may determine the heterogeneity, the duration it takes to become a full-blown cancer, the severity of the cancer, and its response to drugs.

3.2.2 Concept of cancer stem cells—The widely accepted clonal theory of the origin of cancer postulates that all the progeny of a cancer are descendents of a parental cell that has acquired the most beneficial mutations to spawn a successful cancer. A fundamental question that still remains to be addressed is that within a full-blown cancer, are all cells equally tumorigenic?

Two assays routinely used to assess tumorigenicity involve (a) seeding cancerous cells *in vitro* in soft agar that prevents attachment to the substratum and thus measure anchorage-

independent growth of cancer cells – a fundamental property of solid tumours, and (b) injection of human cancer cells subcutaneously into the flanks of immunocompromised mice and assess tumour formation (also called xenotransplantation assays). It has been found that less than 1% of cells derived from solid tumours generate anchorage-independent, 3D colonies when seeded in soft agar (Reya *et al.* 2001). Further, in xenograft mice experiments, it takes injection of millions of cancer-derived cells to generate a tumour of 1 cc over a period of 1–2 months. What is apparent from both these observations is that only a few cells of a cancer appear to contribute to tumorigenicity when assayed in either of these assays. The ‘stochastic model’ explains this conundrum by suggesting that all cells of a cancer possess tumorigenic properties; however, the specific cells that contribute to the final outcome is determined by chance or stochastic mechanisms, much akin to several choices made during the course of normal development. In contrast, the ‘hierarchy model’ posits that to begin with there is a functional heterogeneity within the cancer cells, that some cells, and perhaps very few, are endowed with the potential to generate tumours, while others lack this property (Reya *et al.* 2001). However, both models remained experimentally untested for a long time.

In the past decade, a small subset of cells has been identified within several cancers that alone seem to possess the ability to generate tumours in xenotransplantation assays, thus lending support to the hierarchy model. First identified in leukaemia (Lapidot *et al.* 1994), such tumour-initiating cells have now been uncovered in several solid tumours including brain, breast, skin and colon cancers (Visvader and Lindeman 2008). In contrast to earlier studies that required the injection of millions of cells for tumour formation, injection of as few as hundreds of these tumour-initiating cells sufficed for tumour formation in xenotransplantation experiments, while injection of a vast majority of the ‘bulk’ or rest of the cells failed to do so, supporting the notion that only few cells to begin with possess tumorigenic properties. Many of these tumour-initiating cells have been identified based on their cell-surface marker expression. For example, in the context of human breast cancers, only the subpopulation of CD44^{high}/CD24^{low} cells had tumour initiating properties when injected into immunocompromised SCID mice, whereas the other three combinations (CD44^{high}/CD24^{high}, CD44^{low}/CD24^{high} or CD44^{low}/CD24^{low}) failed to do so (Al-Hajj *et al.* 2003). Thus, several cancers possess a subset of cells that alone carry in them the potential to generate a new tumour. Further, this sub-population of tumour-initiating (and thus, tumorigenic) cells carried in them the potential to generate non-tumorigenic cells (i.e. ‘differentiate’), as well as these could be serially xenotransplanted (long-term replication potential) – properties that go hand in hand with stem cells. Together, these properties soon gained the name ‘cancer stem cells’. So, just like normal tissue wherein stem cells occupy the tip of the hierarchy, and replenish old, worn-out cells thus maintaining normal tissue homeostasis, CSCs appear to occupy the tip of the cancer iceberg (figure 4b) and help in the maintenance and propagation of cancer (Dalerba *et al.* 2007). The terms ‘tumour-initiating cells’, ‘tumorigenic cells’, and ‘CSCs’ all refer to the subset of cells within a full-blown cancer that possess the potential to re-initiate a new tumour, and should not be confused with the term cell-of-origin of cancer, which refers to the ‘normal’ cell within which cancers may arise.

Metastasis, the spread of cancer cells from one organ to another, requires cancer cells to traverse through the blood or lymphatics, colonize and re-initiate a tumour at a distant site. If only the CSCs possess the ability to initiate a new tumour, then cancer metastasis must be mediated by a subset of CSCs; such cells have been termed as migratory or circulating CSCs (Brabletz *et al.* 2005; Baccelli and Trumpp 2012). Indeed, such metastasis-initiating circulating tumour cells with cancer stem cell properties have recently been identified from the blood of patients having luminal breast cancer as EPCAM⁺CD44⁺CD47⁺MET⁺ (Baccelli *et al.* 2013). Interestingly, this study showed that CD47 expression was detected mainly in the circulating tumour cells and metastases, and only rarely in the luminal non-metastatic primary breast tumours, suggesting that the expression of CD47, which serves as a 'don't eat me signal' to overcome immune evasion (Chao *et al.* 2012), may be triggered during the initiation of tumour cell dissemination into the circulation. Thus, metastasis-initiating cells may be a subset of CSCs within the circulating tumour cells.

Further, cancer cells undergoing epithelial-to-mesenchymal transition, which involves the shedding of epithelial characters and acquisition of mesenchymal characters, and which is considered by many to be a prerequisite for solid tumour metastasis, have been shown to acquire stemness properties (Mani *et al.* 2008). Indeed, mere over-expression of EMT-inducing transcription factors such as Twist or Snail sufficed to increase the stemness features of immortalized epithelial cells, suggesting that the process of EMT involves the acquisition of stemness properties. Interestingly, stem cells overexpress ABC transporters that have been implicated in multidrug resistance due to their ability to efflux a variety of chemotherapeutic drugs. In tune with this, CSCs have been shown to overexpress ABC transporters, which may be one possible mechanism for their intrinsic drug resistance (Dean *et al.* 2005; Alison *et al.* 2012). Indeed, this very property of increased expression of ABC transporters has been exploited to isolate stem cells (both normal and cancer) based on the efflux of small molecules such as Hoechst whence the stem cells present themselves as Hoechst low side population (Goodell *et al.* 1996; Setoguchi *et al.* 2004). Further, studies from our laboratory have shown that the very transcription factors that trigger an EMT can also directly bind to the promoters of ABC transporters and increase their expression (Saxena *et al.* 2011). Taken together, these various observations reveal an intricate relationship between stemness and EMT on the one hand, and cancer aggressiveness and drug resistance on the other, explaining why cancers with higher number of CSCs exhibit aggressiveness, drug resistance, and correlate with poor prognosis.

3.3 Cancer stem cells and therapeutic implications

The notion that not all cells, but only a small subset of cancer cells, are critical for the maintenance, progression, and initiation of new cancers has immense therapeutic implications. It has brought about a paradigm shift in the design of anticancer therapeutics. For years chemotherapy has aimed at reducing the cancer burden. Is it possible that this approach successfully kills the bulk of the cancer cells, thus shrinking the cancer, but leaves behind the more dangerous cancer stem cells, which then lead to cancer relapse? Indeed, *in vitro* experiments have demonstrated that cancer stem cells are resistant to commonly used anticancer drugs such as doxorubicin, paclitaxol, etc. Further, cancer patients treated with chemotherapy have revealed an enrichment of the CSC population (Alison *et al.* 2012),

suggesting that to begin with, cancers may possess a subset of cells that fail to be curtailed by the present day chemotherapy which targets the bulk of the cancer. Thus, the CSC hypothesis predicts that newer generations of anticancer drugs must target the CSCs if cancer were to be effectively controlled.

3.4 Challenges, controversies, and what lies ahead

Although the stem cell hypotheses of cancer has changed our ways of thinking about cancer initiation, progression, and treatment, several questions still need to be addressed. Can adult stem cells accumulate mutations, given the prediction of the immortal strand theory that stem cells retain both the parental DNA strands? If the CSCs bear resemblance to the normal stem cells, then can CSCs be targeted without affecting the normal stem cells of the body? Is the subpopulation of CSCs within cancers rigid or interconvertible? Indeed, recent studies have shown that non-CSCs can get converted into CSCs when given the appropriate environment (Chaffer *et al.* 2011; Gupta *et al.* 2011). Are CSCs within cancers rare or plenty? While most studies have demonstrated that CSCs are few within cancers, at least one study demonstrated that given a different host environment, CSCs are plenty (Quintana *et al.* 2008). Are the xenotransplantation assays used to identify CSCs valid, or do they impose a selection on the nature of cells that can survive in the chosen host animal (Fillmore and Kuperwasser 2007)? Will the elimination of one sub-population of cancer lead to the emergence/surfacing of another, more resistant and more aggressive one? Indeed functional heterogeneity has been observed among CSCs with not all of them initiating tumours with similar kinetics (Alison *et al.* 2012).

Thus, are CSCs a reality or a myth, evasive or illusive, an entity or a state, or a misnomer (Zipori 2004; Gupta *et al.* 2009; Maenhaut *et al.* 2010; Welte *et al.* 2010; Li and Laterra 2012)? A lot needs to be resolved. Addressing these questions in the future will determine whether the newer understanding brought forth by recent research will one day benefit cancer patients.

4. Intrinsically disordered proteins, evolution and cancer

The difficulty lies not in the new ideas but in escaping from the old ones.

– John Maynard Keynes

4.1 Background

Cancer may be envisioned as a rebellious act of a cell against the organism from which it is derived. However, what guides the cell's decision to rebel, how it acquires the rebellious characteristics, and how it negotiates the fitness landscape during its evolution are not fully understood. Perhaps, one explanation for the slow pace of progress in addressing cancer is the assumption that cancer is deterministic, in that, there is a causal relation between the genotype and phenotype. In fact, it is widely held that cancer is a genetic disease and that *all* cancers arise as a result of changes that have occurred in the genome of cancer cells (Vogelstein and Kinzler 2004; Stratton *et al.* 2009). Indeed, such changes are believed to 'mark the genome, such that a cancer's life history appears to be encrypted in the somatic mutations present in the cancer cell' (Nik-Zainal *et al.* 2012). This assumption that

underpins the SMT also implies that the individual clones of cancer cells evolve independently from each other, acquiring the hallmarks necessary for tumorigenesis. However, other possibilities such as evolution of cooperation among cancer cells (Axelrod *et al.* 2006) have not been explored seriously, and several unarticulated paradoxes exist (Mintz and Illmensee 1975; Illmensee and Mintz 1976; Georgiades *et al.* 1999; Sole and Deisboeck 2004; Tang *et al.* 2004; Mrozek *et al.* 2007; Pineau *et al.* 2007; McKenna and Roberts 2009; Sharma *et al.* 2010; Allegrucci *et al.* 2011) that are difficult to reconcile with this deterministic viewpoint.

Perhaps, it would be convenient to have in mind an actual example of the kind of difficulty we are dealing with, and a single case would clarify the difficulty. Thus, per conventional wisdom, cancer cells are so encumbered with mutations that there is no way to return them to normalcy. However, a landmark paper by Felsher and coworkers (Shachaf *et al.* 2004) startled the cancer world by demonstrating that indeed, cancer cells can be reformed. By conditionally turning on the oncogene Myc in mice hepatocytes, the authors induced liver cancer in these animals. They then turned off Myc expression and much to their surprise, and to the surprise of others, Myc inactivation resulted *en masse* in tumour cells differentiating into hepatocytes and biliary cells forming bile duct structures. This was accompanied by a rapid loss of expression of the tumour marker α -fetoprotein and increase in expression of liver cell markers such as cytokeratin 8 and carcinoembryonic antigen, demonstrating how oncogene inactivation may reverse tumorigenesis in the most clinically difficult cancers! However, dialling up Myc expression once again resulted in the same cells redeveloping cancer. Remarkably, however, the genomic alterations that had occurred in the cancer cells overproducing Myc remained unchanged when the Myc-expressing cells cycled between the cancerous and 'normal' states! Clearly, new thinking is required to reconcile the seemingly opposing views on how cancer is initiated, how it evolves, and how it switches phenotypes (e.g. transition from epithelial to mesenchymal, EMT, or from a drug-sensitive to a drug-resistant state).

4.2 Hypothesis

Living systems (such as cells, organisms, and ecosystems), and many non-living systems in the universe (for example, stars and galaxies), are self-organizing systems that exhibit nonlinear dynamics (Kaneko 2006). Self-organization is a process where some form of global order arises out of the local interactions between the components of an initially disordered system. Such systems are non-deterministic and open systems that exist far from equilibrium.

Individual molecules in a cell and individual cells in the system interact and self-organize to form an ensemble of complex interactive parts with emergent properties whose behaviour is neither obvious nor predictable on the basis of the behaviour of the individual parts. The emergence of the observed macroscopic behaviour of such an ensemble depends on the type and strength of the interactions among the constituent cells and their response to extrinsic perturbations leading to different types of synchronized emergent dynamics. In this viewpoint, we postulate that macroscopic behaviour of the system such as state/phenotype switching (for example, malignant transformation), and evolution, result from rewiring of

protein interaction networks (PINs) driven by intrinsically disordered proteins (IDPs) in the individual cells of the ensemble.

IDPs are proteins that lack rigid 3D structures either along their entire length or in localized regions at least under physiological conditions *in vitro* (Uversky and Dunker 2010). Despite the lack of structure, however, IDPs play important biological roles especially in transcriptional regulation and signalling (Uversky and Dunker 2010). Studies on PINs in eukaryotic organisms from yeast to humans have revealed that hub proteins, defined as those that interact with multiple partners in the network, are significantly more disordered than end proteins, defined as those that interact with far fewer partners (Patil *et al.* 2010). A typical PIN that includes an IDP hub is illustrated in figure 5 using the Myc sub-network as an example. Furthermore, a remarkable feature of most IDPs is their ability to undergo disorder-to-order transitions upon binding to their biological target (coupled folding and binding) (Tompa and Csermely 2004). Structural flexibility and the inherent conformational dynamics are believed to represent a major functional advantage for the IDPs, enabling them to stochastically interact with a broad range of binding partners (Tompa and Csermely 2004).

Consistent with this argument, Myc and several other oncogenes and cancer-associated genes (Iakoucheva *et al.* 2002), as well as the Cancer/Testis Antigen genes (Rajagopalan *et al.* 2011) that are highly overexpressed in many types of cancer, encode IDPs. When overexpressed in response to extrinsic perturbations, the IDPs engage in promiscuous interactions (Cumberworth *et al.* 2013). We posit that stochasticity in IDP interactions allows the system to search through numerous iterations of network interactions and activate previously masked options potentially resulting in a transition from one state (phenotype) to another. It is important to note that this transition is not driven by mutations or genetic alterations. The demonstration by Shachaf *et al.* (2004) that the Myc oncogene can reversibly turn on the cancer phenotype in normal liver cells *despite the genetic alterations* provides excellent support for our hypothesis. Examples of perturbations that could lead to IDP overexpression include stress such as nutrient, hypoxic, and inflammation. Inflammation appears to play an important role in cancer with current epidemiological data indicating that over 25% of all cancers are related to chronic infections and other types of unresolved inflammation (Vendramini-Costa and Carvalho 2012). Indeed, chronic inflammation is now regarded as an ‘enabling characteristic’ of human cancers (Sfanos and De Marzo 2012). Thus, by exploring the network search space, IDPs can rewire PINs to activate previously masked options in response to stress. The resulting outputs drive the macroscopic behaviour of the system (figure 6). While in some cases such emergent properties may be necessary for the normal function of the tissue or organism, in others it may have pathological consequences such as malignant transformation, and enable the transformed cell to ‘learn’ to adapt to perturbed environments while guiding its evolution.

4.3 Learning and evolution

It seems quite reasonable to assume that, in response to the dynamic environments in which they find themselves, organisms acquire useful adaptations during their lifetime. In other words, organisms exhibit considerable phenotypic plasticity. For example, cancer cells can

reversibly switch phenotypes in response to environmental changes (Sharma *et al.* 2010). Such adaptations are often the result of an exploratory search which samples various iterations of potential outputs in order to discern and select the most appropriate ones. Thus, it is plausible that ‘learning’, which can be described as an elaborate and iterative form of phenotypic modification that allows an organism to adjust its response to the same inputs over time based on the outcomes of previous outputs, could have a significant influence on evolution of a new species such as a stem-cell-like cancer cell from a non-stem-cell cancer cell. Therefore, it would seem quite wasteful to forego the advantage of the exploration performed by the organism to facilitate the evolutionary search for increased fitness. An efficient way to achieve this goal would be to transfer information about the acquired (learned) characteristics (new phenotypes) back to the genotype. Indeed, this type of interaction between learning and evolution was independently proposed in the late 1800s by Baldwin (1896), Osborn (1896) and Morgan (1896) and is often referred to as the ‘Baldwin effect’. Sadly enough, the Baldwin effect remained underappreciated because of its Lamarckian connotation, and consequently it was inferred by many that learning *cannot* guide evolution.

However, in 1987, Hinton and Nowlan, using a computer simulation, demonstrated that this inference is incorrect and that learning (they actually meant phenotypic plasticity) *can* be very effective in guiding the evolutionary search. In fact, the authors observed that learning alters (smoothes) the shape of the search space in which evolution operates and predicted that in difficult evolutionary searches that may require many possibilities to be tested – each learning trial can be almost as helpful to the evolutionary search as the production and evaluation of a whole new organism! Thus, logically speaking, the ‘efficiency’ of evolution is greatly enhanced since a learning trial is much faster and far less energy-intensive than that required for the production of a whole organism by random mutations (Hinton and Nowlan 1987). Subsequent studies by Behara and Nanjundiah (1995, 1996, 2004) demonstrated that although the relationship may not be as straightforward as was assumed by Hinton and Nowlan, phenotypic plasticity can potentiate evolution even when more realistic fitness schemes are simulated.

Although these computational studies are tantalizing, the real question is, can cancer cells (or other protists, for that matter) really ‘learn’ or ‘make’ decisions? To describe the cell’s physiological response to a stimulus as learning/ decision-making is perhaps a matter of semantics. However, several observations made in protists that lack even the rudiments of a nervous system, much less a brain, suggest that they possess sophisticated mechanisms through which they respond to ‘anticipate’, and even ‘learn’ from, fluctuations and challenges in their environment (Nakagaki *et al.* 2000; Saigusa *et al.* 2008; Tero *et al.* 2010).

While cancer cells are not protists *per se*, they exhibit several characteristics that are typical of these simple forms of life. For example, cancer cells develop drug resistance, exhibit traits of the persister phenotype (an extremely slow-growing physiological state which makes them insensitive to drug treatment) and quorum sensing (a system of stimulus and response correlated to population density), and display many other collective behaviour capabilities and cooperative strategies necessary for survival under extreme stress (Ben-Jacob *et al.* 2012). These characteristics present cancer cells in a different light – smart

communicating cells – and tend to portray tumours as societies of cells capable of making decisions (Ben-Jacob *et al.* 2012). Thus, we argue that the stochasticity in interactions of IDPs that are overexpressed in cancer cells could facilitate learning by exploring the network search space and rewiring the network.

But how is the organization of the networks specified? What determines the network dynamics? How does this affect learning? We hypothesize that analogous to the computational models developed by Hinton and Nowlan, and Behera and Nanjundiah, the basic design of the PINs is specified by the genome inasmuch as the expression of the critical nodes in space, time and amplitude are concerned. However, the ultimate organization of the PIN and its ground state threshold are determined by learning and adapting to the environment in which the organism finds itself.

4.4 Inheritance of adaptive learning or phenotypic plasticity and reversal of information transfer

For adaptive learning (phenotypic plasticity) to be inherited, one would anticipate that changes in the genome, whether genetic or epigenetic, would be necessary, implying a reversal of information flow from the phenotype. In response to dynamic environmental fluctuations, an organism's PINs constantly process information and organize and reorganize themselves. However, we postulate that in response to 'unanticipated' environmental changes, several IDPs are overexpressed and the organism explores numerous iterations of network connections many of which are due to the promiscuous nature of these interactions (Vavouri *et al.* 2009). This results in a specific output that the organism benefits from, and in resetting the network to a new set-point (threshold). We suspect that information derived from PIN rewiring can operate across diverse timescales. While some of the information, particularly that which operates over relatively short time-scales, may be retained within the PINS, information that operates over long periods such as cellular transformation, development and evolution, is transferred to the genome to effect heritable genetic/epigenetic changes, or a mechanism similar to genetic assimilation proposed by Waddington (1942) and Schmalhausen (1949). Interestingly, several proteins that are involved in epigenetically sculpturing the chromatin are IDPs (Sandhu 2009; Beh *et al.* 2012), hinting that rewiring of PINs can potentially result in heritable epigenetic changes.

Insofar as genetic changes are concerned, emerging evidence suggests that a nexus between transcription factors and chromatin remodellers (Murawska and Brehm 2011), and between transcription factors and DNA repair proteins (Fong *et al.* 2011) that are part of large PINs, can facilitate such changes. With regard to genetic assimilation, Waddington proposed that it is the process in which an environmental stimulus that affects the phenotype has been superseded by an internal genetic factor during the course of evolution. In more recent times several groups have provided tantalizing evidence supporting genetic assimilation (Rutherford and Lindquist 1998; Milo *et al.* 2007). While such mechanisms could potentially account for permanent changes in the diploid genome of the cancer cell or other unicellular organisms, how information to activate such an internal genetic switch is transmitted to the germline for stable inheritance in metazoans reproducing sexually remains an important and intriguing question.

Notwithstanding the molecular mechanisms, however, an equally important question that needs to be considered here is the evolutionary timescale. A key point in Darwinian evolution is that it works very slowly, over millions of years of geological time, through the gradual, incremental acquisition of small differences. Then how can a cancer cell evolve in such a short time? Perhaps, as has been suggested (Eldredge and Gould 1972), under certain conditions evolution could occur more rapidly than previously envisioned. For example, in the extreme case, in a population of just a few individuals, all sorts of unusual mutations could become fixed simply because the number of individuals was so small and each mutation has a much higher likelihood of survival because competition among mutant forms is lower. Through this process a new species can arise in a few generations. However, in either case, mutations that hold the key arise by *chance* and without foresight for the potential advantage or disadvantage of the mutation. Furthermore, the underlying implication would be a unidirectional flow of information from genotype to phenotype.

On the other hand, in the scenario we favour, wherein phenotypic plasticity can guide evolution, genetic mutations arise due to *necessity* and not by chance, and in a few generations, are fixed. Episodes of rapid change – network rewiring to uncover latent pathway interactions in response to environmental perturbations – could lead to genotypic changes in a relatively short order. In other words, a species need not originate in a series of gradual steps, each resulting from a mutation with a small effect, slowly changing ancestor into descendant. Rather, the genetic changes that lead to the formation of new species have large effects and happen over relatively few generations. Thus, in our model, creation of a new species would reflect an emergent property of the system, and informational flow would be bidirectional.

5. Conclusion

We (Kulkarni *et al.* 2013) and others, in particular Huang and coworkers, who have advanced the cancer attractor concept (Huang and Ingber 2006; Huang *et al.* 2009; Huang 2012), and Sevim and Rikvold (2008), have argued that there needs to be a paradigm shift from the prevailing view of cancer that is fundamentally influenced by deterministic approaches. By applying the tools of nonlinear dynamics in combination with experiments to characterize cancer protein network connectivity and functionality, we need to decipher how cancer cells self-organize to generate phenotypic heterogeneity so that, ultimately, this knowledge could be used to better understand cancer and develop more effective therapeutics. Indeed, recent progress in this direction suggests that the IDPs may represent promising therapeutic targets for several medical indications (Anurag and Dash 2009; Uversky 2012; Follis *et al.* 2012).

In keeping with the spirit of the Almora Meeting on Individuals and Groups, we have presented what we believe is new thinking in cancer and evolution. Given the complex nature of the disease that is confounded by a plethora of intrinsic and extrinsic factors, an inclusive rather than an exclusive approach will be necessary to fully understand and address cancer. In our opinion, the IDP theory is compliant with the former. Furthermore, several elements that are germane to the IDP theory such as the universal presence of IDPs across kingdoms, stochasticity, and dosage-sensitive effects of IDPs due to overexpression, are

now well documented. The fact that, to date, no activating mutations are observed in several IDPs overexpressed in cancer strongly suggests that their pathological effects are likely due to their ability to rewire PINs, lending further credence to our hypothesis. Nonetheless, unequivocal evidence demonstrating reverse information flow from phenotype to genotype, or that cells can learn from their past ‘experiences’, will be required to inspire the reader’s confidence. We trust that this new and provocative thinking will help in escaping from the old ideas and trigger a flurry of new activity in this direction.

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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 morphogenetic 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).

Figure 2.

Illustration of SMT and TOFT models: **(a)** The SMT posits that carcinogens lead to mutations in the cellular DNA of epithelial cells, leading to propagation of cells carrying advantageous mutations. Paracrine factors secreted by the growing tumour alter the tumour stroma. The altered or ‘activated’ stroma in turn contributes to cancer progression, enabling a conducive environment for tumour cell invasion and metastasis. **(b)** The TOFT model predicts that carcinogens affect the stroma, leading to altered cell–ECM and cell–cell interactions in the epithelium, which then results in tumour formation in the epithelium.

Figure 3.
Schematic of mammary gland fat pad transplantation experiments.

Figure 4.

Hierarchical organization of cells within tissues: **(a)** Self-renewing adult tissues contain few ‘stem cells’ that divide to make more of themselves (by self-renewal) and also generate cells of more restricted potential – the ‘progenitors or transit amplifying cells’ – which undergo few rounds of rapid proliferation to finally generate the committed/differentiated cells of the tissue. **(b)** Cancer stem cells occupy the tip of the hierarchy within cancers, with capability to undergo self-renewal, and ‘differentiation’ to generate the bulk of the tumour cells.

Figure 5. A Myc sub-network showing its interactions with multiple partners. Myc represents the central hub in the protein interaction network. The image is obtained by accessing the data from the STRING protein interaction database (<http://string-db.org/>). The proteins that are not predicted to be intrinsically disordered are circled in red. The others, including Myc, are predicted to be intrinsically disordered.

Figure 6.

Cancer, evolution, and intrinsically disordered proteins. The cancer cell is regarded as a nonlinear system characterized by protein interaction networks (PINs) with dynamic topologies. **(a)** The coloured balls (nodes) connected by solid lines (edges) within each cell (large circles and squares) represent the PINs. Individual cells in a tumour interact and self-organize forming an ensemble of complex interactive parts with emergent properties. The macroscopic behaviour of the system such as state/phenotype switching (e.g. malignant transformation) in response to extrinsic perturbation depends on the type and strength of interaction among the constitutive cells and their response to external perturbations leading to different types of synchronized emergent dynamics. **(b)** The latter results from rewiring and synchronization of PINs in the ensemble. Intrinsically disordered proteins (IDPs) that are overexpressed in cancer drive this process by exploring the search space and rewiring the PINs (represented by different configurations in each of the cells a1, a2, a3, etc., in phenotype A). **(c)** Rewiring of PINs results in unmasking latent pathways that facilitate state switching (e.g. phenotype A to phenotype B) in some cells within the population (e.g. a7) and their subsequent proliferation (b1, b2, b3, etc.).