Tumor tissue architecture, immune cells and molecular characteristics in relation to immune escape in colon cancer: what rises must fall

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Chapter 2

The need for a systems biology approach in cancer explained

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Abstract

Traditionally, scientists tend to approach cancer research in a reductionistic way: aiming at uncovering underlying separate components in malignant processes. And indeed, a great progress was made by reducing the development of a tumour to single, specific, genes and mutations in them. For instance, familial adenomatous polyposis (FAP) could be reduced to a germline mutation in the Adenomatous Polyposis Coli (APC) gene. Escape of tumour cells from immune surveillance could be reduced to tumour expression of immune checkpoints, resulting in new approaches in tumour therapy by applying immune checkpoint inhibitors. However, a germline mutation in APC is not 1:1 related with colorectal cancer (CRC) and only some patients respond to immune checkpoint inhibitors. The point here is that biological systems, also comprising cancer, have properties that cannot be reduced to single components. The cooperation of the single components results in new, emergent, properties. The outcome of an interaction in a complex network, like the immune system, depends on the many cell types involved and numerous molecules that interact and activate or inhibit pathways. The way the composing elements are organised is a causal factor on itself for any emergent property. The rise of genomic analysis at the end of the previous century, enabling to sequence a full genome at DNA and RNA level, initiated the awareness of the need for 'systems biology': to consider a full system and how it is organised, in all its aspects, to understand biological pathways and their outcome. In this review, we outline the prospects and limitations of systems biology in cancer research and propose a causal framework that integrates upward and downward causation, and multiple realizability to understand emergent properties of tumours that determine the dynamics of tumour development.

Keywords: cancer; systems biology; molecular biology; emergent property; upward causation; downward causation

1. Introduction

Usually, the focus in cancer research is reductionistic: searching for aberrancies in specific genes or proteins to explain tumour growth. The idea behind this is that basic components are responsible for tumour growth. Indeed, the composing elements at a lower organizational level affect properties at a higher organizational level. It may, however, also be an oversimplification as properties at a higher organizational level cannot be simply reduced to the composing elements. The higher-level property is unique, emerging from the collaborative underlying elements due to the way they are structured and interact[1]. For example, when a T cell receptor is activated by a human leukocyte antigen (HLA)-molecule that presents a virus-derived peptide, it triggers a cascade of interactions within the T cell[2]. These are all pure physical interactions between molecules, at a very basic level, in fact between atoms. Physical laws, based on charge, mass and more elementary properties determine the outcome of such interaction between molecules and dictate the next interactive step[3,4]. At this level it is nothing more than interacting molecules. At the level of an individual, however, the outcome is recovering from a virus infection. None of the involved molecules can be held responsible for recovering from a virus infection. The way the composing elements (molecules) are organized is a causal factor itself for the emerging property[1], in this case recovering from a virus infection. A similar example can of course be given for a T cell killing a cancer cell.

Cancer properties such as uncontrolled cell growth in cancer are also emergent properties, resulting from the way the composing elements are organised and, therefore, cannot easily be reduced to mutations in genes or dysfunctional proteins. Genes are involved, and even responsible, but it is the interaction of the elements of the total system that results in the emergent property ‘uncontrolled cellular proliferation’. The way the composing elements are structured, including for instance the timing and level of protein expression, is crucial[5]. Typical for cancer cells is expression of proteins that are normally not or at a lower or higher level expressed. This ruins the highly fine-tuned functional dynamics in a cell, resulting in ‘cancer’ properties; uncontrolled proliferation, infiltration in healthy tissue, and metastasis. ‘Systems biology’, comprising the dynamics of the full functional system, as will be explained, is needed to understand biological processes as tumour development. This review therefore treats cancer as a multiscale system and uses upward and downward causation as a practical causal scaffold to organise mechanisms across levels, including immune and host constraints, to strengthen interpretation and guide systems level research questions.

2. Causation in biological systems

As discussed above, the composing elements and the way they are organized are responsible for an emergent property observed at a higher organizational level. This is called upward causation. For instance, the process apoptosis is initiated by a network of interacting proteins (the composing elements, lower organizational level) and results in cell death (emergent property, higher organizational level). Apoptosis of cells is a unique process that cannot be reduced to any of the composing elements; it is the elements and how they interact that causes the biological outcome: cell death in this case. This upward causation is at the base of biology at any level: from DNA transcription, resulting in protein expression to communicate and cooperate among (or between) organs that make a living organism. However, systems are not only characterized by upward causation. Also, the opposite process takes place: downward causation. Emergent properties are responsible for this downward causation: processes at a higher organizational level cause effects on a lower level. For instance, eating food activates the salivary gland to produce saliva: Cells in the salivary gland respond to molecules from the food in their environment by activating signalling pathways, resulting in production of digestive proteins and their secretion, together with fluid[6]. Therefore, a process at a higher organizational level (eating food) causes effects at a lower level (molecular processes that lead to secretion of saliva). A simple representation of biological upward and downward causation is shown in **Figure 1**.

Emergent properties also include processes that check for integrity of the property and may activate downstream processes enabling to maintain the emergent property. For instance, cells have a system to check for DNA integrity (cell, higher organizational level) and can activate proteins to replace mutated nucleotides (DNA repair, lower organizational level). Another example: dying cells at the top of epithelial tissues (tissue, higher organizational level) cause cell proliferation at the base of the epithelium (single cells, lower organizational level). Together, upward and downward causation maintain homeostasis: the balance between processes at each organizational level needed to keep an individual healthy. For instance, considering having migraine (higher organizational level), which is caused by defective molecular processes (lower organizational level)[7]. Therefore, migraine is due to upward causation. A person with migraine may take medicine to feel better. The drugs will interfere in processes at a molecular level (lower organizational level). Therefore, when the drugs stop the migraine, this is caused by downward causation.

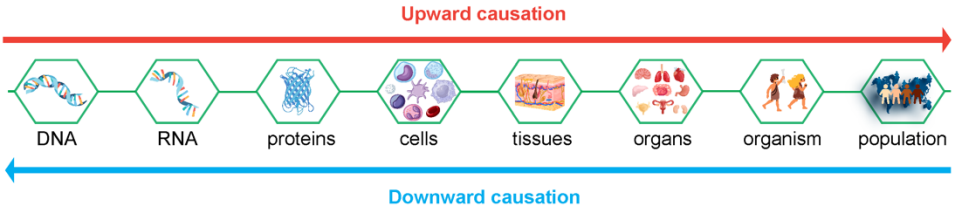


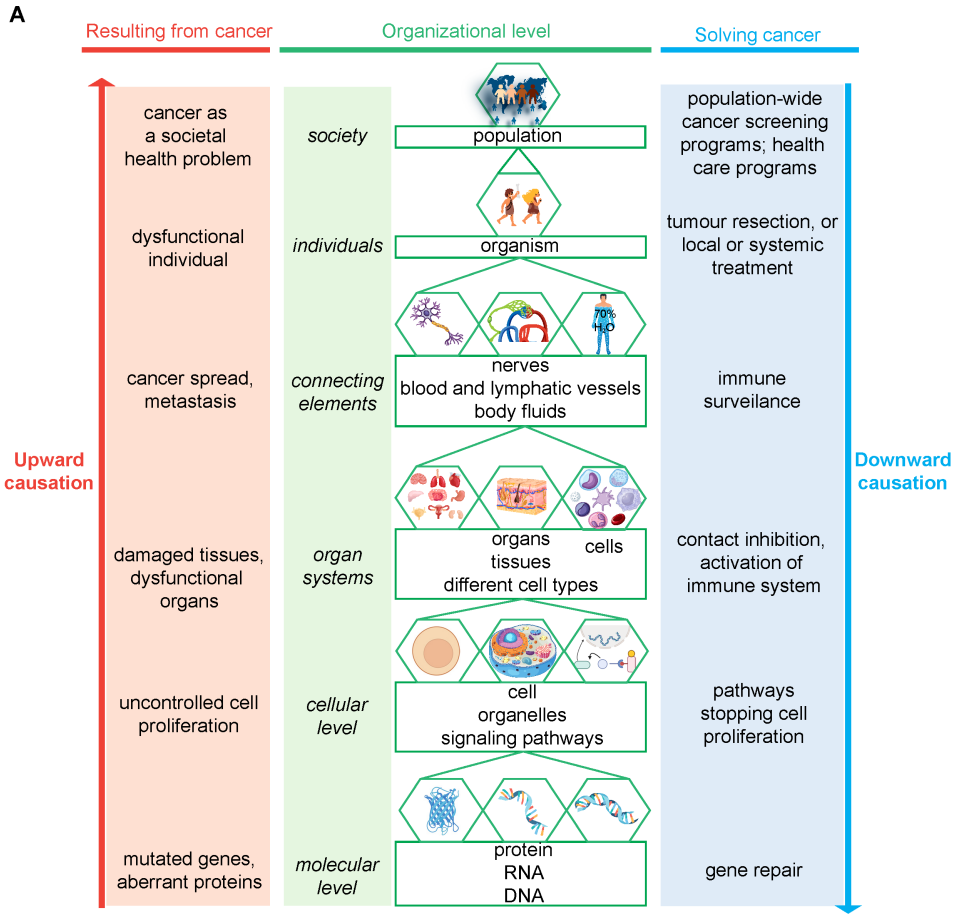
Figure 1. Representation of upward and downward causation in organisms. Each organizational level (boxes) is associated with specific emergent properties. Upward causation: processes at a lower organizational level are responsible for processes at a higher organizational level. Downward causation is the other way around: processes at a higher organizational level cause effects on a lower level. In all cases: emergent properties cannot be reduced to the composing elements; the organization itself is a causative element. Note that the icons depict representative examples at each level rather than an exhaustive list, and the figure can be further extended on both sides.

3. Causation in cancer

The development of cancer fully follows this model of biological causation (see for instance breast cancer[8]). In case of tumour growth, it is both upward and downward causation that should be considered to understand the underlying processes. This because it is not only processes that mistakenly induce cell proliferation but also failing correction processes that lead to tumour development. Cancer development affects emergent properties at many different levels: from molecular interactions to society. In **Figure 2A** this is further explained and illustrated. Any aberrancy at a lower organizational level causes a problem at a higher organizational level; it affects an emergent property or induces a new property, for instance ‘tumour growth’ in an individual; or a company with a problem due to a staff member diagnosed with cancer. At all organizational levels there are downward directed processes intended to control tumour development. Failure at all levels of these control processes, as shown in **Figure 2A**, ultimately results in cancer as a societal health problem.

Therefore, to understand cancer, it is essential to understand both, the processes that cause upwards the problems caused by cancer and the downward-directed processes that are active to control cancer. For each process it is important to consider it in toto as a process that results in an emergent property cannot be simply reduced to the composing elements. Systems biology is in fact the outcome of upward and downward causing processes. At the time a patient experiences complains due to a growing tumour, this tumour had developed due to many upward causing processes and escaped already from many processes of downward-directed control. For effective therapeutic intervention in this tumour

development, a thorough understanding of both, causative processes and failing control mechanisms, is critical. This framework of upward and downward causation as shown in **Figure 2A** will be further discussed below for each organizational level.


A
Resulting from cancer
Organizational level
Solving cancer
cancer as a societal health problem
society
population
population-wide cancer screening programs; health care programs
dysfunctional individual
individuals
organism
tumour resection, or local or systemic treatment
cancer spread, metastasis
connecting elements
nerves
blood and lymphatic vessels
body fluids
70% H2O
immune surveilance
Upward causation
Downward causation
damaged tissues, dysfunctional organs
organ systems
organs
tissues
different cell types
cells
contact inhibition, activation of immune system
uncontrolled cell proliferation
cellular level
cell
organelles
signaling pathways
pathways stopping cell proliferation
mutated genes, aberrant proteins
molecular level
protein
RNA
DNA
gene repair

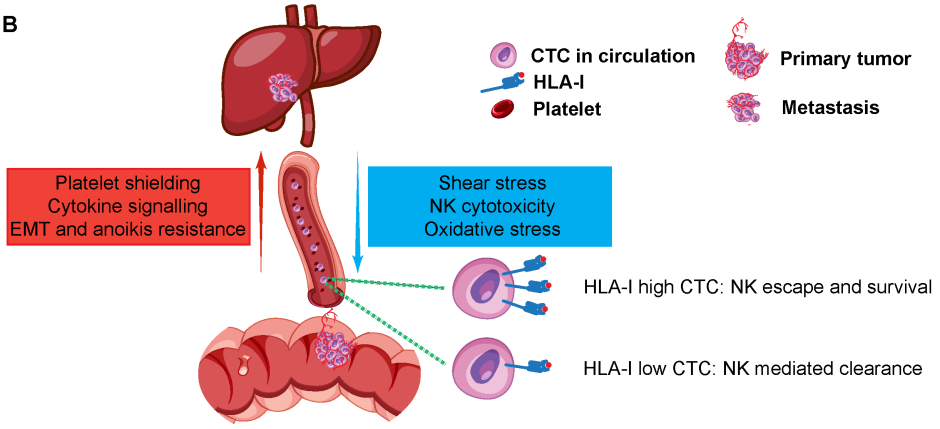

B
CTC in circulation
HLA-I
Platelet
Primary tumor
Metastasis
Platelet shielding
Cytokine signalling
EMT and anoikis resistance
Shear stress
NK cytotoxicity
Oxidative stress
HLA-I high CTC: NK escape and survival
HLA-I low CTC: NK mediated clearance

Figure 2. Upward and downward causation across organisational levels in cancer, with a zoomed view of circulating tumour cells. Panel A summarises how emergent properties at molecular, cellular, tissue, organ, organism, and population levels can contribute to tumour development via upward causation and constrain tumour growth via downward causation. The listed processes are illustrative examples and do not represent all mechanisms operating at each level. Panel B expands the circulation level and depicts circulating tumour cells, escaped from a primary tumour in the intestine, as an interface for opposing forces. Upward enabling inputs include platelet shielding, cytokine signalling, and induction of epithelial mesenchymal transition with anoikis resistance, resulting in growth of a metastasis in the liver. Downward constraints include shear stress, oxidative stress, and NK cell cytotoxicity, which may prevent outgrowth of liver metastasis.

4. Molecular level

At this level molecules like DNA, RNA, proteins, and many more interact. The development of cancer is a multi-causal process. Approaches at this level include protein-protein interaction and signalling network modelling, pathway activity inference from bulk RNA sequencing, and genome scale regulatory network reconstruction to link mutations to network perturbation[9–12]. At the base are mutations in genes, resulting from exogenous, or endogenous factors, or a combination. Mutated genes result in aberrant proteins that do not function properly. DNA mutations can also be responsible for deregulated miRNA networks and epigenetic machinery that turns specific genes on and off, all resulting in deregulated expression of proteins[13]. Proteins themselves can be defect due to a DNA mutation or can be expressed at a too high or too low level or with a wrong timing due to DNA mutations elsewhere. In all these cases the cooperation of proteins in networks is affected, causing insufficiencies in the emergent functional property associated with the network. DNA mutations caused by endogenous factors are probably mainly associated with errors in the DNA sequence during DNA duplication in proliferating cells. That may explain why tumours with the highest incidence are carcinomas: tumours derived from a tissue with a very high proliferation level, epithelium. Exogenous factors causing mutations in genes are environmental factors, including chemical, physical and biological factors.

Chemical factors: for many kinds of chemical substances, it is known they are associated with cancer. For instance, smoke of tobacco (risk on lung cancer, but in fact all kinds of cancer)[14]; asbestos (risk on lung cancer, mesothelioma)[15]; alcohol (risk on many cancer types)[16]; polyaromatic hydrocarbons in burned organic material like barbequed meat (risk on cancers in

skin, lung, and bladder)[17]. Many more can be added to this list. Exposure takes place in the working environment, via nutrition, environmental pollution, consumer goods, or because of a lifestyle.

Evident physical factors that cause cancer are ionizing radiation and UV light. Both types of radiation carry enough energy to damage DNA directly, leading to mutations that can initiate cancer. UV light (from sunlight, tanning beds) is associated with skin cancer[18]. Ionizing radiation (from the environment or as used for medical imaging purposes) may lead to skin cancer and many other types like leukaemia[19], and it may also induce secondary cancers after radiotherapy of a primary tumour[20].

The third class are biological factors. Clearly associated with cancer is the microbiome, being viruses, bacteria, parasites[21]. For instance, human papillomavirus is associated with cervical cancer[22]. Also, bacteria are currently getting a lot of attention for their association with cancer. A clear example is involvement of helicobacter pylori in gastric cancer[23]. For the chemical and physical factors, it is generally accepted that causing DNA mutations is at the base of inducing associated cancers. For the microbiome this is less clear and a topic in research[12,21]. For viruses, integration in the genome or affecting the genome in another way may be an option. For bacteria and parasites, the causal relationship with cancer is not yet known. It is possible that these affect cancer development at a higher organizational level than the genome, for instance by affecting tumour immune surveillance.

DNA mutations are inextricably linked with life. Therefore, it is very logical that evolution has resulted in an extremely sophisticated and diverse system for DNA repair [24]. This check for DNA integrity is at a higher organizational (cellular) level where processes are in place that can activate DNA repair. When successful, DNA mutations are repaired (downward causation), when these processes fail cellular function is disturbed and for instance uncontrolled cell growth may be initiated (upward causation). This check for DNA mutations and DNA repair is also dependent on proteins that are coded for by the DNA and, therefore, also prone to mutations. Indeed, for several cancer types it is obvious that the DNA repair system is affected[25]. For instance, in subtypes of colorectal cancer (CRC) it is the microsatellite DNA repair system that is dysfunctional[26].

5. Cellular Level

At the cellular level, the different cell types function as the living basic elements of an organism. Approaches at this level include single cell RNA sequencing and single cell multi-omics to resolve tumour cell states, trajectory and lineage inference, and mechanistic or probabilistic cell state models to capture phenotype plasticity[27,28]. The genomic status of a cell, i.e. the DNA sequence and among others epigenetic status of promotor methylation and histone modifications, determines which genes will be transcribed in that cell. All components together function in a highly organised manner enabling a cell to function.

The development of a tumour is a complex process. It is typically an outcome of a multiple realizable process: many roads can lead to cancer. At the base are DNA mutations that affect molecular pathways, networks, that control cellular functions[29]. Via many different routes, the result can be uncontrolled cellular proliferation. Defects in repairing DNA mutations are probably at the base of tumour development (upward causation). During tumour cell division, DNA mutations keep on accumulating and explain why a tumour is usually composed of cells with a heterogeneous genotype and phenotype. Next to DNA repair, many other control mechanisms in place (downward causation), like apoptosis, are defective, ultimately resulting in uncontrolled cellular division. The result is a continuum of tumour cell phenotypes, of which those grow out that possess a selective growth advantage.

The exponential growth in the development of -omics techniques, especially single-cell DNA and RNA techniques, have extensively improved our knowledge of inter- and intra-tumour heterogeneity[30–32]. By incorporating profound knowledge of tumour cell heterogeneity in terms of DNA mutations, expression level of proteins, cell cycle stage, etc., into comprehensive computational models, systems biology enables a more holistic understanding of the complete tumorigenic process at the cellular level.

6. Organ Systems

Organs are composed of well-organised specialized tissues that contain different cell types that all together are responsible for the functioning of that organ[33]. Approaches at this level include spatial transcriptomics and multiplexed imaging to quantify tissue architecture and cell-cell interactions, together with digital pathology and ecological modelling of tumour ecosystems[34–36]. Maintaining tissue integrity is crucial for optimal organ functioning. Contact inhibition is a process of downward causation to maintain tissue integrity and stop unnecessary cell proliferation. Especially for epithelial tissue, for instance in ducts, which are

constantly renewing tissues, this is an important process. If the status of a cell enables uncontrolled proliferation, and contact inhibition is also not functioning, a tumour starts growing and may infiltrate surrounding tissue[37]. This will initiate tissue damage, resulting in secretion of specific cytokines and other molecules activating a new level of processes of downward causation in stopping tumour growth. The secreted molecules are necessary to initiate healing of damaged tissue and attract the immune system for immune surveillance[37,38]. Tumours like carcinomas show a very broad spectrum of behaviour and morphology, varying from undifferentiated, hardly distinguishable as being derived from epithelial tissue, to highly differentiated, looking like epithelial tissue, but growing inside the organ[39]. Surrounding tissue responds to this growing epithelial tissue, for instance by inducing growth of supportive tissue, like needed for normal epithelium and visible as stromal tissue, together often referred to as the tumour microenvironment[40]. For a systems biology approach at this level, it is of importance to understand both, the processes in tumour cells and in the reacting normal cells.

7. Connecting Elements

The organs are connected via nerves, blood and lymphatic vessels, body fluids. Via this system, organs can communicate and respond upon signals. Approaches at this level include longitudinal liquid biopsy profiling of circulating tumour cells and peripheral immune subsets, plasma proteomics, and dynamic network models that integrate soluble factors and immune surveillance across compartments[41–43]. Tissue damage signals coming from organs with a growing tumour may alert the immune system and attract immune cells to these sites. If tumour cells are sufficiently recognised by the immune system as aberrant, for instance due to presentation of peptides derived from mutated proteins, these may be killed before becoming clinically evident[44]. However, with genetic instability as driving force, tumour cells may arise that are able to escape from immune control and selectively grow out.

Except for growing into adjacent organs, tumour cells depend on vessels to migrate to distant organs[45]. A part of primary tumour cells may have acquired additional emergent properties needed to metastasize successfully and enter the blood or lymphatic vessels, potentially leading to distant metastases[46]. These tumour cells, then called circulating tumour cells (CTCs), arrive in a new, distinct environment as compared to the primary tumour. These opposing constraints and enabling signals in the circulation are schematised in **Figure 2B**. They are subjected to numerous restrictive barriers, among which immune surveillance, fluid sheer stress, increased oxygen tension, all downward-directed

processes[47]. Next to restrictive barriers, the blood contains a wide range of soluble factors that can affect cell signalling pathways in CTC. These factors compose a complex signalling network and induce a plethora of dynamic signalling cascades in multiple cell types. For instance, the cellular activity of CTC is closely related to multiple cytokines present in the circulation that activate signalling pathways involved in (via upward causation) migration, proliferation, epithelial-mesenchymal transition (EMT), and anoikis/apoptosis resistance[48–50]. Platelets are important producers of these cytokines[51]. However, these cytokines are also secreted by cells from the primary tumour, emphasizing the importance of a systems biology-based approach in cancer research, as CTC behaviour is not solely mediated from within the circulation, but also by the primary tumour itself.

A selection process takes place, and while the vast majority of CTCs will not survive, some CTC clones are able to adapt and survive by modulating properties at a molecular level. Although these adaptive responses are still poorly understood, mRNA sequencing of CTC from CRC patients that are exposed to continuous flow (mimicking shear forces) has demonstrated that several EMT-related genes are upregulated, together with the significant regulation of approximately 100 other genes[52]. These findings suggest that biomechanical forces induce a downward causation process, resulting in transcriptomic changes that, in turn, influence at the cellular level of CTC: increased epithelial-plasticity and morphological changes. Thus, even though the stress factors present in the circulation greatly limit CTC survival, it also results in the emergence of CTC clones with increased metastatic potential, as they can better cope with mechanical- and biochemical stress. Besides, much more genes are differentially expressed in CTC of CRC patients as compared to the primary tumour[53,54]. It has become evident that some CTC clones have acquired an aggressive phenotype, with an increased expression of genes involved in chemoresistance, apoptosis resistance, organ colonization and decreased expression of tumour suppressor genes.

An important barrier for CTC may be the immune system. The interaction between tumour cells and immune cells will be very different in the circulation as immune cell composition differs very much from immune cells present in a tumour. Among others, in the primary tumour environment, NK cells are infrequently present[55]. CRC cells often show downregulation of classical HLA class I (HLA-I), resulting in immune escape from effector T cells[56–58]. However, in the circulation, NK cells comprise 10-15% of the peripheral blood lymphocyte population[59–61]. In this context, metastatic tumour clones that lack HLA-I expression will be eliminated while HLA-I-positive metastatic cells have a survival advantage. Indeed, it has been reported that partial

downregulation of HLA-I in CRC has a worse prognosis than complete loss of HLA-I in CRC patients[62]. Interestingly, the cytotoxic and cytolytic activity of circulating NK cells seems to be impaired in CRC patients, suggesting that the downward directed control process of immune surveillance for tumour cells in the circulation is overruled by downward-directed processes initiated by the tumour, resulting in impaired immune functioning[60,63].

Important for understanding tumour-immune interactions, is the acknowledgement that tumour-immune interactions are inherently multivariate, operating across different time- and spatial scales and cell types. Due to the evident successes of immunotherapy in some cancer types[64,65] and the technological and computational advances in life sciences[66,67], tumour immunology currently stands at the forefront of biomedical research. However, there is an urgent need to distinguish between patients that will respond to therapies and those that will not. Systems biology helps to address this question by integrating knowledge of the interaction between multiple cell types, receptors and cytokines as they travel through different anatomical locations to organize an effective immune response. Studying CTC and peripheral immune subsets is particularly interesting, as blood is very easily drawn from a patient at multiple different time points. Analysing the peripheral immune profile of patients adds yet another level to our tumour biology understanding and investigating tumour-immune interactions on different levels in a patient (for instance, the primary tumour, circulation and metastatic site) enables us to acquire a comprehensive idea of the immune system in relation to tumour progression and therapy response[60,61,68]. A systems biology approach will improve treatment options and enables better personalized care to patients with advanced tumours.

Taken together, once a tumour cell enters the circulation, it becomes exposed to a completely different environment with different selective constraints. Cancer metastasis is a multifaceted progress, unfolding at many biological- and anatomical scales. The molecular characterization of CTC, including also epigenetic phenomena, is vital for deciphering the biological aspects of the metastatic cascade. An evident challenge is to transform the concept of individual events during metastasis to an integrative, multi-scale metastatic framework. While important components governing metastasis have been slowly elucidated in a reductionist manner, all metastatic events and aspects of CTC are by some means connected with each other and it is inadequate to only analyse aspects individually. Systems biology that integrates expertise from different fields (such as cancer biology, genetics, mathematics and bioinformatics) can unravel the organization of the full metastatic cascade and make useful predictions for therapy.

8. Individuals

Every organism has its unique properties in physical and psychological appearance. Approaches at this level include integrated multi-omics for patient stratification, immune profiling, and predictive modelling of treatment response using interpretable machine learning and causal inference frameworks[12,69]. In the first place, this distinction among individuals can be reduced to the lowest organizational level: polymorphisms present in the DNA, affecting via upward causation processes at a higher organizational level. Secondly, each organism experiences different environmental interactions. Signalling from the outer world (by food, education, training, etc.) affects via downward causation the transcription status of genes, leading to further distinction in physical and psychological features among individuals. Due to this process, for example genetically identical twins become different individuals. These processes are also at the base of tumour development: a tumour is characterized by a certain DNA sequence and epigenetic regulated gene transcription in which the environment plays an important role. Therefore, a similar tumour in two individuals will always be different. This justifies the rise of personalized medicine to treat cancer. It was genomic studies that showed genetic and epigenetic variation, and that made people realize the importance of personalised medicine.

In addition, at the level of the individual, co-morbid conditions, such as diabetes, obesity, or chronic inflammatory diseases, interact with cancer development and progression through shared signalling pathways and systemic alterations, including metabolic and immune changes. These represent downward causative processes, where a disease at the organismal level affects molecular and cellular mechanisms that influence tumour growth and therapy response. Conversely, cancer itself may exacerbate co-morbid conditions, illustrating upward causation. Therefore, incorporating co-morbidity into systems biology models is essential for accurate prediction of prognosis and for tailoring personalized treatment strategies[70].

A tumour may be present for a long time before becoming clinically manifest. Once diagnosed, the best treatment option by far is surgical resection. For cancers that are already disseminated through the body, systemic treatment is preferred, perhaps in combination with surgery. The clinical outcome may differ very much between individual cancer patients, even in case of patients with seemingly similar starting points. This may be caused by large differences in the upward and downward causative processes that led to this cancer. Therefore, by considering these processes, for instance by molecular analysis of the tumour

and its microenvironment at all the different levels considered and described here, a better personalised prediction of effective treatment and clinical outcome can be made.

9. Society

The lifetime risk of developing cancer is around 50%[71,72]. Therefore, cancer has a very high impact on our society. The high incidence and early loss of life have initiated many initiatives to cope as society with cancer, for instance by raising funds to support cancer research to understand the development of the disease. Also at the governmental level, initiatives are taken to cope with cancer. All is focused on decreasing incidence and mortality of cancer and improving quality of life of cancer patients. These measures represent downward causation, where collective societal action influences the biological course of the disease through improved prevention, earlier detection, and better treatment. At the same time, biological processes such as rising cancer incidence due to aging populations or lifestyle-related risk factors exert upward causation by shaping societal priorities and policies.

A clear example of downward causation at the societal level is the implementation of population-wide screening programs[73]. This is attractive as most cancers are very well treatable, resulting in complete cure, in early stages. In the Netherlands two population-wide screening programs are running: on breast cancer[74] and CRC[75]. The program on CRC is stepwise introduced starting in 2014. People of 55 to 75 years old are invited to send in a faecal sample for testing on the presence of blood. Essentially, the program reduces CRC to haemoglobin present in faeces. If haemoglobin is detected in the faeces, a person will receive an invitation for a colonoscopy. As such, the program detects CRC at a much lower organizational level (leakage of blood) than at which an individual itself would detect (pain, fatigue, other complains). With this early detection, tumour progression is in an early stage and could have a higher chance of successful treatment. The question is if this indeed happens. In **Figure 3**. the incidence and mortality of CRC for the Netherlands is shown for the years 2000 – 2024[76].

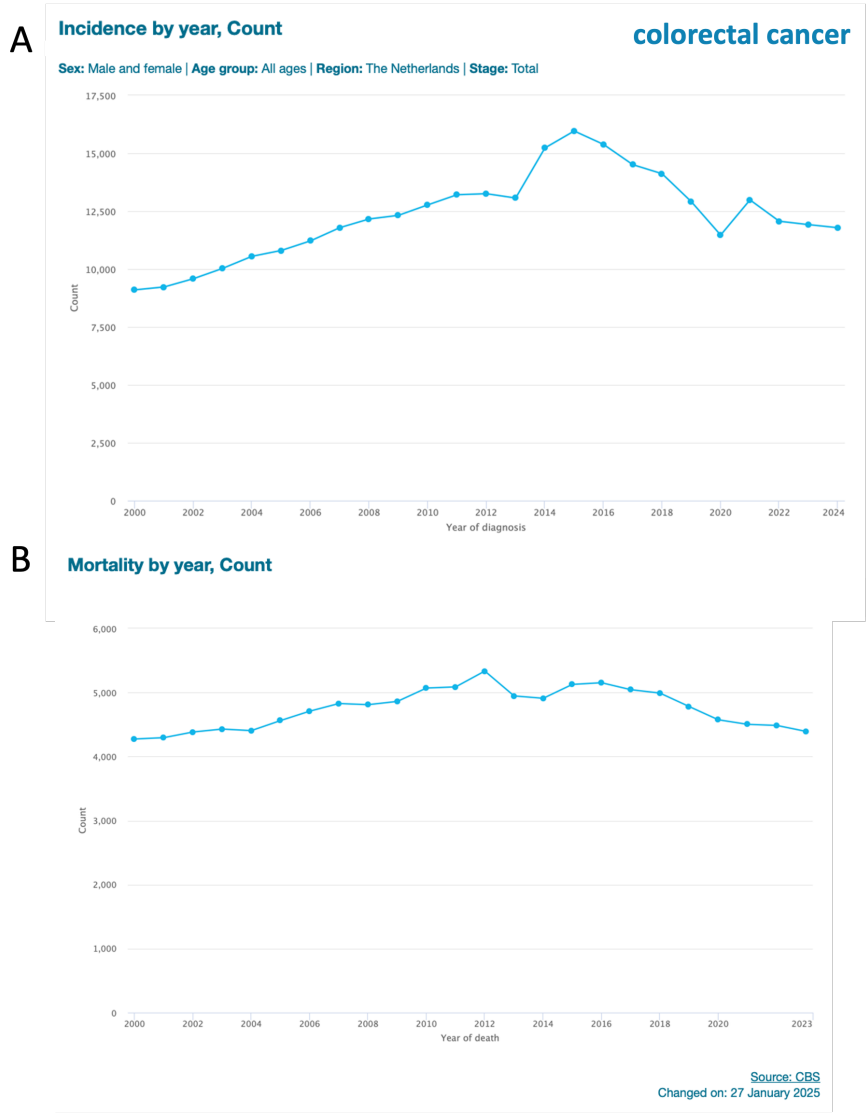


Figure 3. Incidence and mortality of colorectal cancer in the Netherlands. Figures are from the Netherlands Cancer Registry[76]. A, shows data on incidence (year 2000 to 2024) and B, on mortality (year 2000 to 2023) of colorectal cancer. Population-wide screening based on a faecal occult blood test is introduced in 2014 in the Netherlands. The dip in 2012 in incidence is most likely to the corona pandemic at that moment. Due to travel restrictions, less people suspected of cancer visited a hospital during the pandemic.

10. Multiple realizability at all levels

It is important to understand that multiple realizability is operative at each organizational level. Multiple realizability means that a different process leads to the same result. For instance, many different pathways can lead to tumour development. In colorectal cancer 4 main pathways are distinguished that are active in tumour development. And also within these pathways, variations are possible, all with the same result: a tumour in the colon[77]. This is an example of multiple realizability of upward causation. The same holds true for downward causation: many different processes can have the same result. For instance, cure of cancer can be via immune surveillance, but also by therapeutic intervention.

In fact, at each organizational level many different processes of upward and downward causation have the same result. This is a main reason why a reductionistic approach, i.e. reducing a process to its composing elements, would not provide a real insight in the process. Only a systems biology approach, considering not only the composing elements, but also the organizational structure and starting and endpoint, can provide a comprehensive insight in the process and its dynamics as will be explained below.

11. A systems biology approach

Cancer is an exceedingly complicated illness, at all organizational levels, as discussed here. Efforts to profile tumours in the lab have shown a wide range of molecular and cellular changes, but their functional implications have yet to be fully discovered. Characterizing the genetics and epigenetics of cancer and the involvement of specific intracellular pathways to tumour initiation and development has come a long way[68]. The variety of hallmarks available for systems-level probing will continue to expand as underlying cancer biology is more understood. The difficulties encountered when interpreting single parameter research underline the need for understanding cancer as a whole system. While integrative approaches are invaluable for uncovering fundamental cancer mechanisms[12,78], translating them into clinically actionable insights remains a challenge. For systems biology to meaningfully impact patient care, models must be interpretable and practical enough to inform diagnostics, prognosis, and treatment decisions in routine clinical practice. The rising success, as well as challenges, of cancer immunotherapy illustrates this concept well, where a simultaneous awareness of the communication networks within and across cell types in the tumour microenvironment will be critical for extending this treatment modality's efficacy. The intricacy of cancer clearly necessitates a multi-pronged, multidisciplinary approach to understand the full

systems biology involved in cancer development. Systems biology embraces the complexity of cancer by using massive multiscale data sets to construct integrative and predictive models that provide a systems-level knowledge of how molecular-cellular-organic changes interact to promote tumour growth.

One concrete route to make such models predictive is to use explicit in silico formalisms that translate system level constraints into quantitative cell level outputs, thereby operationalising downward causation. For example, genome scale metabolic network models combined with Flux Balance Analysis can implement organismal or tissue level nutrient and oxygen availability as constraints on cellular exchange fluxes, resulting in shifts in feasible intracellular flux distributions[79,80]. These constraint-driven flux reallocations can then propagate upward causation by shaping emergent phenotypes such as proliferative capacity, redox homeostasis, and resistance to apoptosis, and can be compared with transcriptomic, proteomic, metabolomic, or isotope tracing data for validation. To address tumour microenvironment heterogeneity and cell to cell interactions, systems biology increasingly relies on spatially resolved and single cell measurements to parameterise explicit interaction models. For example, agent-based and hybrid multiscale models can represent discrete cell states together with local cytokine, oxygen, and nutrient fields, allowing emergent patterns such as immune exclusion, suppressive niches, and spatially structured therapy resistance to arise from a defined cell status[81]. In parallel, graph-based frameworks and ligand-receptor steered communication models can quantify intercellular signalling and neighbourhood dependent regulation, providing a mechanistic link between cell state composition, spatial organization, and tumour control[82,83]. Network-based models are also central for describing metabolic reprogramming as an emergent system level property[84]. In particular, genome scale metabolic networks and constraint-based modelling formalise how coordinated pathway level flux redistribution emerges from network topology under microenvironmental constraints, and can be extended to multicell settings to represent metabolic coupling, competition, and nutrient partitioning between tumour cells and immune or stromal cells[85–87]. This provides a systems level explanation for metabolic phenotypes that cannot be assigned to single enzymes in isolation.

It is argued here that this systems biology should comprise the full system, even up to the level of society. This is ambitious and currently impossible. However, by considering separate processes of upward and downward causation and how they interact, it may be possible to get a better view on cancer development. In addition, the acknowledgement that the organization of the composing elements cause new, emergent, properties together with multiple realizability of properties is highly important. Together, this will lead to a better understanding and

treatment of cancer. Given the high costs of cancer therapy, it is essential to identify patients most likely to benefit and to avoid ineffective treatment. Systems biology provides predictive tools for patient selection and response prediction, supporting value-based care.

12. Conclusion

As discussed above, systems biology is a complex process of upward and downward operating processes. Therefore, when considering cancer, it requires a systems biology approach to unravel causation, in which many organizational levels can be distinguished. By studying just separate components, at its best a correlation can be established, but no causative relationship. Currently, in molecular research networks of interacting proteins and regulating RNAs are getting more and more attention, which is a very good development, but not sufficient yet as the context in which they operate is usually much broader. Developments in omics technologies and computer hard- and software, including artificial intelligence, will help to better appreciate cancer in a systems biology approach. As discussed, a systems biology approach requires a framework of upward and downward causation, in which emergent properties and multiple realizability are explicitly recognized across organizational levels. A genomic approach provides a concrete means to operationalize this framework by linking molecular variation and regulatory networks to higher-level phenotypes, while simultaneously revealing how environmental, physiological, and population-level constraints shape genomic organization and function.

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Abbreviations

The following abbreviations are used in this manuscript:

APC Adenomatous polyposis coli

CRC Colorectal cancer

CTC Circulating tumour cells

EMT Epithelial-mesenchymal transition

FAP Familial adenomatous polyposis

HLA Human leukocyte antigen

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