frontiers

Stepping from modelling cancer plasticity to philosophy of cancer

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ABSTRACT

In this essay, I suggest that cancer is fundamentally a disease of the control of cell differentiation in multicellular organisms, uncontrolled cell proliferation being a mere consequence of blockade, or unbalance, of cell differentiations. Multicellular organisms are among living systems those whose intricate levels of interaction make their description difficult from an integrated physiology point of view. As a consequence, their experimental and clinical studies seldom yield satisfactory results when one aims to fix issues evidenced as malfunctions, the major of them from a medical perspective being cancer. Cancer cell populations, that can reverse the sense of differentiations, are extremely *plastic* and able to adapt, without mutations, their phenotypes to resist drug insults. I, with others, contend that such plasticity is likely identifiable with the easy reactivation in cancer of ancient, normally silenced, genes. Stepping from mathematical models of non genetic plasticity in cancer cell populations and questions they raise, I propose here a transdisciplinary approach to shed light on this problem from both a theoretical and a practical viewpoints. Theoretically, this approach leads me to a description of multicellular organisms in terms of *multi-level structures*, which integrate function and matter from lower to upper levels. From a practical point of view oriented towards the clinical treatment of cancers, I propose to investigate possible new therapeutic tracks. Cancer is related to the evolution of species, being a disease that appeared as such with the emergence of multicellularity. I here adopt an *evolutionary biology* point of view as an essential structuring element in my proposed methodology. Doing this, I aim at understanding the transition to multicellularity as a design forged by evolution, and at unravelling the mechanisms of multicellularity alterations in disease, which may be rich in consequences for cancer therapeutics.

Keywords: cancer, multicellularity, evolution, therapeutics, philosophy

1 INTRODUCTION

Coherent multicellular organisms are not only cohesive from a spatial, anatomical point of view, but also coherent from the phenotypic and cell-functional point of view of compatibility, cooperativity and division of tasks between cells and tissues. This is mandatory to make possible the achievement of a stable, functional and reproductive whole.

Leaving aside the possibility of spontaneous "emergence of order from chaos", I make here the simpler hypothesis of a system of communication ways between trees of differentiation, relying on the control of

transcription factors that determine differentiations and that I will call “the cohesion watch”. I consider it as a part of the immune system, whose armed force is the immune response, innate as well as adaptive, humoral and cellular, but is not the whole of it. Indeed, I view the immune system as the coordinator of the unity of the organism. Within the immune system in this extended vision that is thus more general than the immune response, the cohesion watch is in charge of the control of compatibilities and cooperations between the anatomical and the phenotypic/cell-functional systems, and also within each of these systems. It is a mandatory component of multicellularity to ultimately lead to an anatomically cohesive and functionally coherent organism.

The immune system in this extended sense should thus comprise: a) the equivalent in all Metazoans of the major histocompatibility complex (MHC) of jawed vertebrates, in charge of characterising all cells of a given individual within its species (here I postulate the existence in all Metazoans of a coding system analogous to the MHC of jawed vertebrates, and present in all its forerunners in animal evolution); b) the immune response; c) the cohesion watch. The latter is here assumed to be a complementary histocompatibility complex in charge of:

- driving indifferently (i.e., in an equal way) in each organism-to-be in a given species the body plan (or Bauplan, described with regulatory mechanisms in (18) for bilaterians, and for older animals in evolution in (59)) by launching morphogens during embryonic development;
- simultaneously, as much indifferently, guiding from pluripotent stem cells attached to the body plan the development of the trees of cell differentiations;
- establishing between the trees and twigs of differentiations that stem from the body plan acellular compatibility regulatory mechanisms, the main compatibility task of the cohesion watch.

These trees, in the Waddington view (inverse, in its three-dimensional presentation, of the tree expansion metaphor), are none else than the epigenetic landscapes, and they are controlled by transcription factors and epigenetic enzymes. All differentiations lead terminally to mature cell types, between 200 and, say, 400 (according to various evaluations) (40) in the human species, but only 20 in the sponges Porifera (57). They are in any event of a fixed number for every organism in a given species. Inscribed, as the body plan, and as the functionality differentiation trees, in the genome of each cell, this cohesion watch should manifest itself materially during development as a net of communications between and within differentiation trees. At the chromatin level, it should control non-expression (in closed-state chromatin), expression or repression (in open-state chromatin) of genes at nodes in the cell differentiation trees, and it should also control the stability of the body plan.

2 MODELLING PLASTICITY IN CANCER CELL POPULATIONS

In a series of papers starting in 2013, a team of mathematicians, to which I belong, at Laboratoire Jacques-Louis Lions, Sorbonne University, Paris, and some followers elsewhere, were initially stimulated by an article published in 2010 (73) that reported reversible drug resistance in a cancer cell culture. The culture, exposed to massive doses of drugs, developed in sparsely distributed resistant subpopulations (named persisters), and such resistance, shown to be of non genetic nature, was completely reversed when the drug was withdrawn from the culture. Driven by this biological observation of reversible resistance in cancer cells, we tackled the question of understanding and predicting the dynamics of these cancer cell populations by using mathematical models. The behaviour of these highly *plastic* cell populations was relevantly described by phenotype-structured partial differential equations. In these equations, the structuring variable, i.e., the parameter-like one that codes for the biological variability of interest, is assumed to store the

heterogeneity of the cell population with respect to the expression of drug resistance. It was chosen to be a positive real variable representing the expression of a resistance phenotype, *continuously* from 0 (totally sensitive) to 1 (totally resistant) (3, 4, 12, 13, 14, 15, 16, 20, 21, 45, 49, 50, 51, 62, 63, 64).

These models, intended to represent the effects of a cancer treatment on cell populations, and ultimately on patients, with the aim to overcome their capacities of resistance induced by the treatment itself, naturally give rise to the proposal of theoretically optimised therapeutic strategies. Such strategies, that have recently been the object of active research (reviewed in (41)) aim at containing or eradicating cancer growth, avoiding the two major pitfalls of treatments in clinical oncology, namely unwanted toxic side effects in healthy cell populations and emergence of resistance in cancer populations (67, 68). These strategies, that are still theoretical, may be too recent to be widely accepted by oncologists as plausibly efficacious and challenged by preliminary experiments, in Petri dishes or in laboratory rodents. In the meantime, many questions arise about the nature of plasticity in cancer cells and cancer cell populations that underlies them (reviewed in (74)). Cancer is a disease of multicellular organisms, that normally are functionally constituted of terminally and irreversibly differentiated cells. ee make progress in understanding the causes and the mechanisms of the reversion of differentiations that make cancer cells so plastic, i.e., able to quickly adapt their phenotypes to a changing environment such as deadly drug pressure, while healthy cells cannot?

3 QUESTIONS ABOUT MULTICELLULARITY AND CANCER

Some motivations for the interest of stepping from such therapeutically oriented models of drug resistance in cancer cell populations, hence of plasticity in cancer, to more general considerations can be set as questions arising from observation facts. Such questions are most of the time dodged, likely being perceived as too complex to be solved by specialists of one domain only, in the field of cancer biology:

- Cancer has been found in all the animal kingdom (1), and beyond, but for instance plants are not letally affected by it (24), so that investigating the earliest stages of multicellularity in animals (57, 58, 59), i.e., searching for its failures, may be a natural way to understand how some somatic cells become cheaters to their established multicellular community.
- The genes that are altered in cancers are the same that serve multicellularity design (Domazet-Lošo & Tautz (22, 23), Davies, Lineweaver and Vincent 2011, 2014 (19, 46, 88, 89)): can we methodically collect these genes?
- What defines a same organism? A ‘self’ that would be conserved during the sequences of differentiations that in Man lead from the first embryonic cell to the about ‘200 terminally differentiated cell types’? Interesting answers are suggested in different works dealing with the philosophy of biology or the ‘philosophy of cancer’ (books by Bertolaso (9), Laplane (44), Plutynski (66), Pradeu (69, 70), and others).
- Can we envision the immune system as not limited to the immune response to pathogens and abnormal host cells, but rather as a law of cohesion for the whole organism construction?
- Would not the immune response be in this extended vision of the immune system only its ‘sword arm’, a police patrol, pale reflection of the law itself, whereas a hidden part of the immune system would be the ‘spirit of laws’ (analogous, *mutatis mutandis*, to Rousseau’s unwritten social contract in human societies)?
- What holds together, normally without conflict, the cell types, and is it not something that governs development from the beginning, something more than what the immune system uses when it recognises as non-self (foe rather than friend) a cancer cell?

- Is there a complementary relationship between the maintenance of such coherence and the major histocompatibility complex (MHC), or rather its likely forerunners in non-vertebrates, yielding early adaptive immunity?
- What is the primary function of the immune system, if not to ensure organism cohesion (of tissues), and how does such coherence (of signals) operate? If it is so, what is the impact of this (extended, i.e., going beyond the classical cellular and humoral immune response and earlier than it in the construction of multicellularity) version of the immune system on cell differentiations?
- Otherwise said, is the immune system the 'glue' (69, 70) that holds together in a coherent way the cells and functions of the multicellular organisms we all are constituted of, until such cohesion/coherence is altered in cancer?

4 THE ATAVISTIC THEORY OF CANCER

4.1 The theory in a nutshell

According to the atavistic theory, cancer is a local regression of a stable multicellular organism (Metazoa 2.0) to an incoherent state of a cell colony (Metazoa 1.0), non-existent as an evolution entity as it is instable and incapable of reproducing itself. Such state is supposed to have preexisted the transition towards established stability that defines a stable and reproductive multicellular organism as a Darwinian selection unit. This point of view has been proposed at least in 1996 (39), likely earlier, but has been popularised in 2011 by Davies, Lineweaver and Vincent (19, 46, 88, 89, 80), then examined from the point of view of the history of genes (10, 17, 22, 23, 81, 82, 83, 92). The atavistic theory of cancer has also recently been compared (47) with the dominating (among cancer biologists) somatic mutation theory (SMT, that is more often compared with tissue organisational theory, TOFT) (75, 76), and popularised in review articles (30, 31). It poses the question of transition to multicellularity, for which we have to elaborate a plausible scenario, not sketched by the above mentioned authors.

4.2 Stage 0, aka Metazoa, the β version

At this elementary stage of multicellularity, in which proliferation limited by apoptosis is the only possible fate for cells (note that the emergence of apoptosis in evolution is studied in depth in (43)), they stick together in the ocean thanks to a form of collagen glue. Note that the existence of collagen implies enough availability of oxygen in the oceans, which dates this episode back to at least -850 million years.These cells are then able to exchange information, either by paracrine communication, or by gap junctions (84, 85, 86), through innexins present, e.g., in Hydra, rather than through connexins (2), or others (56). Gap junctions allow cells to exchange molecules that can be toxic, such as oxygen, toxic indeed before endosymbiosis of mitochondria in eucaryotes. As regards the properties of cells at this stage, we assume only proliferation and its dual property, apoptosis, to be both influenced by environmental factors. We also assume a friend-or-foe recognition system to be present in each cell and able to use intercellular communication, paracrine or by gap junctions. Now, what should be the use of such a system if it would not react when a message testing an external intruder returns foe, i.e., we are under attack? Assuming no specialisation (i.e., no differentiation, no division of work) at this stage, collective fright, fight or flight may be represented respectively by hedgehog-like attitude / secretion of toxins in the environment / collective movement without individual or semi-collective cell motility. Note that the latter is shown by tumour spheres with inverted polarity, TSIPs, sorts of moving hedgehogs or urchins (94) encountered in breast and colorectal cancer cell populations. The genome of each of these cells has evolved to grant them such properties, making them able to resist UV radiation, acidity, cytotoxic molecules, hypoxia (after the endosymbiosis of mitochondria in the case

of animal cells). A bond between them must exist, that defines everyone of them as a member of a colony, a kind of *self* that controls proper cell division. This *self* and the friend-or-foe recognition system are assumed to be remote ancestors of the major histocompatibility complex (MHC, the common law in jawed vertebrates) and of the humoral immune response of vertebrates, immunoglobulins.

4.3 Stage 1, aka Metazoa 1.0

At the following stage, under the pressure of successive hostile attacks from the environment, begins the *reversible* division of work, i.e., differentiation of subpopulations of cells to allow them to perform specialised tasks, not involving the whole cell population in all the tasks. According to John Maynard Keynes and Eörs Szathmáry (54), the first of such specialisations could be the constitution of the germen (germinal cells), in charge of propagating the common genome, as opposed to the stroma (stromal cells), in charge of protecting and preserving the germen by all possible means of further specialisation, e.g., motility, production of secretions, fast communications, etc. Differentiations producing division of work appear then, and they occur according to both molecular determinants inscribed in the DNA and contacts between neighbouring cells, and also according to physical laws of soft matter that determine them in 3D space (26). However, these differentiations are very labile, i.e., reversible; otherwise said, the cells at Stage 1 are endowed with high plasticity with respect to their phenotypes.

Due to such plasticity, that prevents coherent construction of an organised cell colony that could be divided in cooperating subpopulations, no stable structure can emerge at this stage. The sketch of immune system of Stage 1 has not evolved. On the contrary, something of the emerging self may be lost, as cell divisions may be futile, with junk DNA (the common law is easily trespassed and ignored) and existence of monster or non viable cells. No working immune system leading to a stable coherent whole can exist in such cell populations. A Stage 1 cell colony is according to the atavistic hypothesis of cancer (19) characteristic of cancer cell populations found in tumours. Many properties available in tumours, high individual plasticity and adaptability to external insults, loose common self (as all cells are potential defectors - cheaters - with respect to the poor common law of Stage 1), no regulation of proliferation nor of differentiations, are present. Proliferation (fecundity) and apoptosis, are now completed with differentiability and de-differentiability, i.e., extreme cell plasticity. Cooperation between subpopulations may exist (78), however not on a perennial nor consistent basis. From a metaphoric Waddington landscape point of view (36, 37, 38, 90), the scenery is flat, or with unpredictably changing slopes. What can you build with plasticine bricks?

At this stage, the colony of cells is a soft and moving mixed cellular and acellular ‘soup’. To achieve the transition from it to stable multicellularity (61), one can imagine that, if all elements in the genetic roadmap are present at least in some of these cells - in particular if sexed reproduction is also already active, as in yeast cells -, then physical laws of soft matter would drive this soup to a more consistent material. Indeed, mathematical natural gradient dynamics and singularity unfolding (26, 79) can be represented by chemical reaction-diffusion equations (87), at work in morphogen gradient-guided embryology processes. Many attempts to multicellularity may have occurred (and evolutionary biologists tell us that there have been may failed attempts) until a stable cohesion watch (maybe established e.g., on paracrine or Delta/Notch communication, or through gap junctions) can actually emerge and stabilise the structure of the plan. Then any fecundation that launches the division of a fertilised egg can be successful to yield a multicellular organism.

4.4 Stage 2, aka Metazoa 2.0

At Stage 2, an organisational principle emerges from the eddying chaos of Metazoa 1.0, and takes control of differentiations and proliferation. The common law is respected by all cells of the colony, that is able to defend itself as a whole entity against attacks and can now inscribe itself in the fate of Darwinian evolution, maintained as a coherent ensemble by a functional immune system and a nerve communication system. The primitive Urmetazoa as described in (57, 58, 59, 77) may have been some kind of sponges like Porifera. The multicellularity gene toolkit of Metazoa 2.0 (19) appeared at this stage, quite early, and long before the Cambrian explosion, close to a date around -800 million years (59). What is this new collection of genes made of, how has it been hierarchically organised, with respect to preexisting genes of unicellularity (e.g., cell cycle control)? What is the common law that defines an individual as any representative of its species (between-species distinction)? What defines a particular individual within its species (within-species distinction)? These questions ought to be documented, to better understand what support to document the idea of a hierarchical organisation of the genome this point of view may bring.

The immune system is now not only in charge of friend-or-foe recognition and defence of the colony when it is under attack, but most importantly it has emerged as a centraliser principle under the form of a chip present in every cell, ensuring the consistency of the whole construction. This common ‘law’ is inscribed in the genome of each cell. Cheater cells may exist as in every organised society, however they are sensed by a specialised subpopulation of cells (the police, the immune cells) endowed with the mission to contain or destroy them. From a molecular point of view, repeat regions in the genome (in particular LINE-1 (32), in connection with the interferon pathway) could be responsible for such sensing. From the metaphoric Waddington viewpoint (36, 37, 38, 90), an irreversible differentiation potential (95, 96) is now present. As regards the material construction of a stable organism, bricks and enamel are ready to be cooked in oven, perennial Assyrian palaces can now be built. What do such virtual ovens consist of, that will stabilise the multicellular organism during development, we do not know; we can only suppose that some genes are silenced throughout this stabilisation process.

Yet the fact remains that within the developmental stage of this construction, plasticity (reversibility with respect to a differentiation potential) is necessarily present for a limited time. This is the time of embryological development. After that time, the so-called Yamanaka genes (93) Oct3/4, Sox2, c-Myc, Klf4, that can reverse differentiation to produce induced pluripotent stem cells (iPSCs) are normally silenced (they can be revived in cancer, disease in which cells have not been properly ‘cooked’ by gene silencing at some differentiation stage). Nevertheless, we know that some Metazoa, like salamander (or axolotl), are able to locally go back to this developmental stage and regrow a tail or even a limb when it has been severed from the body.

The molecular level at which such control on differentiations is exerted is likely the level of the chromatin, where epigenetic enzymes, themselves coded by epigenetic genes, exert their control on the expression of genes, possibly by controlling transcription factors. The sequence of mutations observed in acute myeloid leukaemia (AML), in evolutionary time firstly on epigenetic control genes, then on transcription and differentiation factors, and only finally on genes of proliferation (34) seems to recapitulate in reverse order the sequence of stages proposed here. One can suspect that a hierarchical relationship, mentioned above about repeat sequences and the immune system, exists among control of gene expression at the chromatin level. Where could exist a repository of an MHC-like common law, i.e., of marks defining not a particular individual, but common to all individuals of a given species and control of differentiations by the immune system is an open question. Indeed, such epigenetic/immune control of differentiations is not documented, to the best of my knowledge, but is likely to exist.

To sum up this stage, there is persistent division of work since it appeared at Stage 1 already, but now it is consistently organised, as *irreversible* differentiation. This constitutes a new fate (added to proliferation, apoptosis and senescence) in the physiological cellular life, in each cell under the control of the cohesion watch during development. Later, added to the cohesion watch, specialised populations of cells, the immune patrol police, have appeared when the organism has been completely built. They are in charge of surveillance and (containment or) destruction of law trespassers. The cell colony, now a Metazoan 2.0 endowed with a functional immune system and able to reproduce itself, can successfully go through the tinkering (40) of Darwinian evolution, from sponges to vertebrates. However, in case of malfunction of any of its parts, due to malfunction of the immune control (insufficient control) on its differentiation fate, this part is likely to revert to Stage 1, aka Metazoa 1.0, according to the atavistic hypothesis of cancer (19). Conversely, when the police patrols (lymphocytes and macrophages) overreact, wrongly interpreting normal signals as trespasses, this may lead to allergies and auto-immune diseases.

5 WHAT IS A FUNCTIONAL MULTICELLULAR ORGANISM?

5.1 A Borromean system responsible for the emergence of Metazoa

The construction of the mind I propose now as common to all individuals in a species thus consists of:

- a) a base for the construction: the anatomical system, sets of genes in charge of the spatial embryological development, i.e., the 3D body plan (5, 59), tissue/organ morphogenesis included;
- b) attached on this base to points that are virtual tissue-specific stem cells, domains of differentiation stemming as tree-like structures (inverted Waddington landscapes) of functionalities, i.e., sets of nodes of differentiations specific of a given functionality, e.g., in vertebrates, digestion, circulation, body covering, that in particular will yield the up to 200-400 functional human cell types (40);
- c) a hypothesised "cohesion watch", complementary histocompatibility control system, a net made of connections - nervous, hormonal, or by cell-to-cell contact - between and within the functionality trees in charge of controlling compatibilities and cooperations within each of the two systems and between the two of them, to achieve a cohesive and coherent multicellular system.

The whole construction should possess the characteristics of a Borromean system (endowed with the Brunnian property: removal of any one component unlinks the entire system) of length 3 (8, 11): each subsystem exists independently of the other two, however no common sense can be obtained, in order to achieve the coherent design of a multicellular organism, without the simultaneous participation of all three to the design. Furthermore, if any of them dissolves in the environment or fails its task, the other two may continue their separate existences, however not leading to a viable organism, or else an impaired one. For instance, in the case of failure of control on the human body plan only, and in increasing order of gravity, possible limb agenesis, partial rachischisis (spina bifida), anencephaly, nevertheless except in the latter case, viable organisms.

The case of cancer, a disease specific of multicellular organisms, and, in as much as it may destroy the whole organism, specific of animals (aka Metazoa, characterised by heterotrophicity among multicellular organisms; cancer exists in plants, but remains localised and is not letal (24)), is the result of primary partial (local) failure of the compatibility control system (the cohesion watch) on the phenotypic coherence of the organism. In cancer, the body plan (in an extended sense, i.e., 3D anatomical shape and functional organ morphogenesis) is usually respected, but failure of control on differentiations (at the level of trees, or inverted Waddington landscapes) gradually leads to incoherence in the cooperation tasks (improper

division of work) between tissues and organs. Then the natural history of the disease leads to dissolution of the organism as a whole (de-unification of the individual, as Thomas Pradeu (70) writes).

5.2 In more detail, why is it a Borromean structure?

Should the cohesion watch be firmly attached to the body plan, but with missing places there for the trees of functionalities relying on phenotypic differentiation, this could (however very unlikely in reality) lead to void shapes that one can figure as development stopped at different embryonic stages, e.g, gastrulation (in triploblastic animals (53, 72)) or neurulation (in vertebrates). If conversely it controls all trees responsible for cell-functional phenotypes, when all necessarily cell specialisations have been achieved, but the body plan is loose, not cohesive, then division of work is there and everything is ready for the emergence of a virtual Metazoan, except that it cannot be embodied in a stable spatial and functional structure and thus cannot exist. Furthermore, the cohesion watch, epigenetically controlled non-cellular system of intercellular communication controlling differentiations must make these differentiations irreversible to yield a stable multicellular organism. Before its appearance in evolution, differentiations were partially or completely reversible, which was in particular useful to make the whole construction able to mobilise enough cells in the colony to face an incoming external aggression. This might be by motility and by specialisation into protecting cells, precursors of immune cells, facing it by fight, flight or fright. In the metaphor of the Waddington landscape, such irreversibility is ensured by the establishment of high epigenetic barriers that prevent de-differentiation or transdifferentiation. Indeed, evolution cannot build anything perennial on moving ground, non-moving meaning here a permanent spatially and functionally organised support within which cell subpopulations can cooperate to establish an individual able to feed on its environment, avoid destruction from it, and secure its reproduction.

The cellular immune system cannot appear out of the clear blue sky, but could emerge from a specialisation from a primitive immune-like cell type in the initial cell colony, then yielding cells and signalling molecules able to recognise both the MHC or rather its forerunners in evolution, by tagging an individual in a given multicellular species. It will also be able to recognise common markers, tagging the species, in any cell of the colony. The next stage would be to validate them as faithful elements of the ensemble, or else to destroy them or reject them from the cell colony by making use of an armed force, the (cellular and humoral) immune response. These specialised immune cells should then take control for all other cells of both the anatomical development system (the materially established body plan) and of the epigenetic system of differentiations rendered irreversible by the cohesion watch, and then can emerge during early embryogenesis a truly stable Metazoan. Note that I envision here the cohesion watch as a set of intercellular communications that I assume to be present in all cells of a Metazoan 2.0 (including the emerging immune cells) under the form of a program that is the basis of the ‘common law’ of the species. Such a dual event, preexisting cohesion watch in all cells - the common law - and enforcement of the cohesion by the materially constituted cohesion law and by the emerging immune cells during embryogenesis - the sword arm, the police - is highly evocative of the constitution of an emergent Borromean system. Before its emergence can only exist tumour-like Davies’s and Lineweaver’s Metazoa 1.0, and after it is constituted, a cohesive and stable Metazoan 2.0 (a true Metazoan).

5.3 The basic anatomic system: the body plan in development

The structure of the body plan (18, 59) is not easily defined, as it has evolved along with the evolution of species. However, one might define it, independently of the animal species under consideration, as the anatomically based collection of all of the organism functionalities. Well known by embryologists for quite a long time, long before the emergence of genetics and the knowledge of the roles of body plan genes,

the embryological development of animals has been described from the blastula stage (a 2-dimensional sphere made of undifferentiated cells) until constitution of forms that depend on the species. These forms resort to diploblastism (two layers: endoderm and ectoderm) in elementary Metazoa such as placozoa, ctenophora or cnidarians, and later triploblastism (three layers covering a 2D-sphere: endoderm, ectoderm and between them, mesoderm) in all others. Triploblastic animals appeared between 1 billion years and 600 million years ago and were later structured by a hard skeleton during the Cambrian explosion, beginning 541 million years ago, and lasting about 13 to 25 million years. In triploblastic animals, particularly in vertebrates, gastrulation and neurulation are dynamic phenomena in which cells follow flows that will constitute their anatomic structures. They have recently been described from a physicist's point of view by Vincent Fleury (26), and from a mathematician's point of view, much earlier by René Thom (79). No genes are present in the points of view of these authors. However, the explanation of the formation of embryological layers due to the dynamics of morphogen gradients, firstly predicted by Alan Turing (87), now identified as, e.g., Wnt, and controlled by, e.g., Hox, is presently the norm, all the more so as knock-out embryos (mice, flies) for these genes are currently documented to help us understand their precise roles in anatomical development (5).

5.4 The trees of cell specialisations controlled by transcription factors and epigenetic enzymes

Cell functionalities, relying on functional cell phenotypes, were developed in a cell colony with the emergence of transcription factors (55). Their combinations forming gene regulatory networks (GRNs) may have occurred very early, as many transcription factors were already present as early as 1.5 billion years ago, in LECA, the last eucaryotic common ancestor (55). One may assume that, likely due to the necessity to develop functional capabilities to make individual cells able to adapt to changing and often hostile environments, transcription factors have gradually combined into GRNs, constituting the biological support of the expression of functional phenotypes. Furthermore, differentiations are by nature *epigenetic*, insofar as they occur, leading to very different terminal cell types, on the basis of the same genome, which naturally sets a role for epigenetic enzymes at the level of chromatin, partly unravelled in (6) in their relationship with transcription factors, and more recently in (7).

Such differentiation phenotypes, achieved by specialisations, branching points in the trees, that before the emergence of Metazoa 2.0 are likely all reversible, are modules of elementary adaptation to the external environment, already present in unicellular constituents:

- germinal or somatic nature (duality germen/soma, in sexed reproduction),
- motility or attachment to a matrix,
- emission / reception of (fast or slow) communication between cells of the colony,
- means of absorption of fueling matter and of elimination of toxic residues,
- activator-inhibitor dynamics, leading to space/time periodic behaviour of tissues and of intracellular/intercellular signalling pathways, mandatory to maintain continuity of flows in a limited space,
- friend-or-foe recognition and elimination of (or fight from) foes,
- etc.

These cell phenotypes, before the closure of the Borromean node, i.e., before the actual emergence of Metazoa 2.0, are still not fixed by epigenetic constraints, and thus are widely reversible. In other words, the epigenetic landscape is flat. It will be hilly when some newly established differentiation potential (95, 96),

ensured by the cohesion watch that I hypothesise to be part of the immune system, will force differentiations to become irreversible.

5.5 The working immune system involves a cohesion watch in charge of compatibilities

Indeed, the immune control of cell differentiations should consist of firstly checking their coherence (i.e., that cells follow a coherent differentiation path according to simple rules in terms of the complementary histocompatibility complex, the cohesion watch hypothesised earlier in this construction) and secondly of making these differentiations irreversible. The latter implies the constitution of a potential (95, 96), or of an entropy, at its highest level in stem cells of the tissue (e.g., haematopoietic stem cells for blood) and at its lowest level in the ultimately differentiated cells of the lineage. Among the differentiated blood cells are lymphocytes, in charge of the control of surface antigens of all other tissues.

In more detail, the task of the hypothesised cohesion watch, part of this extended version of the immune system, and that must exist already virtually, as inscribed in the self-extracting archive of the genome before fecundation, is thus to ensure compatibilities:

- a) between morphogens of the body plan, able to drive it actually from the zygote in an irreversible way within the 3D space of cells of a given individual (defined by its MHC in vertebrates, by some equivalent forerunners in non-vertebrates);
- b) between phenotypic functionalities, ensuring compatibility between differentiation trees that yield lineages within a given subpopulation, and ultimately between cooperating subpopulations (division of work) of terminally differentiated cells;
- c) between the body plan space distribution and the time distribution of phenotypes in each epigenetic landscape attached to the body plan.

One can think of this cohesion watch as being in charge of irreversibility of differentiations along each tree stemming from the body plan (vertical cohesion), but also of compatibility at each developmental stage between neighbouring functionality trees. This involves transversal cohesion, failed for instance in cervical cancer, due to histological uncertainty between two different epithelial coverings, likely resulting from impaired differentiation of immature renewing cells in one or both lineages, and it is mandatory to form a cohesion net, knit node after node in all relevant directions.

To mentally illustrate this construction, I propose as further metaphor the wickerwork basket. Starting from a circle endowed with lots of connections between its elements, that is supposed to represent the body plan, functional willow-like twigs stem from each of these elements, representing the great physiological functions of the organism. If no weaving is made between these twigs, the whole set will consist of just flexible differentiation functionalities of a family of cell types, floating freely in the surrounding space, unrelated to each other. No cohesion, no division of labour can result from such unwoven twigs and trees. The task of the cohesion watch is to ensure such weaving during development, until tips that are terminally differentiated cells. This naturally includes the solidity of the willow twigs (breaches along the vertical axis resulting in blocked differentiations, as is the case with acute myeloid leukaemia, AML), but the main part of the cohesion watch is to ensure compatibility between (spatially and functionally) neighbouring twigs.

Could such hypothesis be tested by evaluation of coherence in the expression of transcription factors responsible for the differentiations of mandatorily compatible tissues at different stages of their differentiations? This could rely on the investigation of intercellular communication means regulating GRNs in different cells, as described in (25, 65).

The emerging capacities of the whole system consisting of the three subsystems, body plan, trees of phenotypic functionalities giving rise to lineages from virtual pluripotent stem cells, and the cohesion watch, will now endow the multicellular organism-to-be (after fecundation), in a coherent and stable way. This will consist of making use of division of work and cooperations between the subsystems, with functionalities, relying on survival means based on the elementary adaptation phenotypes (non exhaustively) listed above, and later producing "the great physiological functions" taught to students in medicine and physiology. These capacities will make precise, if not define it, a common representative of a well-defined species. Such capacities of the whole organism may be:

- boundaries with external environment, in both the anatomical spatial and phenotypic (protection) senses,
- strategies to feed on the environment by ingestion of preys
- friend-or-foe recognition and surveillance against predators,
- abilities to react to hostile environments, whole organism motilitiy (flight) being one such ability,
- integration of all cells by fast intercellular communication networks,
- reproduction facilities (sexed reproduction by germinal / somatic cell specialisation),
- and many others, such as cognitive processes.

Cognitive processes are indeed among the mandatory functionalities of an evolved multicellular organism (not only vertebrates, and certainly, for instance, octopuses) under the control of the hypothesised cohesion watch. Conversely, could there exist a support for a possible control of cognitive processes on the immune control of proliferation and differentiation that might explain some inexplicable spontaneous cures of cancer? If so, would the classic immune response (cellular, humoral) be responsible for it, or could it be an effect of the cohesion watch? All physicians are aware of such stories of cures that cannot find any explanation within the corpus of medical knowledge except by a timely intervention of the immune system. An example of a mild one is a plantar wart that was about to be surgically excised and that completely disappeared in one night without any trace on the morning of the intervention; others exist about cancer, usually not reported as medical observations, being beyond the scope of contemporary science. The mention here of such facts is meant to say that even though the existence of a cohesion watch is primordial for the stability of the organism, it may itself become a part of the organism under the control of a superior integrative control, of nervous origin, that unifies a particular individual within a given species with respect to the maintenance of its stability in behavioural life. Note about this point that Michel Jouvet has proposed the interesting hypothesis that the physiological meaning of cortical activity during paradoxical sleep, i.e., dreaming, is a neuronal reprogramming of the individual, a consultation of its genetic programme together with its past life personal history, aiming at adapting its behaviour to be ready to solve issues it will likely meet in its immediately forthcoming future ((42), cited by Tobie Nathan in (60)).

6 PERSPECTIVES IN CANCER THERAPEUTICS

Within this evolutionary perspective of the design of a multicellular organism, developmental diseases like the briefly mentioned ones above (limb agenesis, etc.) are diseases of the immune system control of the body plan. Assuming a cohesive body plan, which is usually the case, cancer appears as a loss of control of the immune system on the trees of differentiations and on compatibility connections between them. Cancer may thusbe due to flaws in the means of control, or to incoherences in the control subsystem itself. As regards auto-immune diseases, they are clearly due to incoherences in this controlling immune subsystem.

From a cancer therapeutic viewpoint, as stated by Davies, Lineweaver and Vincent (46), attacking cancer by blocking its proliferation using chemotherapies or radiotherapies is clearly short-sighted. It may completely work in some cases, most often partially and for some time only, but as long as the epigenetic system of control on differentiations fails, the dynamics of cancer will prevail again. This may be avoided if the immune response keeps residual cancer cells in check, preventing them from excessive proliferation; this is usually called cancer dormancy, not clinically distinguishable from cure if it is indefinitely prolonged.

Notwithstanding this limitation, relying on the existing cell-killing therapies, that may be (cytotoxic) chemotherapies, (cytostatic) targeted therapies, or immunotherapies, mathematical models have been developed, with corresponding theoretically optimised treatment strategies, representing monotherapies or more successfully combination therapies in cancer (41, 67, 68). This was my starting point, that - inscribed in the time scale of a human life, not in the billion-year perspective presented above - aims at being immediately useful in the clinic. Taking advantage only of what we know presently of the behaviour of cancer cells exposed to cytotoxic and cytostatic drugs in the framework of a cell population, and not of the history of their making - as is the goal of the presently proposed billion-year perspective for therapeutics-, it has been briefly described in the first section of the present study. Among modern immunotherapies, immune checkpoint inhibitors (ICIs), by boosting the immune response by lymphocytes that attack tumour cells, e.g., in the case of melanoma treated with the combination ipilimumab+nivolumab, may be successful with about 60% of of objective response rates in patients, amid which 20% of total cases can even reach complete long remissions. Unfortunately, there may also more rarely exist total failures, resulting in non responders in 30% of cases, and even in so-called hyperprogressors (i.e., experiencing accelerated tumour growth defined by at least two-fold tumour growth rate increase compared with pre-immunotherapy rate) in the remaining 10% (27, 48, 52). Such cell-killing strategies may be successful by mending a breach in the control of cell proliferation, but if a fragility remains in the control of differentiations somewhere in the organism, a relapse may occur, possibly with cells that will have been selected for their robustness and will be less sensitive to the treatment.

This should induce us to enhance our understanding of the role of the immune system (more precisely, of the cohesion watch) in the hypothesised Borromean system on which relies a physiologically well-constituted animal. Rather than fighting uncontrolled proliferation, could we repair altered control on differentiations? Cell-killing strategies, whether they rely on chemotherapies or on modern immune cell-enhancing drugs, miss the basic targets,which are differentiation sites. I know of only two successful non cell-killing therapies:firstly, imatinib in chronic myelogenous leukaemia (CML) (35), where imatinib (or drugs of the same family of tyrosine kinase inhibitors, TKIs) blocks the ATP pocket of a chimeric protein, BCR-ABL, that itself is due to a fusion of genes, normalising proliferation. Secondly, all-trans retinoic acid (ATRA)in acute promyelocytic leukaemia (APL=AML3 in the old French-American-British, FAB, classification of acute myeloid leukaemias) (33), where ATRA degrades the PML-RARα chimeric protein (that also results from a fusion of genes) that blocks maturation of the myeloid lineage at the promyelocytic stage. As far as I know, many redifferentiation strategies close to this one have been tempted, and all the others have failed.

Nevertheless, I imagine that this could be the future of cancer therapeutics: intervention at the differentiation sites on transcription factors or on factors that control them, i.e., enforcing the cohesion watch connection, rather than killing cheater cells; otherwise said, mending a net with a hole in it rather than trying to kill sharks that have escaped its containment. Alternatively, I will illustrate this goal with a sociological metaphor. This is indeed relevant as, after all, in the hierarchy of levels of organisation that goes from genes to cells and from cells to multicellular organisms, the next level is evolving societies of living

multicellular individuals. Hence, rather than killing cheater cells by cannonade (i.e., by chemotherapies) or by enforcing the aggressiveness of the police (i.e., by immune checkpoint inhibitors), would it not be better to imagine how to enforce the law? The law here is the cohesion watch that exists as a plan in the genome before embodiment in development and later as an acellular communication network between tissues and organs. This could be done by repairing broken local social bonds between functionalities (expressed after embodiment as tissues and organs), as neither the army nor the police are the best means to establish harmonious working links of cooperation between citizens. Citizens in multicellular organisms are here somatic cells in tissues and organs, normally organised towards a common goal: preservation of the genome towards reproduction, and to that purpose, preservation of the health of the global society of cells. To be able to do this, a better understanding of the mechanisms of control of differentiation at the level of local transcription factors and at the level of chromatin is needed. The development of epigenetic drugs is promising, widely relying on inhibitors of DNA methyltransferases (iDNMTs) or of histone deacetylases (iHDACs) (71). They could be a starting point, provided that the interactions between epigenetic enzymes and transcription factors can be unravelled (6). This could lead to future differentiation-repairing cancer therapies that would be precisely targeted at the best possible sites of multicellular organisms and would leave cell-killing therapies, except to accelerate a clearance process, as with ATRA, that is usually delivered together with an anthracyclin, resulting in a complete cure of APL (33), in a remote past of cancer medicine. Another track to explore might be to examine, following Davidson's works on intercellular communication means that regulate consistency beween intracellular GRNs during development (25, 65), to target and reestablish such impaired intercellular signalling.

7 CONCLUSION

Far from considerations on evolution of a cell population at the time scale of a human life - my starting point - , that nevertheless undoubtedly present a high interest in therapeutics, such as initially advocated by me about mathematical models designed to optimise strategies based on combined cell-killing therapies (41, 67, 68) and by Robert Gatenby and his colleagues at the Moffitt Cancer Center in Tampa (28, 29, 91), I have presented in this essay an evolutionary point of view on cancer in a billion-year perspective that, from questions on plasticity in cancer, guided me to develop ideas resorting to what is now named *philosophy of cancer* (9, 44, 66, 69, 70). I thus begin to accompany philosophers of cancer, treading a long and winding path towards a fundamental understanding of multicellularity and of its alterations in cancer. Ultimately, following this path should lead to correct impaired control of differentiation, rather than, or at least together with, control of proliferation. I am aware of the fact that much of this presentation, although as much as possible relying on published observations or opinions, is of speculative nature, in particular with respect to the exploration, discovery and generalisation of non cell-killing therapies, that remain elusive so far in the clinic. Nevertheless, in a time when humanities, mathematics, biology and medicine unite their efforts to overcome the cancer disease, I hope that this approach is timely.

CONFLICT OF INTEREST STATEMENT

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTION

I, Jean Clairambault, wrote this article alone.

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