CANCER

A COMPLEX DISEASE

Octavio R. Miramontes Vidal ⊙ Elena R. Alvarez-Buylla
Editors

Authors
Elena R. Alvarez-Buylla • Juan Carlos Balandran • Jose Luis Caldu-Primo • Jose Davila-Velderrain • Jennifer Enciso • Enrique Hernandez-Lemus • Lucia S. Lopez Castillo • Juan Carlos Martinez-Garcia • Nancy R. Mejia-Dominguez • Leticia R. Paiva • Rosana Pelayo • Osbaldo Resendis-Antonio • Octavio Valadez-Blanco

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FOREWORD

THE study of complex systems and their related phenomena has become a major research venue in the recent years and it is commonly regarded as an important part of the scientific revolution developing through the 21st century. The science of complexity is concerned with the laws of operation and evolution of systems formed by many locally interacting elements that produce collective order at spatiotemporal scales larger than that of the single constitutive elements. This new thinking, that explores formally the emergence of spontaneous higher order and feedback hierarchies, has been particularly successful in the biological sciences where it is known under the name of systems biology. Life, an extraordinary, marvelous and amazing emergent natural phenomenon that has so far its only known example on Earth, contains also its own natural seeds towards its end, its own *Nemesis*: diseases and death.

One particular life-threatening disease in humans, overwhelmingly common in the modern world is cancer. It is regarded as a collection of phenomena involving anomalous cell growth caused by several factors including genetic instability with the potential to spread to other parts of the human body. From a particular point mutation in the genome (an small genetic alteration such as nucleotide deletion or insertion may be enough) to the full developed metastasis, this disease is a complex system in its own right. It is about the hierarchical organization emerging from functional multilevel networked interactions between the environment, life styles and molecular complexes coming from genetic and or epigenetic factors that have the capability of triggering the disease by means of generating abnormal spreading tumors. In a basic summary, it may be the case of a extremely small disturbance that gets amplified to reach the worst possible scenario at the body scale. A new scientific paradigm would see this initially molecular-level process as similar to many non-linear phenomena in other areas of science, ecology or physics, for example, where small instabilities get non-linearly amplified following the metaphor of the *butterfly effect*. In this line,

it is not surprising that cancer may be regarded as a complex ecosystem where competing "cell species" interact. Furthermore a recent study found that metastatic migrating cells travel following what physicist call a Lévy walk pattern. These travel trajectories display few long steps interlaced with more frequent short steps. Lévy walks are an optimal locomotion strategy found in most animals when searching for resources and having a strong theoretical physics foundation: These mobility patterns are fractals with precise mathematical laws that also seldom apply to the movement of not living particles. In this way, as these examples show, interdisciplinary collaborations are a must when dealing with the complexity of cancer.

It is estimated that, at the present time, nearly 90 million people all around the world are affected by cancer with a rate of about 14 million new cases per year. It is the cause of death for about 9 million people. When considering the costs of dealing and treating the disease, it is a considerable burden for the world economy and for the personal finances of the affected individuals and so it is also an intricate multidimensional economical, social, anthropological and political issue with considerable consequences. Regrettably, the disease is far away from being controlled or eliminated. It is true that there has been and important progress on the diagnostics and treatment involving the most cutting-edge modern technologies available, yet no definitive solution is on the horizon for the years to come. New ways of thinking are needed, new approaches must be explored and that is the main reason why the methods and concepts of the science of complex systems is a promising hope.

In the present book, a group of well recognized specialists discuss new ideas about the disease. These authors coming from solid backgrounds in physics, mathematics, medicine, molecular and cell biology, genetics and anthropology have generously dedicated their time to write an authoritative text published under the open access philosophy: quality academic texts that are worldwide free to access and read. The efforts of collaborative academic teams when producing openly available scientific texts and so exchanging freely new ideas, would be in the successful front-line struggle against cancer, a complex disease.

Octavio Miramontes & Elena R. Alvarez-Buylla
UNAM, Mexico City
November 2018

CANCER IN ATTRACTOR LANDSCAPE MODELING: A SYSTEMS BIOLOGY PERSPECTIVE OF THE DISEASE

Jose Luis Caldu-Primo[†c], *Jose Davila-Velderrain*[±], *Juan Carlos Martinez-Garcia*[‡] *and Elena R. Alvarez-Buylla*[†c*]

[†]Instituto de Ecología, UNAM, Mexico City, Mexico
[‡]Departamento de Control Automatico, CINVESTAV-IPN, Mexico City, Mexico
[c]Centro de Ciencias de la Complejidad C3, UNAM, Mexico City, Mexico
[±]MIT Computer Science and Artificial Intelligence Laboratory, Cambridge, Massachusetts, USA

Cancer is a degenerative chronic disease that can be interpreted as a robust process intrinsic to human development. Traditional cancer research has considered it a genetic disease and focused on finding genetic mutations causing it. This genocentric approach has inherent limitations as it does not take into account the complex processes involved in the determination of phenotypes from a given genotype. In the field of systems biology, it has been established that cell lineage commitment and differentiation are governed by the dynamics of an underlying complex gene regulatory network (GRN). In this way, development and cellular differentiation can be understood using the epigenetic attractors landscape metaphor as originally proposed by C. H. Waddington. From this perspective it is possible to study the mechanisms underlying cell differentiation through the computational modeling of dynamical GRNs. Recent advances in cancer research have deviated their focus from the identification of cancer associated genetic mutations to the analysis of underlying complex GRNs to reach a mechanistic explanation for the emergence of cancer. In this chapter we review some advances in cancer modeling from the attractor landscape scheme, highlighting aspects of the disease that can be explained from this perspective. Our intention is to show the advantages of this systemic approach over a purely descriptive genetic approach, and its necessity to reach a mechanistic understanding of cancer.

[*]Correspondence: elenabuylla@protonmail.com

INTRODUCTION

THE multiplicity of genetic, environmental and physiological factors involved in cancer appearance and progression makes its comprehension elusive through reductionist perspectives [1–3]. Nevertheless, mainstream cancer research still considers it a genetic-based disorder: a diverse group of diseases that result as a consequence of changes in the DNA [4]. This genocentric conception of cancer is reflected in reductionist approaches aiming at identifying genetic mutations as the causal factors for the disease [5, 6]. In spite of the genetic evidence associated with cancer, this approach has not been able to achieve a complete understanding of the disease. There is mountful evidence pointing at the necessity of a systemic perspective that departs from genetic mutations as the only explanation for the origin of cancer 1. For example: cells can become cancerous in the absence of mutations through trans- or dedifferentiation [7–9]; cancer cells manifest morphological and transcriptional convergence independently of the tissue of origin [10]; cancerous cells can be 'normalized' by several experimental non-genetic approaches [11–13]. These observations and the fact that carcinogenesis invariably recapitulates processes normally occurring during embryogenesis [14, 15], call for a developmental, rather than an entirely genetic, view of cancer.

A perspective on cancer coming from systems biology, seeks not to find the immediate molecular explanations for the appearance of a given kind of cancer, but to understand the generic mechanisms underlying cellular or tissue malignant transformation [2, 16]. Developmental transitions between cell types are a fundamental property of multicellular organisms, that can occur in the absence of genetic changes. A systemic approach implies that cancer is a developmental disease guided by the same mechanisms involved in cellular differentiation, that normally produce the diversity of cell types in multicellular organisms during development [2]. The epigenetic landscape proposed by Conrad H. Waddington is a scientific metaphor used to understand the regulatory constraints underlying development and cell differentiation [17]. From this perspective, the existence of multiple distinct phenotypic states (cell types) arising from clonal cell populations is explained by the dynamics of an underlying multistable gene regulatory network (GRN), as a complex dynamical system. This dynamical system is the mathematical formalization of the epigenetic landscape, and cancer is conceived as a special feature of it. This idea has already been proposed and developed by Stuart Kauffman in the 1970s, when he hypothesized that cancerous cells could be conceived as abnormal attractor states behaving like abnormal cell types [18]. This idea has been further expanded by Sui Huang, who defined cancer as a disease associated with the evolution of multicellularity, summarizing his idea with this phrase: "think of cancer as the price we pay for the capacity of evolving and developing a multicellular organism with one genome" [2].

The field of systems biology has developed a mechanistic methodology to study development and cell differentiation by building gene regulatory network (GRN) models from experimental evidence, and computationally simulating their

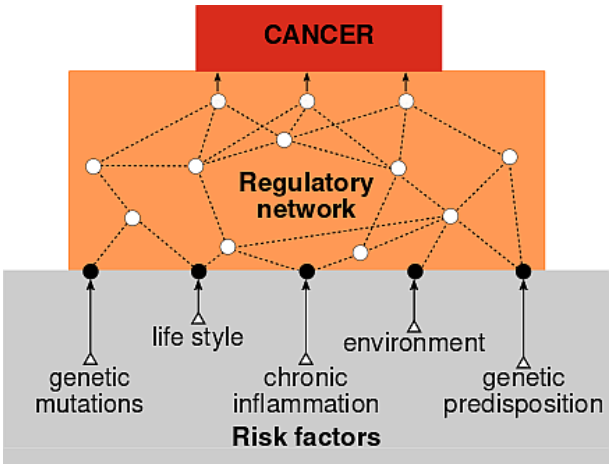

Figure 1: Cancer as an emergent process. A systemic approach to cancer must consider it as an emergent process from the interrelationship of genetic, environmental, and developmental processes.

dynamics in order to reach the attractor states corresponding to observed cell types [19]. These network modeling approach has been already applied to the study of cancer, aiming to reveal the core regulatory mechanisms for its genesis and development, as well as to generate qualitatively different predictions from those coming from the somatic mutation theory [3]. This kind of research has already been undertaken by multiple research groups studying different aspects and kinds of cancers. In this chapter we pursue to make a review of this kind of models to have an overview of the current stage of knowledge in the GRN modeling for cancer.

GRN MODELING CAN EXPLAIN CELL TYPES AS ATTRACTOR STATES AND FORMALLY REPRESENT THE EPIGENETIC LANDSCAPE

Dynamical modeling of GRNs has become a well-established framework for the study of differentiation and cell type specification during development. In this framework, a GRN that represents mutual gene regulatory interactions is modeled as a multistable dynamical system. Given the nonlinear character of the GRNs, its dynamical behavior reaches different stable states, i.e. states where the regulatory constraints imposed by the network make the expression of each gene to stay unchanged [19]. Borrowing concepts from nonlinear dynamics, the stable stationary states are called attractors, and these states operationally correspond to configurations of gene expression or protein activation that underlie or correlate with cellular phenotypes. Dynamical modeling of GRNs can be done using either discrete algebra (e.g. Boolean or multi-valued logic) or a continuous approach using differential equations. Dynamic discrete models do not require kinetic pa-

rameters, which makes them more computationally feasible and allows them to be constructed using qualitative biological data. Regardless of the method used for their dynamical modeling, GRN models assume that the structure of the biologic networks they describe is more important than the kinetics of individual reactions and acquire their richness through the large number of interactions included in them.

GRN modeling has been extended not only to explain cell types as attractor states, but to formally represent the epigenetic landscape. The key for this formalization is to consider that, as well as generating the cellular phenotypic states (attractors), the GRN dynamics also partitions the whole state-space –the abstract space containing all the possible states of a given system– in specific regions (basins of attraction), restricting the possible trajectories from one state to another one. In this context, the number, depth, width, and relative position of the basins of attraction would correspond to the hills and valleys of the metaphorical epigenetic landscape. For a more profound explanation of the methodology for GRN dynamical modeling and the inference of attractor epigenetic landscape refer to Davila-Velderrain *et al.* 2015 and references therein.

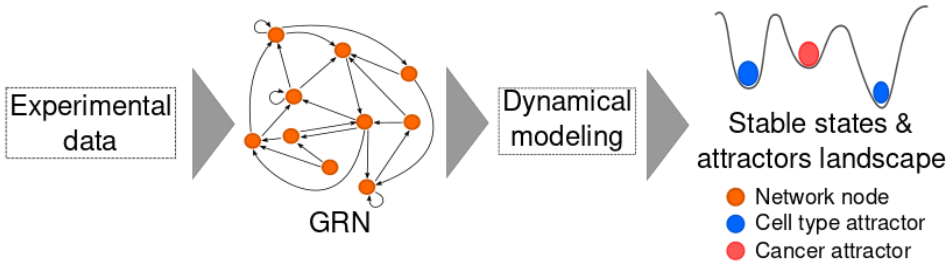

Figure 2: General framework for attractor landscape modelling of a cancerous process.

CANCER APPEARANCE THROUGH EPIGENETIC LANDSCAPE MODELLING

Here we will review some research papers tackling the problem of understanding cancer from the attractor landscape modeling methodology. In general, these methods propose a GRN based on experimental evidence, infer its associated epigenetic attractors landscape by exploring the network's dynamical behavior, and from this model test some hypotheses related to cancer (Figure 2). We focus on different aspects of cancer that have controversial explanations from the mainstream genocentric approach and in which epigenetic landscape gives alternative explanations. Specifically, we focus on the cell heterogeneity in cancer tumors, an explanation for treatments that "normalize" cancer cells, and the spontaneous mutation free appearance of cancer and its association with chronic inflammation (Figure 3).

Sources of cell heterogeneity in gastric cancer

Phenotypic and functional heterogeneity arising in tumoral cells is a shared feature of many types of cancer [20]. Mainstream cancer research offers two possible explanations for this phenomenon: clonal evolution [21] and cancer stem cell theory [22]. The clonal evolution hypothesis states that tumor heterogeneity is the result of heritable genetic and epigenetic variation, in other words heterogeneity comes from different mutations that appear in cancerous cells. The cancer stem cell hypothesis states that within a tumour there are cancer stem cells that give rise to various differentiated states.

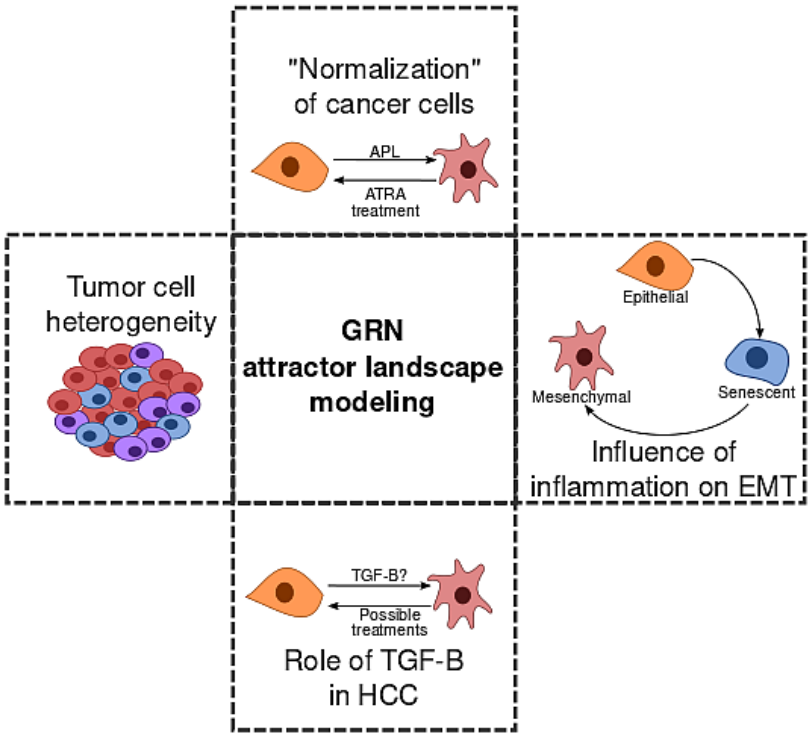

Figure 3: Different phenomena with a complicated explanation from a genetic perspective can be explained by the analysis of GRNs underlying cancer.

Ao Ping's research group approached the question of cellular heterogeneity in cancer from the epigenetic landscape perspective. In particular, they addressed the issue of tumorous cell heterogeneity in gastric cancer by analyzing the attractor landscape of the associated GRN [23]. In their work, Li and collaborators follow the typical attractor landscape methodology. First they built a GRN including transcription factors, growth factors, cytokines, signal transduction pathways, and the interactions among them. They modeled the dynamics of such GRN using a

continuous approach, and found the stable states and possible transitions among them. The system's dynamic simulations reach 8 stable attractors and 13 saddle point states corresponding to transition states among the basins of attraction. They further validated the attractor states by modeling their system with Boolean discrete dynamics finding the same attractors, showing that the primary properties of the attractor landscape are defined by the network structure rather than by specific parameters. The attractors recovered represent 4 general cell states according to the activity of known molecular markers: cell cycle arrest (three attractors), proliferation (two attractors), cell death (one attractor), and stress response (two attractors).

Human gastric cancer cells have been classified into two phenotypes based on their gene expression: a gastric and an intestinal epithelial cell types [24]. Comparing their obtained attractor states with experimental expression data, they determined that cell cycle arrest states correspond to normal gastric epithelium and the proliferation states correspond to gastric cancer cells. The expression state of the two proliferating attractors corresponded to the gastric and intestinal types found in gastric cancer. These two attractors are maintained by two different feedback loops: the *Gastrin-Wnt/β-Catenin-Cdx2* loop and the *Sox2-SHH* loop, responsible for intestinal and gastric differentiation respectively. From this analysis, they showed the existence of two kinds of gastric cancerous cells. Furthermore, the multiple proliferative attractors recovered by the model can be explained by a regulatory mechanism intrinsic to the underlying GRN.

Expanding their network analysis, they looked for other possible sources of cell heterogeneity by looking for the possible paths a normal cell can take to arrive to the cancerous state. They explored this possibility by analyzing the transition routes from the normal gastric state to the two cancer attractors finding 16 different trajectories in the state space for transitions between attractors. A normal cell can pass through different attraction basins driven by non-genetic alterations, like fluctuations in gene expression or environmental noise. The existence of 16 different trajectories to cancer indicates that in the road to become cancerous, a gastric cell can pass through different transitory states and thus result in diverse cancerous cell states. As long as the phenotypic heterogeneity in the cancer cells does not affect the feedback loops that keep them in the cancer state, there can be heterogeneity among cancer cells.

In summary, Li and collaborators found two probable origins for gastric cancer heterogeneity, without the need to invoke de novo genetic mutations or cancer stem cells. It is important to highlight that this systemic explanation does not deny the appearance of new genetic mutations in cancer tumors. In fact, these alterations can be easily incorporated in the model but they are not necessary for the appearance of cancer nor its associated cellular heterogeneity.

The effect of an efficient treatment for acute promyelocytic leukemia

Acute promyelocytic leukemia (APL) is a special type of leukemia because, unlike other types of leukemia, there is a therapy for treating it that "normalizes" leukemic blasts back to granulocytic differentiation. This therapy is based in treating leukemic patients with a combination of all-trans retinoic acid (ATRA) and arsenic trioxide (ATO) [25]. The genetic explanation for the origin of APL is the t(15;17) translocation, causing a *PML/RAR*α gene fusion [26]. Despite the existence of a genetic explanation for the disease and an efficient treatment for it, there are still open questions to have a complete understanding of what happens in APL (Yuan *et al.* 2016 and references there in).

With this concerns, Ao Ping's group applied a dynamical network methodology to try to understand APL and how ATRA treatment causes its remission [27]. They built a regulatory network including molecules and molecular pathways critical for normal hematopoietic development and physiology. Their dynamical analyses found 18 attractors, which were classified in three groups according to their genetic expression patterns: proliferating-like attractors, differentiated-like attractors and attractors with apoptotic signatures. Among the proliferating-like attractors, they identified one attractor as a normal neutrophil progenitor and an APL-like attractor. They also identified in the differentiated-like attractors cell types of the hematopoietic hierarchy, according to their expression configuration [27].

After identifying APL and normal neutrophil attractors, they dynamically analyzed the network states around them to find possible trajectories in the attractor landscape to pass from a normal progenitor state to APL, and vice versa. In this way, the authors were able to identify the critical regulators mediating such attractor transitions. In order to pass from normal progenitor-like attractor to the APL-like one, it is necessary to induce a down-regulation in *BMP* signaling and upregulate *NRF2F2* and *SHH* signaling. On the contrary, passing from APL-like to normal progenitor-like is possible by down-regulating *SHH* and up-regulating *RUNX1* and *BMP* simultaneously; or by down-regulating *VEGF* and up-regulating *RARs*. These theoretical "normalizing" trajectories are consistent with the known effects of ATRA and ATO therapy, and constitute a mechanistic explanation of how this therapy works. In particular, ATO inhibits both *SHH* [28] and *VEGF* [29], while ATRA up-regulates *RARs* [30]; these effects are concordant with the activity changes found theoretically.

Their analysis also reveals that *SHH* and *NR2F2* are important molecular players in the maintenance of the APL cell state. Since *SHH* and *NR2F2* are important inducers of angiogenesis [31, 32], the authors propose that the APL-like attractor formation may be linked to angiogenesis. Angiogenesis is an important process during early embryonic development, so under this interpretation, APL might be considered as an erroneous reversion of an adult hematopoietic phenotype to an endothelial/mesenchymal one necessary during fetal development [27].

This work shows the existence of different network modules necessary for the attainment of an APL state, involving molecular pathways considered specific for embryonic organogenesis and mesenchymal development. In this way they ex-

pand the explanation for the origin of APL from a purely genetic basis caused by the t(15;17) translocation, to a systemic view in which APL is a regression to a fetal state necessary for angiogenesis. They are also able to show a probable mechanistic way of action of ATRA and ATO treatment to inhibit the normal-to-APL transition and enable an APL normalization, expanding the understanding of APL origin and treatment.

Spontaneous appearance of epithelial cancer

In a recent work from our research group, we aimed to find a core GRN underlying a conserved process observed in epithelial cell cultures *in vitro*, in which epithelial cells acquire first a senescent-like state that later evolves to a potential tumorigenic mesenchymal stem-like phenotype [33]. This process is characterized by a series of cell-state transitions, accompanied by the appearance of patterns of cellular promotion and progression, characteristics of epithelial carcinogenesis. We studied how spontaneous immortalization via EMT emerges from the regulatory interactions between molecular players with known contribution to the tumorigenic transformation of epithelial cells.

Our proposed network consist of a set of 41 molecular players (12 transcription factors and 29 signaling molecules) related to epithelial or mesenchymal cell differentiation, cellular inflammation, senescence, DNA damage, cell cycle, or epigenetic silencing; as well as 97 regulatory interactions between them. We analyzed the network dynamics with a Boolean approach and found that it converges to three stable attractors, corresponding to the epithelial, senescent and mesenchymal stem-like phenotypes according to their expression profiles. We tested 6 different mutant conditions (specifically, loss– and gain–of–function of *ESE–2*, *Snai2*, and *p16*) and show that our model is able to recover the experimentally grounded phenotypic consequences of these mutations.

After validating the model, we tested the effect of inflammation in EMT, as it has been recognized as one of the key drivers in carcinogenesis, partly due to its implication in EMT [34]. To do this, we simulated a forced activation of *NF–κB* node in the GRN and observed the changes in the attractors landscape. We found that cellular inflammation increased the size of the mesenchymal stem–like attractor basin from 56.25 to 75% while decreasing the region of convergence of the epithelial attactor (from 17.97 to 6.25%), and of the senescent one (from 25.78 to 18.75%). Thus, the model correctly recapitulates that cellular inflammation increases the probability of a cell to enter the mesenchymal stem-like attractor, and provides a mechanistic explanation for such increase.

Finally, we used our model to study the probable sequence of attractor attainment using the stochastic methodology proposed in by Alvarez-Buylla and collaborators [35]. This analysis indicates that, considering only the regulatory constraints of the GRN, the epigenetic attractors landscape is structured in such a way that the most probable flow for a population of cells starting in the epithelial phenotype is to transit to a senescent phenotype and then to a mesenchymal stem-like

phenotype, corresponding to the cell-state transitions observed *in vitro* [7–9].

Together with the analysis of the effects of inflammation in the epigenetic landscape, this model shows that although an epithelial cell can acquire a mesenchymal stem-like phenotype even under mutation-free, unperturbed physiological conditions, the likelihood of reaching this state is increased when pro–inflammatory conditions are present. Thus, providing a systems–level mechanistic explanation for the carcinogenic role of chronic inflammatory conditions [7, 36].

MODELING THE CANCEROUS EPIGENETIC LANDSCAPE CENTERED AROUND SPECIFIC GENES

GRN dynamical modeling also enables analyses to be focused on the effects that alterations on specific genes have on the epigenetic attractors landscape. In this section we will review two works that use network modeling and attractor landscape analyses to better understand the role of specific genes with important activities in cancer. We want to highlight these approaches because they show a way in which epigenetic attractor landscape modeling can incorporate and explain the role of mutations with known effects in cancer.

TGF-β activity in hepatocellular carcinoma EMT

Epithelial-to-mesenchymal transition (EMT) is known to be a central process in cancer progression and metastasis [37]. In the previous section we revisited a study that found a mechanistic explanation for EMT in epithelial cells *in vitro*. Now we will review work from Reka Albert's research group in which they focus on the role of *transforming growth factor-β(TGF-β)* in EMT, specifically in hepatocellular carcinoma (HCC) [38]. They center their work around this gene because TGF signaling is a conserved driver of EMT in epithelial cancer models [39].

To understand *TGF-β*'s role in HCC, Steinway and collaborators built a network model incorporating growth factors, receptors, signal transductions proteins, and transcription factors involved in EMT, and used Boolean modeling to simulate its dynamics, focusing on the systems behavior upon *TGF-β* activation [38]. An important detail of their model is that they take into account the different time scales of the interactions involved in their network, signal transduction events take seconds, while transcriptional events in minutes [40], by implementing a stochastic asynchronous updating scheme with a ranking system in their simulations [38]. Using this dynamical model, they find that their network reaches two attractor states corresponding to epithelial and mesenchymal phenotypes.

As mentioned above, their work is centered on the role of *TGF-β* has in EMT. They validate this hypothesis by simulating its activation in the epithelial stable state causing the system to transit to the mesenchymal attractor. Since their dynamical model uses asynchronous stochastic update, after *TGF-β* activation the system follows different transitory routes toward the mesenchymal state. Still, no matter what trajectory the system follows, it always reaches the mesenchymal

state, demonstrating that *TGF-β* activation is a sufficient condition for EMT. Their simulations also allow them to dissect the way *TGF-β* activation leads to a mesenchymal state, showing that it is driven by the joint activation of *WNT* and *SHH* signaling pathways. They validate their theoretical results testing the activation of the *WNT* and *SHH* pathways *in vitro*, measuring transcript levels of pathway markers after *TGF-β* induction in epithelial cell lines, confirming experimentally their computational results [38].

On a later work by the same group, they use their previously constructed HCC EMT network to identify molecular targets that could suppress *TGF-β* driven EMT [41]. Their network model allows them to test the systemic effect of thousands of individual and combinatorial node knockout perturbations, something that would hardly be possible experimentally, and measure their effects on the system behavior after *TGF-β* activation from the epithelial state. The knock down simulations are done by setting one or a combination of nodes permanently inactive and running the network dynamics. They test for all possible one, two, three and four-node combinations, giving them hundreds of thousands of possible combinations. Surprisingly, they only find 13 node combinations that inhibit EMT: seven single node and six combinations of two nodes knock downs. The seven single nodes correspond to the direct *E-cadherin* regulating transcription factors, an expected result given that loss of *E-cadherin* is widely considered a hallmark of EMT [42]. All six double-node combinations that inhibit EMT include the inhibition of *SMAD*, highlighting the importance of this protein in EMT but also the necessity of combinatorial interventions for its inhibition.

They also use their single knockout simulations to explore changes in the attractors landscape after node perturbations on the network that are not capable of inhibiting EMT. They show that knocking down single nodes that do not inhibit EMT in many cases causes the appearance of a new attractor intermediate between epithelial and mesenchymal phenotypes. The existence of these hybrid states with epithelial and mesenchymal features had already been reported in experimental models [43–45], but this network analysis gives an explanation for the appearance of these hybrid states in cancer. As with their previous work, they verify their results *in vitro* by testing their EMT inhibitory combinations with siRNAs in epithelial cell lines [41].

This systems biology approach was able to integrate available regulatory information to understand the mechanism through which *TGF-β* causes EMT in HCC and to identify ways to inhibit it. The large number of molecules and their possible combinations involved in EMT makes a thorough experimental screening for targets to inhibit EMT practically impossible. Alternatively, Albert's group demonstrate the utility of a computational dynamical systems approach to tackle this question and reach a testable set of candidate targets.

Differences in *p53* network drive cellular fate choice before and after cancer

Another approach to the effects certain genes have on the epigenetic landscape is the one explored by Choi and collaborators as they studied the effects of DNA damage on the attractors landscape of a GRN centered around *p53* under normal or cancerous conditions. In their paper they are not trying to explore the origin of cancer, but instead the effects of a mutations associated to cancer in the underlying GRN dynamics. The authors assumed that *p53* has an important role in cancerous cell lines, and then analyzed the differences in the attractors landscape of a GRN with and without genetic alterations associated with breast cancer. In this sense, even though they are coming from a genocentric perspective, assuming genetic mutations are the cause of a cancerous state, they use the attractor landscape approach to understand why *p53* is an important player in cancer and its role in the network dynamics controlling cell fate [46].

Summarizing their results, they modeled the dynamics of a GRN module simulating *p53* activation by DNA damage. Afterwards, they modify the network incorporating alterations associated with breast cancer, modeled as up- and down-regulation of network nodes, and once again model the dynamics under DNA damage. The phenotypes they studied are the different behaviors a cell can undertake, which are: proliferation, cell cycle arrest, cell senescence, or cell death, and correspond to the different attractors of their epigenetic landscapes. They find that after DNA damage, *p53* activation makes normal cells enter a state of either cell death or cell cycle arrest, whereas cancer cells avoid entering cell death and instead stay in a senescent or cell cycle arrest state.

Through the state-space analysis, they not only elucidate the differential *p53* dynamics that modulate the cellular response to DNA damage, but also show that attractor landscape analysis can serve as a framework to identify the regulators that can be target of novel therapies. This is achieved by simulating alterations in the activity of different nodes of the network, and selecting those that make the system transit to the apoptotic attractor of the cancerous attractor landscape. It is important to mention that their analyses were coupled with experiments to empirically validate the predictions of the dynamical model [46].

CONCLUSIONS

We examined different studies using an epigenetic attractors landscape modeling approach to cancer. This highlights the contribution of a systems biology approach to the understanding of cancer far from the genocentric view. Several evidences point to the necessity of leaving the mutational box to understand cancer and reach a wider and better understanding of the disease etiology and progression [2, 14, 34]. Following this idea, epigenetic attractor modeling of cancer is an opportunity to achieve a better insight on cancer and a way to understand it as a developmental disease, unchaining it from a solely genetic determinism.

As the examples presented above show, GRN modeling of cancerous processes

gives a formal interpretation of phenomena, like tumour heterogeneity and cancer normalization, that are difficult to explain from a gene-centric approach (Figure 3). Also, the GRN models can easily incorporate genetic mutations as one, but not the only or the most important, cause of cancer. Finally, they can be useful to propose therapeutic targets [41]. In this way, we underscore the importance of systems biology modeling approach to reach a better understanding of the disease, find ways to reduce its incidence in the population and find new treatments when cancer is already present.

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A COMPLEX PATH(WAY) TO CANCER PHENOMENOLOGY

*Enrique Hernández-Lemus**

Computational Genomics Division, National Institute of Genomic Medicine, Mexico
Center for Complexity Sciences C3, UNAM, Mexico City, Mexico

Cancer has been largely considered a paradigmatic instance of complex diseases. The set of conditions and constraints that may cause or at least determine, the onset and progression of cancer is extremely large and disparate. Associated factors range from a plethora of mutations of several types, structural and epigenetic changes, clonal and sub-clonal selectivity biases, gene expression differences and, in general genomic instabilities and abnormal regulatory programs; but also large metabolic changes, changes in the tissue architecture, both in the tumors and on their surrounding micro-environments; to a quite relevant influence of exogenous environmental factors. All of these layers of complexity interact with each other in forms that are still elusive to our complete understanding.

In view of the enormous complexity of cancer biology, the naïve simplicity and reductionism of most of the current therapeutic approaches to cancer remains a paradox. Perhaps even more surprising is the fact that some of these therapies actually work in some cases, although admittedly their impact is still quite limited. Here we present an overview of some of the complexities related to our current view of cancer as well as some preliminary ideas on how this knowledge may help us to improve on our understanding, prognosis, diagnostics and therapeutics of cancer.

INTRODUCTION

CANCER is, no doubt, a complex pathology. The molecular origins of cancer may be traced back to such diverse processes as DNA genomic alterations and gene expression deregulation, but also to hormone disruption, metabolic changes, protein mis-folding, and signaling pathway alterations [1–4]. There is also a strong association with lifestyle and other environmental influences that can participate

*E-mail: ehernandez@inmegen.gob.mx

in the onset, development, and likely affect –to a degree– the outcome of both, primary tumors and their associated metastatic events [5].

Recently, the rise of high-throughput omic technologies has provided us with novel tools to study many of these processes in high detail, becoming central instruments of discovery in basic and clinical research that is also gaining an important role in translational medicine and personalized therapeutics [3]. There are many challenges yet as how to interpret and optimize the results, but quite especially, in how to analyze the data produced in such massive experiments. Aside with the technological limitations and shortcomings and other methodological issues –that are expected to be *tamed* anytime soon– there is a deeper reason of concern which is the multidimensional nature of the disease, its complexity. Cancer complexity, as we will analyze in this chapter calls urgently for new ways of reasoning, that may allow us to consider all different angles of the disease with a fresh view [6].

One of such, relatively new approaches which considers biological phenomena –and diseases, including cancer– as integrated entities, is called *systems biology*. Systems biology is thus the study of biological systems as integral units whose constituents parts interact, often in a complex nonlinear fashion, giving rise to emergent phenomena. As we will see, cancer is quite obviously an emergent phenomenon, that becomes possible only by the complex confluence of many factors. As such, systems biology and complexity theories may have a lot to say about it [3, 6].

Cancer is a neoplasm –i.e. an abnormal growth of tissue– with highly heterogeneous molecular origins. The biological processes involved in oncogenesis –the formation and establishment of a tumor– include DNA damage and proliferative increase of malignant subclonal cell populations. However, such processes do not occur in isolation. Oncogenesis is almost always associated with a number of deregulated high-level biological processes, including cell metabolism, hormone regulation, DNA repair, transcriptional control, and inflammation, and other changes that have been termed the *hallmarks of cancer* [1].

As already stated, oncogenic processes may involve thousands of molecular players –and thousands or millions of interactions among them–, which makes the use of omic technologies and computational analytical approaches mandatory. This is yet another instance in which cancer complexity is evidenced. High-throughput omic experiments provide us with the tools to probe complex biological processes –such as cancer– at an unprecedented scale and with very high detail. However, the analysis of such large datasets, often requires advanced mathematical and computational techniques and new theoretical approaches to rationalize and prioritize the results with view to a deeper understanding of cancer phenomenology [6, 7].

CANCER: A MULTIDIMENSIONAL MALADY

Cancer is a distinctive (somehow even a little misguiding) name given to a series of physiological and molecular alterations at the organismal level that usually starts when a single cell –or a particularly small population of cells– of the very many billions in a human body, begins to proliferate without control in what has been described as a *life-defying microscopic accident*: some mutation in the genome of this one cell, a weird change in the regulation of its cell cycle, then uncontrolled growth, evasion of cell death mechanisms, changes in the local metabolic rates, abnormally high inflammatory activity –often involving revascularization–, unusual oxidative stress management; after some time this leads to the presence of a shapeless, dominant tissue population (a malignant clone) that under strange circumstances become selectively advantageous and is sub-clonally enriched in a dynamic cellular environment.

In time, this microscopic accident overtakes surrounding cell populations in the tumor micro-environment, then the tumor keeps growing and growing until its presence turn systemic, often at the organismal level. How can such an intricate chain of events happen, may take place within the (relatively) tightly regulated and robust healthy cellular environment? How can this microscopic accident of a single cell (and its siblings) may turn into what has been called by the famous oncologist and writer Siddartha Mukherjee *...the most relentless and insidious enemy among human diseases, capable of striking virtually every organ and tissue of the body and of outwitting all our defenses...* [8].

In the remaining of this section we will further discuss some of these issues at a deeper level of detail in order to try to convey a somehow comprehensive view of cancer as an integral entity that results from the confluence of many factors interacting in non-trivial ways.

Cancer as a genetic disease

Starting from the seminal works of Knudson in the early 1970s [2], regarding the role that mutations in the retinoblastoma gene have in the development of the disease, it has been widely recognized that cancer development has an important genetic load [4].

After the completion of the human genome project in the early 2000s and with advent of massive amounts of sequencing and molecular profiling data, the genetic complexity of human malignancies has been progressively unveiled. Within a cancer cell, hundreds of genes may be aberrant, either in their sequence –via point mutations, changes in structure or in the number of copies–, while thousands of genes may be differentially expressed when compared with normal tissue [1].

Some familial cancer genes with high-penetrance mutations have been discovered and validated in these years. One may though acknowledge that the contribution of low-penetrance genetic variants –either rare or polymorphic in some population– to the risk of non-familial cancer development is still quite unclear.

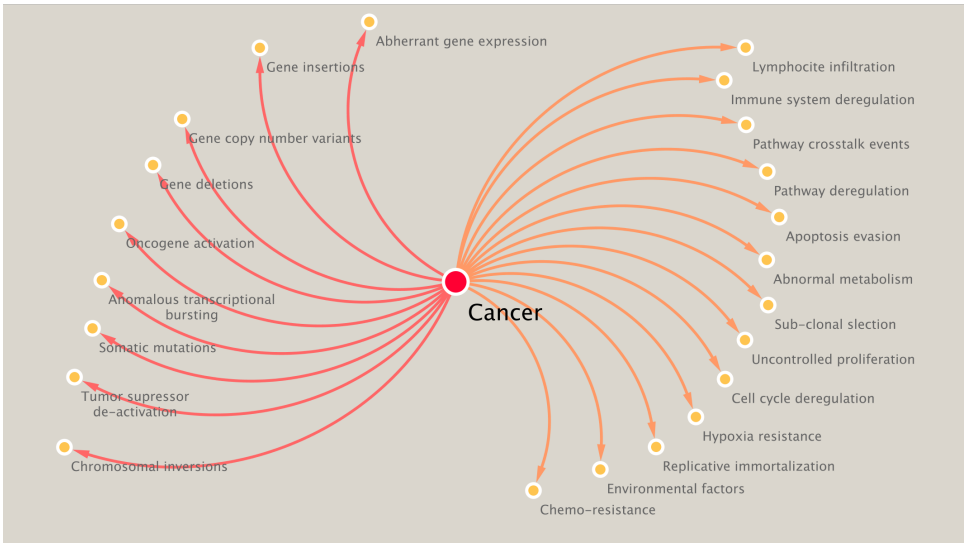

Figure 1: Some factors contributing to cancer complexity. There is a number of genetic factors (red edges) –including structural and functional alterations– and non-genetic factors (orange edges) –including cellular, organismal and environmental features– that are indeed mutually interacting and interdependent at several scales leading to emergent phenomena in cancer complexity.

By probing the complex somatic and germline mutation events that take place in the emerging cancer cell will be beneficial to prioritize variants which are able to confer increased susceptibility [1, 4]. A deeper understanding of carcinogenesis aims at the development of novel statistical and computational approaches for the analysis of the genomic regulatory programs and the related signaling networks behind individual cancer susceptibility and tumor behavior.

Oncogenes and Tumor Suppresors

The traditional view in clinical oncology has been that some particular molecules, termed *oncogenes* (OGs) [9] and *tumor suppressor genes* (TSGs) [10] are indeed the main players in cancer biology. DNA mutations in OGs and TSGs are quite often present in tumors, both at the germline and somatic levels. Among these changes, single nucleotide variants (SNVs), regional variations and structural changes leading to copy number variants (CNVs) and chromosomal rearrangements, giving rise to the so-called *fusion genes* (or their associated *chimeric proteins*). Tumor mutation rates are abnormally high due to *genome instability*, which is one of the already mentioned hallmarks of cancer. In this regard, mutations in OGs and TSGs are thus believed to carry on a fundamental carcinogenic role [11].

Tumorigenesis is –under this view– solely triggered by the interplay of OGs

and TSGs. As mentioned, OGs are frequently mutated (with gain of function changes), overexpressed or both, in cancer cells. OGs are thus, molecules whose activity is abnormally high in malignant cells. There are other processes able to change the state of an inactive form of the molecule –a proto-oncogene–.

It is then desirable to study the biomolecular reactions between the proto-oncogene and its activator molecules. These interactions, along with the physo-cochemical structure of the proto-oncogene and the activity of the oncogene are –according to this classical view– fundamental pieces to understand the phenomenon of carcinogenesis. TSGs, on the other hand, tend to be mutated in such a way that there is a loss or reduction of its function, i.e. their activity is repressed or absent in tumors. Looking for processes that lead to the inactivation of the tumor suppressor molecule and characterizing such interactions as well as the structure and function of the tumor suppressor will also trace back the origins of cancer under this framework [9, 10].

Well known cancer initiator events include the interplay between oncogenes such as RAS, MYC, EGFR, VEGFR, WNT, ERK, TRK, etc. Fusion oncoproteins like BCR/ABL, as well tumor suppressors such as p53, BRCA, PTEN, CD95, and others. It is already known though that there are many other processes and molecules involved in the development and sustainment of tumor phenotypes. However, the classical oncogenetic theory faces important challenges and shortcomings that have shifted and broadened the scope of cancer research, in particular since the advent of whole genome approaches to cancer genetics. One of these partially unsolved issues is related with the distinction between tumor associated events and causal events [1, 11].

Driver and Passenger mutations: On circular causality

The approach we have just outlined has been successful to some extent to reveal a number of foundational principles in cancer biology, is it, on the other hand, far from providing a complete picture of cancer. As reviewed by Hanahan and Weinberg [1], one of the tenets of cancer is genome instability [12]. Malignant cells often present wider and broader variations in their genomes, with respect to non-tumor cells. Such variants occur both in the sequence, in the form of chromosomal rearrangements, abnormal copy number variants, quantity and localization of point mutations and structural changes (inversions, deletions, insertions, etc.), as well as at the gene and protein expression levels. It is not that clear, however, what are the distinctions between so-called *driver events* and *passenger events*.

It has resulted almost impossible to distinguish systematically sets of abnormalities that *may cause* cancer from those that mainly appear *due to* cancer. The onset of cancer has thus resulted an elusive, unless you actually take action to initiate it (for instance, in animal models) in which case, any causal observations will be by necessity biased.

One related issue in uncertain causality is given by the actual role of OGs and TSGs. Likely, the most cited example of a TSG, is the p53 protein, encoded by the

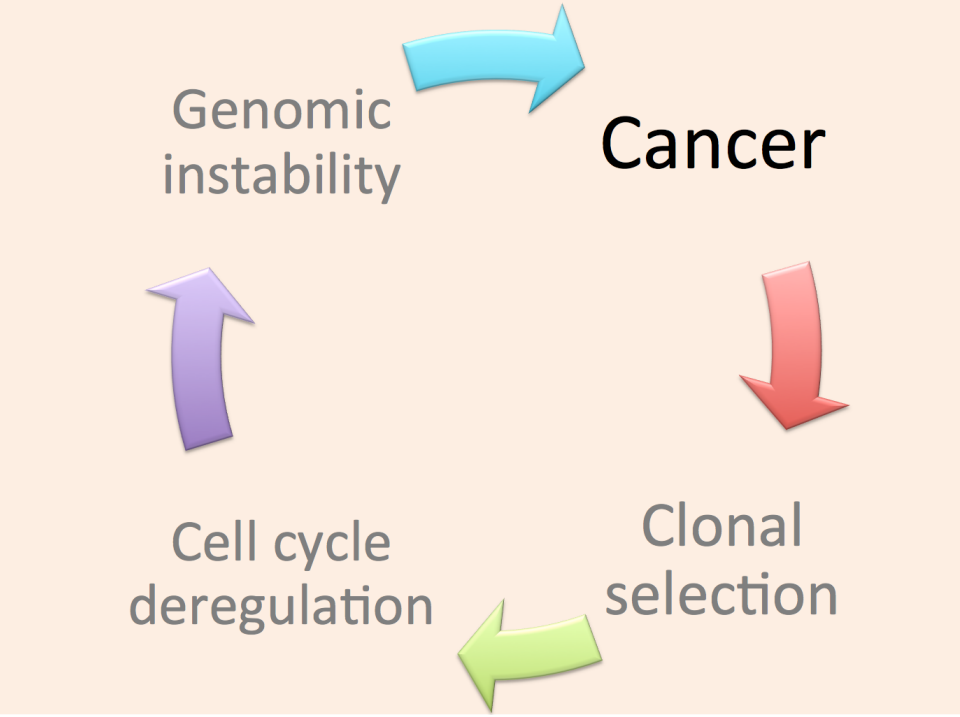

Figure 2: Some factors contributing to cancer circular causality. Cancer is known to be caused by mutations and is also a cause for mutations; cancer cells are known to be sub-clonally selected and also tumors are environmental modulators of cellular fitness; tumorigenesis is associated with cell cycle deregulation, but abnormal tumor metabolism leads to cell cycle changes. These are just a handful of examples to show that causal attribution in cancer is a very tricky issue, that have shown to be ultimately useless in practice.

TP53 gene. TP53 is either mutated, deleted or abnormally functioning in around 50% of human tumors –which by the way means, is NOT presenting any abnormality in the other 50 % of tumors).

Even if p53 has been widely studied for decades, there is still no substantial advancement in cancer prognostics and diagnostics based only on it. The reason is that while it is known that DNA damage mechanisms (in which repair p53 is involved) are fundamental –although not necessarily causal– in carcinogenesis, it is clear that this is not an independent process [13, 14].

In this regard, it is not clear if (or better when) DNA damage may lead to genome instabilities compromising the DNA repair machinery and then leading to cancer, or cancer leads to DNA damage, in particular in genes associated with DNA repair thus leading to genome instabilities [15, 16].

Sub-clonal selection: drivers and passengers revisited

An open problem in cancer genomics is how to identify driver mutations and distinguish them from passenger mutations, in terms of clonal growth advantages. In a nutshell, of the many distinct mutational events that may occur in cell lineages and are not reversed by DNA repair mechanisms, there are some that may remain at the level of normal genome variations –even if they become fixed in the population–, let us call them *passenger mutations*; while there are others that may have some essential functional roles that can be clonally selected and may eventually conduct –cause?– to the development of cancer, those are *driver mutations*. The problem is: how to tell one set from the other?

A usual approach consists in looking at mutation frequency, mutual exclusivity of mutations between gene sets, and pathway/network information. Development of methodologies to identify drivers from passengers is an active area of research. These methods include, frameworks based on network enrichment analysis, evolutionary population dynamic models and the search for genes with low mutational frequency due to epistatic interactions [1, 15].

Data driven approaches: thinking out of the box

Recently, the so-called data-driven approach has gained importance in the study of complex diseases like cancer. This has been pushed forward, by the enormous complexity of cancer, but also due to the technological breakthrough brought by high throughput 'omic technologies, and the advent of high computing data analysis capabilities (the so-called Big Data revolution) [6].

Under this view, cancer can be studied systematically by means of the genome wide analysis of tumor samples and healthy controls. By considering statistically significant differences in their molecular profiles for DNA alterations, differential gene expression, as well as proteomic and metabolomic differences between cases and controls, supplemented with computational classification it has been possible to find for molecules and pathways relevant to cancer [3].

High throughput data-driven approaches have led to the identification of novel cancer-related molecules and pathways beyond the traditional OG or TSG classifications. Interestingly enough, most of these processes were previously not even considered to be oncogenic, thus improving our knowledge about the molecular origins of cancer. Abnormal inflammation, energetic deregulation, immune system adaptability, hypermutated pathways and genome instability, are some of the processes that have emerged in the consideration of the origins of cancer [1, 4, 8].

Other processes such as aging, apoptosis evasion, autophagia and senescence to mild proliferative states (that later turn into highly proliferative stages are also being commonly found under neoplastic conditions in a series of data-driven studies. The extent to what metabolic abnormalities participate in oncogenesis has been also long debated. In the past, it used to be considered that big metabolic changes were a mere consequence of the growth of cancer cells with no functional oncogenic role. However, there is strong evidence that these changes may be extremely

important in very early stages of tumor development.

Cancer as a network disease

It has been largely discussed that complex diseases such as cancer arise from the interplay of many genes with the environment. However, even if today we are in a position to measure and characterize thousands of biomolecules, and millions of changes on their structure and interactions; even when we recognize the synergistic role that arise from this plethora of interactions, there is a fundamental contradiction still present in many studies at the genomic level: most of what one can read in the current cancer biology literature is still strongly reminiscent of traditional genetics: genome wide analyses of mutations, gene expression and structural variants, are performed, only to end up discussing single gene issues –or worse, seems that some are still looking for that *cancer gene* behind all over– or discussing large and involved pathways by invoking the action of individual molecules [17].

Clinical and molecular oncologists are still discussing about phenomena like the one termed *oncogene addiction* claiming that despite cancer's already self-evident complexity, and its entangled origins, the growth and survival of tumor cells –which constitute the ultimate cancer features– can be heavily constrained by the inactivation of a single oncogene – the long-desired, cancer gene! – that may in turn provide a rational, clear-cut, target for molecular therapy.

It has been until relatively recent times that a serious, systemic consideration of gene interaction networks is getting at the center of discussions on tumor biology. We are realizing that the role of gene regulatory networks, for instance, it is far more relevant than individual gene contributions [18–20].

Regarding oncogene addiction, current findings point-out to a more complex scenario for tumors may not be *addicted* to a single oncogene, instead they depend on the action of phenotype-specific pathways and even phenotype-associated gene networks in a phenomenon that has been termed *network addiction* [17].

This shift of emphasis calls for a new way of looking at the molecular origins of cancer. The network addiction paradigm may imply that different components of a cancer network may be globally deregulated at the single cell level, but also at the level of cell populations.

Transcriptional bursting and cancer: the role of master regulators

One striking feature of cancer biology is that often, the biological events finally leading to tumor development occur suddenly, in a fast, orchestrated manner. Such phenomenon may be related to the onset of large scale transcriptional bursts [21–23]. This seem to contradict the idea that tumors –in particular adult age cancers– arise from the slow accumulation of damaging mutations during the lifespan of individuals. How can be reconcile both observations: sudden arise and fast growth of tumors and slow mutation accumulation. One possible answer to this is

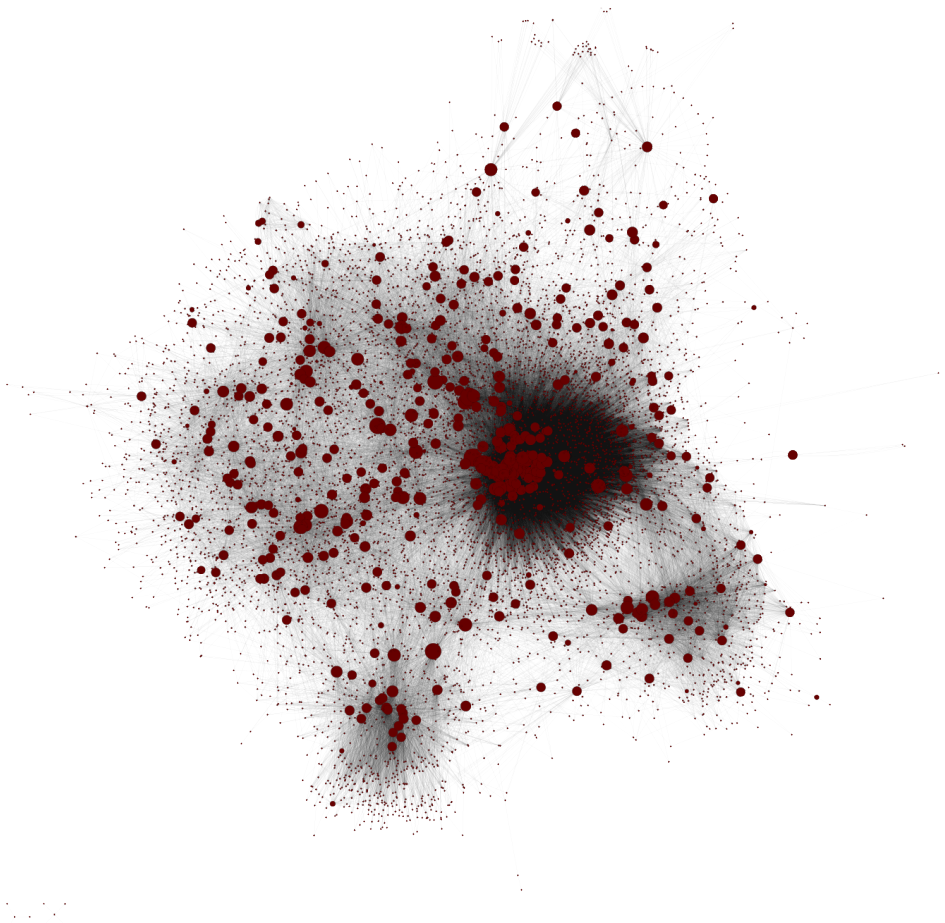

Figure 3: A breast cancer gene regulatory network. The size of the nodes (genes) correspond to their centrality for the whole genome regulatory program. One can notice the large number of molecular players involved in gene regulation for the tumor.

that the definite route to tumorigenesis may depend on changes in genes that have a long range of influence over the genome [24].

It has been observed that a number of large scale transcriptional cascades behind the complex cellular processes involved in tumorigenesis may be actually triggered by the action of a relatively small number of transcription factor molecules known as Transcriptional Master Regulators (TMRs) [3, 25–28].

It has been argued that TMRs may be responsible for the global control of phenotype-specific transcriptional regulatory programs. Hence, by understand-

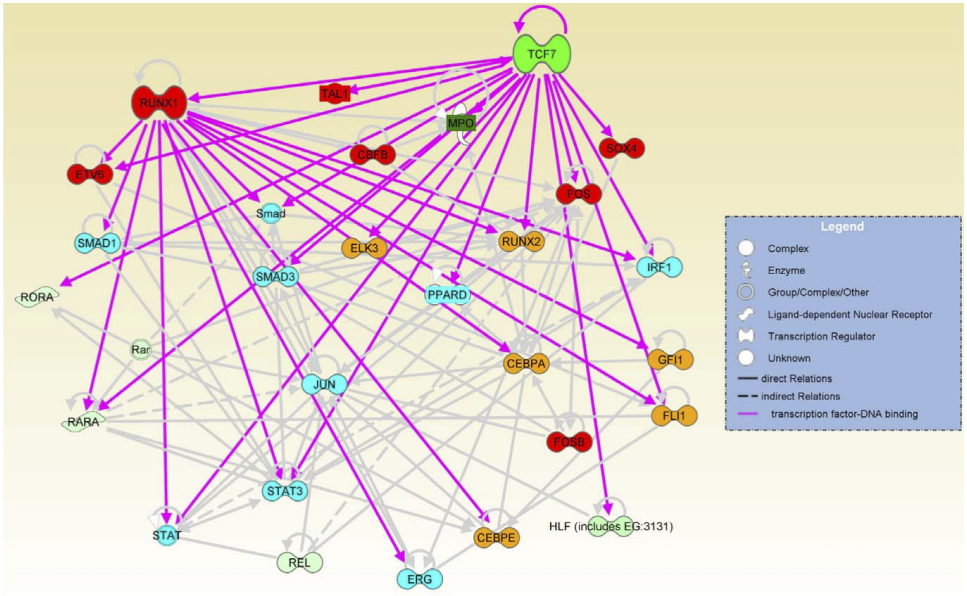

Figure 4: An example of the action of a transcriptional master regulator. *TCF7* is a well known transcriptional master regulator. Here we show how it promotes a series of transcription factor activating events that may lead to transcriptional cascading.

ing the way in which TMR-mediated events are happening, it will be possible to elucidate normal cell physiology –as has been the case of TMRs of the immune system and the differentiation of erythropoietic cells–, but also in the case of complex pathological phenotypes, such as cancer [29, 30]. Dysregulation of TMRs can possible bear a high impact on cancer-related phenotypes, since uncontrolled TMR synthesis may trigger the activation and signaling amplification of several transcriptional cascades.

Cancer as a pathway disease

Due to the multiplicity of deregulated biochemical processes in tumor cells, one can also state that cancer is a pathway-based disease. In the light of this assertion, pathway analysis may become a promising tool in order to understand the complex interactions and reactions associated with this group of pathologies. Pathways are useful representations of said interactions, based on the current knowledge of cellular function at the biomolecular level. Many of these pathways, share many molecules, are able to crosstalk with each other, adding to the already complex scenario of oncogenesis and also –as we will discuss later– having strong con-

sequences for the design of therapeutic approaches [31].

The main hallmarks of cancer are associated with the action of pathways related to cell proliferation, apoptosis evasion, cell-differentiation and in general, to the dysregulation of cell cycle and the alteration of DNA-repairing processes. The phenotype of a cell is determined by the activity of a large number of genes and proteins. Hence, transcriptional regulation lies at the heart of many of the intricate molecular relationships, driving the activity of biological pathways [1].

Considering that such interactions occur in a non-trivial manner, since the processes related to a physiological (or pathological) function may interact in such a way that abnormal changes in one pathway leads to the deregulation of many others. Understanding the mechanisms underlying such entangled biological processes, via a comprehensive, system level analysis might lead to insights into the molecular phenomenology that further the malignant phenotypes.

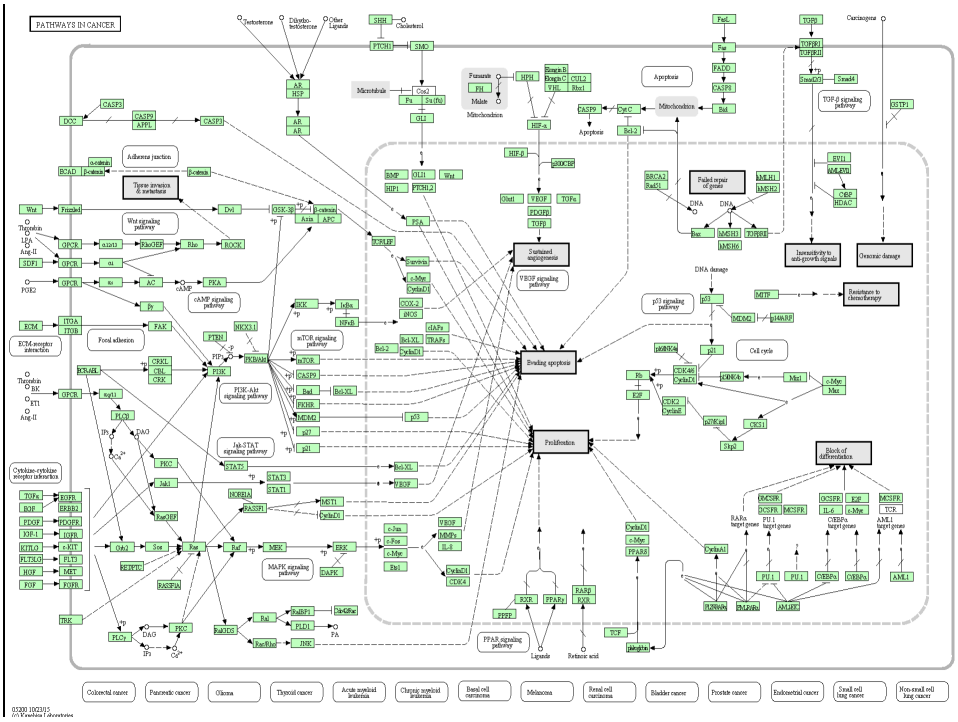

Figure 5: Main molecular pathways associated with cancer. A simplified map of the top molecular pathways associated with cancer in the KEGG database.

Cancer and metabolism

It is known that cancer cells show extreme differences in their metabolic functions when compared to non-tumor cells. Cancer cells can also use distinctive biochem-

ical pathways to supply for their energetic requirements. Hence, neoplastic tissues are able to express some tumor-specific proteins of the family of the glycolytic enzymes (GEs). GEs are able to interact with molecules that play the role of tumor modulators (TMs) adapting their metabolic activity in order to comply with highly proliferative cell growth regimes under hypoxic conditions typical of malignant tumors [32–34].

The interplay of GEs and TMs have attracted oncology researchers to find out whether GE inhibition or TM tuning may deprive tumors from energy, while leaving healthy cells mainly unaffected. Being this the case, the regulation of cancer-related energy production pathways may turn out to be a substantial research area for pharmacological therapy in cancer [35, 36].

Metabolic transformations have been also shown to be involved in tumor survival. This again points out to metabolic pathways as potential pharmacological targets in cancer. The modulation of metabolic pathways may become a promising alternative for cancer treatment. As already commented, metabolic deregulation is also important for the induction of transcriptional instabilities leading to systemic failure, via selective advantages displayed by cancer clones [37]. Therapies must be applied cautiously, however, in order not to annihilate normal cells along with neoplastic ones. In this regard, it has been discussed that a combination of agents that diminished energy production while modulating some aspects of cell signaling could become a multiplexed therapy to target cancer cells with higher selectivity and sensitivity [38].

Tumor micro-environment and cellular heterogeneity

As any experienced oncological pathologist can tell, the molecular heterogeneity of cancer is actually rivaled –or even superseded– by the heterogeneous character of tumors at the cellular and supra-cellular levels. The tumor microenvironment in which the tumor develops –which includes the surrounding vasculature, immune cell infiltrates, fibroblasts and inflammatory cells, as well as wandering lymphocytes, second messengers and other signaling molecules and the vast number and type of the cells constituting extracellular matrix – is constantly interacting with the neoplasm. Tumors exert their influence over the microenvironment via extracellular signals that usually promote tumor angiogenesis and induce immune tolerance inn the surrounding cells. On the other hand, cells from the local tumor environment affect the proliferation and differentiation patterns of malignant via, for instance, immune-editing mechanisms [39, 40].

Other mechanisms of interaction between the tumor microenvironment and the tumor itself include the development of adaptive cellular immunity –often by the action of tumor-infiltrating lymphocytes –, Enhanced permeability and retention in the surrounding vasculature, hypoxia resistance (e.g. via the Warburg effect), inflammation-enhanced angiogenesis in the stroma, fibroblast arrest, as well as more complex mechanisms like macrophage de-activation by myeloid-derived suppressor cells or extracellular matrix remodeling. All such phenomena occur

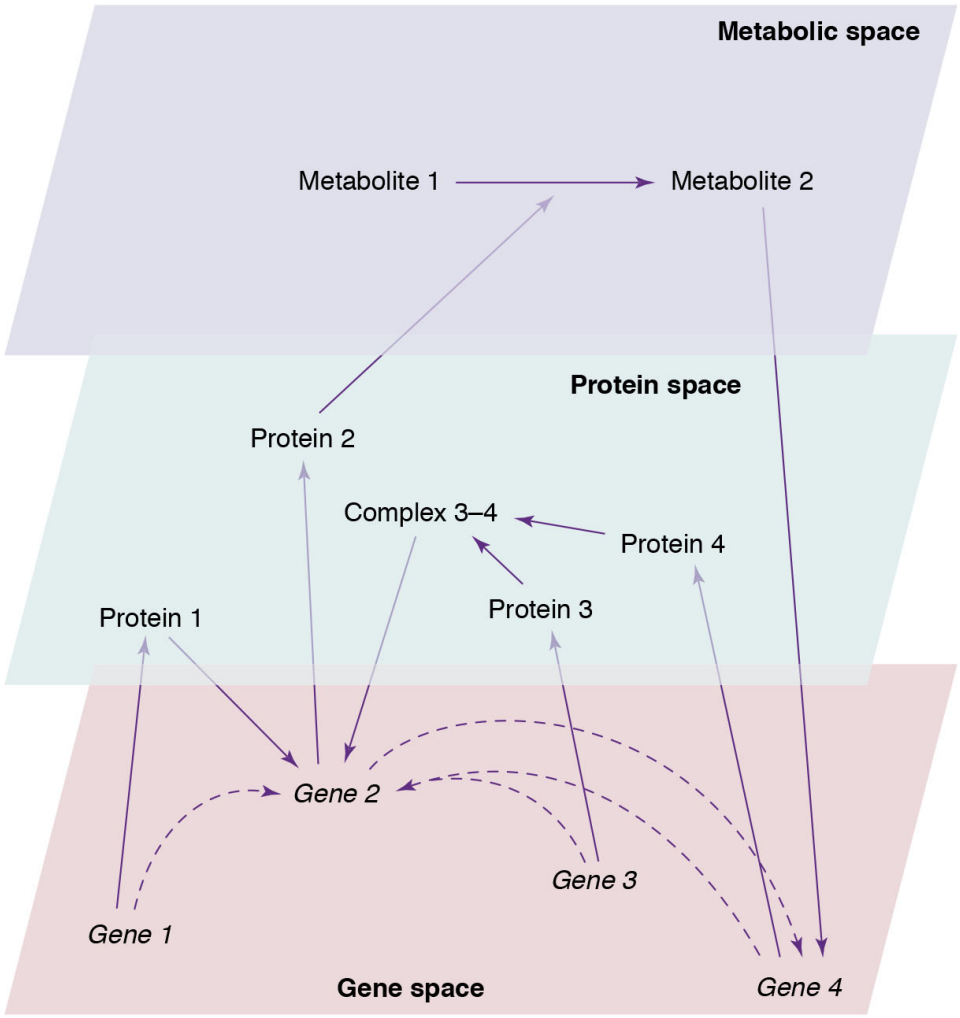

Figure 6: Metabolic networks are not isolated Metabolic deregulation in cancer is not an isolated process, rather it occurs in a multi-scale environment in which transcriptional events, as well as physical and chemical modifications at the protein level interact with the metabolism in a dynamical way.

to an extent *outside the tumor*, turning most of the current studies of cancer –often centered on the population of neoplastic cells –, dubious, to say the least. Tumor microenvironment heterogeneity and the interaction of this ever changing ambient with the tumor is yet another layer of complexity to be considered if we are striving for an integral understanding of cancer development [1].

Cancer and the environment

According with the United States' National Institutes of Health [5], exposure to a number of natural and man-made substances in the environment can be associated to some two-thirds of the cases of cancer in the United States. Such factors include may lifestyle – e.g. cigarette smoking, excessive alcohol consumption, poor diet, lack of exercise, excessive sunlight exposure, and sexual behavior that increases exposure to oncogenic viruses–, certain medical drugs, hormones, exposure to high levels of ionizing radiation, viruses, bacteria, and environmental chemicals in the air, water, food, and workplace.

The contribution of environmental factors to cancer development has been long known. Such contribution it is, however, more complex than initially thought. On the one hand, there is the contribution that gene-environment interactions may have to enhance susceptibility to carcinogens. Genetic variants are associated with differences in therapeutic susceptibility, and their effects can be modulated by changes in the environmental conditions, with unknown impact in the clinical outcomes. Then, the complex interplay between genomic susceptibility and the surrounding environment is one more reason for the unsuccessful results obtained by mainstream cancer therapeutic schemas. Amidst such dynamically changing interactions is the emergence of new traits that confer malignant tumors a highly unpredictable behavior.

COMPLEXITY IN CANCER THERAPEUTICS

As we have seen, recent times have brought a shift in the way we understand neoplastic diseases. Perhaps the more relevant challenge we face today is in how to incorporate all these findings in a rational and useful way that enable us to complete the long-anticipated bench-to-bed goal of translational medicine [41, 42].

Complex systems approaches to oncology must play two roles, in this regard: first of all they may provide a framework to collect, process, and integrate the huge loads of data required for a global understanding of carcinogenesis; secondly, a complex systems view must lead to the development and implementation of integrated models that combine the available information in a form useful in the anti-cancer therapy decision making processes [43–47]. Up to date there has been only a handful of such approaches that we will briefly discuss in what follows.

As we already mentioned, there is an entangled complex phenomenology at the onset of cancer. Such complexity is also reflected by the way in which cancer cells live and survive and consequently has implications in the way they respond to treatment. We will briefly discuss just a couple of examples in which the intricacies of cancer biology impact current therapeutic approaches.

Pathway crosstalk and drug-resistance mechanisms in cancer

In the section of cancer pathways it was already mentioned that there is an abnormally high number of biomolecular pathways deregulated in tumor cells. Aside

from the complexity derived from thinking how to treat each of these processes in order to *set them back to normal levels* looking to recover homeostasis, there is an additional non-trivial layer of complexity which is derived from the fact that all of these pathways are not independent from each other, but are coordinately modulated by a dense cloud of common regulatory processes –which may be to some extent devised if we analyze the complex structure of cancer gene regulatory networks– [31].

This interconnectivity of different molecular pathways –some of which provide the basis for targeted pharmacological therapy and even shape the response to cytotoxic chemotherapies– has important consequences for the development of resistance to anti-cancer therapies. As a first instance –the simpler one!– let us consider the phenomenon of pathway crosstalk caused by molecule sharing. As we can see in the figure 7, if two pathways share one molecule, the response of this molecular probe to therapy –let us say to the action of a drug– will affect *both* pathways in which the molecule participates. This quite simple and pervasive phenomenon will have enormous implications, for instance in, the response to direct pharmacological target therapies [31].

For instance, in our group we have studied, how the modulation of the estrogen receptor –an important trigger of proliferation in breast cancer cells– by anti-estrogen therapy may diminish in efectivity due to the fact that the estrogen receptor pathway (the biological process target of the therapy) presents a large number of crosstalk events with other pathways that ultimately led to the activation of the pathway even in the complete absence of its triggering membrane receptor.

To illustrate this, figure 8 shows how different breast cancer subtypes, two of which are not expressing the estrogen receptor –the ones in panels C and D–, have all quite active the estrogen receptor pathway that leads to high proliferative rates in breast cancer.

Sub-clonal selection and chemo-resistance

We have already discussed that cancer cells are able to enhance their fitness, even under stressful cellular conditions. This selective advantage has also negative consequences of the therapeutic interventions, in particular for those at the pharmacological level.

One common avenue to chemotherapeutic resistance (common to both, direct targeted therapies and cytotoxic treatments) is the fact that from a whole population of cells, only a percentage (hopefully large) of the individual cells responds to treatment, either by modulating its oncogenicity or by dying. However, the remaining, unresponsive tumor cells are the ones that will continue to proliferate [12, 48]. These remaining cellular niches of unresponsive, proliferating cells are thus enriched in factors that allow them to overcome the action of the anti-cancer therapy, so that the coming generations of tumor cells are more likely to *inherit* such traits that in this context provides them with a selective advantage.

Subclonal selection is thus an important mechanism for the development of

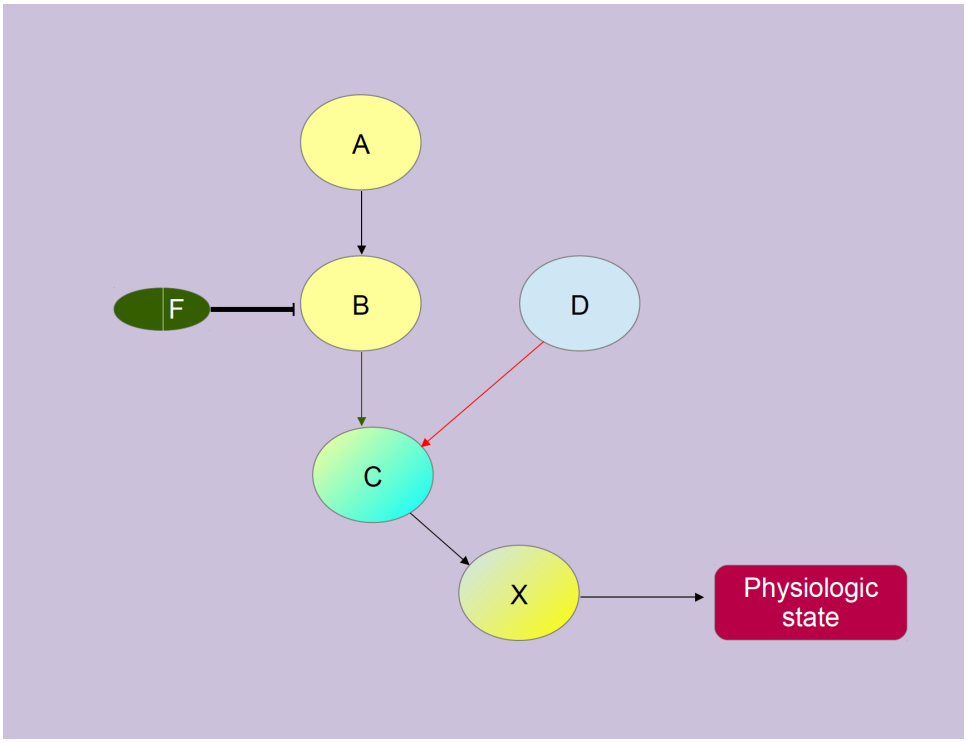

Figure 7: A toy-model of pathway crosstalk. Depicted is a pathway (call it Pathway 1) A $\rightarrow$ B $\rightarrow$ C $\rightarrow$ X $\rightarrow$ [Physiologic state]. One way to stop the physiological state to occur is by the action of a drug F that blocks the action of molecule B. There is also, however another pathway (call it Pathway 2) D $\rightarrow$ C $\rightarrow$ X $\rightarrow$ [Physiologic state]. Since Pathway 1 and Pathway 2 crosstalk to each other by means of molecule C, the activity of Pathway 2 may also induce the physiologic state, even if molecule B has been inhibited by the drug F, thus leading to pharmacological resistance mechanisms.

pharmacological resistance to anti-cancer drugs. One possible way to overcome tumor subclonal selection is by changing the type of anti-cancer therapy over the time, hoping that cells that were unresponsive to one treatment, become responsive to a different succesive therapy. However, there is no guarantee that this will be a lasting situation, since cancer clonal selection mechanisms keep on changing as the tumor develops [49, 50].

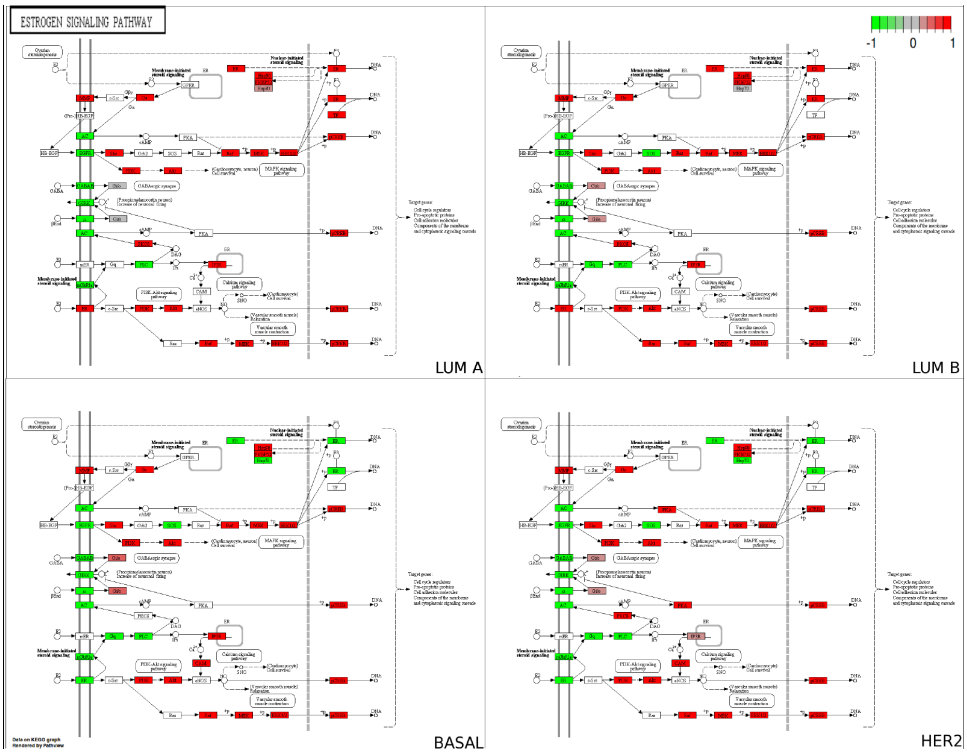

Figure 8: Estrogen signalling pathway in breast cancer. One can see that the estrogen signaling pathway leading to proliferation is active in all four cancer subtypes, even when the HER2 and BASAL tumors are not expressing the estrogen membrane receptor.

NOVEL APPROACHES TO CANCER RESEARCH

It is worth mentioning that in recent times, some new approaches to understand how cancer works, that may complement the efforts from the cancer biology and clinical oncology communities have been developing. Some of these new lines of inquiry arise from the quantitative sciences and are, in some sense injecting fresh air to the more established disciplines studying cancer. Two such approaches to cancer are computational and mathematical oncology as well as physical oncology. We will briefly discuss them in what follows.

Computational Oncology

Computational oncology has been broadly defined as the study of cancer biology with the aid of computer-implemented tools, usually borrowed from the quantitative sciences. Two main computational oncology branches have been established

in recent times: one of them –that one may term the *bioinformatic approach to oncology* or *cancer bioinformatics*– is related to processing, storage, retrieval and analysis of data generated by high throughput technologies (mainly from the omics and imaging). The second one consists in the development of descriptive and predictive methods to translate such enormous quantities of data into rational models, one may call this approach *cancer systems biology* or even *systems oncology*. able to drive experimental research by generating new questions. More importantly, computational oncology develops applications in the clinical setting in order to improve diagnosis, treatment selection and prognosis.

Computational oncology may be also classified on the basis of the disciplines from which its applications are derived: *Mathematical oncology* is rooted in the mathematical and computational sciences. Its goal is the development and implementation of algorithms for the analysis and management of biological data. *Physical oncology* comes from the application of ideas derived from physical models to oncology problems and it is aimed to create a mechanistic view of cancer [6].

Physical Oncology

Physical oncology has been applied mainly to the generate large-scale, mechanistic-driven models based on molecular and physiological cancer data. Models are designed to be constantly refined though interactions with experimental derived data to improve on its physical insight, without relying so much on intuition. One of its strengths is that a number of relevant problems in oncology can be mapped to some equivalent physical problem for which a solution –at least partial– already exist. However, quantitative physical oncology will become increasingly important, only to the extent that new technologies to probe tumors in the preclinical and clinical setting are developed.

Tumor growth modelling begin with kinetic models of cell proliferation. Tumor growth is modeled as a quasi-chemical kinetic process for which the competition and cooperativity among between environmental constraints and the cell program (often including a coarse-grained view of genomic, signaling and metabolic regulatory functions), with the more recent models have advanced by taking into account the interactions between the tumor itself, with its local microenvironment [3, 6].

SOME IDEAS AND (IN)CONCLUSIVE THOUGHTS

In order to integrate all the vast corpus of information that exists (and also the one that is being currently generated) into a complex systems approach, one must strive to develop theoretical frameworks that allow to analyze the data to unveil hidden interactions that may lead to the onset of emergent phenomena (e.g., hormone mediated drug resistance mechanisms) under a multidimensional, multiscale approach. Of course, this is easier said than done.

Major obstacles consist not only in solving the individual parts of the cancer puzzle (an already overwhelming task) but also how to put all the pieces of information together in an intelligible form, able to deal with the enormous heterogeneity of individual tumors, useful in both, the research and the clinical setting while at the same time will develop into an integrated, somehow general, theory of cancer biology. At this point, we have of course more questions than answers... there is a long, long way to go, but at least we are starting to move into what seems to be the right direction.

ACKNOWLEDGMENTS

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THE PATHOBIOLOGICAL COMPLEXITY OF CHILDHOOD CANCER: ACUTE LEUKEMIAS AS A PARADIGM OF STUDY

Jennifer Enciso[†‡c], *Juan Carlos Balandrán*[‡±] *and Rosana Pelayo*[†*]

[†]Eastern Biomedical Research Center CIBIOR, IMSS, Puebla, Mexico
[‡]Biochemistry Sciences Program, UNAM, Mexico City, Mexico
[c]Centro de Ciencias de la Complejidad C3, UNAM, Mexico City, Mexico
[±]Molecular Biomedicine Program, CINVESTAV-IPN., Mexico City, Mexico

INTRODUCTION

CANCER has been one of the leading concerns of global health for the last decades, inspiring a continuous and intensive research. The prospective isolation of primitive tumor initiating cells, along with novel theoretical and experimental integrative approaches outlining the interplay between transcriptional networks and microenvironmental signals that control early cell fate decisions, have been critical to advance our understanding of the pathobiology of cancer.

As a result, the perspective of cancer has moved forward to a multifactorial, dynamic and interactive complex system where subjacent elements such as genetics and epigenetics are in constant interaction with micro- and macro-environmental emergent factors that contribute to the etiology and evolution of malignant cells.

Of note, pediatric oncology has been recently defined as a biomedical priority, with acute lymphoblastic leukemias being the most frequent childhood malignancies and a foremost cause of mortality worldwide. Decreasing overall leukemia mortality in children requires a comprehensive notion of their clinical and biological pathology.

In this book chapter, we focus on current and emerging knowledge on malignant hematopoietic differentiation that provides a more integrated

[*]Correspondence: Rosana Pelayo. Eastern Biomedical Research Center CIBIOR. Km. 4.5 Carr Atlixco-Metepec. 74360 Puebla, Pue., Mexico. E-mail: rosana.pelayo@imss.gob.mx; rosanapelayo@gmail.com

view of the disease complexity.

CANCER COMPLEXITY: THE MICRO AND MACRO PERSPECTIVES

Cancer is one of the most complex biological systems and may result from the interaction of underlying mechanisms, emergent conditions and external factors, contributing to the empowerment of malignant evolution (Figure 1). The clinical, molecular and biological heterogeneity of cancer diseases, indicating an unsuspected multiclonal diversity, has highlighted their complex pathobiology.

Within the dominant subjacent mechanisms, driver or passenger translocations and mutations control crucial cell fate decisions and compromise homeostasis, differentiation:proliferation or death:survival rates. Strikingly, an emergent context where normal microenvironment, intercellular communication or most immune surveillance mechanisms are impaired, cooperate to initiation of damage. Moreover, external inducing factors, such as life style-associated biological elements: diet, radiation, chemical exposure or infections, capable of remodeling normal microenvironments or exposing primitive cell populations to transformation, has been suggested as key players. Of special interest has been the hierarchical theory of cancer development sustaining the notion that cancer stem cells support the emergence and maintenance of tumors and may also be responsible for migration and development of metastatic tumors if an inductive milieu co-exists. Thus, novel malignant progression mapping should consider inter-dependent, multi-system and multi-level biological relationships [1–3] (Figure 1).

The initiation stage of malignant transformation mostly occurs as a consequence of failed capabilities of DNA damage repairing. Further promotion stage is characterized by genetic aberrations and loss of regulatory processes concomitant to poor immunosurveillance mechanisms. Finally, malignant cells move forward the progression phase, endowed with substantial tumor growth and its potential metastasis to distant organs [1].

To date, there are more than 140 recorded genes whose intragenic mutations contribute to promotion of selective growth advantage, known as "driver mutations"[4]. Divergence of multiple of such mutations leads the transformation of normal cells into malignant or cancer initiating cell [4, 5]. Interestingly, while melanomas and lung tumors exhibit around 200 mutations and common solid tumors between 33 to 66 mutations, pediatric tumors and leukemias harbor approximately 9.6. Between two and eight driver mutations are critical for tumor development, although many pas-

senger mutations are generated along cancer progression [4, 6]. The net final balance is survival of cancerous cells due to apoptosis evasion, whereas normal cells are generally induced to death after DNA damage and chromosome breakage [4].

Driver mutations generally target genes encoding for antigrowth factor receptors (e.g. TGFβ), signal transduction mediators (e.g. Ras proteins, PTEN), cell-cycle regulators (e.g. p16, Rb), supervisors of genome integrity (e.g. Chfr, MLH1, ATM), transcriptional regulators (e.g. VHL, GATA3), adherence mediators involved in tumor metastasis (e.g. E-cadherin) and of recent importance, epigenetic regulators (e.g. DNMT1, MLL3, TET2). Among all type of cancers, the most common mutated gene is p53, which encodes a tumor suppressor protein involved in cell cycle progression, activation of DNA repair machinery and apoptosis [5].

Multiclonal diversity and the continous emergence of subpopulations with heterogeneic mutations, producing an intratumoral competence with the resulting selection of the best fitted clones, have suggested a macroperspective where intratumoral competences between normal cells from the same and other tissues take place.

The clear association of major peaks of incidence and the average life expectancy has represented one of the strongest evidence of genetic instability as a consequence of aging processes, such as telomers shortening, high reactive oxygen species (ROS) and accumulated damage resulting from chronic exposure to carcinogens. In contrast, childhood cancer is rare (1% of all individuals with cancer) although representing the major cause of death by disease around the world, with just 5% of cases caused by an inherited mutation [7–9], suggesting that micro and macroenvironmental signals are critical cooperating cues.

THE PARADIGM: HEMATOPOIETIC CELL DIFFERENTIATION IN CONTEXT

Because malignant tissues resemble their normal counterparts in a number of phenotypic and genotypic properties, a comprehensive sight of normal differentiation biology is essential to understand cancer progression. The hematopoietic system has been considered the paradigm of complex differentiation systems, while leukemic hematopoiesis is currently the best cancer model. Normal hematopoiesis replenishes all blood cell categories throughout life by a tightly regulated hierarchical process that starts and progresses within bone marrow (BM) in a conspicuous cell fraction of hematopoietic stem cells (HSC) endowed with self-renewal and multipotential

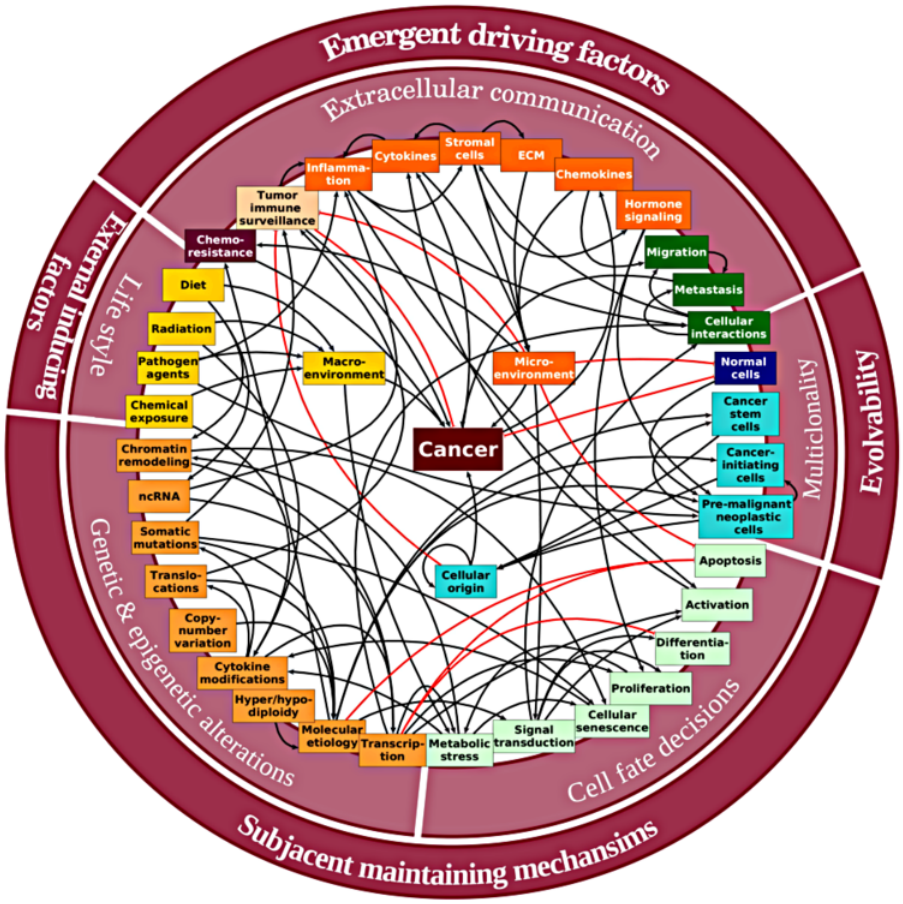

Figure 1: The multi-factorial and complex pathobiology of cancer: a macro perspective. The continuous interplay between external inducing factors perturbing underlying mechanisms involved in cell fate decisions, promote the emergence of aberrant conditions that may result in clonal evolution.

properties. These seminal cells differentiate gradually and continuously toward early progenitor and precursor cell compartments where lineage commitment and specification take place until further mature cells are completely formed. To protect the limited pool of stem cells from exhaustion, the largest proportion of the population is kept in resting or quiescent state. Quiescence and self-renewal are finely balanced to keep homeostasis, allowing HSCs to intermittently exit their quiescent state to self-renew or initiate the differentiation process. Cell fate decisions are intrinsically regu-

lated by the expression of a large network of lineage-specific transcription factors, in cooperation with epigenetic-controlled elements. Cell-to-cell intercommunication is also essential for the proper progression of primitive hematopoietic cells, and the whole BM microenvironment, including the 3 main components -the hematopoietic, the stromal and the soluble-, coregulate the fate of stem and progenitor cells: quiescence, retention or proliferation and expansion.

Downstream the pathway, multipotent progenitors differentiate toward myeloid- or lymphoid-biased oligopotent progenitors to further replenish the two main hematopoietic lineage compartments: myeloid and lymphoid [10–13]. Multiparametric flow cytometry has been a powerful tool for the prospective identification, isolation and tracking of primitive and differentiating cells within bone marrow (BM). While the very seminal stem cells reside in the human CD34+ compartment, multipotent progenitors lose this marker and concomitantly express CD38. Further display of CD45RA, CD10 and recombinase RAG1 marks the early steps of the lymphoid program in early and common lymphoid progenitors.

Myeloid cells include megakaryocytes, erythrocytes, granulocytes and myeloid-derived dendritic cells, whereas the lymphoid lineage pool contains B cells, T cells, natural killer (NK) cells, lymphoid-derived plasmacytoid and conventional dendritic cells and innate lymphoid cells. Although most early stages of lymphoid differentiation take place within bone marrow (BM), full maturation of B cells is completed in secondary lymphoid organs. Moreover, T lymphopoiesis emerges from early thymic progenitors that arise from BM after a sequential mobilization-seeding-colonization process. In comparison with the myeloid differentiation, lymphocyte cell production appears to be a process with more microenvironmental requirements within and out of the marrow. As further discussed, specific niches provide a series of structural and interactive cues and different concentrations of chemokine CXCL12 and interleukin-7 that are essential for regulation of the lymphoid cell differentiation and expansion [14]. When hematopoietic cells establish a feedback loop with their environment through soluble factors: receptors axes, intracellular pathways directly modulate cell fates by transducing the extracellular signals through kinases (e.g PI3K/Akt, MAPK/ERK, JAK, GSK3β), cyclin regulators (e.g. p53, p16, p21, p27), anti- and pro-apoptotic molecules (e.g. PUMA, Bcl-2/Bcl-xL, Bax/Bak, caspases), transcriptional factors (e.g. Pu.1, E2A, Pax5, Gfi1, Runx1, Fox proteins) and epigenetic regulators (e.g. CoREST, LSD1, Dnmt3, miRNAs) [15].

The extrinsic factors regulating hematopoiesis are mostly provided by

BM endothelial cells, osteoblasts, osteoclasts, mesenchymal stromal cells (MSC), adipocytes, monocytes, Schwann cells and sympathetic neuronal cells, besides other cellular immune components of the microenvironment. Direct short and long distance cell-cell communication is mediated by integrins, chemical synapses, gap junctions, extracellular vesicles exchange and nanotubular structures. Together these mechanisms facilitate the adhesion, selective transfer of small molecules, proteins, DNA, RNA and organelles to safeguard cellular survival. Some of the involved soluble factors include stem cell factor (SCF), fms-related tyrosine kinase 3 ligand (FLT-3L), granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), fibroblast growth factor 2 (FGF2), Notch ligands, delta-like ligands (DLL), chemokines and cytokines like CXCL12, TGF-α, IL-7, IL-10, IL-15, and IFN-γ and molecules of the extracellular matrix like fibronectin, osteopontin and others. The combination of cellular and soluble components, shape tridimentional structures known as hematopoietic niches [16, 17], and at least three have been recognized: the osteoblastic or endosteal, the reticular or perivascular and the sinusoidal or vascular. In a simplified view, close to the bone endosteum, the osteoblastic or endosteal niche regulates quiescence and the intermittent entrance to cell cycle of HSC, while vascular or endothelial niche may have a function in the exit and migration from the marrow of the committed and mature cells. Right in the core, the reticular niche is substantially formed by mesenchymal stromal cells that produce high amounts of the chemokine CXCL12, SCF and IL-7, which attracts B-cell progenitors and allow their proper recombination of their BCR, differentiation and proliferation. Of note, oxygen tension, ionic strength and pH gradients are apparently contributed by the heterogeneous niche structures.

The still incomplete understanding of multi-population dynamics in context of multiple regulatory mechanisms within the various BM compartments represents a challenge in the study of normal and malignant hematopoietic complexity.

HIERARCHY VS STOCHASTICITY IN CHILDHOOD ACUTE LEUKEMIA ORIGINS

Pediatric oncology has been recently defined as global Health priority, and the need to better control disease outcomes and to improve treatment decisions in low and middle income countries has been highlighted [18]. Among childhood malignancies, acute leukemias are the most frequent and account

for 30 to 40% of cases. Acute lymphoblastic leukemia (ALL) is the most common cause of morbidity and mortality of relapsed pediatric patients (75-85%), while approximately 20% correspond to acute myeloid leukemia (AML) [19]. Nearly 80–85% of ALL cases have a B-cell immunophenotype and 15% show a T-cell immunophenotype. According to international classifications of lineage and differentiation stages B cell ALL can be Pro-B, Pre-B or a mixed of ProB and PreB cells. In Mexico City, B-ALL incidence has been found among the highest in the world [19, 20].

The uncontrolled production of hematopoietic precursor cells of the lymphoid series within the BM is the prominent feature of ALL, resulting from a complex network of intrinsic and extrinsic factors that may influence early hematopoietic differentiation and cooperate to make aberrant cell fate decisions that sustain tumor development and progression at the expense of normal blood cell production. Indeed, genetic profiling has shown that acute leukemias constitute a heterogeneous group of diseases associated with a large number of aberrations, including translocations, somatic mutations, somatic copy number alterations, hyper (>50 chromosomes) and hypodiploidy (<45 chromosomes). Three major cytogenetic alterations are dominant in childhood ALL: hyperdiploidy and translocations E2A-PBX t(1;19) and TEL-AML1 t(12;21), resulting in detrimental function of the transcriptional network commanding hematopoietic cell homeostasis. Of interest, less than 5% cases of acute leukemia are related to congenital disorders such as Down syndrome, ataxia-telangiectasia, Wiskott-Aldrich syndrome, Bloom's syndrome, Fanconi anemia, Kostmann's syndrome, neurofibromatosis, Noonan syndrome or Nijmegen breakage syndrome [19, 21], and a considerable frequency of cases show normal karyotype, with no apparent chromosomal abnormalities, highlighting the role of additional extrinsic and microenvironmental factors in the disease. A third and less frequent type of acute leukemia is the mixed-lineage or biphenotypic leukemia, characterized by the hyperproliferation of blast cells with co-expression of lymphoid and myeloid lineage markers and poor prognostic [22].

The cell root of ALL is still on debate. Of note, current information indicates that pre-malignant cells giving rise to cancer initiating cells evolve from the normal counterparts under selective anomalous differentiation pathways. This is true for AML, where primitive cancer stem cells (CSCs) are the only minor fraction capable of recapitulating leukemia in transplanted mice, showing remarkable similarities to normal HSCs, including slow-cycling, self-renewal, differentiation potential, gene expression program, surface phenotype and resistance to conventional chemotherapy [23–

25]. In contrast, the cellular origin of ALL is less clear. Leukemia initiating cells (LICs) with immature phenotypes and the various B-cell differentiation stages are able to recapitulate the disease, challenging the hierarchical stem cell model and suggesting that the self-renewal property is maintained in B-committed cells. Moreover, the unsuspected genetic diversity within LICs and an increasingly complex pattern of acquisition of mutations in B precursor cells support the multiclonal evolution of leukemogenesis [23].

In pediatric patients, the pre-leukemic origin is thought to occur with an initial mutation induced in utero and subsequent accumulation of secondary driver events that promote the malignant transformation of a very primitive hematopoietic cell or the re-acquisition of a stem cell-like program of a lineage-compromised cell. The pre-natal origin of pre-leukemic cells is supported by data from monocygotic twin studies, showing high probability of leukemia occurrence with the same first genetic alteration in two identical twins [21]. Sequential secondary genetic events determining the emergence of leukemia initiating cells (LIC) may be concordant to a postnatal latency period [26]. Upon appearance of LIC, the course of the disease is characterized by continuous evolution of subclonal architectures giving rise to intra-tumor heterogeneity. Although there is no conclusive evidence that correlate with childhood leukemia induction, a contribution of parental or new-born exposure to carcinogens, ionizing radiation, chemical mutagens, neonatal administration of vitamin K, parental use of medications and drugs, and proximity to electromagnetic fields to transformation into malignant cells [26] has been suggested. Furthermore, the 'delayed infection' hypothesis proposed by Greaves includes recurrent childhood infections as factors inducing secondary genetic modifications and further cellular transition to malignancy [27, 28]. By exposure to pathogen-derived antigens, peripheral B cells may initiate BCR affinity maturation and somatic hypermutation catalyzed by activation-induced deaminase (AID), which in synergy with RAG activation might drive secondary mutations and clonal evolution in pre-malignant acute leukemia cells. Interestingly, hematopoietic primitive cells are also responsive to Toll-like receptors (TLRs) signals [29]. Under this presumption, stimulation with lipopolysaccharide (LPS) of mice pre-malignant B cells carrying the ETV6-RUNX1 fusion gene or with PAX5 haplo insufficiency, works as leukemia inductor [30].

Thus, while a hierarchical structure is clear for AML and a growing number of solid tumors (e.g. breast cancer, ovarian cancer, prostate cancer, melanoma, pancreatic cancer, colon cancer, brain tumors and hepatocellular carcinoma) [24, 30], ALL may fit on a stochastic model with non-stratified

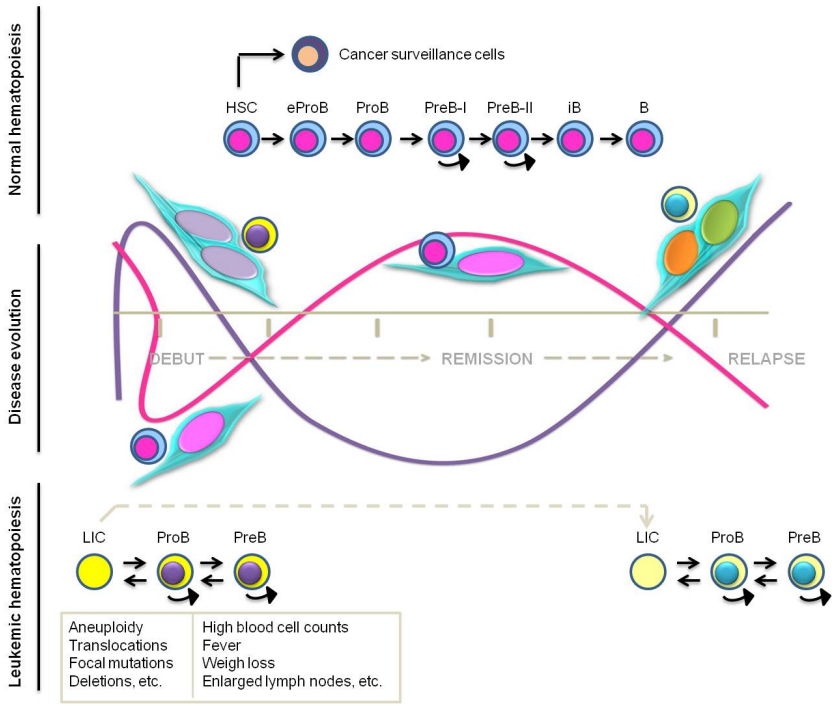

Figure 2: Disease evolution in ALL results from alternate waving of leukemic lymphopoiesis at expense of its normal counterpart. Normal development of hematopoietic stem cells (HSC) gives rise to early B cell precursors capable of differentiating into B lineage cells under a tightly regulated process, concomitant to the production of innate and adaptive cancer surveillance cells (upper panel). While at debut malignant differentiation is prevalent, normal hematopoiesis is crucially compromised. Over time and upon chemotherapy, tumor cycling cells are controlled and a gradual reconstitution of normal cells takes place. 20 to 30% of cases relapse apparently due to di novo growing of quiescent clones with chemoresistance and stemness properties (middle panel). Receptor assembly, self recognition and proliferation behave abnormally in leukemogenesis and the molecular diversity and clinical heterogeneity highlight its biological complexity (bottom panel). Leukemia initiating cells (LIC) at debut may come from normal HSC and evolve to a different "relapsed" clone, re-initiating the disease with unique properties. How these events relate to the surrounding and remodeling microenvironment and which are the factors during this cell competition responsible for an effective malignant selection is still under investigation.

leukemic cell populations and the apparent absence of a phenotype-based cellular root [23, 31, 32] but high responsiveness to extrinsic signals. Some

stem-like abilities present in ALL LICs may contribute relapse or remission failure, evading conventional chemotherapy and remaining in low-profile status [23]. The identification of molecular differences between LICs and normal and cancer HSC is a current area of intense research in order to develop selective therapies for LIC elimination with no concomitant damage of the HSC pool [33].

Again, networking between genetics, microenvironment and the coexistent normal and leukemic hematopoiesis is apparently critical for cell fate decisions. Cell frequencies and absolute numbers of all normal progenitor cell fractions along the lymphoid pathway are critically reduced in ALL. Moreover, the yield per input analysis has shown poor capability of producing lymphoid lineage cells per one cell basis. Whether some pre-leukemic cells reside in normal compartments and may initiate malignant behaviors is uncertain (Figure 2). Disease evolution then results from alternate waving of leukemic lymphopoiesis and its normal counterpart. At debut, malignant differentiation is highest at expense of normal development [3, 34–36]. Over time and upon chemotherapy, cycling cells are controlled while gradual reconstitution of normal cells takes place. 20 to 30% of cases relapse apparently due to di novo growing of LIC quiescent clones with chemoresistance properties (Figure 2). How these events relate to microenvironment and which are the factors during the cell competition responsible for such a selection, are still open questions.

TUMOR MICROENVIRONMENT: CAUSE, CONSEQUENCE OR COINCIDENCE?

Aside from the role of intrinsic programming, cell-to-cell intercommunication is essential for the proper progression of normal and malignant hematopoiesis. Three major components of BM microenvironment –the hematopoietic, the stromal and the soluble–, control quiescence, retention or proliferation and expansion of differentiating cells (Figure 3). In the setting of leukemia progression, the increasing numbers of uncontrolled proliferating cells invade BM niches and displace normal hematopoietic cells that may result in clinical manifestations of early BM failure. Theoretical research by competition modeling of normal and leukemic populations suggest a BM transit from a “steady state” where normal and few leukemic cells coexist to a sudden alteration of homeostasis deriving into the acute progression of leukemia[3, 34–36]. Such biological disturbance may relate to reception/transduction of microenvironmental regulatory cues, niche affinity or avidity, efficiency for resource utilization, intracellular response to

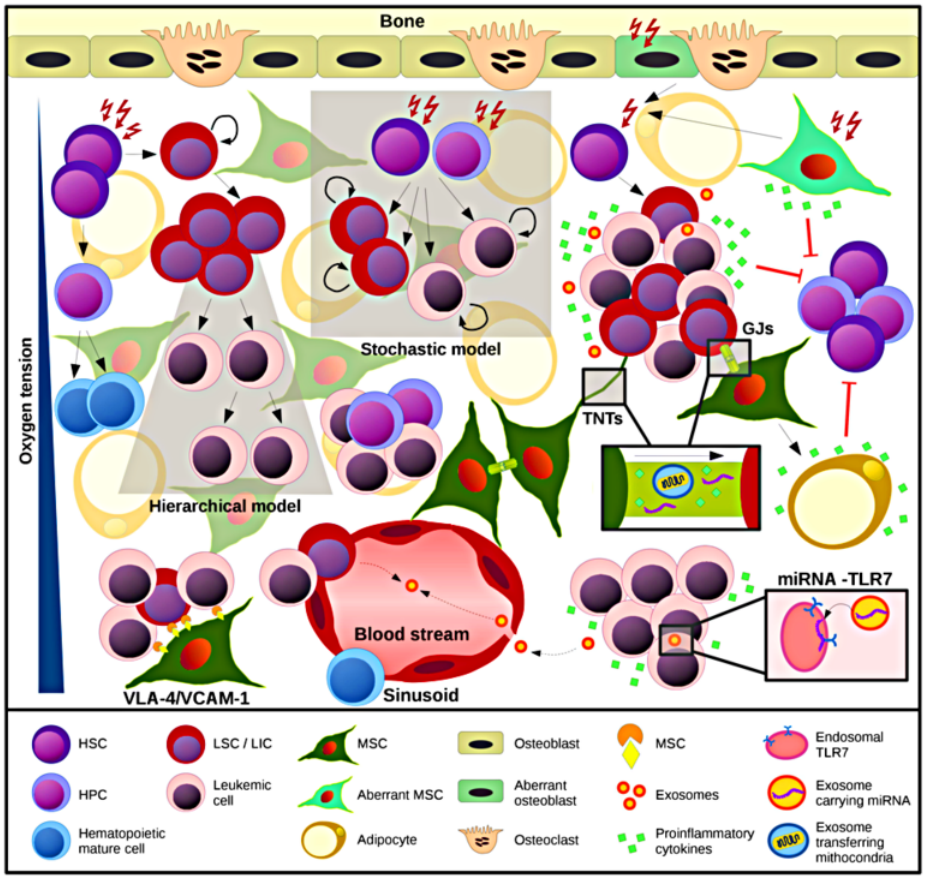

Figure 3: Schematic representation of leukemic bone marrow complexity. Multiclonal populations of leukemic cells co-exist within the bone marrow with normal hematopoietic cells, which are suggested to progressively get exhausted by a leukemic microenvironment unable to support their normal functions. An abnormal microenvironment might result from intrinsic or induced damage in stromal cells. The communication between microenvironment and blastic cells is a two-way dynamic process involving diverse intercommunication mechanisms mediated by soluble factors secretion, gap junctions (GJs), integrins (VLA-4/VCAM-1), tunneling nanotubes, miRNAs and even mithocondrial transference. All together, they provide signals that protect malignant cells from a hostil surrounding, including chemotherapy.

apoptotic signals or remodeling of hematopoietic niches that decrease their capacity to support normal cells. Competitive BM repopulation experi-

ments by xenotransplantation of normal and leukemic cells into recipient irradiated mice, suggest that both cell types have the same initial niche requirements [37–39]. However, it is not clear whether leukemic and normal cells share common niches or they spatially segregate each other creating specialized structures. Leukemic proliferation promotes a malignant microenvironment unable to preserve its normal functions [38]. Furthermore, ALL cells are capable of inducing aberrant signaling and niche disruption by producing a pro-inflammatory secretome (e.g. IL-1β and TNFα), as well as through vesicle secretion and paracrine communication by channels and tubules, that ultimately alter normal biology of hematopoietic and stromal cells [40]. Leukemic niches may also result from intrinsic damage of stromal cells, contributing a "microenvironment-induced oncogenesis" rather than a more conventional "malignant-induced microenvironment". Accordingly, an activating mutation of β-catenin and the null mutation of Dicer1 in osteoprogenitor cells lead to myelodisplasia with development of secondary AML [41, 42], whereas genetic aberrations in BM mesenchymal stromal cells (MSCs) from subsets of ALL patients, record common chromosomal alterations to leukemic blasts [43, 44].

A precise factor triggering local inflammation is uncertain. Pathogen molecular patterns, damage molecules or tumor components may promote a feedback loop where normal and pre-malignant cells are constantly exposed to genetic errors. As recently reported, some miRNAs participate as PRR ligands and are capable of stimulating TLR8 within endosomes with the consequent activation of the NFkB pathway and the substantial induction of pro-inflammatory cytokines [45]. The direct interaction miRNA-TLR was first observed in lung cancer cells where miRNA-21 and miRNA-29 bound to human TLR8 increased secretion of pro-inflammatory cytokines and increased metastatic potential of cancer cells. Considering the disregulated expression of miRNAs by leukemic cells, they represent another possible and unexplored mechanism that may be participating in the pro-inflammatory promotion and microenvironmental remodeling.

Thus, NFkB dependent tumor-associated inflammation co-participate in malignant progression by functioning as the driving force from a repressive niche to a permissive niche, where crucial intercellular communication axes are perturbed. Strikingly, aging related to inflammation in cancer progression is a topical issue. In the hematopoietic system, the induction of mesenchymal stromal cells (MSCs) adipogenic differentiation is a senescence related change concomitant to loss of hematopoietic support [46–49] and the production of pro-inflammatory cytokines.

Finally, the leukemic niche may also function as a highly hypoxic sanctuary for LICs, by protecting them from conventional chemotherapy and allowing drug-resistance and metastasis potential [50–52]. Accordingly, BM of leukemic mice reveal expanding hypoxic zones that correlate with tumor burden [53] and hypoxia inducible factor α (HIF1α) has shown to be activated in LICs even in normoxic conditions. An additional mechanism operating in the stromal-mediated chemoresistance of leukemic cells consist in the leukemic-stromal reciprocal cell activation of NF-κB induced by the interaction between vascular cell adhesion molecule 1 (VCAM-1) expressed by the stroma and very late antigen-4 (VLA-4) expressed on leukemia cells [54].

Taking together, data indicate that leukemic cells are strongly dependent on their microenvironment, apparently even more than their normal counterparts. The altered levels of communication molecules contribute a microenvironment where malignant cells take advantage of normal mechanisms resulting in preferential support of leukemic maintenance. Critical communication paths, other than soluble factors, benefiting malignant cells include Gap junctions, tunneling nanotubes and exosomes. Gap junctions by connexin proteins are one of the simplest forms of direct intercellular communication between neighbor cells and their defects disrupt the niche composition by decreasing the abundance of functional CXCL12-expressing cells [55, 56]. Remarkably, a recently discovered mechanism through which MSC provide a protective effect involves mitochondria containing microvesicles transfer to host cells [57]. Organelle transport can be also achieved by *de novo* formed tunneling nanotubes (TNTs) [58]. In particular, ALL cells can communicate with the BM stromal cells trough TNTs formed by F-actin, resulting in secretion of cytokines such as IP10, IL-8 and MCP-1, and adding significant insight into the mechanisms of communication in the leukemic niche that may induce cell survival and drug resistance [59]. More recently, Galectin-3, a multifunctional galactose-binding lectin, seems to be transferred through exosomes, and mediate communication and drug-resistance [60].

UNRAVELING THE PATHOBIOLOGICAL COMPLEXITY OF LEUKEMIA FROM THE MATHEMATICAL PERSPECTIVE

In recent years, the study of acute leukemia progression has been addressed with novel experimental strategies with emphasis in intratumor heterogeneity and multi-level interactions, including mass cytometry, high res-

olution microscopy and next-generation sequencing. However, a comprehensive model that allows the study of the complex population competition occurring between leukemia emergent cells, the blast crisis and normal residual cells, is still missing. As a response, systems biology strategies have been applied to the construction of mathematical models that integrate experimental or clinical data with unknown parameters. These theoretical approaches have proved to be efficient for the generation of testable hypotheses.

Population dynamics involving malignant clonal evolution in leukemia has been addressed by different groups through continuous dynamic modeling with differential equations. Most of these models consider differentiation stages as compartments whereby cells move simulating differentiating phenotypes [3]. In mathematical modeling of acute leukemias, many steps may be considered as stochastic processes, like new mutations timing of appearance, the characteristics of a particular mutation (e.g. viable or lethal, "driver" or "passenger") or cell fates.

The model proposed by Kimmel and Corey for the study of neutropenia evolution to AML, consider stochasticity at different levels that may derive in the emergence of new leukemic clones with an optimized proliferation rate [61]. Stochasticity in mathematical modeling is translated in biological *in vivo* systems in "random" behaviors that may be due to phenotypic variability, mRNA levels, noise or additional microenvironmental signals that are not considered in the model.

Computational simulation of clonal evolution processes has derived in hypothesis that provide insight into the relapse process of acute leukemias, where apparently some pre-existing minor clones before chemotherapy, are responsible for relapse when bone marrow is permissive for their expansion. One characteristic of this surviving clones is their low-proliferation rate, that is consistent with the hypothesis of LIC population as responsible of leukemia rebounding [62].

More recently, systems biology also exploits acute promyelocytic leukemia (APL) for the study of leukemogenesis through the construction of networks including cell cycle molecules, apoptosis, growth factors, differentiation, immune response, stress response, extracellular matrix, and nuclear receptors. Its simulation as a semi-quantitative dynamical model may be powerful to observe intermediate stages in the transition from health to development of APL [63]. As with experimental models, also in computational approaches, modeling of whole hematopoietic system in leukemia is still in progress. However, mathematical modeling has shown to be a use-

ful tool for a more systemic and integrative study of malignant transformation in acute leukemia. By developing and simulating Boolean systems, the biological consequences of microenvironmental perturbation due by temporal TLR signaling on crucial communication networks in the BM niche, have been investigated [64], entailing pro-inflammation with unstable behaviors of niche inter-communication. Moreover, how common alterations in ALL cells may induce BM microenvironment remodeling, has been explored, confirming that NF-κB mutation in HSPC may perturb HSPC-MSC communication [64].

Although much has been learned about subsystems, including leukemic multiclonality, coexistence with normal hematopoietic cells and microenvironment dependence, there are still a number of questions regarding the sequence of events that drive to the acute phase of disease and the possibility of the microenvironment remodeling to trigger a transformation event. Whether the microenvironment may lead to normal hematopoiesis replacement, which conditions would induce extinction of the leukemic cells, and what make childhood cancer unique are still ongoing theoretical investigations.

CONCLUSION

While it has long been recognized that intrinsic and underlying abnormalities in seminal and maturing hematopoietic cells may trigger hematological malignancies, it is becoming clear that their intercommunication with external and emergent factors regulate the oncogenic activity and lead to a heterogeneous disease. Future progress from the network complexity standpoint will be decisive to understand the health-disease transitional stages and to contribute to the Precision Cancer Medicine.

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SINGLE CELL GENE EXPRESSION: A WINDOW TO ASSESS THE INTRATUMOR HETEROGENEITY IN CANCER

Lucia S. López Castillo[†], *Nancy R. Mejía-Domínguez* [‡] *and Osbaldo Resendis-Antonio*[†‡*]

[†]Human Systems Biology Laboratory, Instituto Nacional de Medicina Genómica (INMEGEN),Mexico
[‡]Red de Apoyo a la Investigación (RAI), Coordinación de la Investigación Científica, UNAM, Mexico

INTRODUCTION

CANCER is a complex disease characterized by an uncontrolled cell proliferation. The transformation from normal to neoplasic state is accompanied by a variety of genetic and metabolic deregulations constrained by the specific microenvironment in human tissues[1]. The tumorigenesis is characterized by a wide spectrum of genetic and metabolic aberrations that affect multiple processes ranging from molecular activity to inter-cellular communication. In order to organize the mechanism that can trigger cancer, in 2000, Hanahan Douglas and Weinberg Robert proposed the "Hallmarks of Cancer" [2], which are a series of biological traits and processes that differentiate cancer cells from their normal counterparts. These hallmarks include sustaining of cell proliferation, the ability to evade growth suppressors, immune evasion, promoting inflammation, angiogenic potential and metabolic transformation. These traits have served as a conceptual scheme to organize the set of mechanisms that promote cancer independent of its tissular origin. Nevertheless, given the heterogeneous complexity, there is still a lack of comprehension of how these hallmarks combine together to support cancer and how this knowledge can be applied in the development of optimal therapies.

Several population-wide cancer databases, such as The Cancer Genome Atlas (TCGA)[3], have been developed to collect and characterize the genetic profiles and phenotypes of thousands of different tumor samples and

[*]Correspondence author. E-mail: oresendis@inmegen.gob.mx

cancer cell lines. These databases have supplied with valuable sources of information to elucidate the genetic regulatory, signaling or metabolic mechanisms sustaining cancer phenotype[4]. In particular, these analyses have shown that gene expression profiles of cancer cells are heterogeneous not only due the diverse microenvironmental conditions in where they are originated but in addition to the genetic landscape of the patients. Thus, heterogeneity in gene expression and metabolism is an inherent property in all human cancer, and its study has gained importance given its crucial role in the outcome of malignancy and prognosis.

Cell heterogeneity can be split into two categories: intertumoral and intratumoral heterogeneity, see Figure 1. Intertumoral heterogeneity is referred to patient-specific biological variations that occur among individuals with the same tumor type. Primary and secondary tumors also present different biological and genetic features due the evolutionary property of cancer modulated by a different tissular microenvironment. Otherwise, intratumoral heterogeneity is referred to the complex structure of tumoral cells population, and it has emerged as a key feature responsible to induce drug resistance in human cancer[5].

Tumors are not made by a uniform cellular population but by a complex structure of cells exhibiting different phenotypes and collectively interacting among all of them. To explore intra-tumoral heterogeneity in living systems, Makino carried out one of the first studies in heterogeneity by analyzing the cytogenetic profiles in mice tumors[6]. Besides, Fidler and colleagues suggested that formation of subpopulations within tumors play an important role in aggressiveness and metastatic processes[7]. In terms of the origin of the intra-tumor heterogeneity, some conceptual schemes have been suggested. For instance, clonal reproduction of cancer cells has been proposed as the main source of genetic variation of cancer cells[8], carrying differences in mutation rates of individual genes, chromosomal translocations and aneuploidies, changes in gene expression and epigenetic features. In this context, Darwinian evolution has been suggested as the principle that explains heterogeneity due to a preferential selection of clones with optimal fitness, see Figure 2[9]. Additionally, the emergent Cancer Stem Cell Paradigm ended with the gene-centric view of cancer cell phenotype and add the idea that the growth, progression and phenotypic distinctions within tumors are the outcomes of a stem population of cells[10].

Giving the vast complexity of tumoral tissues, the origin of heterogeneity probably can be explained by a combination of all the previous hypothesis, complementing it with the influence of intercellular communication

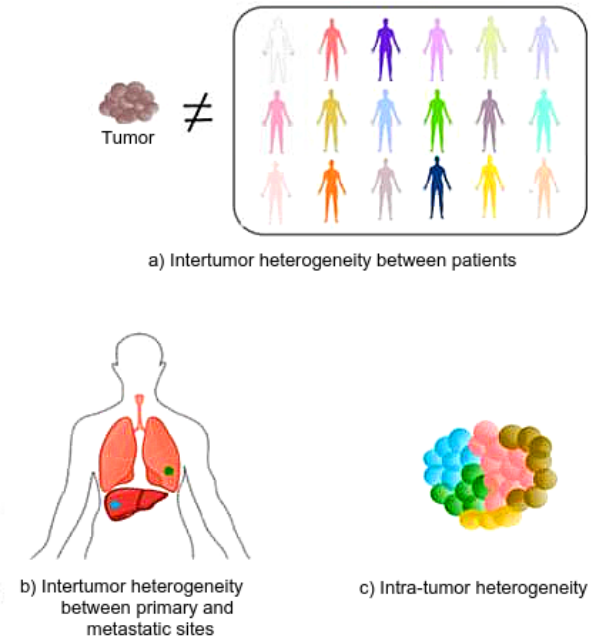

Figure 1: Intra and Inter tumoral heterogeneity in cancer. Heterogeneity in cancer can be defined at different scales: a) Heterogeneous genetic profile can be seen between patients with the same type of tumor at the same stage of advance. b) In same patient it is possible to observe a heterogeneous genetic profile of cell in primary and secondary tumors. c) Inter-tumoral heterogeneity emerges inside the cells or a tumor due its clonal expansion that are subject to the microenvironment conditions.

and microenvironment. In this context, intratumoral heterogeneity is a dynamic trait that change in time and space, and constantly is influenced by diverse factors throughout the tumor development. Thus, tumor evolution are shaped by changes in local microenvironment, spatial location and communication with stromal, endothelial, inflammatory and immune cells[11]. In this conceptual scheme, the maintenance and development of a tumor tissue requires a solid network of cooperativity and successful communication between different cell types[12], see Figure 3. The emergence of cancer cells subpopulations with specific biological properties may be the result of particular niches that favor some of the functional properties of the given tumoral subpopulation, this coexistence provides great advantages for maintenance and development of the neoplasic state.

Diverse methodologies have been used to investigate molecular het-

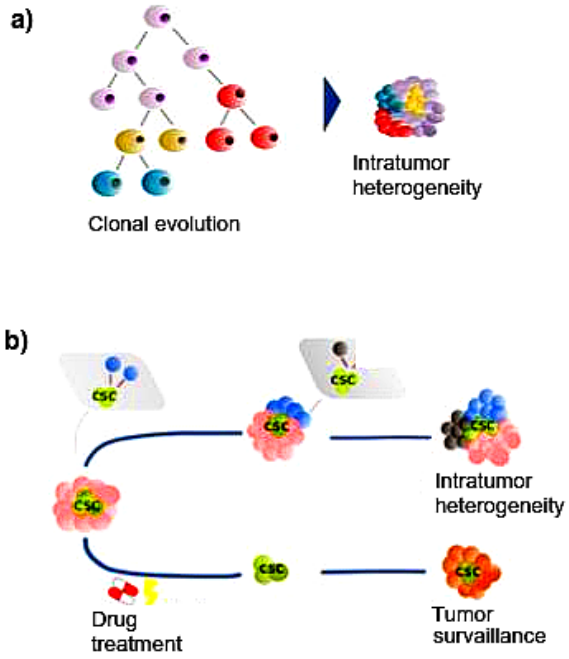

Figure 2: Origin of tumor heterogeneity. a) Clonal cancer cell duplication, a central mechanism to explain the heterogeneity in tumors. b) Cancer stem cells drives heterogeneity generating subpopulations with different phenotypes and have strong influence in reincidence of tumors after clinical treatments.

erogeneity at intratumoral level. In situ techniques –such as Immunohistochemistry (IHC), FISH and Immunofluorescence (IF)– are the preferred methods due to the easy detection and the semiquantitative assessment for the interested biomarkers, in biopsies studies. One of the great advances in imaging technology is the monitoring of multiple biomarkers expression in the same cells, or even in the same slide, through multicolor IF. However, despite the existence of systems capable to analyze multiple biomarkers that map topological locations in a cell population[13], there is a great need of improvements in methods to achieve more complete visualizations of tumor architecture in order to understand tumor complexity.

Recent advances in Next Generation Sequencing Technologies (NGS) have opened the possibilities to deeply study heterogeneity in tumor tissues at genomic scales. Currently, RNAseq technology applied in medicine have had a tremendous impact to explore the biological mechanisms that share and distinguish different types of cancer at different biological scales.

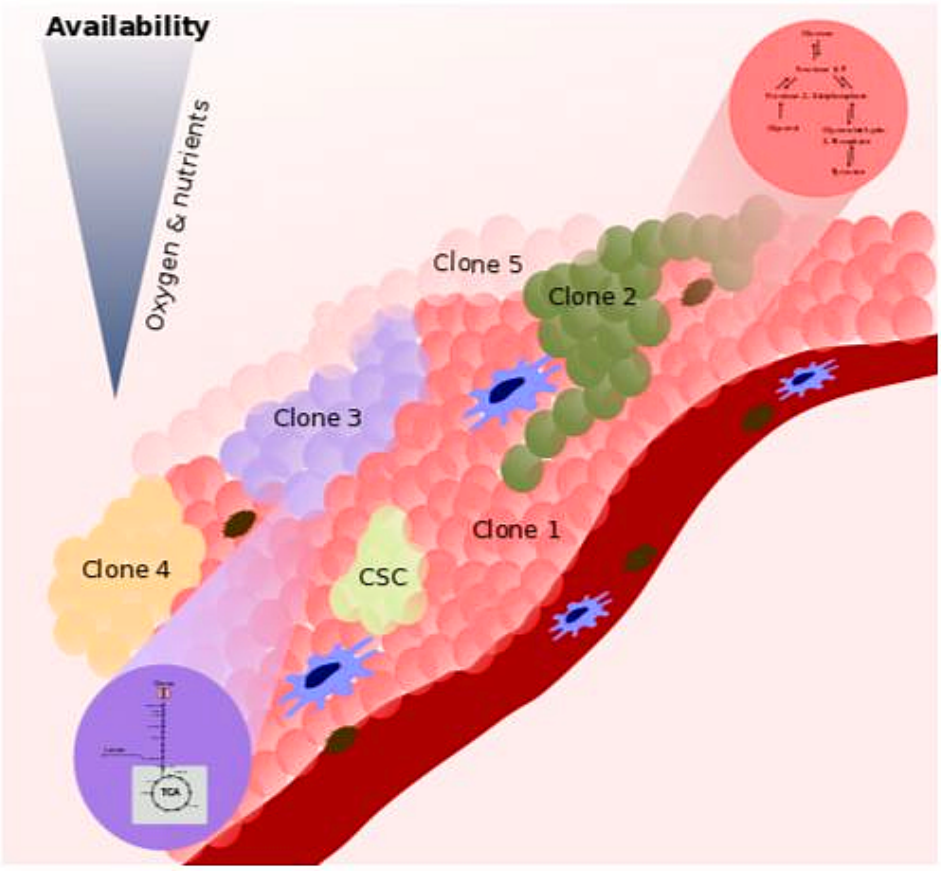

Figure 3: Tumor complexity. Solid tumors are poorly vascularized, and therefore surrounding environment and spatial localization of the cells determine access to oxygen and nutrients. The coexistence of diverse subpopulation of cells, shaped by diverse features, define tumor complexity and determine the success or failure of clinical approaches.

In all of these reports, the study starts from a bulk of cells extracted from the tissue or a bulk of cancer cell line. In these RNAseq studies, the gene expression profiles correspond to an average over all the cellular populations included in the tumor. Even though gene expression profile of bulk tumor samples has been extremely useful to elucidate biological mechanisms in tumors as a whole, this technology overlooks the single cell information and mask the presence of important cancer cell subtypes conforming the tumor.

Application of bulk NGS approaches to single-cell levels makes possible to overcome the previous limitations to explore the intra-tumoral heterogeneity. Specifically, single-cell transcriptomics using next-generation sequencing (single cell RNAseq) is emerging as a powerful tool to profile cell-to-cell variability into genomic scale. Notably, single cell RNASeq technology currently is making significant contributions in biomedical research, offering the possibility of measuring and identifying particular transcriptional behaviour across hundreds or thousands of cells and examine the subpopulation composition in tumors. In next sections, we will present two computational methods that have contributed to quantify single-cell gene expression in cancer samples and explore its implications in biological pathways. Overall, we highlight the need to combined single-cell RNAseq data and computational analysis to survey the crosstalk between different cancer subpopulations in tumors and its role to maintain the neoplasic state[14].

COMPUTATIONAL ANALYSIS FOR SINGLE-CELL RNASEQ

Bioinformatic and statistical analysis in single-cell RNA-seq data

In order to explore the cellular heterogeneity in a biological sample, single-cell RNA-seq should overcome some challenges related with the library preparation, the bioinformatic analysis and their biological interpretations. For instance, given the low amounts of mRNA within an individual cell, the library is constructed by amplifying the initial RNA material by more than 1 million folds to ensure that these would be captured in the sequence machine. From an experimental point of view, fails on the amplification processes on RNA can contribute to an important source of noise by affecting the original relative transcript abundances in a cell. Thus, failures in PCR-based amplifications or linear in vitro transcription amplification can distort the relative transcript abundances in cell, and their consideration is crucial to split noise from signal and conduct a proper biological interpretation. Given that classical bioinformatic approaches applied to bulk RNA-seq data do not have the statistical power needed to deal with the level of noise coming from the library preparation on single cell RNAseq, there has been an interest to develop bioinformatic algorithms that overcome this limitation and contribute to infer the biological activity of an individual cell[15].

In this chapter, we will focus on two computational methods for the analysis of single-cell gene expression data: SCDE (Single-Cell Differential Expression Analysis)[16] and PAGODA (Pathway and Gene Overdisper-

sion Analysis)[17]. From one side SCDE uses a statistical algorithm that considers the rate of outliers in samples to estimate the probability to identify true expression difference than stochastic variability in gene expression. In the core of this algorithm, there exist the assumption that as the gene is observed at high expressions in other cells, it is more indicative of a true gene expression. Thus, the identification of outliers events in gene expression magnitudes, as well as its correct consideration in subsequent statistical analysis play an important strategy in this computational method[16]. On the other hand, PAGODA is a bioinformatic method that allows assessing the heterogeneity on traits associated with well defined biological processes, a required step to explore the effects that noise have into in biological pathways. Overall, the workflow conformed by these two algorithms contribute to characterize the heterogeneous cellular composition associated with normal or dysfunctional tissues.

Statistical approaches for the proper analysis of single-cell RNA-seq data

The main outcome of RNAseq technology is a set of short pieces of transcript sequences called "reads"[18]. When a genome of reference exist in a database, the profile of gene expression is estimated by mapping the reads onto the genome of reference and counting the number of reads associated with a gene region sequence[19]. Thus, genes expression is estimated from discrete counts data rather than continuous measures of expression levels; therefore, it is more appropriate to use a discrete probability distribution to analyze this kind of data. The Poisson model provides a natural framework for identifying differentially expressed genes, however the higher technical noise can affect the final inference. For example, in some early RNA-seq studies the results from the goodness-of-fit test suggested that, for a small proportion of genes, the variance is not equal to the mean, i.e. data may exhibit more variability than expected by Poisson distribution[20]. This extra-Poisson variation is called overdispersion and it means that the variance exceeds the average. In order to include this effect in our statistical models, a natural extension is given by the negative binomial distribution which represents an adequate approach for modeling the overdispersion observed in "bulk" and single cell RNA-Seq measurements[21].

SCDE (Single Cell Differential Expression Analysis)

During the RNA amplification step, technical noise can emerge and modify the original proportions of relative mRNA-quantities in single-cell. As

a result, single-cell RNA-seq data can have a low number of read counts, a large number of outliers, a strong dependence on expression magnitude, and a substantial increase of technical noise relative to bulk RNA-seq. From an experimental point of view, there is evidence that expression of genes in a single cell is affected by factors related with the sample preparation, such as the reverse transcription step and the library amplification required for sequencing[22]. Specifically for single cell RNAseq, these noise factors can lead to an abundance of dropout events (events in which the expression of a gene is measured at high expression in one cell but is not detected in another cell) and induce a zero-inflation data set[23], see Figure 4. For this purpose, SCDE suggests a formalism in which the gene expression in a single cell is represented by two probabilistic processes: a negative binomial and a Poisson distributions for representing an effective and defective amplification process respectively, see Figure 4[16, 17]. Briefly, SCDE mainly is divide in three steps. First, the identification of a set of genes with high correlation in expressions along all the cells in a specific physiological condition. Then, based on this set of genes, the second step is to build an error model for determining the probability of the dropout events for each cell. Finally, the error model is applied to estimate the real differences in gene expression between two cells subpopulations, for instance cells obtained from health and cancer tissue.

PAGODA (pathway and gene set overdispersion analysis)

Having determined a set of genes whose single cell expression is differentiated expressed between two samples, an immediate question is to determine the subpopulation coexisting in each group of cells and explore their probable functional roles. To this end, a crucial issue to solve is the implementation of statistical methods capable to include the high level of technical noise for classifying cells in terms of their single-cell gene expression profiles. One method capable to include noise factor is PAGODA, which uses multivariate analysis to quantify the expression variability of genes in annotated pathways or gene sets and identify subpopulations with similar transcriptional profiles and functional roles[17]. To explore the relevant aspect of transcriptional heterogenetity in cells, PAGODA comprises the following steps. The analysis starts with the error models obtained for each cell in SCDE. With these models, we perform a normalization and batch corrections into the observed expression variance of each gene. After normalization and batch correction, residual variance associated to each gene is modeled through a chi-squared distribution with adjusted degrees of

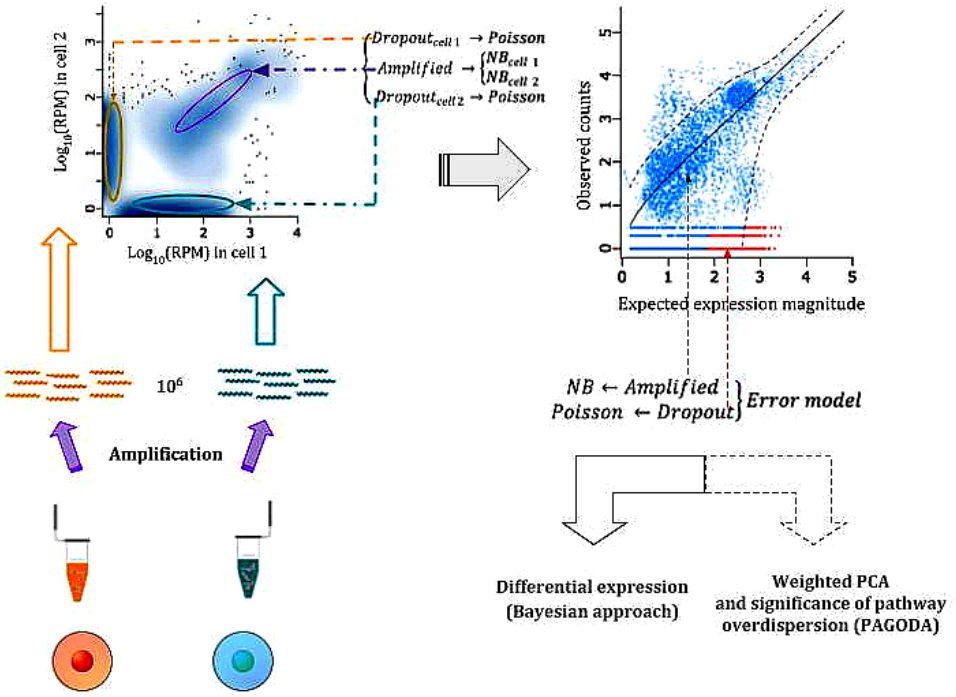

Figure 4: Statistical approach for single Cell RNA-seq. As a consequence of error in amplification single cell RNA-seq, zero-inflation and large number of outliers in the gene expression data is observed, left side. For each cell, Poisson and negative binomial distributions are used to model dropout events and amplification process, respectively. Once the single-cell error models are adjusted, the differential expression or multivariate analysis can be executed (right side). Modified from [16] and [17]

freedom and appropriate weights for magnitudes expression based on the dropout probability of genes. At the end, the analysis of the residual variance is a crucial step for distinguishing subpopulation-specific genes and determining the contribution of each gene in posterior calculations. Then, weighted PCA is performed on gene sets (annotated pathways, gen ontologies categories or set of functional genes) to identify genes with a significant contribution in the transcriptional variation. If the level of variance explained by its first principal component in a set gene displays significant variations, the gene set is cataloged as "overdispersed". Posteriorly, PAGODA clusters the "overdispersed" gene sets with similar behaviors in the population and define them as "aspects of heterogeneity". The evalu-

ation of the transcriptional behavior of gene sets is a central point during this latter step. Gene sets are integrated by genes associated with annotated pathways in databases such as KEGG, REACTOME or MSigDB. Finally, having identified important features of heterogeneity in a cellular population, clustering gene sets with similar transcriptional profiles can distinguish subpopulations with specific functionalpathways.

HETEROGENEITY IN GENE EXPRESSION PROFILE AND ITS FUNCTIONAL IMPLICATION IN CANCER

Tissue specificity of normal human cell emerges from precise transcriptional regulation of particular genes in space and time, acquiring a unique signature activated during development[24]. Unlike healthy human cells, cancer cells alternate their regulatory program through a variety of genetic mechanisms that contribute to a heterogeneous composition of cell population inside the tumor. Notably, evaluation of transcriptional patterns of single cancer cells allows us to estimate the subpopulations composition and trace the events shaping the functional and biological consequences of intratumoral heterogeneity. In this section, we will discuss some important biological signatures that correlated with the heterogeneous composition of tumors and whose specific phenotype has a crucial role in clinical outcomes.

Metabolism signature

In order to supply the metabolic demand inside tumors, cancer cells rewire their energetic metabolism toward the optimal production of aminoacids, proteins and fatty acids[25, 26]. The most studied metabolic alterations during neoplastic transformation is the Warburg Effect, which indicates a preference of degradating glucose through glycolysis instead of using oxidative phosphorylation, even in the presence of oxygen[25]. Despite oxidative phosphorylation is a pathway more efficient than glycolysis in terms of ATP production, this latter pathway generates crucial biosynthetic intermediates to the maintenance of redox balance (NADPH) and energy to support cancer phenotype.

Metabolic alterations in cancer are the result of two simultaneous mechanisms: genetic alterations that overall activate oncogenes and repress tumors suppressors; and the specific microenvironmental conditions prevailing in the tissues. For instance, some solid tumors are poorly vascularized, and therefore surrounding environment and spatial localization of the cells

can determine oxygen availability and access to nutrients (such as glucose, lactate, pyruvate and glutamine)[27, 28], see Figure 3. In this context, the adaptation of cancer cells to these dynamic and microenvironmental conditions are crucial to tumor development. Notably, cellular metabolism is highly interconnected with other several pathways and may trigger additional important responses of tumoral subpopulation properties. For example, glycolytic metabolism and lactic acid production of tumor cells not only provide with a growth advantage to cancer cells but also has been associated with other cellular responses, as the suppression of immune system, tumor growth, and metastasis[29, 30]. Similarly, high levels of hypoxia can trigger complex adaptative cellular responses through hypoxia-inducible factor (HIF) transcriptional regulators, mTOR complex 1 (mTORC1), endoplasmic reticulum (ER) stress responses and other oxygen sensing mechanisms[31].

Specifically, hypoxia propitiates the development of biological processes such as autophagy, an important stress response mechanism to protects cancer cells from low nutrient supply, probably promoting a tumoral area of survival cells or drug resistance[32]. Differences in an active state of pyruvate kinase also have been associated with proliferating and non proliferating cell populations in breast cancer, revealing an influence of glucose metabolism on local tumor formation and proliferation [33]. Therefore, it indicates that cooperativity and communication of tumor subpopulations are necessary to handle spatial heterogeneity of environmental conditions and nutrient availabilities. Finally, these findings make evident the role of the microenvironment in the progression of cancer and open a window to explore its influence to define the heterogeneity in cancer[33, 34]. Hence, deciphering the interplay between microenvironment and metabolic heterogeneity among intratumoral subpopulations may serve as the critical factor underlying tumor resistance and may help to determine therapeutic targets to get maximal drug efficacy.

Metastasis Signature

Metastasis is the process by which primary tumor cells invade the host stroma, penetrate blood vessels and colonize distant organs in the host. Understanding the biological mechanisms by which this process emerges have strong implication in clinical areas given that it is the cause of deaths in 90% of humans with solid tumors. The progression of metastasis involves a variety of genetic and metabolic mechanisms in the tissue that contribute to shape the structure of the cellular matrix and confer the qual-

ities of a mesenchymal-like phenotype. Inside a tumor, only a fraction of cells with specific genetic background are capable to proceed the metastasis. Recently, there has reported that metastatic cells propitiate this ability through specific genetic alterations in DNA-check points[35]. Like occurs with metabolism, metastasis capability in cancer cells depends on two primary factors that induce heterogeneity in tumors: genomic instability and the selective pressure imposed by the microenvironment. Both mechanisms maintain a relationship in such a way that the metastasis is seen as a process where a heterogeneous genetic cancer cell population evolve under different selective pressure imposed by the microenvironmental conditions in the tissue. For instance, hypoxia-inducible factor 1 alpha (HIF-1a) is a gene that mainly is upregulated under hypoxic conditions, a gene that participates in cancer metastasis by promoting the epithelial-mesenchymal transition[36]. In addition, extensive experimental evidence has shown that platelets support tumor metastasis probably by shaping the microenvironment. It has been proposed that when a cancer cell entry into the circulatory system triggers platelet-mediated recognition, causing that platelets guard them against immune elimination and promote their arrest at the endothelium, supporting the establishment of secondary lesions. These contributions of platelets to tumor cell survival and spread suggest platelets as a new avenue for therapy[37]. Besides, early identification of pre-metastatic subpopulation within a tumor can lead to improvement of drug strategies to contain tumoral cells and avoid metastasis.

Immune response

Development of cancer strongly depends on the ability to exploit and take advantage of normal physiological processes in the host, one of the most remarkable examples is given by the immune system. The main function of immune system is to monitor tissue homeostasis, protect against pathogens, and eliminate damaged cells. However, it is well recognized that immune evasion is a common property in all cancer, and there is an imperant need to develop new strategies, such as the use of microbiome composition, for recovering its original function in our body[19, 38] . Immune cells are classified in two major groups: adaptative and innate cells. Dendritic cells (DCs), natural killer cells (NK), macrophages, neutrophils, basophils, mast cells and eosinophils belong to the innate immune cells given its functions as the first line of defense when tissue homeostasis is perturbed. Innate cells induce mobilization and infiltration of leukocytes into the damaged tissue, besides macrophages activate vascular and fibroblast responses in

order to eliminate invading organism. On the other hand, chronic activation of innate immune infiltrations has been associated with the activation of several pathways that contribute to tumor development, such as tissue remodeling, angiogenesis and anti-tumor adaptive immune responses. For instance, tumor-associated macrophages (TAMs) is the most common innate immune infiltrated and their presence correlated with patient survival.

Furthermore, innate immune cells can suppress antitumor adaptive immune responses, allowing tumor escape from immune surveillance. Curiel and colleagues identified in tumor-associated macrophages (TAMs) a chemokine which mediates trafficking of regulatory T cells. T-cells may influence in suppressing tumor-specific T-cell immunity resulting in a decrease of survival in patients[39]. Influence of immune cells in tumor elimination or tumor promotion is a complex process that involves multiple signal pathways, mostly influenced by cytokine and other factors expressed by tumoral cells, immune cells and other non-cancerous cell types present in the microenvironment. Cytokines, chemokines and proangiogenic mediators, such as tumor necrosis factor-α (TNFα), transforming growth factor-β (TGFβ), VEGF, and interleukins 1 (IL-1) and 6 (IL-6) are most common factors founded in the microenvironment that play an important role in intercellular communication. For example, VEGF is one mechanism by which tumor-infiltrating leukocytes increase angiogenesis and promote tumor development, such as transforming growth factor (TGF)β are the main regulator of immunosuppressive effects of tumor-associated macrophages[40]. In addition, strong evidence supports the argument that tumor cells influence the immune response, for example through the tumoral cells production of transforming growth factor (TGF)β-1[41], so that deciphering the interaction of cancer cells and immune cells recruitment, as well as identification of cancer cells responsible for this communication, open a possibility to design new treatments again cancer.

CLINICAL IMPLICATIONS AND FURTHER DIRECTIONS

Heterogeneity population within a tumor has a great impact on the accuracy of several approaches in diagnosis and driven treatments[42]. Intratumoral heterogeneity has become a feature to take into account in medical and clinical applications owing to its suspected role in the failed responses of therapies[43]. In global terms, "trunk-branch" model has been proposed as the conceptual scheme explaining the resilience of tumors, it produced by specific tumor subpopulations capable of resist and over-

growth after targeted specific application of drugs[44]. An example of subclonal resistance to drugs is given by patients with non-small cell lung cancer (NSCLC) treated with gefitinib, an inhibitor of epidermal growth factor receptor (EGFR) and whose somatic mutation in this receptor can be able to confer resistance under the drug action [45]. Nevertheless, heterogeneity also affects in clinical diagnostics. Biomarkers reflect biological or genetic properties in tumors, and variations in the frequency and spatial location of them could cause interpretative errors and suboptimal therapeutic decisions. [46–48]. For instance, glioblastoma (GBM) is the most aggressive primary brain tumor with a survival rate less than 15 months, in which only 5% of patients survive[49]. Despite surgical resection, chemotherapy and radiation, local tumor recurrence occurs with high frequency. Glioblastoma displays a highly heterogeneous profile in the expression of many biomarkers, such as EGFR and PDGFRA, presenting particular regional closeness of cancer cells with endothelial tissue (EGFR) and with poorly vascularized regions (PDGFRA) respectively[50, 51]. In order to enhance the outcomes of therapeutic approaches, the spatial location of biomarkers and the presence of subpopulations with different functional properties are not the only factors that should be taken into account. In practice, limited sampling of biopsies in small regions of tumors supply incomplete biological information of the tumor, resulting in technical bias with a strong implication in clinical diagnosis[12, 52]. Another challenge that clinical should face is the fact that primary and secondary tumor follow different evolutionary processes so that the diagnosis should be designed independently and complementary.

Finally, intratumoral heterogeneity is influenced by genetic alterations during clonal evolution and spatial gradients of essential metabolites supporting the uncontrolled proliferation process. With the advent of Single cell RNAseq technology, a new era is opened to characterize the feasible space of possible regulatory, signaling and metabolic mechanism supporting the cancer phenotype[53]. Undoubtedly, the combined efforts among next-generation technologies, computational methods, and physiological knowledge will have a strong impact on the understanding of the rules and mechanism by which tumor resistance emerge. This latter aim is a primary aim to designing optimal therapeutic strategies for defeating the complex battle against cancer.

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TUMOR THERAPIES CHALLENGES: INSIGHTS FROM MATHEMATICAL MODELS

Leticia R. Paiva

Department of Physics and Mathematics, Alto Paraopeba Campus, Universidade Federal de São João Del-Rei,Ouro Branco, MG, Brazil

For the last decades most of the cancer research had focused on studying individual events, such as how mutations in cancer-critical genes affect specific regulatory pathways, or the effect that mutating one protein has on a biological phenotype, with the ultimate goal of identifying drug targets.

But a tumor may contain ten of thousands of cells with different genetic mutations, and each cell may interact with their neighbors and with their surrounding environment. Hence, cancer progression involves several intertwined phenomena and events, and cancer complexity emerges from this large number of interactions that occur at a wide range of spatial and temporal scales. In addition, drug-cell interactions themselves can be view as a complex system.

In this way, we can use both tools and concepts from physics to provide a different approach to investigate cancer, as developing mathematical models of cancer progression and therapies. By a careful study of these models and the validation of their findings by experimental and clinical observations we can provide an integrative approach to cancer research.

In this chapter we discuss multiscale mathematical models to evaluate the efficacy of anti-tumor nanotherapies. In the quest of a reliable understanding of the complex and nonlinear interactions among nanotherapeutics, tumor cells and their micro-environment, quantitative models can provide relevant insights. They have the potential to suggest the major parameters affecting therapeutic outcomes, guide new essays and prevent excessive experimentation needed to develop effective treatments.

INTRODUCTION

THE conventional anticancer therapeutics have collectively saved millions of life-years. However some issues, such as nonspecific distribution and targeting, toxicity and low therapeutic index have motivated the quest for new therapies [1]. The recent advances in nanosciences and nanotechnology suggest that the association of therapeutic agents with nanostructures has the potential to correct most of the deficiencies of traditional therapies [2–5]. This can be done through drug carriers nanovehicles [6], synthetic macromolecular compounds [7], and engineered viruses [8]. Nanotherapies can overcome both epithelial and vascular barriers and achieve targeted drug delivery by means of the well known enhanced permeability and retention effect of nanoparticles in tumors.

Nanovectors for drug delivery include polymer nanoparticles, liposomes and inorganic nanoparticles. Some of these nanovehicles are in clinical trials and at least five drugs have already been approved by the U. S. Food and Drug Administration [9]. Other therapy focus on genetically modified virus to target, replicate and kill cancer cells. To date, there are dozens of ongoing clinical trials with engineered viruses with promising reported results, and some virotherapeutics have been approved and are available worldwide (in China, Latvia and, more recently, in U.S.) [10–12].

Although nanotherapies are one of the most promising strategies against cancer, the current underlying principles of nanoparticle targeting have not been translated into the desired clinical outcomes. For instance, elucidating the specific mechanisms involved in nanoparticle-tumor interactions has been pointed out as a key issue to improve the nanoparticles delivery efficiency [13]. In a literature survey over the past 10 years, Wilhelm *et al.* [13] recently highlighted the importance of the community involved in cancer nanotherapies take a step back and re-evaluate the principles that have guided the field. Therefore, the nonlinearities from the drug-cell or virus-cell dynamics are key issues to the therapy success and can be investigated through mathematical models, providing relevant insights to the underlying mechanisms of these process and the key issues to be considered on the quest of successful therapies.

In this chapter we will focus on modeling the anti-tumor therapies based on polymer nanoparticles and oncolytic virus, which have been extensively studied during the previous few decades.

CANCER GROWTH AND THERAPIES AS A COMPLEX SYSTEM

Complex systems are nonlinear systems with a large number of independent interacting individuals that usually exhibit global emergent patterns and behaviors resulting of these local interactions. Cancer progression as well as cancer-drug interactions are complex processes, where local interactions give arise to emergent phenomena.

Cancer progression and therapies can be investigated through network analysis[14] and mathematical modeling [15–19]. Mathematical modeling has the potential to indicate what is the minimum amount of components and interactions between them which can give arise to the observed emergent behavior. In the case of cancer treatments, we would be able to underly the main mechanisms of the investigated therapy and then give clues to which features should be modulated in order to increase effectivity.

Modeling cancer development and treatment can involve discrete or continuum techniques, or even a combination of both (multiscale or hybrid models). Moreover, multiscale models are increasingly turning most frequently used, as they allow to combine the benefits of both continuum and discrete descriptions [20]. Multiscale models have been proposed for cancer progression [21–24] and a range of anti-tumor therapies [25–29]. In the next section, we will review a model for avascular tumor growth proposed by Ferreira *et al.* [22] as well as multiscale models for anti-cancer nanotherapies.

A MULTISCALE MODEL FOR TUMOR GROWTH

It is well known that there are many features responsible for the distinct tumor morphologies observed, as environmental constraints and cell kinetics parameters. Nonetheless, Ferreira *et al.* [22] proposed a model which do not explicitly take into account any cell-cell or cell-medium force, and a range of morphologies is obtained changing the nutrient availability.

In this model, the authors consider a 2D-slice of a normal tissue, where a single cancer cell is introduced. The tissue is considered as a regular arrange of cells feed by a single capillary vessel. The nutrients were divided into two groups: those, as glucose or iron, that limits cell replication ($j = 1$) and those essential to cell survival ($j = 2$), as oxygen. Both are described by continuous fields $\phi_j(\vec{x}, t)$, which evolve in space and time according to

$$\frac{\partial \phi_j(\vec{x}, t)}{\partial t} = \nabla^2 \phi_j(\vec{x}, t) - \alpha^2 \phi_j(\vec{x}, t)\sigma_n(\vec{x}, t) - \lambda_j \alpha^2 \phi_j(\vec{x}, t)\sigma_c(\vec{x}, t) \quad (1)$$

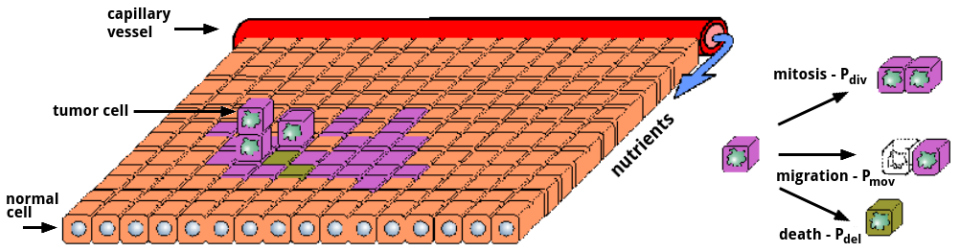

Figure 1: Schematic representation of the multiscale model for avascular tumor growth proposed by Ferreira *et al.* [22].

here, λ_j take into account distinct nutrient uptake rates for normal (σ_n) and cancer cells (σ_c), and α sets up a characteristic length scale for nutrient diffusion in the normal tissue. Eq. (1) obeys a periodic boundary condition along the direction parallel to the capillary and a Neumann boundary condition at the border of the tissue. In Figure 1 a schematic representation of the model is shown. One can see normal, cancer and dead cells, and the capillary (where $\phi_j = 1$) from which the nutrients diffuse.

The functional form of the division and death probabilities where chosen as

$$P_{div} = 1 - \exp\left[-\left(\frac{\phi_1(\vec{x},t)}{\sigma_c(\vec{x},t)\theta_{div}}\right)^2\right], P_{del} = \exp\left[-\left(\frac{\phi_2(\vec{x},t)}{\sigma_c(\vec{x},t)\theta_{del}}\right)^2\right]. \quad (2)$$

And the local migration probability is given by

$$P_{mov} = 1 - exp\left[-\sigma_c(\vec{x},t)\left(\frac{\phi_2(\vec{x},t)}{\theta_{mov}}\right)^2\right]. \quad (3)$$

The parameters θ_{div}, θ_{del} and θ_{mov} controls the shape of P_{div}, P_{del} and P_{mov}, respectively.

Essentially, while in a full nutrient scenario there are a homogeneous microenvironment and rounded morphologies are favored, if nutrients are scarce, the medium heterogeneities enable branches and ramified morphologies are frequent. Some morphologies can be seen in Figure 2. Also, these morphologies follow Gompertz growth curves. Moreover, the tumor gyration radius, as well as the number of peripheral cells, obey power law

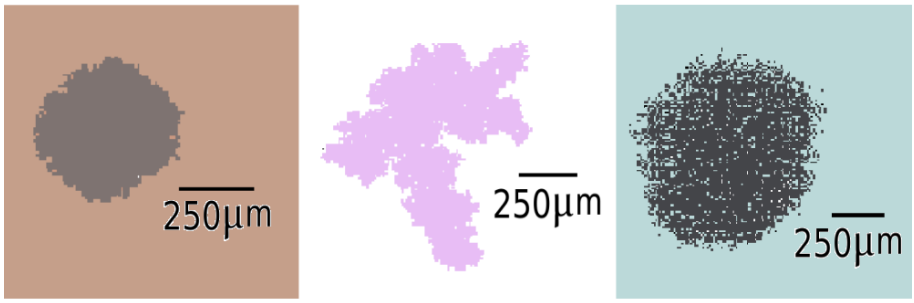

Figure 2: Some morphologies obtained with the multiscale model for avascular tumor growth [22].

scaling when considered as a function of the total number of cancer cells. Finally, the simulated tumors incorporate a spatial structure composed of a central necrotic core, an inner rim of quiescent cells and a narrow outer shell of proliferating cells in agreement with biological data.

MODELING ONCOLYTIC VIROTHERAPY

This cancer therapy was recently highlighted due to FDA's approval of the first oncolytic virus, for use in melanoma patients, in October, 2015. To date, different oncolytic viruses have been developed against a range of tumors and the clinical results are encouraging [4, 11, 30].

In this therapy, genetically modified virus which can kill specifically cancer cells are administrated. As the virus can make copies of themselves only inside the cell, an amplification dose in the tumor region enables that waves of infection eradicate tumors with few side effects.

We proposed three approaches for virotherapy [27–29], based on the model for tumor growth previously described [22]. In addition to divide, move and die, cancer cells can become infected with a probability that depends on the local virus load σ_v,

$$P_{inf} = 1 - \exp\left[-\left(\frac{\sigma_v(\vec{x},t)}{\sigma_c(\vec{x},t)\theta_{inf}}\right)^2\right]. \tag{4}$$

Once a cancer cell is infected, it can only die with a probability

$$P_{lysis} = 1 - \exp\left(-\frac{T_{inf}}{T_l}\right), \tag{5}$$

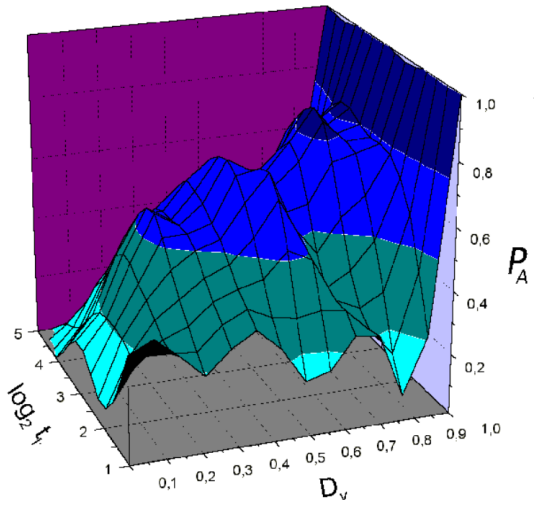

Figure 3: Probability of a successful therapy against a solid tumor (40 samples, $\gamma_v = 0.01$, $\theta_{inf} = 0.03$) obtained from the model proposed by Paiva *et al.*[27] with a continuous description of viruses.

which is maximum after a characteristic time T_l after the infection. Here, T_{inf} is the time elapsed since the cell infection. When an infected cell dies by lysis, virus are locally released, which can infect neighbor cells.

Discrete vs continuous description of virus

Wild viruses have burst sizes around 10^4 [31], what means that the virus would release 10^4 copies of itself after cell lysis. Also, oncolytic virus are very small particles (sizes 20 nm - 400 nm [8]) in comparison to cells (diameter around 10 μm). Thereby, the continuous description is suitable for these viruses, and they can be described by

$$\frac{\partial v(\vec{x},t)}{\partial t} = D_v \nabla^2 v(\vec{x},t) - \gamma_v v(\vec{x},t) \tag{6}$$

Here, D_v is the virus diffusion constant, γ_v is the viral clearance rate and the infected tumor cells act as sources of viruses at lysis.

We investigate this approach against solid tumors and found interesting results [27]. Besides tumor eradication, we found the fail of therapy with the tumor growing at a similar (or even a bigger) rate than before treatment, and a co-existence of cancer cells and viruses with an oscillatory behavior

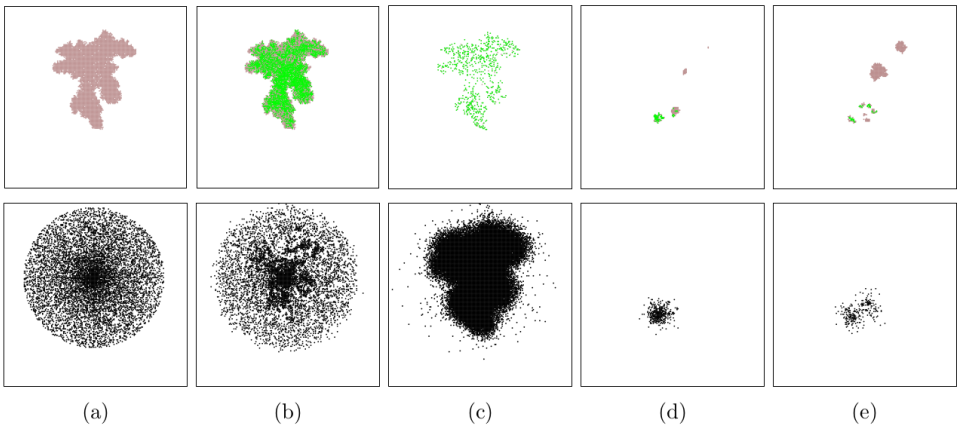

Figure 4: Uninfected (brown) and infected (green) tumor cells are shown on top and the virus population (black) is shown in the bottom figures: (a) at the time of virus injection (t^*); (b) 12h after that; (c) 52h after t^*; (d) 1 week after t^*; (e) 17 days after t^*. Dead and normal cells are not shown. For this simulation the parameters were: $\gamma_v = 0.03$, $\theta_{inf} = 0.01$, $MOI = 1$, $T_{lise} = 16$h, $T_v = 100$h, $bs = 50$.

of both populations. Concerning the typical period to lysis, T_l, we found an optimal range where the efficacy of the therapy is maximum (see Figure 3).

However, some oncolytic virus have much smaller burst sizes[32], ranging from 10 to 100. In order to appropriate describe virotherapy with these burst sizes it is important to consider the individual nature of the virus. Thus, we propose a more detailed model, where each virus particle performs a random walk on the tissue. Furthermore, we take into account the amount of virus which infect each cell (n_v) and the corresponding number of virus released with the lysis.

As before, for compact tumors we found an optimal range for the characteristic time to lysis. In addition, we found that enhancing the viral entry dramatically increases the probability of tumor eradication.

Additionally, we investigate the optimal traits for oncolytic viruses required to eliminate different tumors. These traits depend critically on the tumor growth dynamics, but our simulations reveal that, for all tumors investigated, the antitumor efficacy is determined primarily by its efficiency of entry, its replicative capacity, and its ability to spread over the tissue. Therefore, our results highlights that the design of efficient oncolytic viruses must take into account the particular dynamics of their target tumor.

Modulating the immune response

The immune response plays a key role on the virotherapy. As the immune response can eliminate virus which are potentially able to eradicate the tumor, some researches argue that the immune response should be suppressed in order to improve the therapeutic success.

To suggest how the immune response should be modulated, we include in our model with the agent-based-description of virus [28], an explicit immune response [29]. The innate immune response (represented by the removal rate γ_v) acts immediately after the virus injection. The antigen-specific response will be elicited latter, when antigen-specific B cells and $CD8+$ T cells are recruited to the infected tissue. Antibodies are released by these B cells and also infiltrate in the tissue through the capillary vessel.

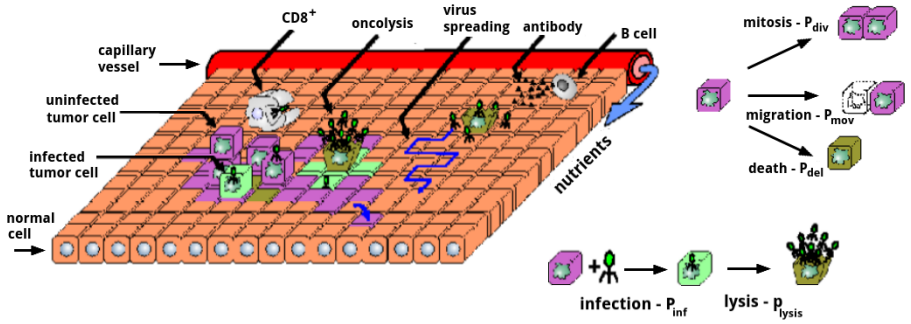

Figure 5: Schematic representation of the model for virotherapy including explicitly the immune response

Our results gave interesting clues to immune response modulation. Besides halting the recruitment of lymphocytes, our results indicate that if we can make the lymphocytes slower in the tumor region the virus can eliminate the tumor before been neutralized. This last approach is based on reprogramming the immune microenvironment in tumors in order to strongly reducing the rate of migration of lymphocytes infiltrating the affected tissue.

MODELING TUMOR CHEMOTHERAPY BASED ON NANOPARTICLES

There are well known anti-tumor drugs, as doxorubicin (Dox), which are highly effective against tumors, but have important side effects, mainly due to nonspecific targeting. To overcome this, one strategy is encapsulate these drugs into nanoparticles of chimeric polymers (CP-Dox)[33].

In this model [34], the tumor grows from a malignant cell introduced in a normal 2d-tissue. Normal cells die due to drug cytotoxity or nutrient deprivation with a probability P^n_{die}. In turn, cancer cells can divide, die or move with the following probabilities

$$P^c_{div,die} = 1 - exp[-V^2(\phi(\vec{x},t)/\sigma_c(\vec{x},t)\theta^c_{div,die})^2], \quad (7)$$

$$P^c_{mov} = 1 - exp[-V^2(\sigma_c/\phi(\vec{x},t)\theta^c_{mov})^2], \quad (8)$$

where $\phi(\vec{x},t)$ is the nutrient concentration and $V = 1/[1+(C_3(t)/IC_{50})^p]$ is a function dependent on the intracellular level C_3 of the drug. $\theta^c_{div}, \theta^c_{mov}, \ldots, \theta^n_{die}$ and IC_{50} are model parameters.

The chemotherapy consists of periodic administrations of CP-Dox at a dose C_0. In the blood, the nanoparticles concentration evolves as

$$\frac{dC_1}{dt} = -k_1 C_1 + C_0 \delta(t - n\tau), \quad (9)$$

where k_1 is the drug's removal rate; $n = 0, 1, , \ldots$ and τ is the interval between administrations. CP-Dox leaking from the capillary into the tissue diffuses as

$$\frac{\partial C_2}{\partial t} = D\nabla^2 C_2 - \sum_{\vec{x}} [\beta_n \sigma_n(\vec{x}) + \beta_c \sigma_c(\vec{x})], \quad (10)$$

with diffusivity D and endocytic rates β_n for normal cells and β_c for cancer cells. The boundary conditions are periodic along the horizontal axis and null flux at the tissue border. At the capillary, the concentration is $C_2(t) = k_2 C_1(t)$. Finally, at each time step a quantity $\delta_{n,c} = \beta_{n,c} C_2(\vec{x},t)$ of CP-Dox is endocytosed per cell. The internalized nanoparticles degrade and release $\sim 68\%$ of their drug load [33], increasing the intracellular free Dox concentration.

Our results indicate that this therapy fails in eradicate solid tumors primarily due to the small nanoparticle endocytic rates. Effective treatments should rely on nanovehicles exhibiting long residence time in the bloodstream, high selectivity for and large endocytic rates by cancer cells.

Modeling the chemotherapy against a 3D highly vascularized tumor

As nanoparticles delivery is a key issue on this therapy, we investigate a 3-dimensional version of the model previously described [35]. In contrast to the single capillary vessel proposed on the last model, we propose a simple, static vascular network [35].

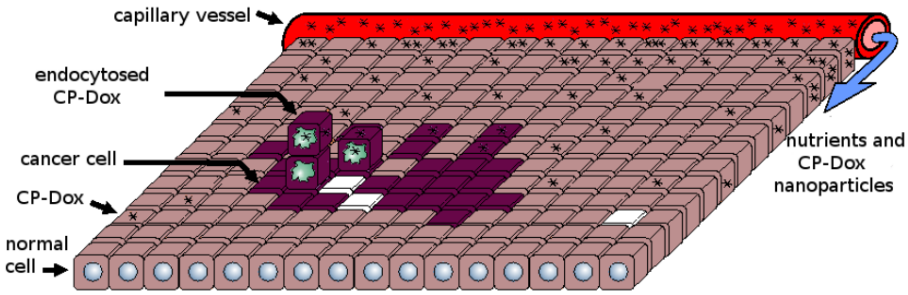

Figure 6: Schematic representation of the model for chemotherapy based on CP-Dox nanoparticles. The cancer (normal) cells are shown in dark brown (brown), dead cells in white and the capillary in red.

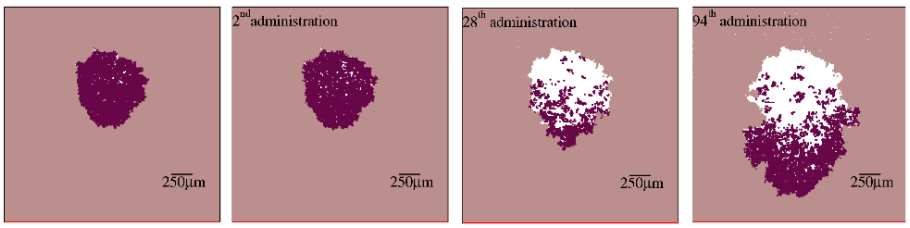

Figure 7: Progression of a micrometastase treated with perfectly selective CP-Dox NPs ($\beta_n = 0$) and endocytosis rate of $3 \times 10^{-9} mMh^{-1}$. $C_0 = 500\mu$M and $\tau = 12$ h were used. The cancer (normal) cells are shown in dark brown (brown), dead cells in white and the capillary in red.

Our simulation results indicate that the efficacy of nanochemotherapy is strongly dependent on tissue vascularization. Actually, highly vascularized tumors demand more aggressive therapies than poorly vascularized ones. The reason is that a dense capillary network ensures high interstitial concentrations of nutrients and CP-Dox nanoparticles, but the drug concentration at the capillaries decays fast after its administration. So, cell division will overcomes cell death unless an aggressive therapy is applied.

Furthermore, our results indicate that the nanoparticles endocytic rate by tumor cells is the major factor that determines the therapeutic success, similarly to the role of virus entry in oncolytic virotherapy [28, 34].

THE FUTURE OF *in silico* CANCER RESEARCH

Although all progress achieved in *in silico* medicine, it is unlikely that it will replace *in vivo* experimentation or clinical trials. They are complemen-

tary routes to a deeper understanding of the complexity of cancer-drug or cancer-virus interactions. However, it seems that multiscale models will play a major role in future medical research, transforming medicine in a highly computer-intensive science.

This is in commitment to the vision exposed by Hanahan and Weinberg[36], where they argue that "we foresee cancer research as an increasingly logical science, in which myriad phenotypic complexities are manifestations of a small set of underlying organizing principles."

In order to propose a simple but realistic model able to capture the underlying mechanisms, reproducing experimental data and providing reliable predictions, a huge effort must be done in building a multidisciplinary approach. As a complex system, which is more than the sum of their parts, if physicists, mathematicians, biologists, physicians and engineers work together to investigate cancer growth and anti-cancer therapies, a new science can emerge. This complex system point of view can be the start of the winning in the old battle against cancer.

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CANCER AS CIVILIZING PROCESS: ANTHROPOCENE, CAPITALOCENE AND COMPLEXITY

Octavio Valadez-Blanco

Institute of Anthropological Research, UNAM, Mexico City

INTRODUCTION

In the global discourse about disease, such as that arising from the International Agency for Research on Cancer, the "basis of cancer" are understood in biological terms: affected genes, cells, tissues and organs that produce cancerous symptoms[1]. Even though there are a wide variety of cancers, at least two biological processes seem to define this diversity: neoplasms and metastasis. The first refers to the excessive proliferation of cells, and the second to the migrant and invasive abilities of these cells to affect other body tissues. From this biomedical perspective, cancer can be considered a physical and biological object and, as such, a phenomena that can be explained with models and born in molecular and cell biology [2], in the physics of complex systems[3], and in the methodologies of experimental medicine[4]).

In recent decades the consideration of cancer as a genetic disease has been changing towards a discourse that places it as a "complex" disease. Initially the "complexity" of cancer referred to the diversity of both molecular interactions (not just one gene) and environmental factors that were crucial to understanding the etiology and development of the disease[5]. With the recovery of environmental perspectives and the importance of epidemiological studies, cancer was assumed to be a preventable disease associated with lifestyles, habits, work spaces and specific social conditions[6]. Thus this environmental complexity of cancer was directed as an overcoming of genocentric models, such as a fight against the disease not only in terms of a biomedical cure, but also in terms of health policy and prevention (quitting tobacco, changing diet or having access to certain vaccines). I named

this view of cancer as a biological process a "naturalistic perspective" of the complexity of cancer, that seeks to explain the nature of the disease upon three temporal frames:

- The first is the *ontogenetic frame* where we can understand the mechanisms that produce the processes of cellular and histological cancer.
- The second is the *epidemiological frame* where we analyze exposure of populations or individuals to certain types of environmental factors.
- Finally *evolutionary frame* where the disease develops in the history of organisms, and therefore this is expressed in terms of inter- and intra-species questions.

For example, the question of the role of stem cells in the development of the disease is located in the ontogenic explanation of multicellular organism[7], while the study on changes in the frequency of certain types of cancer in different environments is located in epidemiological frame[8]. The last question has become more important when we want to understand the uniqueness of cancer on the biological stage. Under these frameworks, human history seems to be present just as a part of the evolutionary process, or as a narrative explanation of epidemiological data: human history and culture does not change the nature of cancer, but it does modify its incidence[9].

In this chapter I want to expand the importance of this historical time in cancer research, which I call “civilizing frame”, in order to analyze the establishment and expansion of cancer in modern capitalism. This is because this civilizing process acquires the dimension of a force capable of changing climatic variables and the dynamics of life on the planet. In fact, interdisciplinary categories such as “anthropocene”[10] and “capitalocene” are examples of empirical studies that seek to indicate, without falling into anthropocentrism, the impact of humankind on the planet and life (anthropocene) as well the transformation guided by capitalist system.

I want to show the ontological importance of the fact that the high incidence of cancer is a product of modernity, and not just a “natural” phenomenon. My goal is to use an interdisciplinary perspective, where biomedical sciences, humanities and the voices of those affected are needed to outline more articulated actions and research programs, under the idea that the complexity of cancer is not only physical and biological, but also historical.

My approach is as follows: First, I will present some of the contemporary discussions on the antiquity of cancer, from which it is argued that the high incidence of cancer worldwide is a product of the dynamics of modern societies. Secondly, I will discuss briefly the importance and distinction of the categories "anthropocene" and the "capitalocene" to show that the global dimension of cancer is a symptom of a global civilization. Finally I will discuss briefly the implications of this framework in our understanding of the complexity of cancer.

FROM BIOLOGICAL TO HISTORICAL COMPLEXITY OF CANCER

From a "naturalist" perspective, the historical or social reality of cancer is a secondary issue: cancer was a disease among Egyptians, and it existed in medieval Europe. Cancer is a disease of the body that resists and remains in different cultural contexts. I call this a naturalistic perspective of the disease, because it assumes that the ontology of cancer is "biological" (and therefore also physical and chemical). With this perspective we gain some kind of objectivity about cancer; it is seen as a biological process independent from history". However, something is lost in this abstraction: the degree of historical transformations that our own bodies entails, as well as the historical dependence of our own "natural" understanding of the disease.

An example may help clarify this: for decades cancer was considered a genetic disease that could be caused by the mutation of one or more genes[11]. After several debates and failed promises in research, it has been shown that rather than being reduced to a few mutated genes, cancer should be understood as a phenomenon that includes histological entities[12], biomechanical processes and environmental issues[13], among others social factors[14].

In addition to this naturalist project that claims to have discovered the basic ontology of the disease, there have been approaches that emphasize the cultural aspects of cancer, and remain skeptical about the invariant and univocal entities of the disease because it has been shown historically that fundamental ontologies change over the history of science[15]. With an historical approach, we can obtain a diversity of necessary perspectives that includes social, economical and cultural determinants of cancer, but at the same time we deal with a risk of reducing cancer complexity to an irrational dynamic of power struggles between various research programs [16].

In this chapter I want to recover the objectivity regarding the disease, as

well the its historical determinants. This cannot be achieved by appealing to the universality of an ahistorical perspective of cancer, nor falling into a relativistic approach that denies the biological materiality of the disease. Instead I propose to understand cancer as a biological object, but also as singular bodies, from human and nonhuman beings, that deserve respect, love and dignity[17]. In what follows, I will bring in voices that could help us to recover the material content of cancer as a biological-evolutionary phenomenon and also as a cultural-civilizing phenomenon.

FROM EVOLUTIONARY TIME TO HISTORICAL TIME: THE HIGH INCIDENCE OF CANCER AS A MODERN PRODUCT

In the biological sciences, cancer is assumed to be a diachronic phenomenon in the history of humanity and even as a condition not reducible to the human species. From paleoncology, cancer has been described as a phenomenon present in vertebrates, very rare in amphibians and birds, and a little more common in fish, reptiles and mammals, increasing its frequency in captive animals [18]. Hypotheses about this continuity of neoplasms in vertebrate organisms are varied, but some point towards survival strategies where tumors may have some adaptive advantages [19].

No one seems to deny that there has been cancer in other animals, and throughout the history of mankind. However, the debate arises about the origin of the high incidence of cancer in our time compared to other stages of the history of humanity. The most common thesis in this case suggests that cancer has increased its incidence in recent centuries due to increased life expectancy[20], and although the role of environmental factors of our time is recognized, the emphasis is on longevity, i.e. cancer is the negative effect of mankind's effort to live longer.

Some paleopathologists such as Capasso [18] coincide with the thesis that associates the longevity of modern man with high rates of cancer, but he emphasizes that age is not the only factor. The urban way of living and industrialization processes could better explain the presence of cancer in humans and domesticated animals:

> The impressive increase in cancer prevalence documented in human populations over the last century is associated with modern man. It is a completely new phenomenon and has no precedents in the history of animals on the Earth. The high prevalence of cancer contributes to limiting the increase in life expectancy, and seems to be associated with the modern lifestyle. This lifestyle is characterized by living

> in a completely artificial environment (i.e., a prevalently indoor and metropolitan life in an environment in which we undergo prolonged exposure to environmental carcinogens associated with an increase in carcinogenic pollution). The high prevalence of cancer in vertebrates that share this new lifestyle with us in our almost completely artificial environments (i.e., domestic dogs and birds) seems to confirm this picture [18].

However, this type of evolutionary conclusion is criticized for the lack of fossil record of neoplastic, invasive and metastatic processes (many of the studies were usually made of bone tissue) and the lack of population statistics on the disease.

In October 2010 a brief discussion about the age of cancer was presented in the journal Nature Reviews of Cancer. Based on studies of mummies from around the world, paleopathologists Rosalie David and Michael R. Zimmerman [21] concluded that, although it was present in virtually the entire history of humanity and other species, it was not until modernity that cancer became a serious and significant problem. The crucial point of the article was the strength of the arguments: in studies of mummies can apply similar criteria to those currently used by pathologists, and we have enough historical documents of these cultures to understand more about their material living conditions.

Life expectancy in Egypt was approximately 50 years for high classes and 30 for the low classes. This was very similar to mid-sixteenth century England. In addition, in contemporary times various cancers occured in young people, so if there was a pattern of incidence in ancient and modern cultures, it should be observed. The work of Zimmerman received two replies. The first was Wang *et al*, who showed historical documents of China in the fifth century BC, where the etiology of some cancers is described in detail[22]. The second criticism was made by Faltas who contrasted epidemiological studies where some cancers were indeed more frequent in antiquity that in societies of early twentieth century. They concluded that cancer could not be taken as "man-made", but as a disease prevalent in many societies[23]. Zimmerman *et al.* replied that they did not claim that cancer was just a disease made by man, but that its high incidence is a modern product. The relevant comparison is not between ancient populations and others in the late nineteenth century, but between the incidence of cancer in the mid-nineteenth century and the mid- and late twentieth century. The relevant comparison should include populations exposed to snuff and products generated by the petrochemical industry, which characterize con-

temporary societies. With this, both authors maintained the thesis that most cases of cancer that occur in our modern populations are "man-made"[1].

Thus, cancer processes acquire two ontological connotations: it is a biological process, i.e. independent of human history, and at the same time we can only understand its high incidence depending on the historical processes that were developed in the twentieth century. We move from a consideration of cancer as the cause of symptoms and signs in the biological body towards cancer as a biological symptom of a social process that requires explanation.

EPIDEMIOLOGICAL TIME: CANCER AS A GLOBAL DISEASE

Global cancer statistics show the spread of cancer as a phenomenon that is present in every country in the world. In fact, the World Cancer Report mentions that despite the efforts, the global burden of cancer has increased in recent years and its impact will grow especially in developing countries:

> In 2012, the worldwide burden of cancer rose to an estimated 14 million new cases per year, a figure expected to rise to 22 million annually within the next two decades. Over the same period, cancer deaths are predicted to rise from an estimated 8.2 million annually to 13 million per year. (...) As a consequence of growing and ageing populations, developing countries are disproportionately affected by the increasing numbers of cancers. More than 60% of the world's total cases occur in Africa, Asia, and Central and South America, and these regions account for about 70% of the world's cancer deaths, a situation that is made worse by the lack of early detection and access to treatment[1].

Cancer is a global problem, and its globality should also be understood in terms of the unequal structure of the contemporary world. Therefore, statistics and analyses presented in this and other reports always conclude with this dual role of cancer: is globalized, and at the same time is differentiated depending on the geopolitical place where it is faced.

> As countries transition to higher levels of human development, their populations tend to increasingly adopt behavioral and lifestyle habits that have become conventional in prosperous and industrialized countries. A changing prevalence and distribution of several reproductive,

[1] Cancer certainly existed in antiquity, as said before, but the rarity of that diagnosis in the tens of thousands of skeletal remains and thousands of mummies that have been examined supports our view that most cancers in our modern populations are due to man-made factors (David and Zimmerman, 2011).

> dietary, and hormonal risk factors has the effect of increasing the risk at the population level of certain cancers associated with affluence; these include female breast cancer, prostate cancer, and colorectal cancer in both sexes. The net effect of this shift to lifestyles typical of industrialized countries –occurring largely in countries traditionally classified as "developing"– is a steady increase in the overall incidence rates of cancer, as well as a change in the spectrum of the most common cancers towards those observed in most of the highly developed industrialized countries[1].

So, first, the burden of cancer is increased depending on "lifestyles", which only confirms that cancer is no longer seen as a disease that can be described and explained only in terms of biological structures and processes. Second, the greatest impact of this disease will be in low and middle human development countries, i.e. regions where more than 2.2 billion people live[24]. As usual, in the reports of the UN and WHO, the historical origin of this structural inequality between developed and underdeveloped countries is not part of the explanation, interpretation and scientific discussion. Third, the report presents a paradoxical fact: developing countries seek and tend to adopt the behavior and lifestyles of a "developed" and industrialized world, even though these habits and conditions are associated with cancer[25]. Thus, with these evolutionary and epidemiological studies we confirm that the severity or high incidence of cancer is mainly an effect of modern society, and this depends not only on an increased life expectancy, but on lifestyle and contemporary industrialization. However, the origin and expansion of this "industrial society" and structural inequality between developing and developing countries are questions that remain separated from naturalistic approaches, reducing the analysis to global symptoms of a historical disease that we understand less and less. In the following section I propose a series of axes which can serve to explain the "modernity" and "globality" of cancer that these naturalistic approaches only suggest.

CIVILIZING TIME: ANTHROPOCENE, CAPITALOCENE AND MODERNITY CANCER

Statistics and etiology demand to be interpreted: how can we understand this universalized condition of cancer beyond disconnected epidemiological data and social factors?. Without being exhaustive, I believe that a necessary step should start from the recognition that the global spread of cancer presupposes capitalist globalization, and thus the exposure of human and

other species to environments that are contaminated or modified by an instrumentalization and exploitation of life primarily in terms of economic profit. Two arguments can be retrieved for this purpose. The first is related to the concepts of anthropocene and capitalocene, and the second is related to the much better known category of "modernity". With both categories, the environment of cancer acquires a natural-cultural reality marked by a capitalist history.

Anthropocene, capitalocene and cancer

Although there are categories that have sought to speak of the earth as an articulated system such as Gaia or Biosphere [26], the term anthropocene claims, from geological and ecological studies, that the geological Holocene epoch has ended and that we are now in a epoch marked by humanity as a geological force. The basis for these claims refer to the study of changes in global warming, ocean acidity, mass extinctions of species, and generally a growing impact on the geological dynamics of the planet:

> Between 1800 and 2000, the human population grew from about one billion to six billion, while energy use grew by about 40-fold and economic production by 50-fold. The fraction of the land surface devoted to intensive human activity rose from about 10 to about 25–30%. The imprint on the environment was also evident in the atmosphere, in the rise of the greenhouse gases CO_2, CH_4 and nitrous oxide (N_2O). Carbon dioxide, in particular, is directly linked to the rise of energy use in the industrial era as it is an inevitable outcome of the combustion of fossil fuels[10].

As has been pointed out by the economist D. Chakabrarty [26], the anthropocene implies the articulation of at least three histories that had previously been narrated as if they were separate: the history of the Earth system, the history of life and the history of industrial civilization. In the articulation of these narratives new political, economic and moral challenges emerge about the future that we can build in these historical constrains. For Latour [27], for example, the category of the anthropocene represents a kind of awareness of the planetary boundary and therefore an awareness of living in a war to occupy and take over resources, territories, and resources on a limited planet.

However, some authors consider that this category collapses in the "*anthropos*" an unequal diversity of agents and processes, and that while mankind is responsible for many of the effects caused by modes of production and consumption, it is also true that a large percentage of these effects has

been generated by a small proportion of that humanity[28]. In addition, some authors claim that rather than talking about anthropocene, we should place the human being in the context of a capitalist system that, although produced by the "*anthropos*", is in no sense the civilizing nature of humankind[29].

The capitalocene would be the category that historicizes the anthropocene to avoid fetishization and naturalization of a way of civilization which reached its hegemony and dominance at the expense of war and exploitation of labor force and diverse ecosystems.

How can we understand the role of cancer in this global historical process? As we have seen, we can begin by articulating the high incidence of cancer in the postindustrial world, not only with the change in certain habits and exposure to certain factors, but as a disease associated with the capitalocene. For example, there has been deeper research that not only establishes causal relationships between radiation and certain human populations, but also analyzes the complex effects and the political, economical and ecological processes that were deployed by a few countries to make hundreds of nuclear bomb tests during a half a century, producing long time effects in a global scale at the expense of life on the planet[30].

From capitalocene to postcolonial modernity

The "anthropos" in the anthropocene, have its own diversity and history, and therefore it is not enough to add geological, geographical, ecological or economic data to understand our time.The capitalocene warns us of the occultation of the pernicious transformation made by the logic of capital and this represents a critique of the fetishism of geology, that is a criticism of those scientific or political discourses that assume natural causes for historial transformations. It is from this global and historical view of the global nature of cancer that we can begin to build a transdisciplinary approach to research that dialogues between those discourses and practices that seek to address and transform the civilizing projects of capitalistic globality[31]. Science is not above this historicizing perspective, but it itself has subjects, bodies and places that make it a product and a producer of ideas and civilizational forms. That is why it is not appropriate to separate the idea of capitalist civilization from the modern European project that founded it, while these ideas and their practices are closely related in the history of the expansion of modern capitalism.

The analysis of this modernity can be described based on two perspectives:

i) a historical vision of modernity, where it represents an ideal form of totalization of human life, whose main notes are: humanism (triumph of "technical reason" over magical thinking), rationalism (predominance of the cognitive domain over the practical), progressivism (linear and inevitable progress time), urbanism (big cities as opposed to rural "barbarism"), individualism (individual identity over the community), economism (the individual incorporated into the State for the common enrichment), and patriarchy (subjugation, control and management of women and sexual diversities)[32–34].
ii) diachronically, modernity can be understood only in the light of the emergence of the world system from the European conquest of the Atlantic and Mediterranean seas[35, 36]. With both perspectives, modernity simultaneaously implies capitalism, Eurocentrism, colonialism and patriarchy, in a complex and open story that needs to be built and, even more, transformed. If cancer has gradually become the leading cause of death worldwide, should we consider this disease an inescapable sign of the anthropocene, capitalocene or capitalist modernity? What implications does the articulation of geological, evolutionary and civilizing temporality on our understanding of cancer?

THE CIVILIZING COMPLEXITY OF CANCER

With the inclusion of these historical frames in cancer research, the complexity of the disease becomes a nature-culture process. Thanks to the historical contingency of this complexity, we can not only criticize the high "natural" incidence of cancer, but try to change the "cultural" component of this prevalence[37]. Anthropocene, capitalocene and modernity are terms that provoke debates about the necessary convergence of health, ecological, political and economic problems that underlie the globalization of cancer. In addition, through the critical content of these categories we can avoid collapsing the globalization of cancer into an ahistorical naturalism, as well as an interpretive relativism, as both extreme views dilute or obscure the response-ability that science has in the fight against injustice and material and ecological violence. Cancer research could join with other voices and social movements that are trying to understand and to overcome the civilizing project of heteropatriarchal, neo-colonial and capitalist modernity [38].

By including these cultural and historical perspectives, the challenge to both recognize the diversity of bodies, environments and stories opens, but

also the challenge of understanding that this diversity is not separated and isolated, but related materially in a world and a civilizational discourse that is ever globalized.

So, the "civilizing frame" of cancer includes, on one hand, the diversity and multi-causality of disease, and simultaneously the homogenizing dynamic of a political, economic and cultural process driven largely by the capitalist accumulation of gain. As we have seen, this nature-culture of cancer is not an inherent feature of this disease, nor an exclusive effect of modernity: cancer can be traced in other historical and geologic stages; however, my approach starts from the important fact that the high incidence of cancer is a product and an effect of the globalization of modern lifestyles, so cancer itself is already a symptom of the transformation of our bodies and environments in the capitalocene.

In sum, when we add "civilizing time" to the ontogenetic, evolutionary and environmental narratives, cancer can no longer be explained only as the object of biomedical physical sciences (a cell, an organism), but as a variety of natures-cultures converging on the borders of a finite planet and a dominant civilizational project. This is not just "another" perspective, but a whole ontological reconsideration of the globalization of cancer, which is manifested in the diversity of experiences that the disease causes at the anonymous patients from different cultures, in our near families, in our own bodies and even in the bodies of other species[37].

Cancer ontology could become a pernicious naturalization if the historical contingencies of both scientific discourse and social reality are hidden or assumed[39]. On the other hand, when social and historical explanations downplay and deny the physical-biological materiality of bodies, cancer may become a pernicious idealization where the disease is reduced to a mental image of a disembodied narrative subject. My work seeks to walk in between these extreme perspectives.

The complexity of cancer involves dialogue and debate on the diversity of these ontologies (genes, cells, organisms, cultures, institutions, worlds) based on the fact that we share a finite planet, as well as the recognition of multiple voices (science, humanities and those affected by the disease) that are facing, from (still) different worlds and bodies, the global dynamic of the capitalocene.

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