Current concepts in cancer research

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Abstract: Cancer research is an extremely broad topic covering many scientific disciplines including biology (e.g. biochemistry and signal transduction), chemistry (e.g. drug discover and development), physics (e.g. diagnostic devices) and even computer science (e.g. bioinformatics). Some would argue that cancer research will continue in much the same way as it is by adding further layers of complexity to the scientific knowledge that is already complex and almost beyond measure. But we anticipate that cancer research will undergo a dramatic paradigm shift due to the recent explosion of new discoveries in cancer biology. This review article focuses on the latest horizons in cancer research concerning cancer epigenetics, cancer stem cells, cancer immunology and cancer metabolism.

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Introduction

Cancer is a dreaded disease. It is estimated that at least 12 million people are diagnosed with cancer every year, and more than half of them will die because of this disease. This is equivalent to one person dying from cancer every five seconds daily. Among the different types of cancers, lung and breast cancers are by far the deadliest with more than 1 million deaths due to lung cancers and half a million deaths in women due to breast cancers annually. This is followed by stomach, liver and colon cancers which register more than half a million deaths every year.

Over the past 150 years, rapid advances in cancer research have generated a rich body of knowledge, revealing cancer to be a diverse and dynamic disease involving changes in the genome (Figure 1). The discovery of mutations that produce dominant gain-of-function oncogenes and recessive loss-of-function tumor suppressor genes has been demonstrated repeatedly in human cancers. The recent explosion of new discoveries of the diverse molecular and biological changes underlying cancer development and progression has also changed our understanding of the complex pathways that regulate cancer cell survival, the interactions of tumours with their microenvironment, and the mechanisms that normally restrain tumourigenesis. Importantly, these insights are transforming cancer diagnosis, prognosis and therapies in every facet of clinical practice.

This review aims to provide a comprehensive overview of important advances and trends in cancer research in recent years and focuses on the emerging concepts in cancer stem cell biology, cancer epigenetics, cancer cell metabolism and cancer immunology.

Hallmarks of cancer

Cancer cells are recognised to have dysregulation in the cell and molecular circuits that govern normal cell survival, proliferation and homeostasis. There are more than 100 distinct types of cancer, and different subtypes of cancers can be found within specific organs in the same patient. In other words, cancer is highly heterogenous. This complexity provokes a number of fundamental questions: How many distinct regulatory pathways must be disrupted within a normal cell to become cancerous? Does the disruption of the same set of regulatory pathways give rise to different neoplasms in the human body? Which of these pathways operate on a cell-autonomous basis, and which are driven by surrounding microenvironment within a tissue? Can the large and diverse collection of cancer associated genes be linked to the specific regulatory pathway that could be targeted for therapy?

To answer these questions, Hanahan and Weinberg proposed eight hallmarks of cancer that must be acquired during the multistep development of human tumours and collectively dictate malignant growth. They include self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of programmed cell death,

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limitless replicative potential, sustained angiogenesis, tissue invasion and metastasis, deregulation of cellular energetics and evasion of immune destruction (Figure 2).[1,2] Underlying these hallmarks are two recently recognised enabling characteristics of cancer cells - genome instability and inflammation.

Genomic instability is the most prominent enabling characteristic in cancer cells that confers selective advantage for their survival, growth and eventually, domination in a local tissue environment. As such, multistep tumor progression can be portrayed as a process of clonal expansions triggered by the acquisition of successive enabling mutations. Because heritable phenotypes can also be acquired through epigenetic mechanisms such as DNA methylation, histone modifications and microRNA expression, tumourigenesis may also be triggered by non-mutational changes affecting the regulation of gene expression.[3-5]

The second enabling characteristic of cancer involves the immune system which drives the inflammatory state of the premalignant and malignant lesions to promote tumor progression. The presence of immune cells and inflammation is common in most neoplastic lesions.[6] Originally, it was thought that the immune responses were triggered as an attempt by the immune system to eradicate tumours. However, recent studies on the inflammatory cancer pathogenesis revealed an unanticipated, paradoxical effect of immune cells to enhance tumourigenesis and progression.[7-10] Indeed, numerous evidences have demonstrated that inflammation plays an important role in tumour progression by supplying bioactive molecules such as growth factors that sustain cell proliferation, survival factors that inhibit cell death, proangiogenic factors that facilitate angiogenesis, extracellular matrix-modifying enzymes that promote invasion and metastasis, inductive signals that activate epithelial-mesenchymal transition (EMT) and many other programmes that promote tumourigenesis.[7-9,11] In addition, inflammatory cells can also release chemicals (e.g. reactive oxygen species) and cause mutation at the nearby cancer cells, accelerating their genetic instability toward malignancy.[8] As such, inflammation is considered an enabling factor that contributes directly to the acquisition of many hallmarks of cancer.

Cancer Epigenetics

Normal development usually takes place through a unidirectional process characterised by step-wise decrease in developmental potential, from the stem cells state which differentiates into specialised cell types. Once the cells are differentiated, sequential activation and silencing of specific genetic programmes in a cell-type-specific manner must be maintained even after the inductive differentiation signals have disappeared so that the cells will maintain their fate. This genetic programme must be maintained throughout the life of the individual in normal development, and epigenetic mechanisms, which are defined as heritable patterns of altered gene expression that are mediated by mechanisms that do not affect the primary DNA sequence, are ideal for regulating such events.[4,12-16]

Classically, cancer was thought to be solely a consequence of genetic changes in key tumour-suppressor genes and oncogenes that transform normal cells into malignant cells.[2,4,17] However, recent studies have shown that human cancer cells harbour global epigenetic alterations such as DNA methylation, histone modification and micro-RNA expression.[4,5,15,18,19] Although the molecular mechanisms that regulate the cancer epigenome are only beginning to be elucidated, the best understood component is the transcriptional repression of a growing list of tumour suppressor genes. This suppression is commonly associated with hypermethylation of DNA at specific CpG islands around the promoter regions of tumour-suppressor genes.[12,14-16] By this epigenetic silencing mechanism, the expression of tumour-suppressor genes in the cancer cells can be reduced or eliminated as an alternative mechanism to genetic mutation.[12, 14-16]

Numerous studies have demonstrated that the abnormal epigenetic events can occur at any time during tumour progression, but it occurs most frequently during the early stages of tumourigenesis.[13,16-18,20,21] Similarly, as the cancer cells undergo metastasis, it is also likely that epigenetic mechanisms are activated to allow critical and transient changes in gene expression patterns for cancer cells to disseminate from the primary tumours, invade local tissues, survive in the blood stream and metastasise to distant sites. One gene that may promote survival in one physiological condition (e.g. primary tumour site) may be deleterious in another physiological condition (e.g. metastatic colony site). As such, altering gene expression patterns epigenetically could account for transient silencing of a specific set of gene expression under one condition yet allow for the re-expression of these genes should its function provide a selective advantage later in cancer progression.[22]

The central conundrum in epigenetics remains unsolved: what causes the epigenetic changes in cancerous cells? One hypothesis suggests that aberrant epigenetic changes in cancers could be caused by repeated exposures to 'epimutagens', agents that cause epigenetical changes without causing genetic mutation.[23] Indeed, there is mounting evidence for such an environmental influence on epigenetics in both normal tissues and cancers.[23,24] For example, diets that are deficient in folate and methionine lead to DNA hypomethylation [25,26]; exposure to heavy metals, such as arsenic[27], cadmium[28], lead[29], nickel[30,31], and chromium[32], is linked to changes in the expression of DNA-methylation enzymes, histone acetyltransferase (HAT), and histone deacetylase (HDAC); and *Helicobacter pylori* infection has been shown to alter DNA methylation patterns and contribute to gastric cancers.[33] It is also worth noting that chronic inflammation is a universal accelerator of DNA methylation, as indicated by studies in preneoplastic colon[34], esophagus[35], liver[36] and lung.[37] Tumours that arise in the setting of chronic inflammation in the colon (e.g. ulcerative colitis) are more likely to be hypermethylated[38]; again linking lifestyle and exposures to the phenotype.[34,38] All these point to the importance of environmental factors on the regulation of epigenetic mechanism.

As of the current state, the carcinogenic potential of various exposures is identified mainly through mutagenicity tests. If the alteration of cancer epigenome can be traced unequivocally to environmental exposures, a careful evaluation of the epimutagen concept will become eminent as the current mutagenicity tests might underestimate the carcinogenic potential of exposures that lead to cancer primarily through epigenetic lesions.[39] This issue will have a substantial public health impact.

Unlike genetic mutations, epigenetic aberrations are potentially reversible and can be restored to their normal state which makes them promising therapeutic targets for cancer treatment.[19,40] To date, the most intensively investigated drugs are DNA methylation and HDAC inhibitors. DNA methylation inhibitors, 5-azacytidine (Vidaza™) and 5-aza-2'-deoxycytidine (Decitabine™), are nucleoside analogues that incorporate into replicating DNA and prevent methylation. Both drugs are FDA approved for treatment of myelodysplastic syndromes, acute myeloid leukemia (AML) and chronic myeloid leukemia (CML).[19,41] Zebularine is an improved orally administered DNA methylation inhibitor while Zebularine is currently undergoing intensive investigations in preclinical and clinical trials.[19,42,43]

A growing number of HDAC inhibitors such as suberoylanilide hydroxamic acid (SAHA), depsipeptide and phenylbutyrate are currently under clinical trials.[19,44] SAHA, in particular, has now been approved for use in the treatment of T cell cutaneous lymphoma.[43,45] The antiproliferative effects of HDAC inhibitors are mainly mediated through re-establishing the normal histone acetylation patterns of the genome and hence reactivate silenced tumour suppressor genes to induce growth arrest, apoptosis and differentiation.[46] However, the currently available HDAC inhibitors still show a very broad spectrum of activity which might affect both the normal as well as the tumour cells. Therefore,

it is worthwhile to develop new and better reagents that target individual HDACs and thus improve the specificity of the treatment.[43,45]

Finally, micronutrients such as flavonols, isoflavones and catechins have been shown to regulate chromatin-modifying enzymes activities.[47] For example, (-)-epigallocatechin 3-gallate (EGCG) from green tea has been shown to inhibit DNA methyl transferases (DNMTs), a group of enzymes that primarily regulate DNA methylation and reactivate tumour suppressor genes in cultured human cancer cell lines.[48-50] Similarly, genistein which is isolated from soybean has also been shown to modulate DNMTs and HDACs, and reactivates tumour suppressor genes in prostate[51], esophageal[52], and renal[53] cancer cells. Polyphenols, caffeic acid, and chlorogenic acid which can be obtained from coffee have been shown to inactivate DNMT1 and cause demethylation of retinoic acid receptor β (RARβ) in human breast cancer cells.[54] Therefore, it is possible that bioactive food components can influence DNA methylation pattern and, in turn, regulate gene expression and prevent cancer development.[39,47,55]

Overall, the study of interactions between environmental factors, dietary patterns, nutrients and genetics is a new and important area of cancer research.

Cancer Stem Cells

The concept of somatic stem cells is not new and has been described as early as in the 18th century. The observations that lower organisms can regenerate multiple tissues and organs suggest that cells or subset of cells possess regenerative potential. We now know that the regenerative potential of certain mammalian tissues is mediated by stem cells that are present in those tissues.[56] Stem cells are essential for tissue regeneration and are physiologically regulated to generate either one or both undifferentiated daughter cells through self-renewal division, or to generate specialised cells with limited proliferative ability through differentiation division. Self-renewal divisions are required for regeneration, while a balance between self-renewal and differentiation divisions is required for homeostasis.[57]

According to the American Association for Cancer Research (AACR), a cancer stem cell (CSC) is defined as a cell within a tumour that possesses the capacity for self-renewal and to generate the heterogeneous lineages of cancer cells that comprise the tumour.[58] As such, CSCs can be most rigorously and specifically defined by their ability to produce a continuously growing tumour consisting of cells that resemble those in the original tumour. Experimentally, this can be done by determining the frequency of CSCs in the initial tumour-derived cell suspension by limiting-dilution transplants or other clonal tracking strategies. The tumours that form in primary hosts are again tested for their content of cells with CSC activity through injection into secondary hosts to formally confirm that the initial CSCs have self-replicating capability.[57] Because of the nature of the techniques used to define CSCs, different researchers have used terms such as functional tumour stem cells, tumour-rescuing units, tumour- or cancer-initiating cells (TIC), cancer stem-like cells or cancer stem cells to described the stem-like properties of the putative cancer stem cells.[59-61] The broad concept of CSCs, however, should not be confused by the narrower concept 'can¬cerous stem cells' (nor¬mal stem cells becoming cancerous) as CSC do not necessarily originate from the transformation of normal tissue stem cells.[61-63] Indeed, several lines of evidence indicated that CSCs can also arise from mutated progenitor cells (also known as "transit-amplifying cells") that possess substantial replicative ability, but lack of the self-renewal capacity of stem cells.[64-67] Such progenitor cells must acquire mutations or epigenetic changes to regain the property of self-renewal in order to become cancer stem cells.[68]

Currently, there are two models to explain the origin of cancer cells and tumour heterogeneity (Figure 2). The hierarchical model assumes that tumours are originated from cancer stem cells that give rise to progeny with self-limited proliferative capacity. The

model also suggests that most of the cells in the tumour are genetically homogeneous and do not have tumour-initiating activity. Evidence that support this model comes from the fact that a stable malignant phenotype requires accumulation of a series of rare mutations over several generations of a cell. This makes it unlikely that a cell will accumulate enough of such lesions within the number of cell divisions that are required to be fully differentiated. In contrast, CSCs constitute a reservoir of cells that can undergo self-renewal for many generations. This makes CSCs an obvious candidate for accruing the mutations that are required to generate a fully malignant cell population. The clinical implication from this model is that the elimination of all CSCs is expected to inevitably terminate the tumor growth, and that failure to do so may cause relapse. This is the basis of the CSCs theory.

The second model, the stochastic model (or clonal evolution model), postulates that tumourigenesis is a multistep process that leads to progressive genetic alterations and in turn drives the transformation of normal cells into highly malignant phenotypes.[69] Evidences that support this model include the demonstration of clonal selection of variant cells that show increasing aggressiveness and genetic instability during tumour progression.[2,70-72] Unlike the hierarchical model which assumes tumours as genetically homogeneous entities that have arisen from CSCs, the stochastic model assumes that every cell within a tumour has the similar tumourigenic capacity, i.e. every cell can act as a CSC.

At first glance, it seems that the two models are contradictory. However, it is important to note that the CSC and clonal evolution concepts need not to be mutually exclusive. Indeed, two recent studies have highlighted a high degree of convergence between the two models in leukemia. Leukemia stem cells (LSCs) in AML harbouring the ETV6-Runx1 translocation were shown to be exhibiting different degrees of self-renewing activity *in vivo* as postulated in the CSCs model, and also genetically diverse, supporting the clonal evolution model.[73] In BCR-ABL acute lymphoblastic leukemia (ALL) patients, the LSCs population also displayed profound genetic diversity, with multiple genetically distinct tumour-initiating subclones observed at diagnosis.[74] Together, these studies suggest that CSCs within individual cancer patients can be genetically heterogeneous (as opposed to the hierarchical model which assumes that cancer arises from CSCs and most of the cells in the tumour are genetically homogeneous) and that cellular interconversion of non-tumourigenic cell to reacquire stem cell-like properties and vice versa in a stochastic manner is plausible. Based on these observations, an emerging consensus in the CSCs field is that "cellular state" rather than phenotype is, perhaps, more important when defining a CSC.

Although the CSC concept is interesting from the scientific point of view, its clinical applicability to predict patient response remains a fundamental question. To date, most of the putative anti-CSC therapies have attenuated tumour growth rather than eradicated tumours in preclinical models. To achieve efficacious response, anti-CSC therapies often require concomitant chemotherapy.[62] The standard clinical trials design that use tumour size as tumour response criteria might not be relevant in anti-CSC therapy trials as measurements of tumour size largely reflects tumour response in the non-CSC tumour bulk. Specific response criteria that will provide a readout of response to anti-CSC agents in clinical trials is a pressing need. Tumour sphere-forming assays and measurement of CSC marker expression might provide some clues on the response but are unlikely to be robust surrogate markers in a clinical setting as these premises are highly dynamic and dependent on the CSC microenvironment.[75-78] The measurement of critical properties of CSCs such as self-renewal activity using limiting-dilution transplants or other strategies will almost certainly be required. We speculate that for most tumour types it will still prove necessary to test novel anti-CSC therapies in combination with tumour debulking (non-CSC) therapy, such as conventional chemotherapy.

Cancer Immunology and Immunotherapy using dendritic cells

One of the natural roles of the immune system is to detect and destroy cancer cells.[79,80] Many cancers fail to activate host immune response as most of the cancer cells have the ability to evade recognition by the lymphocytes. The lymphocytes usually does not make immune response to "self" proteins or cells as these cells are carefully selected during their development based on their inability to mount immune responses to host antigens.[81] So, it will be rather difficult to activate host immune system against tumours. The main goal in cancer therapy is to remove and destroy "*all*" malignant cells without harming the patient. Surgical interventions, radiotherapy and chemotherapy can help to reduce tumour load. However, these approaches generally cannot remove and destroy all the tumour cells, which can reinitiate the onset of tumour and metastases. Activating the host immune response against the tumor might be a good way of preventing the recurrence of tumour once the initial mass is removed so that there would be continuous surveillance that can prevent the residual tumour cells from regaining their aggressive growth and spread.

In the past decade, numerous studies have shown that the host immune system is capable of recognizing and destroying tumour cells.[80,81] Several cellular immunotherapy studies have shown that it is possible to stimulate anti-tumour activity in the patient either through the use of dendritic cell (DC) vaccines, autologous and/or allogeneic lymphocytes.[80-83] The aim of the DC vaccine strategy is to harness potent immunological weapons to destroy cancer cells, which can help in the development of promising new strategies against cancer.

Dendritic cells (DCs) were first described by Steinman and Cohn in 1973.[84] These cells are reported to be the most powerful professional antigen presenting cells (APC) that can activate both arms of the immune responses.[85] A number of studies have shown that tumour antigen-loaded DCs (DC vaccines) can be used to treat some forms of cancer such as melanoma[86-89], non-Hodgkin's lymphoma[90,91], leukemia[92], non-small-cell lung cancer[93], prostate[94] and colorectal cancer.[95] In addition, there are several animal studies that show that repetitive vaccinations with DC vaccines can break pre-existing tolerance and achieve clinically relevant anti-tumour immune responses.[96-98] The use of this approach to treat other solid tumours such as breast cancer and colon cancer are still in the early stages of clinical application. These observations suggest that DCs can be developed as a suitable candidate for cancer immunotherapy.

Cancer as a metabolic disorder

Cancer, often characterised by abnormal cell growth, is a disease involving a web of biological interplay at different levels of cellular complexity. Traditionally, it was often believed that genetic instability is the main requirement for the cell to display hallmarks of cancer.[2] However, genes alone cannot account for the various types of mutations and pre-malignancy development. Otto Warburg, in the 1930s, proposed that perturbation in cellular energy metabolism, specifically the tendency for cancerous cells to undergo aerobic glycolysis, is the fundamental problem in cancer.[99] It is important to note that although both aerobic and anaerobic glycolysis produce lactic acid, aerobic glycolysis can arise in majority of cancer cells whereas anaerobic glycolysis only arises in the absence of oxygen.[100] Whereas oxidative phosphorylation would have yielded a larger amount of ATP per molecule of glucose, glycolysis offers the fastest mode for adenosine triphosphate (ATP) generation when energy demand is high. This is not surprising as cancer cell growth, like any rapidly proliferating cells, requires a large amount of ATP.[101] Such metabolic phenomenon in cancer is synonymously known as the Warburg effect.

The role of metabolic reprogramming in cancer development and progression is increasingly been looked at as one of the more important factors in cancer.

This is due to the fact that many signaling pathways are affected by genetic mutations and the need for sustainable energy supply to fuel proliferating cells.[2,102-104] p53 tumor suppressor protein, for example, not only plays an important role in DNA damage and apoptosis but also in the regulation of cellular metabolism.[105,106] p53 is known to promote oxidative phosphorylation and inhibit glycolysis as well as promote mitochondrial respiration. A loss of p53 protein function in cancer cell may be one of the main drivers in metabolic reprogramming towards the glycolytic pathway and accumulation of reactive oxygen species. In addition, cancer cells are able to upregulate glucose transporters thereby increasing glucose uptake and utilization.[104] Activation of oncogenes such as myc and ras, have been shown to be associated with increased glycolysis.[104,107] Furthermore, ras oncoprotein can increase levels of transcription factors HIF1α and HIF2α, which are involved in cellular gene expression changes at low oxygen conditions leading to upregulated glycolysis.[102] The switch to glycolytic pathway in cancer cells was suggested by Potter in 1958, later by Vander Heiden *et al* (2009), as a way in which cancer cells proliferate by increasing biosynthesis of macromolecules and organelles through diversion of glycolytic intermediates into various nucleosides and amino acids-generating pathways.[108,109] Evidence that certain tumour cell types exhibit symbiotic behavior are indicative that these cells are capable of proliferating regardless of the energy source that is available in their environment, be it glucose or lactic acid.[110] As such, the ability of cancer cells to reprogramme cellular energy metabolism is regarded as one of the emerging hallmarks of cancer.[2]

Concluding Remarks

Cancer research is an extremely broad topic. Like other scientific disciplines, key discoveries most often involve the work of many investigators, fusion of ideas, models, experimental evidence and acceptance of theories based on a body of work. This article is by no means intended to cover the broad spectrum of cancer research and discoveries, but instead aims to highlight the main and recent concepts in order to convey a perspective on how cancer research will move in the next decade. Some would argue that the search for the origin, diagnosis and treatment of cancers will continue in much the same way as it is by adding further layers of complexity to the scientific knowledge that is already complex and almost beyond measure. But we anticipate that cancer research will undergo a dramatic paradigm shift than the type of science we have experienced over the past 25 years. Much of these changes will be apparent at the technical level, but ultimately, the more fundamental changes will be conceptual and translational.

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Table 1: Mechanisms contributing to the hallmarks of cancer.

Hallmarks	Example of Mechanism[1]
Self-sufficiency in growth signals	• Aberrant secretion of mitogenic growth factors (e.g. EGF, PDGF, TGFα) • Receptor overexpression (e.g. EGFR, HER2) • Receptor mutation lead to ligand-independent hyperactivation (e.g. RAS, PI3K)
Insensitivity to growth-inhibitory signals	• Disruption of the pRb pathway • Avoiding differentiation through c-myc mutation • Activation of the APC/β-catenin pathway that block differentiation
Evasion of programmed cell death	• Overexpression of pro-survival BCL2 • Inactivation of p53
Limitless replicative potential	• Activation of telomerase • Maintenance of telomere length
Sustained angiogenesis	• Overexpression of VEGF and/or FGFs • Downregulation of thrombospondin-1 or b-interferon
Tissue invasion and metastasis	• Changes in expression of CAMs • Inactivation or E-cadherins • Expression of MMP • Activation of EMT
Deregulation of cellular energetics	• Reprogramme energy metabolism to "aerobic glycolysis" • Upregulating GLUT1 glucose transporters • Gain-of-function mutations of isocitrate dehydrogenase 1/2 (IDH) enzymes • Upregulation of HIF1
Evasion of immune destruction	• Paralyze infiltrating CTLs and NK cells, by secreting TGF-β • Recruitment of inflammatory cells that are actively immunosuppressive (e.g. Tregs, MDSCs)
Tumor-promoting inflammation	• Supplying bioactive molecules to the tumor microenvironment (e.g. growth factors, angiogenic factors) • Release mutagenic chemicals (e.g. ROS)
Genome instability and mutation	• Inactivation of DNA repair mechanisms • Inactivation of DNA damage response mechanisms

[1]*CAM, cell–cell adhesion molecule; MMP, matrix metalloproteinase; EMT, epithelial-mesenchymal transition; ROS, reactive oxygen species; Tregs, regulatory T cells; MDSC, myeloid-derived suppressor cells*

Figure 1: Milestone in cancer research.

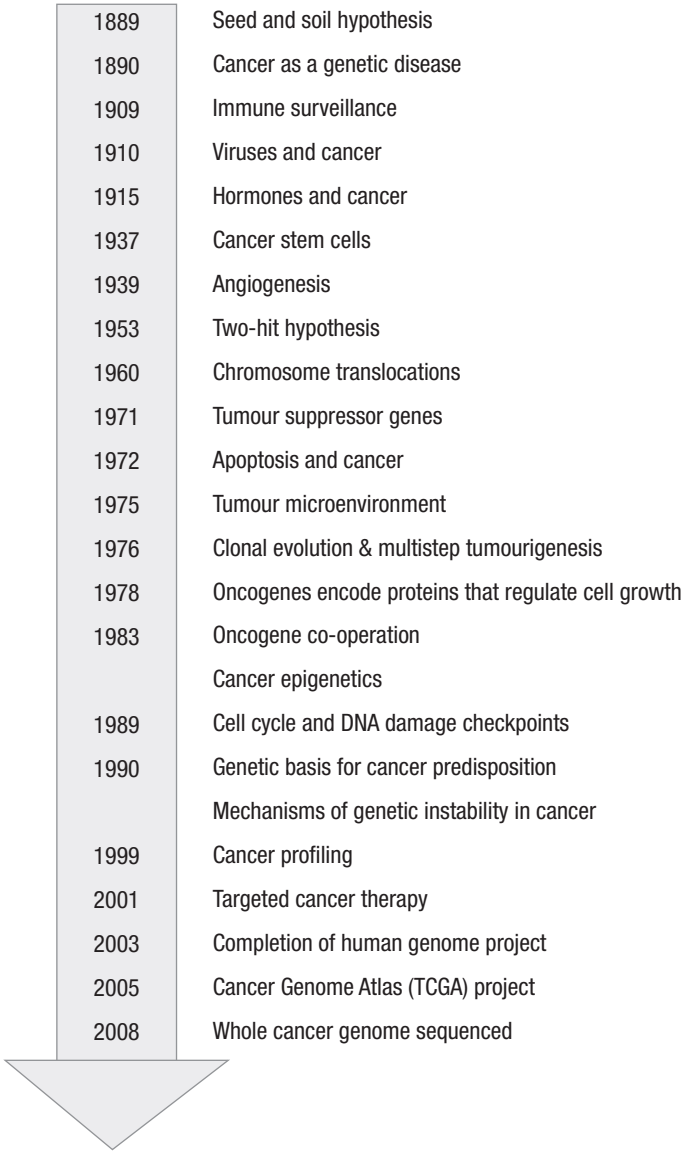
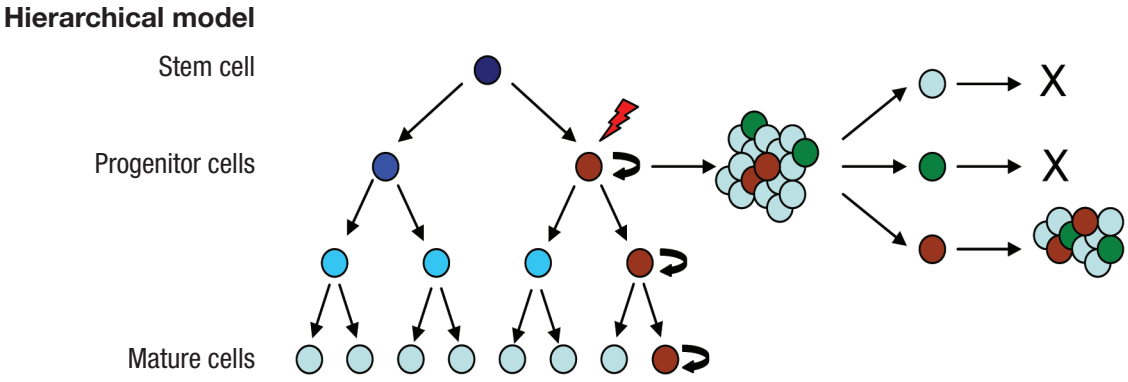


Figure 2: Two model for tumor heterogeneity.

(A) The hierarchical model (or cancer stem cells model) assumes that tumours are originated from a small subset of cell that gained the ability to sustain tumourigenesis and generate heterogeneity through differentiation. For example, when a mutated progenitor cell (brown) obtains stem cell-like properties and become cancerous, this cell will undergo self-renewal and give rise to a range of different tumour cells (light blue and green), thereby leading to tumour heterogeneity.



(B) The stochastic model (or clonal evolution model) assumes that tumour originates from cells through accumulation of mutation that lead to a selective growth advantage. For example, a normal cell might acquire a series of mutations (red) and produce a dominant clone with growth advantage. This clone will then give rise to tumour cells (red and orange) that share similar tumourigenicity. Other cells (light blue) may become non-tumourigenic due to stochastic events. Collectively, this diverse cell population will eventually make up the whole tumour and causes tumour heterogeneity.