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Towards a systemic paradigm in carcinogenesis: linking epigenetics and genetics

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Abstract For at least 30 years cancer has been defined as a genetic disease and explained by the so-called somatic mutation theory (SMT), which has dominated the carcinogenesis field. Criticism of the SMT has recently greatly increased, although still not enough to force all SMT supporters to recognize its limits. Various researchers point out that cancer appears to be a complex process concerning a whole tissue; and that genomic mutations, although variably deleterious and unpredictably important in determining the establishment of the neoplastic phenotype, are not the primary origin for a malignant neoplasia. We attempt to describe the inadequacies of the SMT and demonstrate that epigenetics is a more logical cause of carcinogenesis. Many previous models of carcinogenesis fall into two classes: (i) in which some biological changes inside cells alone lead to malignancy; and (ii) requiring changes in stroma/extracellular matrix. We try to make clear that in the (ii) model genomic instability is induced by persistent signals coming from the microenvironment, provoking epigenetic and genetic modifications in tissue stem cells that can lead to cancer. In this perspective, stochastic mutations of DNA are a critical by-product rather then the primary cause of cancer. Indirect support for such model of carcinogenesis comes from the in vitro and vivo experiments showing apparent 'reversion' of cancer phenotypes obtained via physiological factors of cellular differentiation (cytokines and other signaling molecules) or drugs, even if the key mutations are not 'reversed'.

Keywords Carcinogenesis · Genetics · Epigenetics

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Cancer as a genetic disease: the somatic mutation theory

> The revolution in cancer research can be summed up in a single sentence: cancer is, in essence, a genetic disease [1]

The genetic basis of cancer was first recognised in 1902 by the German zoologist Theodor Boveri, who postulated that chromosomes transmitted inheritance factors; proposed the existence of cell cycle check points [2]; suggested that mutations of the chromosomes could generate a cell with unlimited growth potential which could be passed onto its descendants; observed aneuploidy in cancer cells that had acquired the potential for uncontrolled continuous proliferation [3]; speculated that cancers might be caused or promoted by radiation, physical or chemical insults or by pathogenic microorganisms [4, 5]. The hypothesis of cancer as the result of accumulated mutations to a cell's DNA was first proposed by Nordling in 1953 [6] and better formulated by Knudson in 1971 [7]. In the last 30 years, at least since Feinberg and Vogelstein reported the first mutation known to result in a human transforming gene (the *c-Ha-ras* oncogene) [8], cancer has definitely been considered as a genetic disease and the somatic mutation

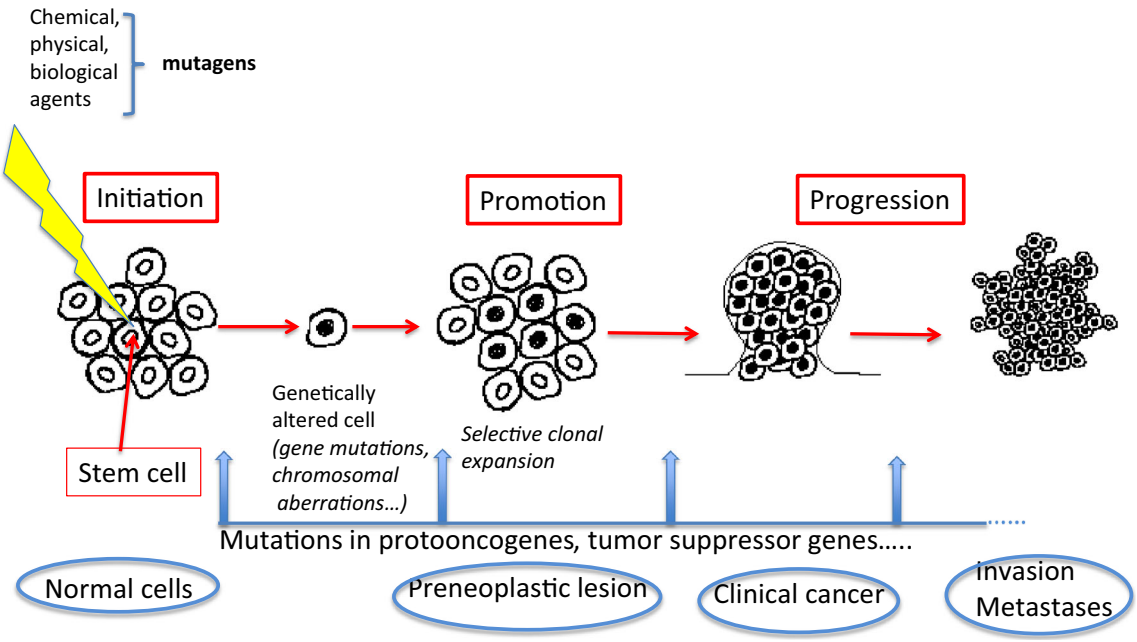

Fig. 1 From the somatic mutation theory (SMT) to the stem cell theory of cancer

theory (SMT) became the dominant paradigm in carcinogenesis. In SMT carcinogenesis is generally conceived as a multistep process, including initiation, promotion and progression: a multifactorial pathology characterized by the accumulation of a multitude of genetic and cytogenetic alterations [9] leading to malignancy [10, 11] (see Fig. 1).

The SMT's fundamental assumption is that there is no cancer without genetic or chromosomal harm. It is true that in the cells of the neoplastic clone, gene mutations and/or chromosomal aberrations, genomic instability and aneuploidy are always present, albeit differently interpreted and connected to each other. In 1991 Loeb, elaborating the thesis proposed by Nordling on the tendency of actively proliferating cells to accumulate mutations, formulated the theory of *mutator phenotype*, stating that an intrinsic tendency of the neoplastic clone is to accumulate genetic and chromosomal alterations [12].

The Hallmarks of cancer

In 2000 Hanahan and Weinberg [13] defined, in one of the best known, most cited and recently revisited [14] paper in this field, the basic features conferred on a tumor cell by the progressive accumulation of mutations. A total of eight/ten "hallmarks" were noted to be 'acquired capabilities shared by most and perhaps all types of human cancers': self-sufficiency in growth signals, insensitivity to inhibitory signals, evading apoptosis, limitless replicative potential (by reactivation of telomerase and immortalization), sustained angiogenesis, tissue invasion, tendency to metastasize, genomic instability (as a facilitator of tumor progression), metabolic reprogramming, and evasion of the immune system.

Curiously, one fundamental hallmark of cancer cells, the *loss of differentiation* (or, according to an epigenetic and dis-ontogenetic model, a *lack of differentiation*), was not considered, even though it is arguably a main feature of almost all cancers, as properly stressed by some authors [15] who considered it a primary and essential discriminant between benign and malignant tumors, supported by a measurable expression switch of all categories of genes [16] and a robust diagnostic index for some tumors [17].

By this way the SMT took its definite shape, according to which cancer is the consequence of the onset, in one or more somatic cells, of stochastic mutations in some key genes [18], responsible for the control of proliferation, programmed cell death (apoptosis) and DNA repair, i.e.: *oncogenes* [19] and *tumor suppressor genes* [20].

In the SMT one can discern the transposition, at the cellular level, of the classic, neo-Darwinian evolutionary model [21, 22]: among the myriad of mutations continuously damaging our DNA, some are able to confer to a cell clone a selective advantage to ultimately prevail and stabilize the cancer clone [23, 24].

The SMT also interprets the increase in human cancers in post-industrial societies, characterized by a significant lengthening of lifespan: it assumes that DNA gradually destabilizes with age, due to an accumulation of oxidative damage [25–28].

Criticism of the SMT

Criticism of the SMT has become varied and significant, although it is still not enough to force its supporters to recognize its shortcomings.

First of all, in many cases, cancer appears to result from a complex process involving a whole tissue: many carcinogens initially act by disrupting the normal interactions taking place among cells in the parenchyma and stroma [29, 30]. Genomic mutations, although variably deleterious, are in this model not directly responsible for the development of a neoplasia, instead representing epiphenomena unrelated to cancer causality [31].

Many of these criticisms of the SMT have their origin, as we will later see, in earlier theories of cancer as a product of distorted development of a whole tissue. It is undeniable that the SMT fails to recognize the key role played by the microenvironment [32–34] (stroma [35–39], endothelial cells [40], activated macrophages [41] and the surrounding tissues [42–44]); or the distorted developmental course followed by the neoplastic tissue [45–47].

Other arguments against the SMT arise from the fact that it is often not possible to demonstrate either the existence of specific mutations ending in a well-defined neoplastic type [48, 49], nor a clear relationship between mutations and tumor progression. Even in the classic Vogelstein-model of colon cancer, no mutation is necessary and sufficient to determine the transition from one to another stage of neoplastic progression [50]. Moreover the SMT is not able either to adequately clarify the action of non-mutagenic carcinogens, nor to adequately explain many complex tumor phenotypes and carcinogenic processes [51].

Even though stochastic mutations have generally been recognized as the main source of genome instability in cancer, one well-known alternative hypothesis suggests that *aneuploidy* is the primary cause of genome instability, rather than a simple consequence of the malignant process (a thesis already proposed by Bovery more then a century ago) [3]. Some researchers documented both the early occurrence of aneuploidy in neoplastic cells lacking mutations [52] and the high frequency of tumors in syndromes characterized by karyotypic abnormalities (trisomies, disomies) [53, 54]. We should bear in mind that the *Down Syndrome* is characterized by increased tumors peculiar in type, location and shape, which are better interpreted as the product of a genomic unbalance, than as the result of stochastic mutations [55]. Also in this case mutations and chromosomal aberrations of various kind and type, more or less specific for different forms of cancer, would be considered a consequence rather than a cause of cancer.

Also arguing against the SMT, and in favour of a model of direct induction of genetic and epigenetic reactive-adaptive changes by the information coming from the environment, is the enormous prevalence of neoplastic processes affecting tissues persistently exposed to pollutants (skin, pulmonary, gastrointestinal, uterine epithelia) [56] and/or solicited by continual exposure to these agents to react (above all immune and neuro-endocrine systems). These considerations together reduce the validity of the SMT which is gene-centric and explicitly stochastic, demeaning at least in principle any direct causal role of environmental factors in carcinogenesis [57].

The SMT is partially undermined by epidemiological studies, which increasingly (and with better quality) demonstrate a continuous increased frequency of tumors in populations exposed to low levels of many agents [58] not well explained by increasing population age or diagnostic improvements; analogous trends for the immune-mediated, endocrine-metabolic and neurodegenerative diseases that are, even more clearly linked to changes in the environment and lifestyles [59]; a wide incidence variation of diverse cancers (and chronic diseases in general) in different regions of the world, shown to be linked to lifestyles and exposure to toxic agents, particularly through the food chains, rather than to genetic predisposition [60, 61]; an increase of cancer and chronic inflammatory and degenerative diseases in areas affected by high rates of environmental pollution [62–64]; the significant increase in childhood cancer during the last 40 years, which radically contradicts carcinogenesis via the stochastic accumulation of mutations [65].

In this regard, among the most alarming epidemiological data is the annual increase of 2 % in infant cancer established by the recent ACCIS project, a large scale monitoring conducted by the IARC, using 63 cancer registries throughout Europe [66]. Moreover the age of peak incidence of cancer in children occurs during the first year of life, in infants, and at least the first stages of the malignant process are already present at time of birth. The importance of genetic events in utero has been long suspected, for many years, on the basis of the correlation studies on twins with leukemia [67]. That some cases of leukemia originate in utero is also shown by genetic studies from blood samples (Guthrie cards) taken from infants who subsequently would develop leukemias, translocations and gene sequences corresponding to the fusion genes later found in leukemic blasts [68]. Moreover pro-leukemic translocations and clones are found in foetuses with a much higher frequency than the incidence of leukemias: the common leukemic fusion genes, TEL-AML1 t (12; 21)(p12; q22) or AML1-ETO t (8; 21)(q22q22), are found in the cord blood with a frequency 100 times greater than the corresponding risk of leukemia [69]. This is usually interpreted as

evidence of the fact that translocations do not necessarily determine the onset of leukemia, which would require additional genetic events during the postnatal period [70]. An equally interesting interpretation is to hypothesize that as less than 1 % of children who "produce" a translocation will develop leukemias, the translocation is an active, potentially positive, adaptive genomic change, e.g. a response to toxic exposures in utero.

Towards a new model in carcinogenesis

It is evident that all these epidemiological data—above all the constantly increasing rates of cancer in the last century (especially among the younger) and the global shifting burden of illness toward chronic diseases (endocrine-metabolic, inflammatory, immunologic, neurodevelopmental, neurodegenerative, oncologic, etc.)—could be better explained by growing epigenetic discrepancy in *tissue programming* (Developmental Origins of Health and Disease (DOHaD) theory [71]), rather than by genetic stochastic mutations. Such relevant epidemiological transitions are far more consistent with epigenetic changes, induced by environment and microenvironment, which occur orders of magnitude more frequently than DNA sequence changes. Genomic instability too is actually better conceived as the result of epigenetic changes (wide-ranging DNA hypomethylation and retro-transposon mobilization, hypermethylation of CpG islands at tumor suppressor gene promoters, microRNAs networks changes) [71] induced by persistent stressors, rather than as a consequence of stochastic mutations

To put it briefly, the SMT is based on the linear model of genomic information created by molecular biologists in the 1960s; in which the fundamental part of the genome are the *genes*—ordered sequences of nucleotides conceived as single entities random distributed along the huge DNA molecule that composes the human genome—regarded as the key determinants of form and function. Smoothly fitting into this simple model is the idea that stochastic DNA mutations would determine the malignant fate of cells by damaging some key genes (oncogenes and tumor suppressor genes) coding for proteins deputized to cell cycle control, differentiation, receiving and processing signals from the outside, apoptosis. Many of the assumptions associated with this molecular-reductionist approach are no longer accepted by scientists [72]: the discovery of alternative splicing RNA editing, small regulatory RNAs and post-translational protein modification added layers of complexity which are not controlled by genes [73]; epigenetics has shattered the central dogma that the genetic information flow is unidirectional, forcing all to critically reconsider the role of DNA and the current evolutionary and pathogenic models. The genome is more and more considered as a complex, composite and dynamic molecular frame, in which DNA is both stable component (inheritance), and changeable in response to signals from (its) environment. The "classic" model of DNA as a *Read-Only Memory (ROM)* program, subject to change only by copying errors and accidents (random mutations), is going to be replaced by a dynamic model of genome, which is a sort of *Read-Write (RW) data storage system*, which not only uses coded instructions, but incessantly receives and elaborates signals, (re)actively changing its software (the epigenome) and, in the medium to long term, its hardware (DNA) [74].

In such a context, the models of carcinogenesis are changing too, and cancer is more and more considered as a complex, systemic disease [75] starting from stem cells rather than from well differentiated somatic tissue cells and involving the entire tissue, if not the organism [76, 77]. Information coming from the environment (and in particular from the cellular microenvironment) is actively processed by the epigenetic "software" of tissue stem cells (still characterized by great developmental plasticity) pushing them to change in an active, responsive and potentially adaptive way [78]. In such a perspective carcinogenesis should be considered a potentially adaptive and a dys-ontogenic/evolutionary process: the product of a tissue differentiation process gone awry, rather than a loss of differentiation from random genetic mutations [79]. In such an "evolutionary context" the main molecular changes (mutations, translocations, duplications, etc.) with cell transformative potential (driver mutations) should be considered (in ontogeny as in phylogeny) as potentially adaptive events, rather than simple stochastic mutations and/or chromosomal aberrations.

Perhaps the most emblematic example is given by translocations: considered within the SMT as "chromosomal aberrations", these rearrangements between definite coding and/or controlling regions in different chromosomes are often related to particular exposures and produce—as we have already seen in the context of childhood leukemias—specific and significant effects.

It is not possible to review here this issue in depth, but remind what seems to occur in areas with persisting pollution, as in Seveso (Italy), where decades after the industrial accident which released dioxin contamination over a wide area, many residents show a high number of translocations 14:18 (typical of follicular lymphoma), although without developing lymphoma [80]. The same translocation is frequent in long-time pesticides applicators [81]. This is clear evidence that it is not the chemical agent that directly determines the specificity of the genome change, but the active reaction of the genome itself to the environmental change

If we consider that in this, as in other pro-leukemic or pro-lymphoma translocations [82], an oncogene (*BCL-2*–an anti-apoptotic gene, physiologically activated in memory lymphocyte populations [83] and in B lymphocytes "immortalized" by Epstein Barr virus [84]) is constantly activated because of the juxtaposed enhancer sequence of the gene coding for the immunoglobulin heavy chain, the hypothesis of translocations as genomic active, adaptive (potentially defensive) changes, not simple "chromosomal aberrations", acquires greater meaning.

A new carcinogenesis paradigm: from genetics to epigenetics

Those who adhere to the paradigm of stochastic mutations and more generally to a linear and gene-centric model of DNA, have evidently some difficulty in accepting a carcinogenesis model in which the "information flow" goes from the (micro)environment to DNA, genes constantly need to be told to switch "off" and "on" and gene expression regulation is not controlled by DNA, but by many epigenetic networks reacting to environmental information [85]. In such a systemic, non-linear model the environment should be considered as a continuous stream of information interacting with cells, forcing their epigenome to adapt: the 'transparency' of the chromatin for transcription factors being largely a matter of nucleosome structure, dynamically modulated by histone methylation, acetylation, and phosphorylation [86, 87].

In the last decade the research on cancer has highlighted the prominent role of an altered epigenetic regulation of gene expression [88, 89]. In 2006, Feinberg et al. suggested that epigenetics and genetics should be combined to achieve better understanding of cancer, which latter is the product of "a polyclonal epigenetic disruption of stem/progenitor cells, mediated by tumour-progenitor genes" [50]. This early *paradigm-crack* since gained currency, as it became evident that *epimutations* can outnumber genetic abnormalities, and they often occur early in cancer development [90–92], representing the adaptation of cells to a sustained *stress* environment [93].

Aberrant methylation of genes controlling the cell cycle, proliferation, apoptosis, metastasis, drug resistance, and intracellular signalling have been identified in multiple cancer types [94]. Recent advancements in whole-genome analysis of the methylome have yielded numerous differentially methylated regions, the functions of which remain largely unknown [95, 96]. However, as a general rule, we can say that in cancerous and pre-cancerous cells, *global DNA hypomethylation* (particularly of regulatory sequences), leads to genomic instability, loss of imprinting (LOI) [97], activation and mobilization of retrotransposons, transcription of proto-oncogenes [98, 99] and other genes encoding proteins involved in genomic instability [100] and metastasis [101]. Whereas, on the other hand, *hypermethylation of the promoter sequences of various tumor suppressor genes (TSGs)* blocks transcription factor-binding sites, causing their transcriptional silencing [102–106]. Moreover recent cancer genome analyses have identified an impressive and growing number of epigenetic enzymes that are deregulated in many types of cancers [107], whereas most (although not all) miRNAs are under-expressed compared to normal tissues. Finally some experiments demonstrated that even within a single lineage, distinct patterns of miRNA expression can be detected that reflect an extensive range of mechanisms of transformation, further supporting the idea that "miRNA expression patterns encode the developmental history of human cancers" [108].

Another remarkable discovery of some years ago contributed to overthrow the conventional view that cancer always develops in a steady, stepwise progression. In at least one in forty cancers (of the common types) the genome can be shattered into hundreds of fragments in a single cellular catastrophe, wreaking mutation on a massive scale [109]. This new model of massive rearrangement acquired in a single cataclysmic event has been named *chromotripsis* and represents a sort of crisis of the entire genomic system: something very different from the slow and gradual accumulation of stochastic mutations described in the SMT.

Such evidence obviously requires the acceptance of a radically new model of the genome, considered as a unitary and complex, dynamic and responsive molecular network [110], directly and incessantly responsive to information coming from the (micro)environment. *In such a model*, epigenetic (global DNA hypomethylation, hyper-methylation of promoter sequences of tumor suppressor genes, global down-regulation of miRNAs) [71], genetic (genomic instability, mobilization of transposable sequences [111]) and chromosomal mutations (translocations) could be seen as steps of a failed or distorted [112] evolutionary (adaptive and essentially defensive) [113] process, determining the progression of cancer. In such a context, epigenetic modifications are the main mechanism by which cells convert the information coming from the environment in phenotypic alterations [114]. This also accommodates the studies presenting cancer as a product of aneuploidy and consequent chromosomal and genetic instability, independently of gene mutations. Finally, the neoplastic process is considered to be the consequence of persistent environmental stress.

Different theories in carcinogenesis: a simplified starting point

At this point we must ask if there is a theory in carcinogenesis strong enough to counteract the SMT. Vineis et al.

have significantly clarified the field, suggesting that carcinogenesis theories can be classified according to two models i.e. (i) some biological changes in cells alone may lead to malignancy and (ii) changes in stroma/extracellular matrix are necessary for malignancy [115].

Relying on this simple scheme we can establish that if the SMT is the prototypical (i) model, we should put in the (ii) class every theory that recognizes cancer as a disease of the entire tissue (organism); i.e. "epigenetically" induced by information coming from the microenvironment that are:

- sufficiently persistent;
- recognized as potentially harmful by tissue stem cells (physiologically appointed to the repair and remodeling of the tissue itself);
- able to push the genome of these cells to implement potentially reactive and defensive processes: at first epigenetic (DNA large-scale hypomethylation and retro-transposons' activation, hypermethylation restricted to promoter sequences of the tumor suppressor genes, etc.), then genetic and chromosomal (e.g. translocations).

Evidently, in this context, the thousands of stochastic mutations that characterize most of the neoplastic cell lines and that undoubtedly play an important role in its development and stabilization should be considered as a consequence, rather than the cause of this epigenetic activation and the subsequent (progressive) genomic instability.

The Embryonic rest theory and 'field' theories of cancer

It is interesting, at this point, to remind that the idea of cancer as a *dis-ontogenetic* disease originating in *tissue stem cells* (the "*tissue remnants of embryonic cells*" endowed with enormous reparative potential, but also able to differentiate in many ways, depending on the information coming from the microenvironment) was already proposed more than a century ago by some German pathologists. This was the *Embryonic rest theory of cancer* [116, 117], which definitely represented a perfect (ii) model of carcinogenesis.

On observing cancer tissues under the microscope, Virchow and other well known pathologists, noted the similarity between embryonic tissue and cancer, and suggested that tumors arise from embryo-like cells [118]. On this basis, some Virckow's followers [117, 119] formulated the theory that adult tissues contain *dormant embryonic remnants* that could be activated to become cancer. Remarkable for his era, the hypothesized trigger of the process would be a change in the environment, a "disequilibrium" in the surrounding tissue, that would induce these embryonic remnants to resume cell proliferation and to produce masses of cells that resembled fetal tissues (*field theory*).

Virtually all these researchers were aware that the "field" (the tissue microenvironment) plays a key role in the genesis of cancer. Conheim hypothesized that the benign or malignant character of a tumor depends on the behaviour of the remainder of the organism [120]; Ribbert in 1904 proposed that the critical factor for expression of the malignant phenotype of cells is their isolation from a normal controlling environment [121, 122]. Moreover, since then and for many years, a relationship was recognized between developing systems and tumor growth, and more specifically between cellular mechanisms of normal growth and differentiation in the embryo and irregular growth and abnormal differentiation in cancer.

In fact the basic paradigm of embryology, the idea that gave it structure and coherence, was the *morphogenetic field*, first postulated by Boveri, designating areas of embryological information, bound by physical substrates [123]. The components of these fields create a web of interactions such that any cell would be defined by its position within its field. Like an electro-morphogenetic field, the term denoted both informational and regional relationships.

The abandonment and resurfacing of the *embryonic theories* of carcinogenesis

However by the turn of the 20th century, the embryonic rest theory was generally discredited, simultaneously with the abandonment by Morgan and other biologists of the embryological context of evolution in favour of the genetic context. Morgan, who had once been second only to Child in his publication record on gradient fields, began to consider such work "old-fashioned and not good science": he was convinced that genetics would lead evolutionary biology out of natural history into 'objectivity', and this way of thinking had an important influence both in the context of evolutionary thought and among cancer researchers [124].

In some way the concept of "*field cancerization*" was re-introduced in 1953 by Slaughter [125], who discovered the existence of histologically abnormal tissue surrounding oral squamous cell carcinoma and explained in this way the development of multiple primary tumors and locally recurrent cancer. Organ systems in which field cancerization has been described since then are: head and neck (oral cavity, oropharynx, and larynx), lung, vulva, oesophagus, cervix, breast, skin, colon, and bladder.

Concurrently, studies on teratocarcinoma led to a reassertion of the Embryonic rest theory, in the form of the stem cell theory of cancer [126]. More recent molecular findings (DNA amplification techniques, immunohistochemistry, in situ hybridization) supported a carcinogenesis model in which the development of a field with genetically or epigenetically altered cells plays a central role: loss of heterozygosity (LOH), microsatellite alterations, chromosomal instability, and loss of expression (or mutation) of the tumor suppressor genes (mainly the *TP53* gene) are the main "*markers*" used [127].

The great lesson of teratocarcinoma and the stem cell theory of cancer

The best model for exploring the developmental origins and the implications of distorted differentiation in cancer is the teratocarcinoma, a malignant tumor characteristically containing undifferentiated stem cells known as embryonic carcinoma (EC) cells—which are the malignant counterparts of embryonic stem (ES) cells derived from the inner cell mass of blastocyst-stage embryos [128]—as well as differentiated elements of many cell types.

As early as in 1907 some scientists had noted that teratoma cells could differentiate into normal somatic tissues, composed of normal embryonic germinal layers [129]. In the 1960s it was observed that, in teratocarcinomas, the stem cells not only generated more stem cells but also differentiated cells that gave rise to non-tumorigenic tissue.

In the 1980s it became clear that the transplantation of pluripotent or embryonic stem cells into adult mammals, frequently leads to the growth of teratomas, which can turn into malignant teratocarcinomas [130]. However, intriguingly, putting the teratocarcinoma cells into an early mammal embryo at the blastocyst stage [131], they became incorporated in the cell mass, generating normal tissues (including gonads) in viable mosaic individuals resulting from this manipulation [132]. This meant that, in subsequent generations, normal offspring could result from a cell that was previously a cancer cell [133].

The microenvironment was central to these paradigm-breaking findings. The origin of the teratoma appeared to be a dissonance, a mutual "*misunderstanding*" between young donor cells and surrounding adult cells (the so-called *niche*) of the recipient. In some subsequent experiments, normal germinal stem cells that became cancerous, showed the potential to revert to normal cells if placed in an embryonic tissues, which confirmed that the "field" where the stem cells were located established their normal or malignant potential.

The main questions, at that point, were: is the teratocarcinoma model only an exception in carcinogenesis? If the carcinogenic process is, in essence, the ontogenic development gone awry, what are the signaling molecules, i.e. the physiological inducers of the correct differentiation process (and can we use them to obtain the reversion of the neoplastic process)? Is it possible to establish an experimental model system to examine whether malignant cells could be reprogrammed to revert back to "normal" cells, showing normal growth control? [134].

Virtually all studies conducted over the past half century to respond to these questions returned consistent results, summarized as follows:

- the main cause of cancer is a block in cell differentiation programs (the "hallmark", inexplicably neglected by major theorists of SMT);
- the best way to reduce cancer would be removing this differentiation block and switching on programs that are "the reverse of malignant transformation pathways";
- inducing differentiation by normal signaling molecules (cytokines etc.) configures an epigenetic suppression of malignancy, which bypasses the genetic abnormalities in tumor cells;
- in this way cancer cells may re-establish the distorted molecular circuits and revert several processes resulting in malignancy (chromosomal instability, translocations, oncogenes activation and loss of tumor suppressor genes) [135].

The first cell culture system in which normal hematopoietic cells were programmed to develop, in the presence of feeder cells, into different cell lineages was established a half century ago [136]. It soon became evident that the inducers of hematopoietic cell clones, present in conditioned medium, were produced by the feeder cells [137–139]: this allowed the identification, purification and gene cloning of the main *cytokines* regulating hematopoiesis [140]. It was also shown that, at appropriate in vivo locations, hematopoietic stem cells could differentiate into glial cells [141], skeletal muscle [142], liver cells [143] and epithelial cells [144]; showing considerable plasticity and the capacity of reprogramming their gene expression in response to right combination of "niche" signals.

By the 1990s, the principles of the *stem cell theory* of cancer had been clearly established. However for a long-time the teratocarcinoma-cancer stem cells-field theory was considered to be an exception to the rule; and the results of chemical carcinogenesis studies were used to support the SMT and *de-differentiation*, rather than an epigenetically induced and reversible blockage in differentiation programs [145] with the potential to reverse many cancers.

Only recently have some scientists positively returned to the *embryonic rest theory* and the *field theories,* reminding us that "histogenesis occurs during embryonic and fetal development as an orderly process regulated by cell-to-cell

interactions" within "morphogenetic fields'' [124]. In particular, describing cancer as an emergent phenomenon resulting from a flawed interaction among cells and tissues, Soto and Sonnenschein have build up their *tissue organization field theory* (*TOFT*) of carcinogenesis, asserting that the causal role of the SMT on carcinogenesis is "substantially undermined, if not practically falsified" [31].

The original embryonic rest, the stem cell and the TOFT theories of cancer are based on four major concepts: cancer arises from stem cells that are present within all tissues since the first stages of ontogenesis [146]; *oncogenesis is ontogenesis gone awry* [147]; cancers are composed of the same types of cells as are normal tissues—stem, transit amplifying, and terminally differentiated cells [127]; some persistent stress signals triggers the malignant transformation of these cells [148].

All the data and theories exposed above lead us to propose an interpretation of cancer as a para-physiological process (reactive, defensive, reparative, adaptive) that becomes pathological because of the persistence of some tissue inducers and/or environmental trigger and the gradual acquisition by cells of some necessary, but potentially dangerous, features. The block in differentiation programs appears to be central to carcinogenesis.

Tumor reversion: an epigenetic task

Critically, all these non-SMT theories consider cancer as a potentially *reversible process* to the extent that the "field" is again to send correct, epigenetic signals (in practice the signals of a correct ontogeny) to the cells *"perverted"* by the process of malignant transformation.

In summary: for 40 years we have known that a block of cell differentiation programs is a main flaw in tumor cell populations; for 30 years we have known that this block can be solved, at least in some cases, by providing the correct "information" (differentiation signals) to the tissue; 10 years ago we discovered some epigenetic mechanisms that allow "reversal" of the neoplastic phenotype. In fact the best evidence that cancer should be considered primarily an epigenetic disease, due to alterations of the differentiation and proliferation programs (reactive, adaptive, defensive changes in gene expression) rather than to stochastic mutations of DNA, comes from several experimental and clinical proofs of a apparent "reversion" of the cancer phenotype, obtained either by physiological factors of cellular differentiation (cytokines and other signaling molecules, microRNAs), or by drugs, without even correcting genetic abnormalities. As recently stated by some pioneer scientists in this field, by promoting such a reversion of the malignant to non-malignant phenotype, *"epigenetics wins over genetics"* [149].

Cancer: a cell differentiation therapy?

If we agree that cancers arise from self-renewing tissue stem and progenitor cells—actually a modern version of the *Embryonic rest theory*—then the great expectation should be to treat cancer by inducing terminal differentiation in the cancer stem cells [118]: in such a perspective differentiation therapy should be defined as a strategy aimed at inducing the re-activation of endogenous differentiation programs and, by this way, the maturation of cancer cells and the reversion of the tumor phenotype [150]. The idea of transforming malignant to benign cells as a potential treatment for patients with cancer was first suggested in 1961 by Pierce [151] as self-differentiating teratocarcinomas were observed in his laboratory. In the 70s and 80s several pioneering reports stressed the potential of this strategy as a therapeutic approach for hematological malignancy [152]. The definite transformation of this idea to a real clinical practice was realized in 1984, thanks to the successful employ of all-trans retinoic acid (ATRA) in the treatment of acute promyelocytic leukemia (APL) [153] that transformed this lethal disease in the most curable form of leukemia [154]. In the next two decades progress in understanding the differentiation pathways and the development of differentiation-inducing agents allowed scientists to realize that the abnormal developmental programs typical of malignant cells could be epigenetically reversed [155, 156] to regain normal behavior by the reintroduction of differentiation-associated transcription factors, or by inducing the differentiation of CSCs with normal cytokines, including IL-6, IL-1, GM-CSF, G-CSF and IL-3 [157, 158] and/or specific differentiating agent as retinoic acid (RA, Vitamin A) and in particular ATRA [159]. The various clones of cancer cells have different blocks in their ability to be induced to undergo differentiation [160, 161]. This is not surprising, as many experiments have shown that there are different pathways of gene expression for inducing differentiation [162], and that genetic changes which suppress induction of differentiation by one compound do not affect differentiation by another compound using alternative pathways [163].

Experiments of 30 years ago showed that, after injection of myeloid leukemic cells into mouse fetuses, the cancerous cells undergo normal differentiation to mature granulocytes in apparently healthy adult animals [164, 165], creating the conceptual basis for differentiation therapy of leukemia [166]. Since then many studies showed that an abnormal developmental program in leukemic cells can be reprogrammed by appropriate differentiation that induces cytokines, and that this epigenetic suppression of malignancy bypasses the genetic abnormalities in tumor cells [167, 168].

Various chemicals other than normal hematopoietic cytokines have been shown to induce differentiation in myeloid leukemic cells [169] and erythro-leukemic cells [170], including compounds used for cancer chemotherapy, such as cytosine arabinoside, methotrexate, irradiation and glucocorticoid hormones. Interestingly, if at high doses, irradiation and chemotherapy kill cells by inducing apoptosis, at low doses they may induce differentiation. Other compounds that can induce differentiation in leukemic cells include insulin, bacterial lipopolysaccharide, tumor promoting phorbol esters [162] and, as we have just seen, RA [171].

Thus far, no differentiation-inducing agents have been reported to have an effect comparable in solid tumors to that of ATRA for treating APL. The reason may lie in the fact that APL represents a simple karyotype disease—a "monogenic cancer" primarily, if not exclusively, "driven by a fusion protein PML/RARA" responsible for tumorigenicity—in which the simple reversal of one pathway is sufficient to reverse the tumor phenotype and place it back on the road to normal differentiation; whereas most solid tumors are viewed as heterogeneous aberrant tissues containing a hierarchy of cells originating from CSCs. Therefore, a single-targeted therapy does not appear appropriate to induce the terminal differentiation of solid tumors and the search for combination and multi-targeted therapies is more and more required.

A recent paper by Mack et al. (2014) is a stellar example of an epigenetic model in carcinogenesis [172]. Ependymomas are common childhood brain tumours. Whole-genome and whole-exome sequencing of hindbrain ependymomas reveal an extremely low mutation rate. Conversely ependymomas are characterized by a specific CpG island methylator phenotype (essentially consisting in transcriptional silencing of differentiation genes through trimethylation of H3K27 Polycomb repressive complex 2). It is exciting that methylator phenotype-positive ependymomas are responsive to epigenetic treatment targeting either DNA or H3K27 methylation, in vitro and in vivo.

Conclusions

Curiously, a fundamental characteristic of cancer cells—the loss of differentiation—was not considered by the most leading cancer experts, as a distinct *hallmark of cancer*, though it is a main feature of all cancers. For nearly 30 years, the somatic mutation theory, has remained the prevailing theory on how malignant tumors arise and progress, despite many theoretic weaknesses and much experimental, and epidemiological data contra-indicating it. First of all the ongoing increase of many types of cancer in recent decades, particularly in organs and tissues directly exposed to pollutants or designed to the defence of the whole organism; the astounding increase of cancer in children; the wide incidence variation of diverse cancers in different regions of the world: all contradict the validity of a gene-centric theory which even explicitly diminishes the causal role of environmental factors agents in carcinogenesis. Some recent criticisms and some promising alternative models of carcinogenesis have their origin in a earlier theory according to which cancer could be a late product of distorted tissue development. In the last decade, many studies have highlighted the prominent role of an altered environments ("epigenetic" signals) to regulate gene expression in carcinogenesis, and it is becoming evident that epimutations generally outnumber genetic abnormalities and often occur much earlier in cancer development, basically signifying the adaptation of tissue stem cells to a sustained and persistent environmental stress.

In such a context a more specific hypothesis of carcinogenesis is that most epigenetic (global DNA hypomethylation, hyper-methylation of promoter sequences of tumor suppressor genes, large-scale down-regulation of miRNAs), genetic (genomic instability, mobilization of transposable sequences) and chromosomal (translocations) mutations should be considered as steps of a failed or distorted evolutionary (adaptive and essentially defensive) process determining cancer progression; rather than simple "genetic and/or chromosomal aberrations". This is a radically different model of carcinogenesis, based on a more realistic model of the genome, essentially a complex, dynamic and responsive molecular network of epigenetic control of mobile DNA directed by non-coding RNAs [173]. In this model most of the thousands genetic mutations (variably deleterious, found in advanced cancers) critically contribute to the establishment of the neoplastic phenotype; and so should be considered the consequence of a persistent (epigenetically induced) genomic instability, not the actual primary cause of cancer (Fig. 2).

A final proof that cancer is fundamentally an epigenetic disease—arising from alterations of cell differentiation and/or proliferation programs rather than from stochastic mutations of DNA - comes from several experimental and clinical proofs of "reversion" of some cancer phenotypes, obtained either by physiological factors of cellular differentiation (cytokines and other signaling molecules or microRNAs), or by drugs, *without the correction of genetic abnormalities*. This leads to a final postulate, that epigenetic (informational) therapies would work better than today's inadequate treatments for this terrible disease.

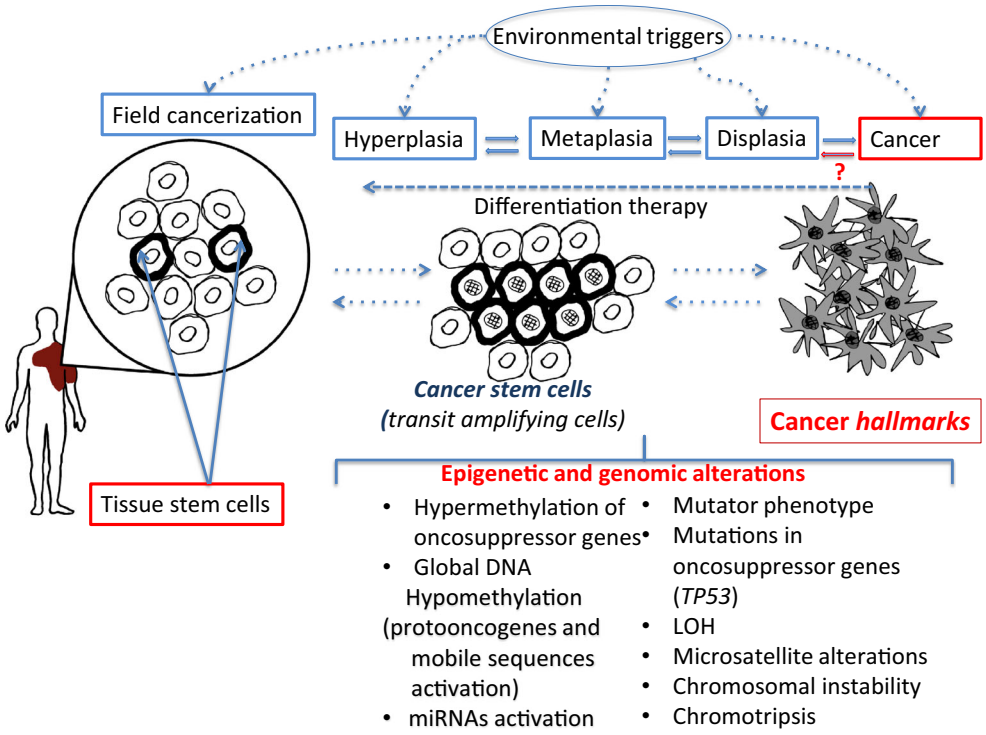

Fig. 2 Towards an epigenetic theory of cancer. Oncogenesis should be seen as "ontogenesis gone awry". In such a context tissue stem cells could be considered as a today equivalent of embryonic rests (see in the text: "*Embryonic rest hypothesis*"); the main alteration is a maturation arrest in stem cells differentiation (rather than de-differentiation); the main changes in the epigenetic and genomic landscape should be seen as responsive to aberrant signals coming from the environment and/or to abnormal interactions between the mesenchyme/stroma and the parenchyma/cells (see in the text: "TOFT—Tissue organization field theory")

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