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The aging of the 2000 and 2011 Hallmarks of Cancer reviews: A critique

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Abstract

Two review articles published in 2000 and 2011 by Hanahan and Weinberg have dominated the discourse about carcinogenesis among researchers in the recent past. The basic tenets of their arguments favour considering cancer as a cell-based, genetic disease whereby DNA mutations cause uncontrolled cell proliferation. Their explanation of cancer phenotypes is based on the premises adopted by the somatic mutation theory (SMT) and its cell-centered variants. From their perspective, eight broad features have been identified as so-called 'Hallmarks of Cancer'. Here, we criticize the value of these features based on the numerous intrinsic inconsistencies in the data and in the rationale behind SMT. An alternative interpretation of the same data plus data mostly ignored by Hanahan and Weinberg is proposed, based instead on evolutionarily relevant premises. From such a perspective, cancer is viewed as a tissue-based disease. This alternative, called the tissue organization field theory, incorporates the premise that *proliferation* and *motility* are the default state of *all* cells, and that carcinogenesis is due to alterations on the reciprocal interactions among cells and between cells and their extracellular matrix. In this view, cancer is development gone awry.

Keywords

Oncogenes; proliferation; somatic mutation theory; stroma; tissue organization field theory

'…One day, we imagine that cancer biology and treatment—at present, a patchwork quilt of cell biology, genetics, histopathology, biochemistry, immunology, and pharmacology—will become a science with a conceptual structure and logical coherence that rivals that of chemistry or physics.'

–Hanahan D and Weinberg RA 2000 The hallmarks of cancer. *Cell* **100** 57–70

'…The historical truth, for William James, is not what happened; it is what we judge that happened.'

–Borges JL 1999 Fictiones. *Biblioteca Borges* 53

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Note added in proof

In a recent article, researchers committed to the SMT conclude that most mutations in genes proposed to be 'drivers' of carcinogenesis, as originally described by The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium, represent instead false positives. This conclusion gleaned from sophisticated data-mining computer models suggests that those alleged driver genes remain elusive and their nature is subject to the premises of the model used to find them (Lawrence MS *et al.* 2013 Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Nature*. doi:10.1038/nature12213).

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> '…understanding how the parts relate to each other is a precondition to understanding process and understanding process is the precursor of uncovering principles.'
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> –Bateson P 2006 *Biol. Philos.***21** 553–558

1. Introduction

Almost a century after Theodor Boveri proposed the original view on what eventually became known as the somatic mutation theory of carcinogenesis (SMT), two highly influential review articles entitled 'The hallmarks of cancer' were published summarizing in about 27,000 words what was claimed to have been accomplished during the second half of the 20th century and what the future has in store for cancer research in the 21st one (Hanahan and Weinberg 2000, 2011). Many reviews were published before and after these, ones assessing who, what, when and why data and interpretation affected our respective views about cancer. The influential nature of these 'Hallmarks' reviews is, however, reflected in the over 7000 mostly laudatory citations that have accumulated in the first decade after the first of these two reviews was published, and by the fact that it spun the publication of a textbook that has been adopted by most graduate programs in the Western world (Weinberg 2006).[1] As further evidence of the influence of these reviews, a 2012 rating of the most popular articles read in the journal *Cell* concluded that the 2011 and the 2000 Hallmarks were first and second, respectively, among the 25 publications examined.

Because of the prominence given to these publications, they could be regarded as central to the dominant strategy applied since 1972 when the War on Cancer was formally declared with the often-referred aim of reaching the 'mechanistic understanding of cancer'. In this regard, startling technical improvements have increased the analytical depth at which genomic mutations can be identified and transcriptional and translational modifications characterized, sequencing techniques were refined to a point that was hardly imagined in 2000 and a whole new slew of microscopic procedures were added to those already available in 1972. The explicit rationale behind the emphasis on using these technological marvels was that they would eventually uncover the lowest possible level of organization at which carcinogenesis takes place, with the clear implication that biological organization could be reduced to machines and its parts. This research program would in turn lead to effective cancer treatments. And yet, by any objective evaluation of the data collected under the Hallmarks I and II approach, the 'mechanistic understanding of cancer' remains elusive, as admitted by committed supporters of the SMT (Garraway and Lander 2013; Vogelstein *et al.* 2013).

Central to the selection of those Hallmarks has been the perception that the appearance of cancers in humans was equivalent to the process of phenotypic 'transformation' that was described in *in culture* conditions starting in the 1950s and 1960s. Among the premises adopted and conclusions drawn in these two Hallmarks reviews was the statement that (a) cancer is a genetic disease and (b) everything that cannot be now linked to a genetic origin will finally become associated to a genetic interpretation of the disease in the near future. These inferences remain central to the current explanations of cancer (Nijhawan *et al.* 2012).

A comprehensive critical evaluation of the content of these influential reviews has never been published according to our bibliographic searches. Nevertheless, during the period 2000–2013, several aspects of those reviews have been unambiguously criticized both on epistemological and biological grounds (Marcum 2005; Bizzarri *et al.* 2008; Bertolaso 2011;

[1]To avoid repetitions of the names of Hanahan and Weinberg and 'The hallmarks of cancer' articles from 2000 and 2011, we will use the acronyms H&W, and Hallmarks I and Hallmarks II, respectively.

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Soto and Sonnenschein 2011; Baker 2012, 2013). For instance, alleged unique properties of individual cancer cells ended up being shared by normal cells during diverse stages of normal development. From such an analysis, we developed 'the death of the cancer cell' concept (Sonnenschein and Soto 2011) and also highlighted the differences between the prevalent SMT and our own tissue organization field theory (TOFT) (Sonnenschein and Soto 1999; Soto and Sonnenschein 2011).

Two main arguments should be offered when criticizing a theory. First, why has the theory failed, and second, what does the competing theory offer that would resolve the unknowns left by the failing theory. The main reason why H&W's views and ours differ substantially is because each of us adopts significantly different premises and, therefore, the methodology used and the conclusions we respectively draw will be necessarily different. Herein, we aim at rebutting the value of the specific Hallmarks identified by H&W as being crucial to the explanation of the cancer process.

2. Background

H&W have aligned themselves with the SMT by stating that '…cancer cells have defects in regulatory circuits that govern normal cell proliferation and homeostasis' and sided with those who consider cancer as a *cell-based* disease caused by DNA mutations. In fact, H&W have been among the most ardent theoretical and pragmatic architects of this view of cancer (Weinberg 1998).

In Hallmarks I, H&W pointedly linked six essential alterations in cell physiology to malignant growths. It is relevant to list verbatim those putative alterations as they were defined in the text. The 'six essential alterations in cell physiology that collectively dictate malignant growth are… self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis…' These alterations '…are shared in common by most and perhaps all types of human tumors…' As recalled above, these conclusions were drawn by H&W under the assumption of equivalence between *in culture* and in animal data that uses genetically modified mice and the process of carcinogenesis in human beings. In Hallmarks II, H&W re-evaluated and expanded their 2000 assessment and increased by two their six original Hallmarks incorporating 'reprogramming of energy metabolism and evading immune destruction'.

During the last decade, many have pondered about the explanations for the initiation and progression of cancers and reached alternative conclusions to those offered by H&W. By using mathematical modeling, Enderling *et al.* (2009) challenged the conclusion reached by H&W whereby evading apoptosis represents a hallmark of cancer (Weinberg 1998). Separately, Lazebnik concluded that H&W fell short in dealing with the complexity of cancer at large by ignoring that benign tumors share five of the six putative 2000 Hallmarks while failing to be associated with the crucial differentiating quality of cancers, i.e. their malignancy (Lazebnik 2010). This latter particular objection relates to a non-trivial aspect of the subject given the toll that this disease has taken along the last half a century especially in the industrialized countries. It is likely that this is, in fact, the main reason why cancer has acquired such social prominence to the point that about two million publications have accumulated over a century and well over 200 billion dollars have been invested into a gargantuan effort to explain, accurately diagnose, prognosticate and, most importantly, eventually 'cure' the disease. The impact of this research contribution on treatment is summarized in a public document drawn by a panel of experts convened by the U.S. Institute of Medicine (a branch of the U.S. National Academy of Sciences) toward the end of 2012 that, among other things, concluded that '…Despite progress in reducing cancer death

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rates, disparities in cancer outcomes persist, problems of overuse and misuse contribute to a lack of evidence-based cancer screening and treatment, and many patients do not experience patient-centered cancer care, such as access to palliative care and use of treatment plans to help with patient–clinician communication and decision making…' (Institute of Medicine 2013).

Before examining in detail the Hallmarks of Cancer, it is worth clarifying some issues prominently alluded to in these two reviews, namely, (a) cell proliferation and its relationship with carcinogenesis and (b) the methodological tools and models used to explore the SMT. The relationship between control of cell proliferation and carcinogenesis stems from the claim that, 'the most fundamental trait of cancer cells involves their ability to sustain chronic proliferation.' Objectively, cell proliferation is a straight forward process whereby two daughter cells are generated from a (single) 'mother' cell. If exceptions to this rule exist, cancer cells have not been recorded as being one of them. Thus, the alleged 'sustained chronic proliferation' abides by the rule that a 'mother' cancer cell always delivers two 'daughter' cells no matter what may be happening within the 'mother' cancer cell carrying the mutated DNA sequence. In short, regardless of whether the proliferation is episodic or chronic, the end product of the event is the generation of two cells out of one. Equally important, this binary process of proliferation can be easily monitored in culture conditions using electronic particle counting. Accrual of cells as a function of time allows for measuring population doubling times during the exponential phase of cell proliferation (Luria 1975; Sonnenschein and Soto 1980; Sonnenschein and Soto 1999). Cells in a tumor have not been shown to proliferate at a faster rate than normal enterocytes or blastomeres (Baserga 1976). Of note, the common practice of using tritiated thymidine incorporation, an indicator of DNA synthesis, as a surrogate parameter of cell proliferation yields or rates is inadequate (see below).

The relevance of using two-dimensional cell culture experiments and genetically modified mice approaches has not been extensively and explicitly debated given that these methods of generating cancer growths are not representative of the way cancers arise in humans or in naïve experimental animals exposed to carcinogens (Durum and Muegge 1998; Pitot 2002; Cardiff *et al.* 2008).

3. The six (plus two) essential alterations in cell physiology that allegedly dictate malignant growth

3.1 Self-sufficiency in growth signals

In Hallmarks I, it is stated that normal cells remain in a state of passive *quiescence* and that they require growth signals to stimulate them to enter the cycle. Additionally, in that review, growth factors and oncogenes are attributed the role of stimulators of proliferation in cancer cells. Further, it is concluded that (a) tumor cells become autonomous due to the dominant character of oncogenes, b) tumor cells proliferate because of their capacity to produce their own growth factors, c) tumor cells over-express growth factor receptors and (d) over-expression of oncogenes stimulate cell proliferation. These conclusions can be challenged on two fronts: first, as explained elsewhere, an important point in this regard is to make explicit which is the premise adopted regarding the default state of cells. Based on evolutionary theory, *proliferation* as the default state is a *sine qua non* pre-requisite for life (Sonnenschein and Soto 1999). Thus, it becomes puzzling to explain how data could have been generated and eventually published claiming just the opposite, that is, that *quiescence* was instead the default state of cells in metazoa. Notwithstanding, despite its implausibility from an evolutionary perspective, explicit references that *quiescence* is the 'default state' of cells in metazoa are still being widely disseminated in the scientific literature (Alberts 2010)

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and in textbooks of biology (Cooper 1997; Lodish *et al.* 2000; Alberts *et al.* 2008). And second, and equally disturbing, no objective published experimental data shows the alleged direct stimulatory effect on cell proliferation of any growth factor and/or of any oncogene (Sonnenschein and Soto 2000). In addition, no knock-out experiments document a proliferative phenotype due to a putative stimulatory effect by a single or a combination of growth factors (Durum and Muegge 1998). In sum, as mentioned above, no cancer cell proliferates faster than normal early embryonic cells, and more chronically than, for an instance, enterocytes in the small intestine, or hemopoietic cells in the bone marrow.

Growth factors have been operationally defined while using an artifactual, far-from-physiological, two-dimensional tissue culture methodology. The vast majority of these models have equated serum starvation with physiological quiescence. Under these non-physiological starving conditions that usually lead to cell death, the effect of putative growth factors was measured as an increase of thymidine incorporation into DNA; *sensu stricto*, this is not equivalent to increased cell proliferation (Drach *et al.* 1981;Wolff and Bodycote 1986). Alternatively, when 'bioassays' were used to characterize growth factors (like in the case of nerve growth factor (NGF) or epidermal growth factor (EGF), the relationship between the bioassay and cell proliferation was equivocal. For example, the purification of EGF was monitored by observing a shortening of the age of eyelid opening and teeth eruption in newborn mice, phenomena that are rather linked to cell death and stromal breakdown, and not to cell proliferation *per se* (Sharov *et al.* 2003). Remarkably, the original seminal founding papers published by Rita Levi-Montalcini and Stanley Cohen, working at Washington University in St Louis, MO, in the 1950s and 1960s suggest that neither growth factor directly stimulated the proliferation of alleged target cells, or any cell for that matter. In fact, these Nobel Prize winners unambiguously reached this conclusion themselves (Cohen and Elliot 1962; Cohen 1965; Levi-Montalcini 1986).

Because cell proliferation and motility are so central to a comprehensive understanding of all aspects of living organisms, clarification of the above-referred issues represent important priorities in developmental biology and carcinogenesis. In sum, based on epistemological and experimental grounds, *proliferation* remains as the default state of *all* cells. Thus, the need to stimulate cells does not exist in order for them to enter the cycle and generate their respective two daughter cells (Sonnenschein and Soto 1999; Harris 2004; Soto and Sonnenschein 2004; Sonnenschein and Soto 2011). Recently, the concept of a proliferative default state re-emerged in the fields of stem cell biology and immunology. In the former, it is referred to as the 'ground state', by analogy to the concept used in physics to signal the state of lowest energy, and the opposite of the 'excited state'. Thus, embryonic stem cells 'have an innate programme for self-replication that does not require extrinsic instruction' (Ying *et al.* 2008). In immunology, the study of lymphocyte quiescence revealed that it is an induced phenomenon, and thus, it implies that proliferation is the default or ground state (Yusuf and Fruman 2003). In conclusion, the regulation of cell proliferation or of motility can only be effectively implemented by inhibiting the expression of these default states.

Comparable conclusions are relevant when considering oncogenes. During the Oncogene Revolution (1975–1985), it was emphatically claimed that these genes or their gene products directly stimulated the proliferation of cells either in culture or in animal experiments. In fact, no such direct stimulation has been verified (Scully and Edwards 1991; Jacobsen *et al.* 2002). Even one of the pioneers in the field of oncogenes admitted two decades ago that '… we have learned very little about the causes of cancer from the study of proto-oncogenes and tumor suppressor genes' (Bishop 1991). Thus, as research on oncogenes expanded, the evidence pointing to the pleiotropic nature of gene effects blurs the assumed specificity of oncogenes as inducers of cell proliferation and neoplastic transformation.

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Review articles about carcinogenesis follow a familiar trend whereby they refer to publications that would have documented the alleged oncogene-mediated proliferative stimulation of cells. For the most part, these references are made in review articles, which, in turn, refer to previously published review articles in which the promised evidence keeps being referenced but the actual data are hard to retrieve (for examples of this practice, see Podlaha *et al.* 2012 and Visvader 2011).

Over-expression of so-called oncogenes relied on phenotypic changes on cells *in culture* conditions, a phenomenon described as 'transformation.' Carefully conducted experiments aimed at rigorously defining the 'transformation' phenomenon concluded that a mere change in culture conditions (changes in serum concentration under which cells were maintained) reliably mimicked those phenotypic changes (for a comprehensive review, see Rubin 2011a). This evidence implied that 'transformation' was an unspecific, trivial *in culture* artifact that could hardly be extrapolated to the complex process of carcinogenesis happening in living organisms (Rather 1978; Pitot 2002).

3.2 Insensitivity to growth-inhibitory (antigrowth) signals

In Hallmarks I and II, it was claimed that, in addition to being self-sufficient in growth signals, cells might also be under the active influence of endogenous and exogenous inhibitory or anti-proliferative signals that would prevent them from expressing either their autonomous and/or their acquired proliferative capacity. This interpretation implies that an effective way to regulate proliferation in multicellular organisms is by actively inhibiting such cells from proliferating. In this context, where no rejection of an alleged stimulatory option is stated, inhibitors of cell proliferation would represent just a balancing act (the *yin*) to neutralize the 'positive' arm of the metaphorical balance represented by growth factors and oncogenes (the *yang*).

Moreover, according to H&W, in order to 'prosper', incipient cancer cells would acquire the capacity to evade these hypothetical inhibitory signals, a process inferred to be mostly funneled through the TP53 proteins and the retinoblastoma protein (pRb) and its two relatives, p107 and p130. Despite acknowledging a lack of consistency regarding the alleged inhibitory function of these proteins, H&W have called them 'canonical suppressor(s) of cell proliferation'. Although in 2000 the mode of action of these molecules was elusive, since then several groups have linked pRb to TGFβ signalling and to either or both cell proliferation and inhibition. Consequent with the premise favoured by H&W that cancer is a cell-based disease, the action of TGFβ and other alleged inhibitors is placed at the intracellular signaling circuitry level. In 2011, the mode of action of TP53 and TGFβ on cell proliferation still remained obscure (Levine 2011; Senturk and Manfredi 2012). What has received much attention in this regard, however, is the participation of TP53, TGFβ and pRb in 'signal transduction' pathways and networks. For example, H&W further inferred that '… the existence of an antigrowth signaling circuitry is clear (Figure 2), as is the necessity for its circumvention by developing cancers' (Hanahan and Weinberg 2000 p 61). No reference was made either in 2000 or in 2011 that this alleged 'anti-growth signaling circuit' is an inferential entity which requires empirical verification. As remarked above when we referred to unsubstantiated claims that growth factors stimulated cell proliferation, what is being challenged is the existence of evidence for an unequivocal proliferative or anti-proliferative effect of these alleged stimulators or inhibitors of cell proliferation and not whether growth factors or the anti-proliferative factors affect signal transduction during either normal organogenesis and/or carcinogenesis. Our objections are aimed, instead, at the unsubstantiated inferences made when these sub-cellular biochemical reactions are considered relevant to a discrete, meaningful proliferative effect due to direct stimulation by

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either growth factors or oncogenes, or an inhibitory one by inhibitors of cell proliferation, showcased by TGFβ.

3.3 Evasion of programmed cell death (apoptosis)

During normal development, apoptosis is an important feature of organogenesis (Gilbert 2010). During carcinogenesis, on the other hand, explaining a net local accrual of cells requires either an increased cell proliferation rate and/or inhibition of cell death. In this latter regard, however, the alleged 'acquired resistance toward apoptosis' as 'a hallmark of most and perhaps all types of cancers' becomes nothing more than another unsubstantiated inference unless experimental evidence is quoted to document such a claim in *in vivo* carcinogenesis models. Once again, as it was remarked above when referring to growth factors, oncogenes and suppressor genes, no challenge is being leveled at the reliability and reproducibility of the multiple biochemical reactions that involve Bcl-2, pTen, c-Myc, FAS, caspases and other proteins together with the hundreds of molecules with which these putative participants interact during the apoptotic process. Criticisms are leveled, instead, at the inference that 'evasion of programmed cell death (apoptosis)' can be reliably linked to the tissue-based events that characterize the initiation and progression of cancer. In fact, Enderling and Hahnfeldt using mathematical modeling and computer simulation found that increasing the rate of apoptosis, while obviously reducing tumor size in the short-term, actually enhances growth in the long-term. They show that tumors can remain dormant for a long time while stimulation of apoptosis can cause the tumor cell population to aggressively invade. Their work suggests that the widely regarded 'evading cell death' as a hallmark of cancer needs to be revisited (Enderling and Hahnfeldt 2011).

3.4 Limitless replicative potential

Regarding this fourth acquired capability, in Hallmarks I, H&W are rather reserved about a significant role, if any, played by senescence in carcinogenesis. After making strong arguments, nuanced by their usual pre-emptive caveats, regarding the rounds of proliferation by human cell populations and the putative role of telomerases in aging and in cancer, H&W made the sobering statement that senescence could be an artifact of cell culture that may not represent an impediment to tumor progression *in vivo*. This represents a significant concession when considering that immortalization was previously viewed as a crucial event in carcinogenesis (Elenbaas *et al.* 2001;Lundberg *et al.* 2002). In Hallmarks I, it was anticipated that, within the following 10 years, research might clarify this subject. In 2011, however, H&W estimated that 'replication-induced senescence as a general barrier (of the neoplastic state) requires refinement and reformulation'. They conclude that '…cell senescence is emerging conceptually as a protective barrier to neoplastic expansion that can be triggered by various proliferation-associated abnormalities, including high levels of oncogenic signaling and, apparently, subcritical shortening of telomeres'. After referring to a number of publications arguing for and against a significant role played by telomeres on carcinogenesis, H&W concede that '… The realization that impaired telomere function can actually foster tumor progression has come from the study of mutant mice that lack both p53 and telomerase function. The proposition that these two defects can cooperatively enhance human tumorigenesis has not yet been directly documented'. Thus, the role of telomerases during carcinogenesis remains to be fully elucidated, which questions their characterization as a *bona fide* Hallmark of cancer.

3.5 Sustained angiogenesis (2000) or inducing angiogenesis (2011)

This fifth acquired capability together with the sixth one (tissue invasion and metastasis) belong to features that take place at the tissue level of biological organization during stages of cancer occurring much later than those of initiation ('progression'). This particular

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'acquired capability' appears as a means to merge the notion of cancer as a cell-based phenomenon (SMT) with features of the *tissue-based* theories of carcinogenesis. In other words, this appears as a 'synthesis' (a premature one at that) or a 'compromise' between the SMT and the premise of the TOFT that states that carcinogenesis is a *tissue-based* disease (Sonnenschein and Soto 1999, 2008; Baker 2012, 2013; Soto and Sonnenschein 2004).

In Hallmarks I, it was already acknowledged that 'induction of angiogenesis will prove to be an early to mid-stage event in many human cancers', and thus it represents a phenomenon that, like 'tissue invasion and metastasis,' i.e. the sixth induced capability of cancers, is triggered once the initial stages of tumor formation have been well underway. Significantly, this angiogenic capability was considered to be the result of a balance between pro-angiogenic (VEGF, acidic and basic fibroblast growth factors or FGF i/2) and anti-angiogenic factors (trombospondin-1, among them). The switch toward angiogenesis would have been triggered by the tumor itself through mechanisms that were not quite obvious in 2000. Nevertheless, a decade before 2000 and since then, the strategies aimed at therapeutically managing angiogenesis during cancer progression were being prominently and successfully hailed to an eager investment community. Critical assessments were published regarding the rationale to offer promises and their impact on the reality of the market, and more importantly in the bodies and minds of patients, their relatives, the medical community and public health officials (Ebos and Kerbel 2011). In 2011, bevacizumab (Avastin) was withdrawn from the market of effective therapeutic drugs for breast cancer (http://www.cancer.gov/cancertopics/druginfo/fda-bevacizumab).

3.6 Tissue invasion and metastasis

From an evolutionary perspective, *motility*, like *proliferation*, is the other default state of *all* cells. On the other hand, ontogenetically, cell and tissue motility and migration are part of a vast array of processes that make normal organogenesis possible (Gilbert 2010). The alleged exceptionality of a hypothetical cancer cell to abide by its constitutive ability to move and to proliferate together with other proposed cancer cells singularities has been a prevalent but misguided notion for a century now. Notwithstanding, aggressive efforts to find what is unique in a cancer cell when compared with its normal counterparts have been unproductive (Sonnenschein and Soto 2011). In other words, if a cancer cell exists as a discrete entity distinguishable from a normal cell, it has certainly been very uncooperative in revealing what it has 'invented' to deserve the fame it has acquired. More to the point, cells isolated from cancers revert to normalcy when placed in a normal microenvironment (Illmensee and Mintz 1976; Maffini *et al.* 2005; Hendrix *et al.* 2007; Bussard *et al.* 2010)

In Hallmarks II, the 'epithelial-mesenchymal transition' (EMT) was attributed to be the means by which 'transformed epithelial cells can acquire the abilities to invade, to resist apoptosis, and to disseminate'. H&W inferred that EMT-inducing transcription factors (including Snail, Slug, Twist and Zeb1/2) can orchestrate most steps of the invasion-metastasis cascade save the final step of colonization. The induction of phenotypic changes on individual cells would be due to 'heterotypic interactions of cancer cells with adjacent tumor-associated stromal cells' and that, next, they would 'be choreographed by one or more of these transcriptional regulators'. By using this metaphorical language, H&W invite the reader to consider the participation of a 'choreographer' during carcinogenesis.[2] Regardless, it remains unexplained how hypothetical 'micro-environmental stimuli distinct from those received by cancer cells located in the cores of these lesions' would fit within the SMT as conceived by H&W. Incidentally, whether EMT plays a role in carcinogenesis and metastases has been compellingly challenged by experienced cancer pathologists (Tarin *et*

[2]In Longo *et al.* (2012), we criticize the use of metaphors in biology.

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al. 2005). The narrative in this segment of Hallmarks II is fraught with contradictory statements that baffle a reader interested in discerning whether, on the one hand, much progress has been made since 2000 as the text declares and, on the other, the admitted failure of this alleged progress to be translated into meaningful improvements in treatment as shown statistically in the Institute of Medicine report, referred to above (Institute of Medicine 2013).

3.6.1 Plasticity in the invasive growth program—In Hallmarks II, it is acknowledged that (a) 'phenotypes of high grade malignancy do not arise in a strictly cell-autonomous manner, and that their manifestation cannot be understood solely through analyses of tumor cell genomes' and that (b) 'the ability to negotiate most of the steps of the invasion-metastasis cascade may be acquired in certain tumors without the requirement that the associated cancer cells undergo additional mutations beyond those that were needed for primary tumor formation'. Moreover, 'the notion that cancer cells routinely pass through a complete EMT program is likely to be simplistic, if plausible at all'. Notwithstanding, plasticity due to the yet-to-be identified 'contextual signals' allegedly responsible for inducing invasive growth (often via an EMT) can, in H&W's view, act also in reverse and become responsible for a reversibility that a mutational event is unlikely to 'negotiate'. Thus, in another *ad hoc* accommodation to empirical evidence, H&W propose that the complex EMT process can be going backward in an equally complex way and become a mesenchymal-epithelial transition (MET). This nimble plasticity argument is used to explain the 'formation of new tumor colonies of carcinoma cells exhibiting a histopathology similar to those of carcinoma cells in the primary tumor that never underwent an EMT'. H&W resort to a dexterous vocabulary to cover all possibilities, even contradictory ones, to explain the same phenomenon, in this case the generation of metastases and curiously the 'reverse migration' of metastatic cells from metastatic sites to the their primary tumor site. These propositions, where no hypotheses are ruled out, make finding explanations an untraceable and unverifiable process incompatible with the aims of scientific discovery (Ayala 1968; Soto and Sonnenschein 2012) (see below).

3.6.2 The daunting complexity of metastatic colonization—In Hallmarks II, H&W ignore the fact that motility is the other important dominant, constitutive property of all cells (see above). Thus, whether *metastatic cells* adapt to ectopic tissue microenvironments in which they either colonize and thrive by forming a tumor or are, instead, prevented from proliferating and remain dormant or die *in situ*, represents a relevant question in stages of cancer progression. In this regard, metastatic efficiency is significantly enhanced by the co-migration of cancer-associated stromal fibroblasts that join migratory epithelial cancer cells (Duda *et al.* 2010). The presence of these mixed emboli reinforces the notion that cancer metastases, as their primary cancers, are not cell-based events but represent, instead, tissue-based phenomena (Sonnenschein and Soto 1999; Soto and Sonnenschein 2011).

4. Emerging hallmarks

When introducing their 7th and 8th Hallmarks of Cancer, H&W qualified them as 'emerging' under the commentary that there are still 'unresolved issues surrounding its (their) functional independence from the core hallmarks'. The new Hallmarks are: 'reprogramming energy metabolism' and 'evading immune destruction.'

4.1 Reprogramming energy metabolism

H&W go on to state that '[T]he chronic and often uncontrolled cell proliferation that represents the essence of neoplastic disease involves not only deregulated control of cell proliferation but also corresponding adjustments of energy metabolism in order to fuel cell

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growth and division'. Thus, in H&W's view, energy metabolism comes to represent a condition necessary to fuel the proliferative process. This notion may be easily extended by stating the obvious, i.e. if there is no fuel or energy source, there will be no proliferation. The architectural tissue dislocation resulting from those initial steps enables an increased proliferation rate of the cells within the tumor which, in order to survive and 'prosper' will require what H&W call the 'reprogramming of energy metabolism'. A simpler and still accurate way to sum up this situation may be by stating that the 'reprogramming of energy metabolism' is one more of the consequences and not one of the causes of carcinogenesis.

The involvement of energy metabolism in carcinogenesis has been predicated initially by Otto Warburg who claimed that, unlike normal adult tissues, a cancer cell produces energy mainly by aerobic glycolysis instead of oxidative phosphorylation. For over half a century, this argument has been cyclically resurrected and abandoned, a common pattern followed by other failed theories and hypotheses of carcinogenesis (see below). The most recent episode in the Warburg effect saga is highlighted by the demonstration that normally proliferating embryonic *Xenopus* retina cells *in vivo* use glycogen to fuel aerobic glycolysis (Agathocleous *et al.* 2012). Again, the locus of energy metabolism resides inside cells. And, as mentioned all along, there has been nothing qualitatively novel inside a cancer cell that would distinguish it from what happens inside a normal cell (Sonnenschein and Soto 2011). The differences, if any, are of a quantitative nature: a cell metabolizes more or less of a given substrate, or a signalling pathway might be more active in a cell within a cancer than in a normal cell (cells may proliferate more often that in 'normal' tissues). However, these quantitative differences show the plasticity of all cells and are not unique to their being part of a tumor. The proliferative and gene expression repertoire of a so-called 'cancer cell' has been proven similar to that of normal cells during one or more stages of normal development. At some of these stages, certain cells proliferate more frequently than others and as a result they require additional sources of energy substrates; these normal cells use the same energy resources that cells within a tumor use when they proliferate.

Given that H&W view carcinogenesis through a reductionist, oncogene-centered perspective, they consider that 'aerobic glycolysis is simply another phenotype that is programmed by proliferation-inducing oncogenes' and because of it would merit being an additional Hallmark of Cancer. However, given the elusive impact that oncogenes have had in explaining carcinogenesis, it might be high time to relegate the Reprogramming of Energy Metabolism to the dust bin of failed hypotheses.

4.2 Evading immune destruction

In Hallmarks II, it is stated that 'the role that the immune system plays in resisting or eradicating formation and progression of incipient neoplasias, late-stage tumors, and micrometastases' is still an unresolved issue. Notwithstanding, as done with Reprogramming Energy Metabolism, H&W introduced Evading Immune Destruction as the 8th Hallmark of Cancer. Ever since the immunological surveillance theory of carcinogenesis was introduced in the late 1950s, a cyclical dismissal and revival has characterized the fate of this controversial theory (Stutman 1974; Fuchs and Matzinger 1996; Dunn *et al.* 2004). In sum, as with the Warburg effect, the H&W narrative provides a merely lukewarm endorsement to this topic as the 8th Hallmark of Cancer.

5. Miscellaneous topics covered in Hallmarks II

In addition to the analysis of the six plus two Hallmarks of Cancers dealt with in 2011, H&W commented on various subjects tangentially related to the core of cancer initiation and progression. In our own analysis of the H&W reviews we have selected a few topics that we

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consider relevant to the controversies surrounding interpretations of those reports and analysis.

5.1 Cancer cells and cancer stem cells

H&W and others have characterized 'cancer stem cells' as entities that have been defined operationally. As with other operationally defined entities inhabiting the subject of cancer research, such as growth factors and oncogenes (see above), cancer stem cells are an elusive and controversial subject that nonetheless generates an extensive body of literature. H&W's narrative on stem cells and cancer stem cells define stemness vaguely. Cancer stem cells easily morph in and out of their stemness at the whim of any convenient situation conceived by H&W. Admittedly, as has happened with oncogenes, another operationally defined participant in carcinogenesis, they may be mutated in certain loci within their DNA sequence, they can be present in a fraction of some cancers, they can play a role in either cell proliferation and in carcinogenesis; however, in all these instances the experimental evidence is rather weak. Despite those blanks and uncertainties in the experimental record, we will be looking forward to the now traditional, yearly-renewed 'future ten-year' period(s) when more experimental evidence might solidify conclusions about the true impact of CSC in carcinogenesis.

5.2 Heterotypic signalling orchestrates the cells of the tumor microenvironment

Theoretical propositions have not been subjected to a rigorous analysis in Hallmarks I and II. For instance, in the latter, H&W proposed to complement the already crowded intracellular circuitry with 'similar diagrams charting the complex interactions between the neoplastic and stromal cells within a tumor and the dynamic extracellular matrix that they collectively erect and remodel'. Objectively, this proposition runs counter to the notion that, according to the SMT, a single cell originated the neoplasm. Nevertheless, regarding their Figure 5, H&W state that '(A) a reasonably complete graphic depiction of the network of micro-environmental signaling interactions (that) is still far beyond our reach, as the great majority of signaling molecules and pathways remain to be identified. We [H&W, that is] provide instead a hint of such interactions in Figure 5, upper. These few well-established examples are intended to exemplify a signaling network of remarkable complexity that is of critical importance to tumor pathogenesis'. H&W openly acknowledge that the melding of their proposed signaling interaction circuitry between cancer cells and their supporting stroma 'clearly complicate the goal of fully elucidating the mechanisms of cancer pathogenesis'. However, they now discharge on systems biologists the task of charting 'the crucial regulatory networks that orchestrate malignant progression'. And as usual, 'it seems likely that understanding these dynamic variations will become crucial to the development of novel therapies designed to successfully target both primary and metastatic tumors'.

6. Therapeutic targeting

Finally, in Hallmarks II, H&W deal with the controversial issue of whether '[T]he introduction of mechanism-based targeted therapies to treat human cancers has been heralded as one of the fruits of three decades of remarkable progress of research into the mechanisms of cancer pathogenesis'. Not everyone in positions to know shares the optimistic views of H&W (Institute of Medicine 2013;Kaiser 2012;Miklos 2005;Tarin 2011). Even H&W acknowledge that their optimism is based on evidence yet to be collected, that is, 'the myriad therapies that are under development or have been introduced of late into the clinic.' While from the SMT perspective, it may be considered a virtue to have aimed at molecular targets, the bedside reality suggests that the 'resulting clinical responses have generally been transitory, being followed by almost-inevitable relapses'. Thus, explanations for the perceived failures are in order and, they happen to be the

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collection of usual suspects, namely, 'partially redundant signaling pathways', 'alternative forms of drug resistance', etc. Thus, regarding therapeutic gains, we also prefer to allow for time to provide the unbiased verdict of whether 'personalized targeted medicine' and additional therapies based on the SMT will become as effective as these Hallmarks reviews anticipate.

7. General comments about theories of carcinogenesis and the role of the microenvironment in them

In Hallmarks I, H&W repeatedly referred to what the future of cancer research in the following decade was going to be. In 2000, H&W forecasted that cancer research was going to resemble a science more akin to physics or chemistry. A short aside is justified here. A recent Editorial in *Nature* magazine evaluated the impact of an experiment conducted at CERN, near Geneva, Switzerland, aimed at finding evidence for or against the existence of the then elusive Higgs boson (Brumfiel 2012). The evidence collected from this experiment so far favors the notion that, indeed, the Higgs boson does exist. The *Nature* magazine commentator concluded that a number of alternative possibilities will have to be re-evaluated and, in all likelihood, some of the theories on the subject will have to be abandoned. This conclusion reinforces a practice in the exact sciences (in this case, physics) where it is acknowledged that not everyone has a piece of 'the truth'. In physics, at least, the tradition of testing theories and dropping them when proven inadequate has been applied and accepted by all players. A welcomed novelty would be the adoption in cancer biology of this centuries-old practice that has been so beneficial to the progress and the prestige of physics and even branches of biology, with the exception of cancer research (Soto and Sonnenschein 2012).

In Hallmarks II, H&W proposed to hybridize their long-held reductionist approach to an organicist one, where the complex microenvironment with its multiplicity of cell types plays a role in carcinogenesis as illustrated in their Figure 4. H&W also introduced a provocative caveat in this hybridizing compromise whereby this combination of reductionism (represented by the SMT and their always *cell-based* variations) and the participation of the microenvironment would be only operative in carcinomas, which happen to represent over 90% of the cancers that bring patients to the clinic. The other 10% of cancers are represented by connective tissue cancers (sarcomas and blood cancers). It is noteworthy however, that the oncogene pioneers, among which Weinberg featured prominently, focused their efforts on so called 'viral carcinogenesis' while using chicken and mouse fibroblasts in culture conditions (Rubin 2011b). The sarcomas resulting from the local inoculation of millions of fibroblast-like cells did not justify their inclusion in this generalization in the genesis of carcinomas, i.e. tumors that have a significantly different histogenesis and histopathology (Cardiff *et al.* 2008).

In Hallmarks I and II, the mounting evidence for a causal role of the stroma in carcinogenesis is incorporated within the oncogene theory by proposing that 'incipient neoplasias begin the interplay by recruiting and activating stromal cell types that assemble into an initial pre-neoplastic stroma, which in turn responds reciprocally by enhancing the neoplastic phenotypes of the nearby cancer cells.' Thus, the increasing participation of the microenvironment in carcinogenesis, an alternative originally unworthy of being considered within the SMT scenario, is now morphed into the problem of how mutated genes recruit otherwise normal neighboring stroma cells. H&W, as noted by J Marcum, 'believe cancer is ultimately and fully explainable in terms of mutated genes'. The increasingly popular compromise of unifying the SMT with events localized in the microenvironment ignores the fact that the premises on which the SMT and the TOFT are founded are incommensurable

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because (a) they occur at different levels of biological organization and (b) they are diametrically opposed to each other (Soto and Sonnenschein 2011; Baker 2012, 2013).

8. An alternative view on carcinogenesis

Our analysis of the alleged Hallmarks of Cancer lead us to conclude that they are not the hallmarks H&W assumed them to be and that their adoption as such are unlikely to remedy, first, the stagnation of current SMT-based cancer research and, second, equally important, the frustrating experiences of patients and doctors in the therapeutic front. But… are there alternatives that may offer a more promising outlook to the failure of the SMT, its variants and the hybridized theories (SMT plus the micro-environmental component of the TOFT)?

A conceptually and pragmatically significantly different strategy to explain carcinogenesis has been based on the premises adopted by the TOFT. To repeat, those premises are (a) cancer is a *tissue-based* disease, and (b) *proliferation* and *motility* are the default states of *all* cells. The rationale for why these premises are reliable and evolutionarily relevant to biological theory has been explicitly described and debated, and more importantly, the theory has been successfully tested at the laboratory bench using *in animal* approaches (Soto and Sonnenschein 1987; Sonnenschein and Soto 1999; Maffini *et al.* 2004; Maffini *et al.* 2005; Soto and Sonnenschein 2011). Briefly, the experimental protocol we designed gave equal chances to both the SMT and TOFT to provide a comprehensive explanation for the generation of mammary gland tumors in rodents. When rats of the susceptible WF strain are injected with the mutagenic carcinogen *N*-nitrosomethyl urea (NMU) at 50 days of age, they develop mammary carcinomas with an incidence of 80–100%. Under the SMT the results are interpreted as an effect of the mutagen in the DNA of epithelial cells; in this view, the epithelial cells are the target of the carcinogen. From the TOFT perspective, the target is the reciprocal interactions between stroma and epithelium; these are the interactions that mediate normal mammogenesis. According to the TOFT, alteration of this reciprocal interaction would lead to carcinogenesis. From this perspective, the stroma could have been the primary target of the carcinogen. Thus, isolated epithelial cells were exposed to the carcinogen or to its vehicle *in vitro*, while the stroma, represented by the epithelium-free cleared fat pad, was exposed *in vivo*. The half-life of NMU is 20 min. Five days after exposure epithelial cells were injected into the cleared fat pads to produce 4 types of recombinants. Cancer only developed in the recombinants whose stroma was exposed to carcinogen. Exposure of the epithelium did not result in cancer outcomes, instead, normal ducts developed from them when recombined with vehicle-exposed stroma.

The generation of adenocarcinomas by the epithelial cells that were never directly exposed to mutagenic agents is compatible with the expectations of the TOFT and incompatible with the predictions of the SMT. Similar conclusions are drawn from the outcome of the groups of rats that received epithelial cells that, while exposed to NMU, did not develop tumors when injected into fat pads that were not exposed to the mutagen (Maffini *et al.* 2004). The overall results from these experiments favour rejecting the SMT while satisfactorily explaining carcinogenesis based on the evolutionary-relevant premises adopted by the TOFT.

Consistent with the notion that carcinogenesis results from the faulty interaction between stroma and epitheliium is evidence generated in other experimental models like the one whereby the fate of transplanted pre-neoplastic hepatocytes depends on whether they are incorporated into either normal or in retrorsine (a naturally occurring pyrrolizidine alkaloid)-exposed livers: in the latter, those hepatocytes generate hepatocellular carcinomas while they become ‘normalized’ when implanted into livers of untreated hosts (Laconi 2007).

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In order to buttress the arguments supporting the adoption of the TOFT, we explored an additional possibility anticipated by this theory, i.e. that cancer is a reversible developmental disease. This notion opposes the inference drawn from the SMT that being a genetic disease due to the accumulation of stable somatic mutations, cancer becomes irreversible. When testing whether cancer is a reversible process, we devised an experimental protocol where epithelial cells from the type of rat mammary adenocarcinomas induced by NMU would revert to normalcy. The results of this experiment showed that the mammary epithelial tumor cells injected in cleared fat pads of older rats and in twice-pregnant mothers became normalized and formed normal ducts at the site of inoculation (Maffini *et al.* 2005). These data strengthen conclusions drawn from experiments conducted during the last four decades while the SMT was becoming the prevalent theory of carcinogenesis. There are multiple examples of normalization of cells that once belonged to a cancer: from teratocarcinoma cells that when injected into blastocysts populate all tissues and even develop into normal gametes (Mintz and Ilmensee 1975; Illmensee and Mintz 1976), to melanoma cells normalized when injected into embryos before the neural crest cells migrated (Hendrix *et al.* 2007), to teratocarcinoma and mammary carcinoma cells that form normal structures when placed into the mammary gland of mice (Bussard *et al.* 2010; Booth *et al.* 2011).

9. Reactions from the scientific community and the public to the failure of the prevalent theory of carcinogenesis

An increasing proportion of the scientific community and the public at large are becoming aware that the well-meant but misguided efforts started in the 1970s are unlikely to render the desirable outcomes. Almost imperceptibly, probably due to this realization, both researchers who have pursued the research strategies of the SMT and organizations that represent cancer patients are sensibly switching the emphasis of their future efforts in the direction of cancer prevention (Interagency Breast Cancer & Environmental Research Coordinating Committee 2013; Vogelstein *et al.* 2013). Lately, this goal is being viewed as more realistic, socially responsive and financially sound when compared to the aggressive therapeutic options of chemotherapy, surgery and radiation. Nonetheless, despite recognizing the shortcomings of a failed research option, the current international consensus continues to emphasize an agenda consistent with the SMT (Varmus and Kumar 2013). Based on this conceptual disconnect, the need to go back to the metaphoric blackboard and re-evaluate objectively and dispassionately what has been done, what has been useful, and what has been superfluous and wasteful is becoming imperative. Searching for pragmatic answers to resolve the cancer puzzle by plodding on the same unproductive ideas advanced by the SMT is unlikely to satisfy the wide array of stakeholders firmly committed to find a solution (Baker 2012, 2013).

10. Conclusions and future vision

Our analysis substantiates the fact that the so-called Hallmarks of Cancer, a byproduct of the century-old SMT, has failed when assessed by the two main pragmatic criteria used to evaluate the success of any theory, namely, (a) it has not contributed to an increased understanding of the phenomenon under study, in this case cancer and (b) there has not been a meaningful beneficial translation of this research program at the site where it counts, that is, in the cancer ward.

The stated purpose of writing the 2011 version of the Hallmarks has been ‘to revisit, refine, and extend the concept of cancer hallmarks, which has provided a useful conceptual framework for understanding the complex biology of cancer’. A cursory analysis of these highly selectively referenced reviews becomes perplexing when comparing claims and contradictions with the reality of the cancer ward. A new conceptual approach is in order.

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We have provided a blueprint on which to execute such novel approach based on the tissue organization field theory. To implement this blueprint, the reductionist philosophy at the core of the SMT and its exclusive focus on bottom-up causation should be abandoned. Its continuous invocation of 'programs' and 'mechanisms' is a reflection of inadequate metaphors borrowed respectively from computer sciences and outdated physics; organisms are neither computers nor machines (Longo *et al.* 2012; Tallbot 2013).[3] Instead, an organicist approach whereby causation occurs in all directions, namely bottom-up, top-down and reciprocal, will best illuminate the complexity of cancer. In this perspective, there are no privileged causes; mechanical forces, electrical fields and molecules all contribute to the formation of a biological structure, be it normal or a cancer (Soto *et al.* 2008). Additionally, rather than looking at complexity as if it was a hindrance, we should embrace it by developing the appropriate theories and tools to study it. Systems biology offers such a possibility. Mathematical modelling and computer simulation offer great heuristic value, and may help practitioners to separate promising hypotheses from the bad ones (Soto and Sonnenschein 2012).

As for cancer being an exceptional disease, a pretentious 'emperor of all maladies', we are looking forward, instead, to seeing cancer research and cancer biology disappear as independent branches of biology and become, instead, an integral part of an enlarged field of developmental biology. A rational discussion of the merits of this alternative to the SMT will provide a refined understanding of normal and neoplastic alterations of development based on reliable evolutionarily-relevant premises. A productive debate within this context may then contribute to a realistic approach to cancer therapy.

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[3]The success of molecular biology techniques when sequencing the human and other genomes exposed the unsustainability of reductionistic, of linear thinking, genetic determinism and additional failed ideologies. These issues are analysed in the book *Genetic explanations: Sense and nonsense* (eds) S Krimsky and J Gruber 2013 (Cambridge, MA: Harvard University Press).

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