Chapter 13
Why Chemotherapy Does Not Work: Cancer Genome Evolution and the Illusion of Oncogene Addiction

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Lay Summary To shift from largely palliative chemotherapy to tumour control and cure, a tumour should be considered through the prism of evolutionary biology. In a tumour, the genome level alterations produce profound phenotype leaps and fast adaptations to micro-environmental stresses that are not achievable through simple gene mutations. Constant chromosome changes create striking intercellular genomic heterogeneity, drive dynamic expression changes of hundreds of genes, rewire metabolic and signalling pathways, and give rise to genetic and phenotypic diversification of tumour cells, which is a basis for cancer evolutionary selection. Overall, genomic heterogeneity can be used as a predictor of tumour evolutionary potential; treatment applied at different phases of genome evolution may have differential impact on tumour evolution and patient survival; efforts directed at pushing the genome of cancer cells towards a stable phase may lower the evolutionary potential of a tumour due to reducing population genetic/phenotypic diversity.

Vadym Kavsan—Deceased

I dedicate this chapter to the memory of my tutor, professor Vadym Kavsan, a prominent Ukrainian scientist (1939–2014). His patience, advice, guidance, and attention to detail were invaluable. His original ideas and striking personality will inspire me in future.

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13.1 Introduction

The long-held and dominating view that cancer is a genetic disease caused by the deterministic sequential mutations in a restricted number of cancer-driver genes (oncogenes and tumour suppressor genes), occurring in a continuous linear pattern of tumour progression (linear clonal model), and directly determining the hallmarks of cancer, has now been disproved. The cancer sequencing studies revealed a large number of stochastic gene mutations and multiple genetic subclones evolving in parallel (branching clonal model) (Fig. 13.1). The majority of somatic mutations are not detectable in all tumour regions; some genes undergo multiple distinct and spatially separated mutations within a single tumour, and diverse mutations emerge

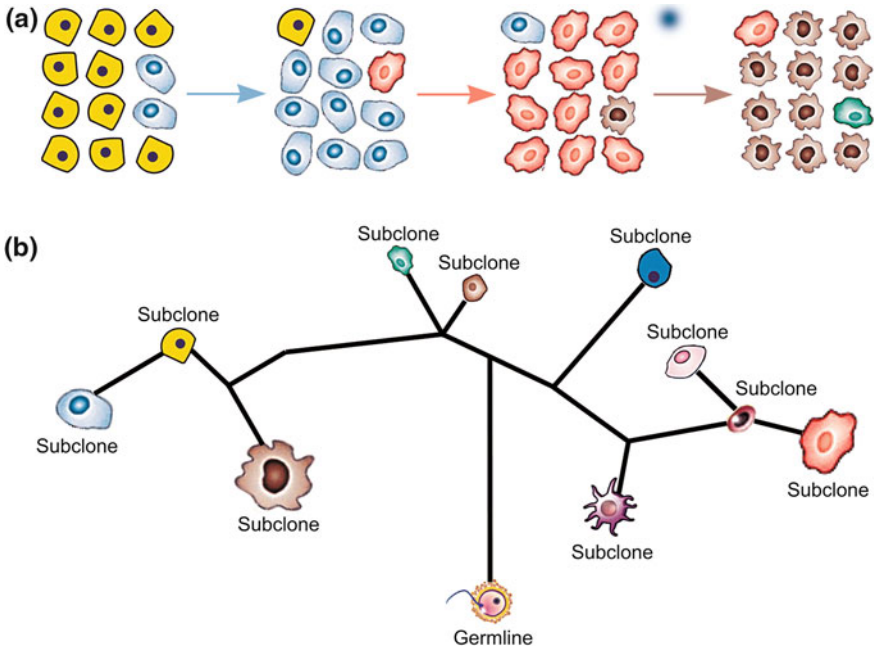

Fig. 13.1 Linear *versus* branching clonal evolution. **a** The linear clonal model of cancer evolution is based on assumption that tumour progression occurs in a continuous linear pattern due to sequential dominant clones (clonal sweep), which accumulate mutations in the key cancer "driver" genes in a deterministic stepwise manner and overwhelm earlier clones carrying only some of the mutations. **b** The branching structure of the phylogenetic tree demonstrates the number of simultaneous subclonal populations (represented by different coloured cell cartoons) within the cancer samples and their genetic relationships. The length of the trunk (represents the complement of mutations shared by all malignant cells within the cancer) and individual branches (represents the complement of mutations shared only by subclones on a branch) is proportional to the number of non-synonymous mutations separating the branching points. The size of a cell cartoon denotes the size/frequency of a subclone. Unique subclones emerge as a consequence of random genome alterations and gene mutations and may individually be capable of giving rise to disease relapse and metastasis. The dynamic clonal architecture is shaped by environmental selection pressures, including cancer treatments. The complexity of branching is underestimated on this figure for simplicity

and disappear during tumour progression to metastases and recurrences. There is a substantial evidence for mutation and amplification heterogeneity of the key cancer genes within individual tumour specimens and among multiple specimens from individual patients. The unprecedented level of inter- and intra-tumour heterogeneity is reflected in the statistics of the COSMIC database (http://www.sanger.ac.uk/genetics/CGP/cosmic/) [1]. The list of cancer genes and mutations within them is constantly growing in the Cancer Gene Census (http://cancer.sanger.ac.uk/census) and the Network of Cancer Genes databases (http://ncg.kcl.ac.uk/; http://bio.ieo.eu/ncg2/) [2]. Thereby, Heng [3] concludes:

> Now it is clear that cancer progression is a stochastic process both at the genome and gene levels, and is not a stepwise process defined by sequential genetic aberrations.

Similarly, Salk et al. [4] deduce:

> The large number and breadth of diversity in genes mutated among individual tumor specimens emphasize the fundamentally stochastic nature of cancer evolution.

Both cancer sequencing and genome instability studies strongly support the view that cancer evolution is not the sequential order of genetic alterations (specific cancer genes and common chromosome alterations) but, instead, represents multiple cycles of punctuated/discontinuous and gradual/step-wise evolution where stochastic (random, non-clonal) genome-level alterations are the primary and most important driving force of genetic heterogeneity and phenotype diversity. Todorovic-Rakovic [5] makes a conceptual deduction:

> oncogene mutation profiling now reveals all the complexity of cancer and provide the final explanation of the oncogenic pathways, based on stochastic (onco)genomic variation rather than (onco)genic concepts

and Brosnan and Iacobuzio-Donahue [6] emphasize:

> The evolution of cancer is not as straightforward as a stepwise series of mutations. As a result of genetic instability… cancers are often a heterogeneous mix of genomes.

Thereby, there is an appeal to shift "from the generally accepted view of cancer as a disease of a gene into that of a genome-based disease" [5], "from cancer gene-focused research to genome-focused research" [7], and from "analysing tumours not just as a ground-up bulk tissue, but as a population of individual tumour cells" [8].

13.2 Research Findings

13.2.1 The Genome Theory of Cancer: Building a Framework for Cancer Biology

There are two levels of genetic information organization: gene and genome. The gene mutation theory of cancer states that cancer somatic evolution is stepwise,

clonal occurring by progressively acquiring more and more genetic and epigenetic abnormalities, whereas the hallmarks of cancer (e.g. self-sufficiency in growth signals, replicative immortality, resistance to growth suppressors and apoptosis, sustained angiogenesis, invasion, and metastasis) are driven by the several key oncogenes and tumour suppressor genes. The advocates of this concept disregard genome instability and judge it largely as a by-product of tumourigenesis. They focus on the identification of the shared gene mutations in cancer cells and on the individual molecular pathways responsible for the initiation and progression of disease. In contrast, the genome theory of cancer is based on the concept that genome-level instability (structural and numerical chromosome aberrations) together with random gene mutations serves as a driving force of cancer somatic evolution by increasing the cell population diversity, which is the raw material for evolutionary selection (reviewed in [9–15]). Genome is not just total DNA sequence (all genes and regulatory elements). Instead, genome is a self-organizing three-dimensional chromatin structure that governs the physical relationships between thousands of genes and regulatory elements through both *cis* and *trans* mechanisms along and between chromosomes (genome topology). It means that the loss of a chromosome not only results in reduction of expression of majority of genes located on that chromosome by half, but also leads to the disruption of the regulatory interactions within the nucleus that were established by that chromosome. Similarly, the chromosome translocation may or may not affect the gene structure at the break point but it inevitably entails changes in the expression pattern of genes located not only on the translocated chromosomes but also on non-translocated chromosomes, by *in trans* mechanism. An altered distribution of chromatin (changes in genome topology) and an aberrant expression of transcription factors favour the deregulation of transcriptome and cellular functions. Genome-level alterations rewire protein interaction network, alter timing and amplitude of signalling response, and change the functions of signal transduction pathways by multiple mechanisms. Altogether, these modifications affect cellular growth, division, migration, death, and other cellular processes. Moreover, transcriptome and proteome including protein–protein interactome are under constant change in cells with unstable genome. Therefore, the genome-level alterations play a key role in cancer evolution [9–15]. Single-cell analysis studies confirmed biochemical individuality of each cancer cell (different types and strengths of protein–protein interactions, proliferation rate, variation in responsiveness to stimuli and drugs, and potential to invade) and showed that a cancer cell relies on a unique series of pathways that ensure survival upon drug treatment [16, 17].

Heng et al. [10] describe the evolutionary mechanism of cancer as encompassing three components: (1) diverse stresses induce genome instability (e.g. mutagenic and non-mutagenic chemical and physical carcinogens, viral/bacterial infection, inflammation, ageing), (2) genome instability produces genetic and epigenetic heterogeneity, which is the raw material for evolution, and (3) cancer somatic evolution, based largely on random genome alterations, results in overcoming the system constraints such as cell death, tissue architecture, or immune system surveillance (Fig. 13.2). There are two phases of somatic genome evolution. One is

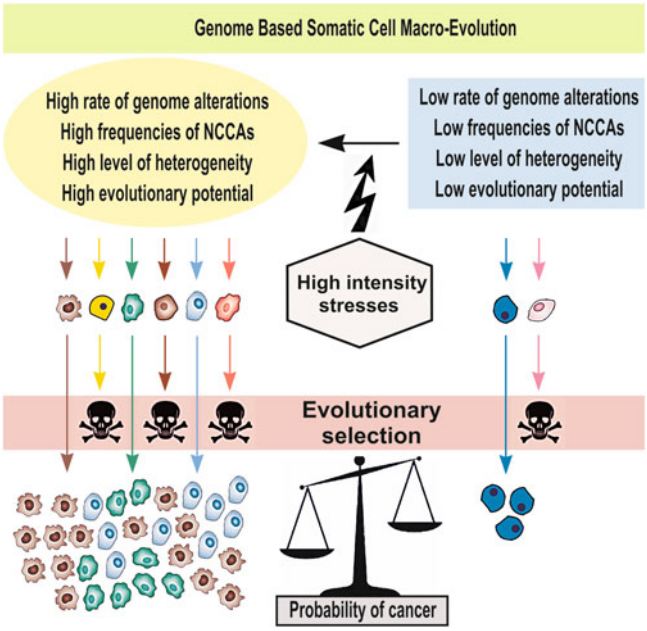

Fig. 13.2 Cancer somatic evolution in the light of the genome theory of cancer. Cancer evolution is based primary on genome-level alterations rather than gene mutations. A cell population without/low genome instability has a low diversity and, therefore, low evolutionary potential to overcome system constraints such as cell death, tissue architecture, or immune system surveillance. However, exposure of cells to high-level stresses (internal: e.g. oxidative stress, replication stress, endoplasmic reticulum stress, metabolic stress; external/environmental: e.g. drug treatment, radiation, viral/bacterial infection, metabolic, mechanical; experimental manipulations: e.g. gene overexpression, gene knock out/down, chemical inhibitors, culture conditions) promotes genome instability and results in an increase in population genetic, epigenetic and non-genetic heterogeneity and, therefore, high evolutionary potential and probability of cancer formation. Due to stress-induced genome chaos, many cells are not viable and undergo mitotic cell death or apoptosis. Eventually, more stable rare cancer-causing karyotypes are selected. NCCAs: non-clonal chromosome aberrations

the discontinuous phase characterized by heterogeneous karyotypes between cells and ongoing progressive karyotype changes; the other phase is the stepwise continuous phase within which the majority of cells share similar karyotypes for a long time. These two phases represent punctuated (or macro-) evolution and Darwinian (or micro-) evolution, respectively. Relationship between these two phases and genome system stability, measured by the level of stochastic genome alterations, showed that the punctuated phase is characterized by genome system instability (high frequencies of non-clonal chromosome aberrations (NCCAs), whereas the Darwinian stepwise phase demonstrates relative genome system stability (dominant clonal chromosome aberrations (CCAs) and low frequencies of NCCAs). Extremely high genome-level heterogeneity in the punctuated phase provides the genetic underpinning of the high degree of heterogeneity universally detected in

cancers. Follow-up experiments evidenced that all factors, genetic/non-genetic, internal/external, functioning as a stress to a given system, can contribute to cancer evolution, either through micro- or macroevolution [10, 17–22].

System stress (CIN-promoting factors such as drug treatment, radiation exposure, metabolic perturbations, oxidative stress, infections/inflammation or experimental manipulations) during the different phases may lead to very different responses [12]. During the stepwise phase, acute stress may increase instability, destabilize CCAs, and abolish growth advantage that those CCAs endowed. While stabilizing genome, selection and spread throughout the population of chromosome aberrations result eventually in the fixation of newly formed advantageous dominant CCAs able to continue tumour progression. During the punctuated phase, acute stress may result in such instability when most of unstable cells are not viable [12]. Actually, high-level aneuploidy has a negative impact on cellular fitness and generates non-neoplastic and nonviable cells [23, 24]. However, genome chaos significantly increases the population genome diversity and the evolutionary potential of tumour. These phases of cancer cell evolution may shed light on the tumour-promoting and suppressing effects of CIN in tumourigenesis as well as on the "paradoxical" relationship between excessive CIN and improved survival outcome in cancer [25, 26].

Karyotypic heterogeneity has been linked to tumourigenicity. All cell lines displaying high tumourigenicity were characterized by high levels of genome heterogeneity (the high frequencies of NCCAs), regardless of which molecular mechanisms were deregulated. In contrast, all cell lines with low tumourigenicity displayed distinctly lower frequencies of NCCAs.

Genomic instability drives resistance in two ways. First, it generates population heterogeneity which increases the probability to survive drug treatment. The more different combinations of molecular mechanisms exist within a cancer cell population, the more likely a population survives. Second, drug treatment-mediated stress may induce genome chaos, which is accompanied by large-scale genome changes and increased population heterogeneity. This favours the emergence of resistant cells. Altogether, cancer genome evolution is the key event in cancer initiation-progression and drug resistance (reviewed in [10–15, 20, 27], see also Chap. 12).

In building a framework for cancer biology, Nicholson and Duesberg [28] conceptualized:

> Neoplastic transformation occurs because carcinogens, including conventional mutagenic and nonmutagenic carcinogens, or activated oncogenes destabilize the karyotype by inducing random aneuploidy. Aneuploidy destabilizes the karyotype by unbalancing teams of proteins that segregate, synthesize and repair chromosomes - in proportion to the degree of aneuploidy. Aneuploidy initiates and maintains karyotypic evolutions automatically because of the inherent instability of aneuploidy… Occasionally, rare cancer-causing karyotypes evolve stochastically… Flexibility and heterogeneity of cancer karyotypes is also the basis for the further, spontaneous evolutions that are known as tumor progression, such as metastasis and drug resistance.

In support of this argument, induction of aneuploidy by carcinogens and activated oncogenes is well documented (reviewed in [29–34]).

Dr. Peter Duesberg and colleagues formulated questions of great experimental and clinic importance that the gene mutation theory of cancer founds difficult answering: why do transgenes produce conditionally reversible hyperplasias and dysplasias early *versus* irreversible cancers late in conditional transgenic mice models? Why do a single transgene or a group of the same transgenes induce diverse cancers with different karyotypes, phenotypes, and transcriptomes in mice models? Why do some cancers of transgenic mice continue growing despite loss of or failure to express transgenic oncogenes? What does maintain the transformed phenotype of transgene-negative cancers? Why is cancer caused by non-mutagenic carcinogens? Why do cancers develop years to decades after the initiation by carcinogens (long latent periods) and follow pre-neoplastic aneuploidy? Why is cancer chromosomally and phenotypically unstable and generates much more complex phenotypes than conventional mutation as well as non-selective phenotypes (i.e. phenotypes that are unnecessary for tumour formation at the site of its origin), such as metastasis or multidrug resistance? Another set of questions referred to (multi-)drug resistance, in particular: why are cancer cells intrinsically resistant or rapidly acquire resistance against numerous drugs, and sometimes loose drug resistance in the absence of drugs? How do cancer cells generate complex resistance phenotypes against dozens of drugs? Why are drug treatment response and the acquisition of drug resistance accompanied by alterations of the genome, DNA methylation, transcriptome/proteome, metabolome, morphology, etc.? These fundamental questions remain unanswered by the advocates of the gene mutation theory of cancer. By contrast, these issues are faithfully explained by the genome theory of cancer (reviewed in [28, 35–42]).

13.2.2 The Illusion of Oncogene Addiction: Why Cancer Models Do Not Recapitulate a Natural Tumour

The term “oncogene addiction” was introduced by Bernard Weinstein to describe the dependency of tumour cells on a single activated oncogenic protein or pathway to maintain their malignant properties [43]. The oncogene addiction concept is based on results derived from tumour culture cell lines and conditional transgenic animal models in which acute inactivation of the overexpressed wild-type or mutated oncogenes resulted in rapid apoptosis or growth arrest and consequent tumour regression. However, many research groups monitoring long-term tumour response in diverse mouse models after oncoprotein withdrawal repeatedly observed tumour relapses. Tumour escape from oncogene addiction upon the primary oncogene inactivation was attributed to the acquisition of the diverse novel genetic lesions through CIN (reviewed in [34]).

The advocates of the concept of oncogene addiction name chronic myeloid leukaemia (CML) and the drug imatinib as the most successful example of this concept application in clinic. However, imatinib is an exception in cancer research and its success does not carry over to most solid tumours (reviewed in [27]). Actually, CML patients in the chronic phase of disease (characterized by a relatively

stable genome and comparable to the benign phase of solid tumours), but not in the late phases (accelerated or blast crisis characterized by highly dynamic genome instability), perfectly respond to imatinib treatment. Imatinib was originally designed to target BCR-ABL fusion tyrosine kinase and a "magic bullet" effect of imatinib was ascribed due to the specific inhibition of BCR-ABL tumour "driver". However, it is now well documented that imatinib also inhibits the receptor tyrosine kinases and non-receptor tyrosine kinases, as well as RAF kinase family members, and the oxidoreductase NQO2. Thus, imatinib should be referred to as a multi-targeted cytotoxic drug (reviewed in Stepanenko and Dmitrenko, 2015). Moreover, recent studies revealed that imatinib fails to eradicate BCR-ABL-positive CML stem cells even at high concentrations. Authors concluded that "cancers are never truly oncogene addicted" [44].

The models, which afforded grounds for establishing the oncogene addiction concept, have obvious shortcomings and pitfalls. Cell lines display the markers of genetic drift and are characterized by low genomic heterogeneity as a consequence of selection and adaptation for cell culture conditions [45]. Furthermore, pure cultures of cells ignores the fact that tumours are surrounded by stromal cells that can provide nutrients and additional signals needed for cell growth [46].

In addition, other simplicities significantly reduce the generalization potential of *in vitro* tests: lack of cells of the immune system; lack of a complex of vessels supplying and removing fluids with many different parameters of flow, pressure, and nutrient levels; lack of spatial and temporal variations in external drivers of cancer evolution and growth; and lack of gradients in oxygen tension, CO_2 levels, applied drugs, etc. Gillet et al. [47] investigated the multidrug-resistant transcriptome of six cancer types in established cancer cell lines (grown in monolayer, 3D scaffold, or in xenograft) and clinical samples and revealed that:

> All of the cell lines, grown either *in vitro* or *in vivo*, bear more resemblance to each other, regardless of the tissue of origin, than to the clinical samples they are supposed to model.

Authors [47, 48] invoke investigators "to be aware of the associated caveats and temper their extrapolations so as not to infer direct applicability to clinical medicine". Furthermore, many anticancer agents have dose-limiting organ toxicities that are not represented in model systems such as cultured cancer cells [49].

Advantages and disadvantages of orthotropic xenografts of human tumours and genetically engineered mouse cancer models are thoroughly considered elsewhere (reviewed in [50–53]). Due to a limited number of initiating genetic alterations, transgenic mouse tumours are typically more homogeneous than human tumours [54]. In transgenic models, the initial conditions (such as the overexpression of specific oncogenes) form a dominant pathway through artificial selection that drastically reduces genome heterogeneity and artificially favours cancer progression [11].

Artificially activated oncogenes, benign levels of CIN, intra-tumour genetic homogeneity, and fostered evolution of tumour cells and their microenvironment make mouse tumour model inappropriate for the targeted treatment of human cancers. As Richmond and Su [53] sharply notice, "If one wants to know whether a patient's tumor will respond to a specific therapeutic regime, one must examine the

response of that human tumor, not a mouse tumor, to the therapy" and further "We can cure many mouse tumors, but there is not a direct correlation between response in the mouse and response in the clinic".

Actually, cancer therapy based on the oncogene addiction concept is palliative rather than curative. Targeted drugs are highly successful from a financial perspective but have little curative impact/beneficial effect on patient survival. The unprecedented level of intra-tumour heterogeneity, the existence of myriads of genetic networks, and the complex protective phenotype response to chronic drug treatment make solid tumours independent from any particular oncogene and clinical utility of many cancer genes as targets for cancer treatment uncertain and challengeable. A failure of targeted therapies in clinical trials and the limited relevance of the oncogene addiction concept for the majority of tumours should lead researchers to abandon the idea of seeking for and targeting the putative addictive oncogene that maintains one's cancer.

13.3 Implications for Policy and Practice

Firstly, genomic heterogeneity may be used as a predictor of evolutionary potential as heterogeneity provides a greater chance to adapt and survive. Indeed, the overall genomic heterogeneity significantly correlates with tumourigenic potential of cells, tumour disease progression, patient survival, intrinsic and acquired (multi)drug resistance, and radio resistance [55, 56].

Secondly, the phase of cancer evolution should be monitored. Treatment applied at the different phases of genome evolution may have differential impact on tumour aggressiveness and patient survival [12, 57].

Thirdly, we should not aim at killing tumour cells with the highest tolerable drug concentrations. High-dose chemotherapeutic-mediated stress may significantly increase tumour evolution by generating novel phenotypes through induction of genome chaos. Therapy-induced genome instability should be avoided; instead, efforts should be directed at pushing the genome of cancer cells towards a stable phase and supporting the immunological system as well as homeostasis of the individual. These should lower the evolutionary potential of a tumour and constrain its dynamics due to reducing population diversity [14]. By contrast, the therapeutic promotion of excessive instability of the genome in tumour cells is a double-edged sword: while the primary objective response in the form of reduced cell viability will be positive, the price for a moderate inhibition of tumour growth will be changes in the genomic landscape, tumour subclonal architecture, and, eventually, promotion of cancer evolution that impacts both the patient survival and the therapeutic management of recurrence [13, 57, 58].

Fourthly, we should stop cataloguing putative cancer genes and classifying them on tumour suppressor genes and oncogenes. Context-dependent antagonistic functional duality of cancer genes is widely documented (reviewed in [16, 59]). The genome changes rewire the genetic network and may result in alterations of the role and function of the same genes and pathways within different genetic background. Cells within the same tumour may differentially respond to a drug, succumbing to senescence and death or on the contrary demonstrating enhanced growth and invasion. These are the instructive examples of "paradoxical" effects of anticancer drugs depending on the cellular genetic background/signalling network [16].

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Glossary

Aneuploidy	Refers to a state of karyotype when whole chromosome(s) or parts of chromosome(s) are lost or supernumerary
Chromosome instability (CIN)	Is a high rate of genome changes of a given cell population (cell to cell variation). It implies a constant process of generation of numerical (loss or gain of whole chromosomes) and structural (loss of chromosome arm, translocations, amplifications, deletions, insertions) aneuploidy variants
Clonal chromosome aberration	Is an aberration found in two cells or more among at least 20 examined metaphases
Dysplasia	Is appearance of the tissue as disordered, with the increased number of immature cells, and great variability between cells
Evolutionary potential	Is a probability of cell population to persist, adapt, and survive the harsh microenvironment, intrinsic or extrinsic stresses
Genetic network of a cell	Includes the whole gene content, RNA, and protein expression and their interaction in space and time
Genetically engineered mouse cancer model	Is a model when a mouse genetic profile is altered such that one or several genes thought to be involved in tumourigenesis are mutated, deleted, or overexpressed

Hyperplasia	Is a condition when cell number increases due to hyperproliferation unbalanced by cell elimination that results to an increase in the amount/volume of a tissue/organ
Non-clonal chromosome aberration	Is an aberration found in only one cell among at least 20–50 examined metaphases
Orthotopic xenograft	Is the transplantation of a primary human tumour mass or the injection of human tumour cell line into a mouse tissue from which a tumour mass/cell line naturally originated
Transcriptome	Is the complete set of mRNA, rRNA, tRNA, and other non-coding RNA transcripts produced by the genome of a cell or a population of cells at any given time
Transgene	Is a foreign gene that has been deliberately transferred into genome of a cell/an organism by the genetic engineering techniques
Transgene-negative tumours	Are tumours formed by cells, which lost a transgene that endowed advantageous traits and accelerated propagation
Somatic evolution	Is the accumulation of heritable (through mitosis) variations such as mutations, epigenetic changes, and sporadic aneuploidy in somatic cells within a body during a lifetime
Stochastic nature of cancer evolution	Implies that cancer is mainly driven by random, non-clonal, and transitional genome alterations, and these dynamic genome changes are neither shared by cells of the same tumour nor by the different tumours

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