Cancer

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Advanced article

Article Contents

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Cancer develops through a process of somatic evolution underpinned by genetic alterations in genomic DNA and epigenetic mechanisms that alter gene expression. Early neoplastic development that typically occurs well before tumour diagnosis is characterized by changes in a relatively small set of driver genes that accumulate in a linear fashion generating little intratumoural genetic heterogeneity (IGH). Thereafter, a process of branched evolution occurs generating cell populations containing subclonal (private) mutations; this increased IGH impedes successful therapy. Founder cells of cancer are most likely normal stem cells, and cancers themselves may have stem cells that could be key cellular targets for tumour eradication. The tumour microenvironment is a significant player in tumour behaviour, but tumour metastasis signals a poor prognosis. Improvements in treatment are underway, chemotherapy with cytotoxic drugs and associated unwanted side-effects often being replaced by immunotherapies such as cancer vaccines or strategies that harness the power of T cells.

Overview

Cancer is a complex disease resulting from genetic defects that are caused primarily by environmental factors. The cancer-causing agents (carcinogens) can be present in food and water, in the air, and in chemicals and sunlight that people are exposed to. Since epithelial cells cover the skin, line the respiratory and alimentary tracts, and metabolize ingested carcinogens, it is not surprising that over 90% of cancers occur in epithelia.

The causes of serious ill-health in the world are changing. Infection as a major cause is giving way to non-communicable diseases such as cardiovascular disease and cancer. Cancer is the second leading cause of death globally and in 2018 there were 9.6 million deaths attributed to cancer. The WHO's target of a 25% reduction in cancer deaths amongst the 30–69 age group by 2025 will require not only better healthcare systems for treatment, but also much -improved prevention strategies. Part of the reason for the increased incidence of cancer is that life expectancy is steadily rising and most cancers are more common in an ageing population. More significantly, a globalization of unhealthy lifestyles, particularly cigarette smoking and the adoption of many features of the modern Western diet (high fat, low fibre content) will increase cancer incidence.

Tobacco use and diet each account for about 30% of new cancer cases, with infection associated with a further 15%; thus, much of cancer is preventable. No individual can guarantee not to contract the disease, but since it is so strongly linked to diet and lifestyle there are plenty of positive steps that can be taken to reduce the chances: eat more fruit and vegetables, reduce the intake of red meat and definitely do not smoke. Carcinogens interact with the individual's constitution, both inherited and acquired, determining vulnerability to cancer induction. This vulnerability is based on how an individual deals with the carcinogens, ideally eliminating them in a harmless form before they do any genetic damage and/or being able to repair that damage.

Epidemiology has identified populations at high cancer risk, for example, users of tobacco products. However, many lifelong smokers do not get cancer, perhaps because of the way they handle potential carcinogens metabolically, and molecular epidemiology explores the contribution of potential genetic and environmental risk factors, identified at the molecular level, to the incidence of disease. Many issues concerning diet and cancer are controversial (e.g. fat intake and breast cancer). This may be because only certain polyunsaturated fatty acids generate damaging free radicals; furthermore, the intake level of antioxidant vitamins that can scavenge these harmful radicals is a confounding factor. Reducing infection, particularly in the poorer countries, will undoubtedly lead to reductions in cancer incidence. Infectious agents and the chronic inflammation associated with them account for 20–25% of all cancers, including hepatitis B and C virus (primary liver tumours), certain subtypes of human papillomavirus (cervix), the bacterium *Helicobacter pylori* in the stomach, Epstein-Barr virus (EBV) for certain lymphomas and nasopharyngeal carcinoma and human immunodeficiency virus (HIV) for cancers in many sites. Other factors with a

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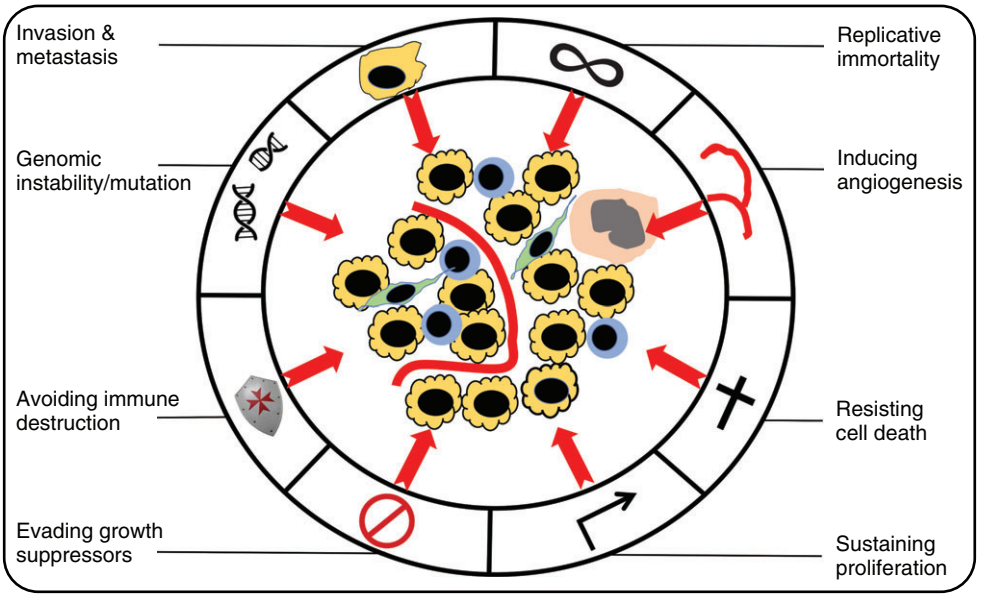

Figure 1 The defining characteristics ('hallmarks') of cancer as envisaged by Hanahan and Weinberg (2011) with modification. These are discussed in the text. The tumour microenvironment (TME) is an enabling factor that promotes many of these characteristics and is primarily composed of tumour-associated macrophages (TAMs), cancer-associated fibroblasts (CAFs) and tumour-infiltrating lymphocytes (TILs). Modified from Hanahan D, Weinberg RA. (2011) Hallmarks of cancer: the next generation. Cell 144(5): 646–674.

bearing on cancer are less obvious, for example the development of colorectal cancer (CRC), the second most common cancer globally, appears to be influenced by the gut microbiome (Allen and Sears, 2019). Gut microbes can cause deoxyribonucleic acid (DNA) damage and affect the epigenome through the likes of DNA methylation, chromatin structure and non-coding ribonucleic acid (RNA), while bacterial fermentation of dietary fibre to short-chain fatty acids such as butyrate may actually promote epithelial cell proliferation and hence tumour development! **See also**: ***Hepatitis B Virus***; **Hepatitis C virus**; **Hepatitis Viruses**; ***Epstein–Barr Virus***; **Human Immunodeficiency Virus (HIV)**; **Papillomaviruses**

The management of patients with cancer is costly, but there is the daunting prospect that 70% of tomorrow's patients are likely to live in countries that between them have only 5% of global resources. Huge steps in improving the prognosis of patients with cancer are almost immediately achievable with present-day technology and sufficient financial resource, and all essentially relate to early detection. Cancer does not develop overnight, instead often evolving over many years with detectable premalignant lesions presaging the development of full-blown malignancy. Malignant tumours not only invade surrounding tissue but are also able to colonize other, often vital organs, a process known as metastasis. Widespread metastatic disease is usually a harbinger of imminent patient death. Thus, immediate referral to the oncologist after detection of any suspicious lump or symptom is paramount; in many parts of the world with poor health education patients present with very advanced disease. In the same vein, cancer screening programs are designed to detect not only early asymptomatic malignant tumours but also premalignant lesions. Even in the richer countries, such programs are a significant financial burden, and the more cost-effective ones target the higher-risk groups, particularly by age.

Over the last twenty years, Hanahan and Weinberg have highlighted the biological characteristics (hallmarks) that typify neoplastic growth (Hanahan and Weinberg, 2011), indicating areas where therapies might be directed (**Figure 1**), and these are all discussed in this article.

Classification and Terminology

In terms of behaviour, tumours are either 'benign' or 'malignant'. Benign tumours are generally slow-growing expansive masses that compress rather than invade surrounding tissue. As such, they generally pose little threat, except when growing in a confined space like the skull, and can usually be readily excised. However, many so-called benign tumours have malignant potential, notably those occurring in the large intestine, and these should be removed before malignancy develops.

Malignant tumours are usually rapidly growing, invading surrounding tissue and, most significantly, metastasizing to distant organs. The ability of tumour cells to detach from the original mass (the primary tumour) and set up a metastasis (secondary tumour) discontinuous with the primary is unequivocal proof of malignancy. Tumours are also classified according to their tissue of origin (histogenetic classification); recognition of the founder tissue in a lymph node metastasis could establish the location of a hitherto undiagnosed primary tumour.

The suffix 'oma' usually denotes a benign tumour, and tumours of glandular epithelia are called 'adenomas' (e.g. colonic adenoma). Tumours of surface epithelia are called

Table 1 Nottingham breast cancer grading system

Feature	Score
Tubule formation (%)	
Majority of tumour (>75%)	1
Moderate degree (10–75%)	2
Little or none (<10%)	3
Nuclear pleomorphism	
Small uniform size	1
Moderate increase in size/variation	2
Marked variation	3
Mitotic count in 10–40× fields	
0–5	1
6–10	2
>11	3
Grade 1 (well differentiated) (sum)	3–5
Grade 2 (moderately differentiated) (sum)	6–7
Grade 3 (poorly differentiated) (sum)	8–9

See also: **Breast Cancer**

'papillomas' (e.g. skin papilloma). However, carcinoma and sarcoma refer to malignant tumours of epithelia and connective tissue respectively, qualified by the tissue of origin (e.g. prostatic adenocarcinoma, osteosarcoma). There are numerous exceptions to this systematic nomenclature; leukaemias and lymphomas are malignant tumours of bone marrow and lymphoid tissue respectively, and malignant melanoma, a highly aggressive tumour, derives from the melanin-producing cells (melanocytes) of the skin.

Tumour Assessment

The management of a patient with cancer is dependent upon a number of pieces of information that can be gathered about the tumour:

Tissue of origin
Benign or malignant
Tumour grade
Tumour stage

Benign tumours can normally be removed by biopsy or surgery. Malignant solid tumours will, if possible, be surgically resected, probably followed and even preceded (so-called 'tumour debulking') by other treatment modalities. More diffuse tumours such as leukaemias with circulating tumour cells require systemic chemotherapy. A histopathologist will 'grade' a tumour in terms of its state of differentiation on a scale from well differentiated, through moderately and poorly differentiated to finally anaplastic. For example, normal colonic epithelial cells form simple tubular glands called crypts; malignant colonic cells largely organized into glandular structures, albeit in a disorderly fashion, would be graded as well differentiated (low grade). At the other end of the spectrum, poorly differentiated (high grade) tumours have only a small proportion of cancer cells forming glandular structures, while anaplastic tumours are likely to have no resemblance to the tissue of origin. Glandular formation is not necessarily the sole grading criterion, for example the Nottingham grading system for breast cancer also includes the degree of nuclear pleomorphism (variation in size, shape and staining) and the number of cells visibly dividing (the mitotic score), each given a score from 1 to 3, making a maximum score of $3+3+3=9$ (**Table 1**). Likewise, soft tissue sarcomas such as leiomyosarcoma derived from smooth muscle are graded (1) according to their resemblance to normal cells, (2) abundance of mitotic cells and (3) presence of necrosis, again with a $3+3+3$ score, 9 being the highest grade. Surprisingly, little or no necrosis scores 1, while over 50% of the tumour cells being necrotic scores 3. Intuitively one would think the presence of excessive cell death signalled a good prognosis, but in fact, portends a poor prognosis because aggressive growth has likely outstripped the blood supply. Poorly differentiated tumours tend to be more aggressive, growing faster and more likely to have metastasized before the patient has presented. Thus, patients with poorly differentiated tumours tend to have a poorer prognosis and might be selected for more aggressive treatment. **See also**: **Breast Cancer**; **Molecular Genetics of Uterine Sarcomas**

Tumour staging is a semi-quantitative assessment of the clinical gravity of the disease. A complete profile can be built up from knowing the size of the primary tumour, the extent of local lymph node involvement and the presence or absence of distant metastasis. In the tumour, node and metastasis (TNM) staging, the larger the primary tumour and the more local nodes involved then the more advanced the stage with a concomitantly likely poorer prognosis. Significantly, the presence of metastatic disease immediately assigns the patient to the most advanced stage, irrespective of the size of the primary tumour, highlighting the importance of early detection and intervention to patient survival (**Table 2**).

Cancer databases such as 'cancerresearchuk.org' or 'gco.iarc.fr' provide a wealth of information on all types of cancers. For example, not all cancers are equally common, in the UK, breast, prostate, lung and bowel cancers made up over 50% of all new cancers in 2017, but globally lung, colorectal, stomach and liver

Table 2 Staging systems for colorectal cancer (CRC)

TNM classification (AJCC)				Dukes' classification	5 year survival
Stage	T	N	M		
Stage 0	Tis	N0	M0		
Stage I	T1	N0	M0	A	
	T2	N0	M0	B1	
Stage II	T3	N0	M0	B2	90%[a]
	T4	N0	M0	B2	
Stage III	T1 T2	N1/N2	M0	C1	70%[a]
	T3T4	N1/N2	M0	C2	
Stage IV	Any T	Any N	M1	D	14%[a]

TNM, tumour, nodes, metastasis; AJCC, American Joint Committee on Cancer; Tis, tumour *in situ* (intramucosal cancer). Stage I tumours have invaded beyond the underlying mucularis mucosae, Stage II tumours have invaded as far as the muscularis propria, Stage III tumours have reached the outermost serosal covering and involve several local lymph nodes, Stage IV tumours have spread to sites discontinuous with the primary tumour (metastasized). [a]Survival data based on an alternative staging system known as SEER (The Surveillance, Epidemiology and End Results: training.seer.cancer.gov) where patients are grouped according to CRC being 'Localized' – no tumour growth outside the colon or rectum; 'Regional' – the cancer has spread outside to nearby structures and lymph nodes; 'Distant' – the cancer has spread to distant parts of the body to sites such as liver and lungs. Other tissues have their own unique staging systems, for example, the *FIGO staging systems* are determined by the International Federation of Gynaecology and Obstetrics (Fédération Internationale de Gynécologie et d'Obstétrique) and are used for cancers of the female reproductive tract. **See also**: **Colon Cancer**

cancers were the most common causes of cancer deaths. Another useful statistic is the survival rate, commonly the 5-year survival rate, but it can be for 1 year or even 10. The 5-year survival rate is defined as the percentage of people in a study or treatment group who are alive 5 years after they were diagnosed or started a treatment for a disease, such as cancer. The disease may or may not have come back. Some cancers such as prostate cancer have a good 5-year survival rate (>80%), other such as lung, liver and brain a quite poor one (∼10%), while patients with inoperable pancreatic cancer have an expected lifespan measured in months. This parameter can be further interrogated by a *Kaplan–Meier* survival curve that describes the probability of an event (e.g. survival) at a given length of time, when time is measured in small intervals over the 5-year period. Kaplan-Meier curves are not only used for overall survival (OS) data, but also for disease-free survival (DFS, time to relapse) and are also useful for comparing one treatment with another, or for comparing OS by stage or grade or, for example, for making a comparison between two groups of patients with the same tumour type, but one expresses a stem cell-like gene expression pattern and the other does not.

Tumour Genetics: A Brief Summary

The ability to maintain genome integrity in the face of DNA damage is critical for healthy survival. At a cellular level cancer is a very rare disease given that an individual has many millions of cells, so normally the repair and/or elimination mechanisms of damaged cells must be very efficient, akin to having a 'caretaker' function. The pathway to malignancy involves the accumulation of many genetic alterations, achieved through successive rounds of alteration and clonal expansion (**Figure 5**). To account for the multiple mutations in cancer cells, much attention has become focused on the mechanisms of DNA metabolism that maintain genome integrity, looking for the so-called 'mutator phenotype'. If the mechanisms of DNA repair are faulty, this leads to 'genetic instability', facilitating an increased rate of alterations in the genome. Most cancers are genetically unstable, providing the genetic plasticity to drive the stepwise progression of genetic changes required for the development of malignancy. This relaxation in genome stability is due to alterations in genes involved in DNA replication, repair, telomere stabilization and chromosome segregation, and can lead to point mutations, deletions or additions of a few nucleotides, translocations, and even losses or gains of whole or parts of chromosomes. **See also**: **Caretakers and Gatekeepers**; **Tumour Suppressor Genes**; **Oncogenes**

The importance of repair processes can be appreciated by studying the rare chromosomal instability syndromes, autosomal recessive diseases where homeostatic mechanisms fail, resulting in multisystem effects including a predisposition to malignancy and immunodeficiency. In Bloom syndrome, the defect is in a DNA helicase; while heterozygotes do not have an increased cancer risk, homozygotes commonly develop lymphomas and leukaemias in their twenties. Ataxia-telangiectasia homozygotes have a 30–40% lifetime risk of malignancy, and the ataxia-telangiectasia mutated (ATM) protein is a member of a family of protein kinases that include DNA-dependent protein kinase (DNA-PK) and ATM and Rad3-related (ATR) kinase that has a prominent role in the DNA damage response (DDR) pathways that respond to genotoxic stress (Li *et al*., 2016). Cells from patients with ataxia-telangiectasia cannot accomplish cell cycle arrest after irradiation-induced DNA damage, referred to as radiation-resistant DNA synthesis. Patients with xeroderma pigmentosum (XP) suffer from a defect in nucleotide excision repair, becoming highly sensitive to ultraviolet light-induced damage with a 2000–fold increased risk of developing skin cancer. Genes that maintain genome integrity can be classified as 'caretaker' genes, and along with many 'gatekeeper' genes that regulate essential cellular functions such as growth or cell death control, can be inherited as Mendelian tumour susceptibility genes

Table 3 Some inherited cancer susceptibility syndromes

Gene	Syndrome	Major tumour types	Notes
APC	Familial adenomatous polyposis	Bowel + others	Gatekeeper
ATM	Ataxia-telangiectasia	Leukaemia	Caretaker
BLM	Bloom syndrome	Generally increased malignancy especially haematological	Caretaker
BRCA1/BRCA2	Familial breast and ovarian carcinoma	Breast/Ovary + others	Caretaker/Gatekeeper
CDH1	Hereditary diffuse gastric cancer	Stomach + lobular breast cancer	Gatekeeper
FANCA/B/C	Fanconi anaemia	Leukaemia	Caretaker
MSH2/MLH1/MSH6	Hereditary non-polyposis colon carcinoma (HNPCC)	CRC + others	Caretaker
NF1	Neurofibromatosis type1	Phaeochromocytoma	Gatekeeper
PTCH1	Gorlin syndrome	Skin basal cell carcinoma	Gatekeeper
PTEN	Cowden syndrome	Multiple	Gatekeeper
RB1	Familial retinoblastoma	Retinoblastoma/Osteosarcoma	Gatekeeper
TP53	Li-Fraumeni syndrome	Multiple	Gatekeeper
VHL	von Hippel-Lindau syndrome	Renal carcinoma + others	Gatekeeper
XPA	Xeroderma pigmentosum	Skin cancers	Caretaker

All of the above mentioned genes are classified as tumour suppressor genes.

(**Table 3**). Studying one of these inherited syndromes, familial retinoblastoma, Alfred Knudson in 1971 formulated his 2-hit hypothesis that suggested most tumour suppressor genes (TSGs) require inactivation of both alleles before a phenotypic change can be realized. Retinoblastoma susceptibility can be inherited or the tumour can arise sporadically, and he noted that where it appeared to run in families, then the disease occurred earlier than in the sporadic cases and was often bilateral. He thus surmised that in familial retinoblastoma one 'hit' (later identified as an *RB1* mutation) was inherited and the second one was acquired, while in sporadic cases 2 'hits' were required for tumour development, thus explaining the later onset. Interestingly, although the genes illustrated in **Table 3** are all considered 'driver' genes, the inherited predisposition syndromes show marked differences in tissue response (Schneider *et al*., 2017). Whereas inherited mutations in mismatch repair (MMR) and *TP53* genes cause tumours in a wide variety of tissues, mutations in the likes of *APC*, *CDH1*, *BRCA1/2* and *VHL* have a much more restricted tissue response. **See also**: **Genetics of the Pathophysiology of Retinoblastoma**; **Chromosomal Instability (CIN) in Cancer**; **Familial Adenomatous Polyposis**; **Nevoid Basal Cell Carcinoma (Gorlin) Syndrome**; **Renal Carcinoma and von Hippel–Lindau Disease**; **Mismatch Repair Genes**; **DNA Repair**; **Human Mismatch Repair: Defects and Predisposition to Cancer**; **Hereditary Breast Cancer Syndromes: Molecular Pathogenesis and Diagnostics**; **Genetics of *PTEN* Hamartoma Tumour Syndrome**; **Neurofibromatosis Type I (NF1)**

Firm support for a 'mutator' phenotype being important for cancer development comes from patients with hereditary non-polyposis colonic cancer (HNPCC) who are very prone to cancer development. As in the other recessive diseases, individuals suffer from the consequences of defects in DNA repair once the wild-type allele is inactivated during tumorigenesis, accumulating mutations in proto-oncogenes and TSGs that are characteristic of cancer. In HNPCC, mutations are present in MMR enzymes, enzymes that recognize and repair distortions of the double helix resulting from a 'misfit' of non-complementary base pairs. Defects in these enzymes are indicated from examining 'microsatellites', regions of chromosomes in which a single base (e.g. A) or a small number of bases (e.g. CA) is tandemly repeated a number of times. Microsatellites are relatively constant in normal cells but can vary greatly in tumours, so-called 'microsatellite instability', a marker of MMR defects in a cell. **See also**: **Hereditary Nonpolyposis Colorectal Cancer**

The whole spectrum of genes whose altered expression is implicated in cancer development is massive and the reader is directed to 'COSMIC', the *C*atalogue of *S*omatic *M*utations *i*n *C*ancer (cancer.sanger.ac.uk) for the world's largest and most comprehensive resource for exploring the nature of somatic mutations in human cancer. Though COSMIC regularly records over 10 000 somatic mutations per tumour, each tumour is believed to carry only about 5–10 driver gene mutations (a mutation that influences a cell's growth as opposed to 'passenger' mutations that have little or no bearing on tumour development).

Unlike steroid-dependent tissues where the ligands are able to diffuse through the plasma membrane to bind to intracellular receptors, tissues that are dependent on peptide growth factors that cannot pass through the plasma membrane must rely on intracellular signalling pathways to convey messages from the local microenvironment to the nucleus. Some of these pathways are particularly important during embryonic development, for example, Notch, but most are involved in tissue homeostasis and are commonly subverted in cancer. Pathways include the mitogen-activated protein kinase (MAPK) pathway, Wnt, hedgehog (Hh), PI3K/Akt/mTOR, Notch, JAK/STAT, TGFβ, Hippo and NFκB. Listed below are some of the genes frequently altered in cancer. **See also**: **Role of the Wnt Signalling Pathway in Cancer**; **Notch Signalling in Cancer**; **TGF-β Signalling and Its Role in Cancer Progression and Metastasis**; **Hedgehog Signalling**; **Role of the JAK-STAT Signalling Pathway in Cancer**;

Hippo Growth Control Pathway and Organ Size; **Phosphoinositides – The Seven Species: Conversion and Cellular Roles**

APC, a TSG whose encoded protein is part of the 'destruction complex' that facilitates phosphorylation of β-catenin prior to its ubiquitination. Loss of APC function leads to enhanced Wnt signalling. *BAX*, a pro-apoptotic gene. *BCL2*, an anti-apoptotic gene. *CDKN2A*, a TSG that encodes $p16^{INK4a}$, an inhibitor of cyclin-dependent kinase 4A. *CTNNB1*, a proto-oncogene encoding β-catenin. *ERBB2*, a proto-oncogene encoding EGFR2, a receptor tyrosine kinase, also known as HER2 in breast cancer. *KRAS*, a proto-oncogene encoding a GTPase, a non-receptor tyrosine kinase involved in intracellular signalling, for example, the MAPK pathway. *PI3KCA*, a proto-oncogene encoding the catalytic subunit-α of phosphoinositide 3-kinase, an enzyme catalyzing the phosphorylation of phosphatidylinositol-4,5-bisphosphate (PIP_2) to PIP_3. *PTEN*, a TSG encoding a phosphatase that dephosphorylates PIP_3 to PIP_2. *hTERT*, a proto-oncogene encoding the protein subunit human telomerase reverse transcriptase, a gene up-regulated in the majority of human cancers (Hannen and Bartsch, 2018). **See also**: **Telomeres and Telomerase in Ageing and Cancer**; **Oncogenic Kinases in Cancer**; **Cell Cycle: Regulation by Cyclins**; **Ras Mutations in Cancer**; **Telomerase: Structure and Function**

Loss or mutation of the tumour suppressor gene *TP53* (commonly known as *p53*), is the most common genetic lesion in human cancer. TP53 is a 53 kDa protein discovered over 40 years ago as the host protein that was bound to the viral oncoprotein 'large T antigen' in Simian virus 40 (SV-40) transformed cells. TP53 is one of three p53 family members, the others being p63 and p73, and it functions as a tetrameric DNA-binding transcription factor that activates thousands of genes, the best known of which act to either promote the elimination or repair of genetically damaged cells. This feature has presumably evolved to reduce the risk of propagating cancer-causing mutations – 'the better dead than wrong hypothesis'. Hence David Lane's description 'p53, guardian of the genome' (Lane, 1992).

In normal cells, a high level of p53 is probably deleterious to cell survival (e.g. it might cause premature ageing), and so p53 is maintained at a safe low level by negative regulators such as mouse double minute 2 (MDM2), a p53 target gene that functions as a p53 E3 ubiquitin ligase to facilitate p53 proteasomal destruction (Dobbelstein and Levine, 2020). In response to stress signals such as DNA damage, oxidative damage, telomere shortening and hypoxia, stress-induced kinases of the DDR such as ATM, ATR, Chk1 and Chk2 are activated resulting in p53 phosphorylation and its stabilization through reduced binding to MDM2. The best-understood functions of p53 are its ability to induce apoptosis through transcriptional activation of pro-apoptotic Bcl-2 family proteins, and to cause a reversible cell cycle arrest, principally through activation of $p21^{Cip1}$, a potent cyclin-dependent kinase inhibitor, in cells with non-lethal DNA damage. Originally *TP53* was considered a proto-oncogene because unlike other TSGs, the *TP53* gene mutation acts as an autosomal dominant gene in that only one mutant allele is needed for an effect. Mutant TP53 exerts a dominant-negative effect on wild-type TP53 because TP53 binds DNA as a tetramer consisting of a dimer of dimers; wild-type and mutant TP53 proteins form heterotetramers that show impaired DNA binding and transcriptional activity. So despite loss of function, mutant TP53 can be detected by suitable antibodies in cells that in their normal state would have undetectable levels of TP53 (Goh *et al*., 2011).

TP53 has been implicated in what has become known as 'Peto's paradox', the observation made by epidemiologist Richard Peto that the incidence of cancer across species does not correlate with increased body size (a surrogate of the number of cells), despite having higher numbers of cells and a longer lifespan. For example, the incidence of cancer in elephants is far less than expected given their huge number of cells and long life, perhaps because the elephant genome contains 20 copies of the *TP53* gene and the increase in *TP53* copy number coincides with the evolution of large body sizes, and an enhanced sensitivity to genotoxic stress (Sulak *et al*., 2016). Though an intriguing association, we should remember that the likes of whales and elephants do not engage in unhealthy lifestyles such as 'bad diets' and smoking!

The roles of TP53 go far beyond that of elimination of cells harbouring potentially cancer-causing mutations and include the control of metabolism and the ability to influence the cells of the tumour microenvironment (TME). For example, TP53 can help cells survive conditions of nutrient deprivation, possibly through temporary activation of p21, while it may also activate catabolic pathways such as autophagy and fatty acid oxidation to sustain cells in the absence of exogenous nutrition (see review by Labuschagne *et al*., 2018). TP53 can also regulate immune responses, for example by increasing the expression of Toll-like receptors (TLRs) making cancer cells more susceptible to agonist-induced cell death, a response lost in many cancer cells expressing mutant TP53. Loss of TP53 also has a profound effect on the TME, affecting cytokine production by the immune cells to possibly favour tumour growth (Blagih *et al*., 2020). Cancer cells are also involved in immune suppression through expression of the immunosuppressive molecule programmed cell death ligand 1 (PD-L1), also known as CD274, and together with its receptor on T cells, programmed cell death protein 1 (PD1), combine to block an anti-tumour immune response. miR-34a, a transcriptional target of TP53, represses PD-L1 expression and so mutant TP53 in tumours can be associated with increased PD-L1 expression that suppresses T cell function; such patients might benefit from immune checkpoint blockade therapy (see section titled 'Prevention and Cure').

Inheritance of one mutant *TP53* allele results in a 200–500-fold increased risk of cancer development (Li-Fraumeni syndrome, see **Table 3**) in ectoderm- and mesoderm-derived tissues (e.g. sarcomas and glioblastomas in young patients), but only a 2–4-fold increase in cancers in endoderm-derived tissues (e.g. lung and colon cancers in old age patients). It has been suggested that the difference might be because the initial (truncal mutation, see **Figure 5**) mutation in ectodermal and mesodermal stem cells results in a preinvasive lesion, while in endodermal stem cells the same *TP53* mutation has no demonstrable effect until after other mutations have accumulated over a long time period (Levine, 2020). Support for this hypothesis comes from the fact that in spontaneous CRC, loss of TP53 function usually occurs only after the accumulation of other truncal mutations such as *APC*, *KRAS*

and *SMAD4*. **See also**: **P53 and Cell Death**; **Gain-of-Function Mutants of p53 and Their Role in Tumourigenesis**; **Li-Fraumeni Syndrome**; **Polyomavirus Infections of Humans**; **The BCL-2 Family Proteins – Key Regulators and Effectors of Apoptosis**; **Ubiquitin Pathway**

In this section, I have so far restricted discussion to alterations in gene expression brought about by changes directly to the genome through the likes of point mutation, gene deletion, gene amplification, chromosomal loss and chromosomal translocation (the latter particularly important in haematological malignancies). On the other hand, changes in gene activity can occur when there is no underlying change to the genetic code – the study of which is called 'epigenetics'. The first significant epigenetic events to be recognized as important to cancer development were histone modifications and DNA methylation, particularly DNA methylation whereby methyl groups (CH_3) can be added to CpG islands within the promoter regions of TSGs, thus silencing the gene. More recently there has been an explosion in our knowledge of non-coding RNAs (ncRNAs) and the reader is directed to several excellent reviews on the subject. ncRNAs include microRNAs (miRNAs) that bind to complementary mRNA resulting in mRNA translational inhibition and/or degradation (Rupaimoole and Slack, 2017). On the other hand, long non-coding RNA (lncRNA) and circular RNA (circRNA) may have roles as molecular sponges, sequestering miRNAs and so protecting mRNAs from miRNA-mediated degradation (Tsagakis *et al*., 2020; Sheng *et al*., 2020). It is likely that manipulation of ncRNAs will be increasingly viewed as a therapeutic target. **See also**: **MicroRNAs in Cancer**; **Long Noncoding RNAs and Tumorigenesis**; **Epigenetic Drivers of Genetic Alterations in Cancer**; **Methylation-mediated Transcriptional Silencing in Tumorigenesis**; **DNA Methylation and Histone Acetylation**; **DNA Methylation and Mutation**; **MicroRNAs in Cancer**

Tumour Evolution

The founder cells of cancer: While a great deal is known about established tumours in terms of the likes of their intratumour genetic heterogeneity, invasion and metastatic properties, along with likely clinical course, relatively little is known about tumour origins – from which cell(s) do individual tumours arise from? The classic 2-stage model of epidermal carcinogenesis developed in the 1950s in which a mutagenic chemical such as the genotoxin 9,10-dimethyl-1,2-benzanthracene (DMBA) was administered to mice, demonstrated that tumour yields were not necessarily reduced by delaying for up to 43 weeks the time of the subsequent application of the necessary promoter of cell proliferation, in this case, the phorbol ester, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) found in croton oil. Thus, it was considered that tumour initiation was largely irreversible. Since cell migration from the basal layer to the surface of the mouse epidermis only takes up to 9 days, an entirely reasonable conclusion is that the successful transformation events must have occurred in long-lived cells in the basal layer that do not join the upward- moving cell escalator, presumably the epidermal stem cells. Stem cells with their in-built powers of continued self-renewal combined with their longevity make them prime targets for permanent transformation, while numerous studies have highlighted the critical importance of cell proliferation in general to the carcinogenic process. For example, the liver and pancreas have very little cell turnover, and most of their tumours arise in a setting of chronic inflammation where cell damage induces a regenerative response. Even in tissues such as the oesophagus, stomach and colon where there is ordinarily a continual turnover of cells, inflammatory conditions such as oesophagitis, gastritis and inflammatory bowel disease (Crohn colitis and ulcerative colitis) pose an increased, if somewhat unpredictable risk of tumour development.

Does a tumour's phenotype (behaviour) indicate its cell of origin? In continually renewing systems (**Figure 2**) such as those of the gastrointestinal tract and haematopoietic system, evidence supports the belief that mutations in stem cells are the most likely to successfully initiate vigorous malignant growth. Pioneering studies in 1967 showed that chronic myeloid leukaemia (CML) is probably initiated in a multipotential haematopoietic stem cell (HSC) because the Philadelphia (Ph) chromosome resulting in the *BCR:ABL1* fusion gene, was found in all erythrocytes and granulocytes. In acute myeloid leukaemia (AML), recurrent *DNMT3a* (DNA methyltransferase 1) mutations have been found in HSCs from patients in remission (Shlush *et al*., 2014). Since these mutated HSCs were able to regenerate the entire haematopoietic hierarchy in xenografts in a manner superior to non-mutated HSCs, it was suggested that the former cells were pre-leukemic HSCs clonally expanded from a normal HSC, and from which AML evolves. However, in the haematopoietic system, the more mature committed progenitors can be the cell of origin of cancer, whereby leukaemia can be induced from granulocyte-monocyte progenitor cells. **See also**: **Chronic Myeloid Leukaemia**

The cell of origin of epithelial tumours (carcinomas) clearly influences their phenotype. In the mouse intestine, targeted deletion of the *Apc* (*adenomatous polyposis coli*) gene in the Lgr5-positive crypt base columnar stem cells is associated with the development of large adenomas with nuclear-associated β-catenin (Barker *et al*., 2009). On the other hand, targeted loss of the same gene in the short-lived transit-amplifying crypt cells only results in small adenomas with stalled growth. In the epidermis, there is also persuasive evidence that the position of the initiated cell within the cell hierarchy has a profound bearing on subsequent tumour growth. Basal cell carcinoma (BCC) is the most common skin cancer in man, and hedgehog (Hh) signalling is critical to its formation. In mice, when either 'loss of function' mutations in the Patched (Ptch) receptor or 'gain of function' mutations in Smoothened (Smo) were introduced into either the cytokeratin 14-positive stem cells/committed progenitors of the interfollicular epidermal basal layer or the more differentiated cells expressing the keratinocyte protein involucrin, then BCCs were readily induced in the CK14-positive cells, but were very much reduced in size and number in the involucrin-positive population (Sánchez-Danés *et al*., 2016). Concluding, it is quite clear that cancer can be readily induced in stem cells but as cells progress towards maturation, cells become increasingly refractory to neoplastic transformation. **See also**: **Colorectal Cancer: Genetics**

Expanding tumours need a blood supply: avascular tumours cannot grow beyond a size of 2–3 mm^3 without new blood vessels. This vasculature is mostly derived from the pre-existing

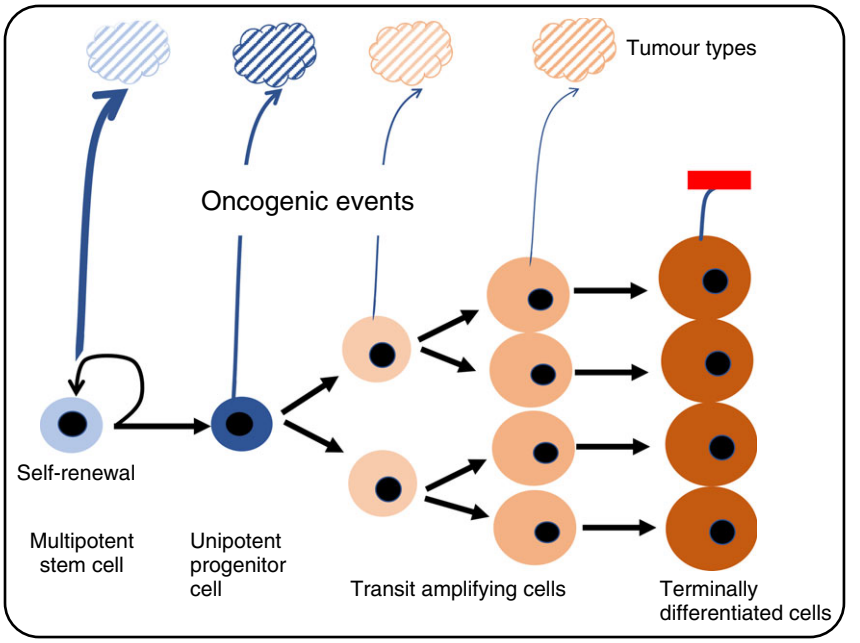

Figure 2 Does tumour phenotype inform on tumour histogenesis? Current dogma suggests that most tumours arising in continually renewing systems (haematopoietic cells, gut lining cells, epidermis) with a hierarchical organisation (as illustrated) are initiated in stem cells or closely related progenitor cells, since ordinarily these are the only cells with a sufficient lifespan to accrue the mutational burden required for a malignant habitus. Moreover, stem cells possess the ability for seemingly continual turnover and are anchored to the niche. Inter-tumoural diversity is unlikely to be because tumours can be initiated stochastically from any stage of lineage differentiation, and is most likely a reflection of either further differentiation and/or blocked differentiation/maturation arrest imposed by the oncogenic regime. Thus, tumours are unlikely to be similar to their cell-of-origin but may possess some of their features, for example, stemness or multipotentiality. The cell-of-origin may be further disguised by antecedent metaplasia, for example, intestinal metaplasia in the oesophagus and stomach, mucinous cell metaplasia in the pancreas or by proceeding epithelial–mesenchymal transition (EMT).

vascular network in the surrounding normal tissue; thus, the endothelial cells that line the blood capillaries can be considered 'gatekeepers' of tumour expansion. The growth of new capillaries is called angiogenesis – vessel sprouting from pre-existing vessels (De Palma *et al*., 2017), and a failure of tumour cells to stimulate angiogenesis may be responsible for the long-term dormancy of some primary and metastatic tumours. Hypoxia is a key driver of this process and under normoxic conditions the transcription factor hypoxia-inducible factor (HIF)-1α is being continually synthesized and degraded, but under hypoxic conditions, HIF-1α is stabilized and forms a heterodimer with HIF-1β. The HIF-1α:HIF-1β complex functions as a transcription factor to control the expression of many genes including vascular endothelial growth factor A (*VEGFA*). VEGFA initiates angiogenesis by binding to VEGF receptor 2 (VEGFR2) expressed on endothelial cells. This results in a shift in the balance of pro- and anti-angiogenic factors towards a pro-angiogenic environment, the so-called 'angiogenic switch' (Bergers and Benjamin, 2003) leading to a major expansion of tumour size. VEGFR2 is a receptor tyrosine kinase whose dimerization leads to autophosphorylation of tyrosine residues and activation of intracellular signalling including the p38 MAPK and PI3K pathways to promote the likes of endothelial proliferation and migration.

Angiogenesis can be summarized as follows:

Under the influence of hypoxia, tumour cells release pro-angiogenic growth factors that, in concert with growth factors produced by the endothelial cells themselves acting in an autocrine manner, stimulate capillaries to sprout side-shoots with endothelial cells becoming motile and invasive and to protrude filopodia. Spearheaded by so-called tip cells, and followed by stalk cells with fewer filopodia, the stimulated endothelial cells release extracellular matrix (ECM)-degrading enzymes such as matrix metalloproteinases (MMPs), urokinase-type and tissue-type plasminogen activators and collagenases to clear a path through the surrounding ECM, as well as inhibitors such as plasminogen activator inhibitor 1. Stalk cells establish a lumen and proliferate to extend the sprouts and neighbouring sprouts anastomose with each other to form vascular loops. To stabilize the new vascular connections, endothelial cells then re-establish a basement membrane by the release of basement membrane components such as laminin, type IV collagen and tenascin, and express ECM receptors such as the $\alpha_5\beta_3$ and $\alpha_5\beta_5$ integrins. The blood vessels in a tumour become organized in a chaotic manner, most likely because of over-stimulation by pro-angiogenic factors, they are also very leaky and this hyperpermeability facilitates the influx of inflammatory cells and their pro-inflammatory cytokines (the original name of VEGFA was 'vascular permeability factor'). Some tumour neovascularization can also be achieved by bone marrow-derived endothelial progenitor cells (EPCs), whereby EPCs are mobilized from the bone marrow and contribute to the creation of new blood vessels (vasculogenesis), or contribute to existing vascular sprouts. High

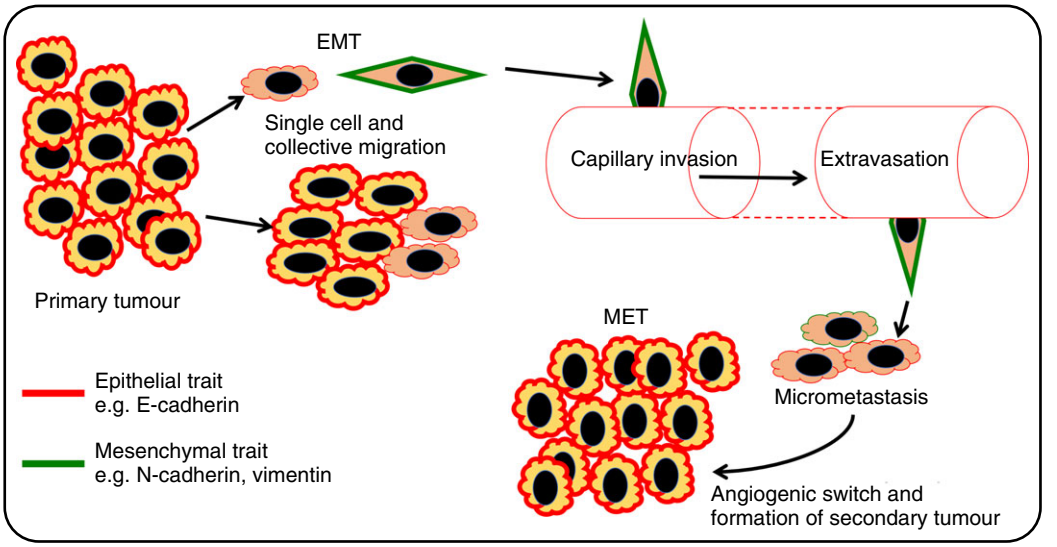

Figure 3 Invasion and metastasis occur either as single cells or cell clusters (collective migration) from the primary tumour, often epithelial cells undergo EMT, loosing contact with their neighbours. Migrating cells secrete matrix-degrading proteases that break down the ECM and facilitate invasion into the lymphatics or bloodstream. The location of extravasation might be the first capillary bed that the tumour emboli become immobilised at, although it may have more to do with the CAMs expressed by the tumour and endothelial cells (see 'seed and soil' hypothesis). Exit from the vasculature may be followed by a period of dormancy as a micrometastasis, but once the angiogenic switch occurs then a secondary tumour can be established.

numbers of EPCs in the bloodstream, expressing the likes of CD133, c-Kit and VEGFR2, can be an indication for poor patient prognosis. **See also**: **Tip Cells in Angiogenesis**

Creation of the tumour microenvironment (TME): the angiogenic switch heralds the rapid formation of the TME in which there is an inflammatory response involving the immigration of innate and adaptive immune cells (Gonzalez *et al*., 2018). In the early phase of tumour development, it is likely that many of the most immunogenic cancer cells are recognized and killed by natural killer (NK) cells and $CD8^+$ T cells. As expected, high levels of tumour-infiltrating T cells often correlate with a better prognosis. However, hypoxia induces surrounding fibroblasts to take on a 'cancer-associated fibroblast' (CAF) phenotype, associated with the release of bFGF, IL-6, PDGF and TGFβ that facilitate evasion of the anti-tumour immune response (Hanahan and Coussens, 2012; Sahai *et al*., 2020). Apart from the immunosuppressive functions of CAFs, they also function to deposit matrix and remodel matrix through the expression of matrix-crosslinking enzymes leading to a stiff ECM. Excessive fibrosis surrounding a tumour is known as 'desmoplasia', and a desmoplastic reaction may not necessarily be a bad thing, possibly a defence mechanism employed by the body to wall-off a tumour to limit further spread. CAFs also secrete mitogenic growth factors such as various FGFs, EGF and HGF, the latter also acting as a motogen for tumour cells.

The other major cell type of infiltrating stromal cells are macrophages, that in the TME take on a special phenotype and are known as tumour-associated macrophages (TAMs). TAMs originate from circulating monocytes, and high numbers of TAMs are associated with a poor prognosis in many cancers. While in a pro-inflammatory M1-like state, TAMs may eliminate the most immunogenic cancer cells, but the TME elicits a switch to a regenerative M2-like state that sustains angiogenesis, promotes tumour cell proliferation, secreting multiple proteases that release further growth factors from the matrix and also releasing immunosuppressive cytokines such as IL-10. TAMs also induce the so-called epithelial–mesenchymal transition (EMT) that aids an invasive and metastatic habitus.

EMT and the formation of metastasis: EMT is activated by many transcription factors, commonly those of the SNAIL, TWIST and ZEB families. EMT facilitates cell motility and so plays an important part in cell migration during embryonic development, thus it can also increase cancer cell motility either as cell clusters or individual cells (Zhang and Weinberg, 2018). Carcinomas are derived from epithelial cells whose defining characteristic is that they form cohesive sheets of cells. Epithelial cells are held together by various junctional complexes, in particular adherens-type junctions that depend on Ca^{2+}-dependent interactions between E-cadherin molecules that span the plasma membranes of adjacent cells (**Figure 3**). Cell-cell adhesiveness is generally reduced in human cancers, both in diffuse-type cancers as well as during invasion. Silencing of the E-cadherin gene (*CDH1*) can occur by many ways including gene mutation/loss, binding of SNAIL to the *CDH1* promoter and probably more commonly by CpG hypermethylation around the promoter region (Hirohashi and Kanai, 2003). **See also**: **Adherens Junctions**

The development of most carcinomas is associated with reduced expression of E-cadherin and up-regulation of mesenchymal molecules such as vimentin, facilitating cell detachment from the primary tumour mass, thus invasion and metastasis. EMT gives cancer cells plasticity, the ability to be able to go back-and-forth between an epithelial (more-or-less

static) and a quasi-mesenchymal (motile) state, being motile for invasion and once at the metastatic site, down-regulating the EMT program and becoming more epithelial-like again – so-called mesenchymal–epithelial transition (MET) (**Figure 3**). Of course, the above modus operandi might apply to individual cells detaching from the primary tumour, but as noted (Brabletz *et al*., 2018), many carcinomas migrate as large coherent multicellular phalanxes of cells (collective cell migration) maintaining their adherens junction connections (Friedl and Mayor, 2017). In such a scenario, the 'leader' cells may have a mesenchymal phenotype while maintaining adherens junction connections with the 'follower' cells. A variation of this scheme was proposed by Labernadie *et al*. (2017), who observed that *in vitro* A431 carcinoma cells can still collectively migrate despite E-cadherin expression, here the 'leader' cells were CAFs that engaged the carcinoma cells (followers) through multiple heterophilic adherens junctions composed of E-cadherin from A431 cells and N-cadherin from CAFs (Dart, 2017).

A metastasis (a secondary tumour) is a tumour implant discontinuous with the primary tumour. Metastases are the major cause of death from malignant disease because widespread heterogeneous metastatic disease, particularly in vital organs such as the liver, is difficult to treat. Tellingly, many patients are diagnosed after metastatic spread has occurred (designated M1) and are immediately assigned to the most advanced clinical stage (expected worse prognosis) irrespective of the size of their primary tumour and degree of local lymph node involvement (see TNM staging). Achieving metastatic spread involves a series of sequential and interrelated steps and a failure at any step can be rate-limiting (Fidler and Kripke, 2015). After invasion beyond normal tissue boundaries, a defining feature of malignancy, cell(s) detach from the primary tumour mass and enter blood or lymphatic vessels before adhesion to a suitable endothelium. Cells must then exit from the circulation, spread locally and once more induce angiogenesis to establish a new tumour.

The distribution of some metastases can be explained on purely mechanistic grounds: tumour cells that are shed into the bloodstream lodge in the first capillary network they meet downstream. For example, the lung is the most favoured site in patients with primary tumours draining into the systemic veins. Also determining patterns of metastasis may be the 'stickiness' of the endothelium, because endothelia in particular organs have organ-specific cell adhesion molecules (CAMs) that determine which cell-cell interactions occur. In particular, members of the immunoglobulin superfamily such as vascular cell adhesion molecule (VCAM) on endothelia may react with specific integrins expressed on tumour cells. Integrins are a large family of receptors mediating adhesion between the cell membrane and either the ECM or other CAMs. Each molecule is composed of two noncovalently associated α and β subunits, and at least 20 heterodimers exist. Integrins are not merely transmembrane rivets linking the cell to the ECM; ECM binding may directly stimulate signalling pathways such as the MAPK pathway, and failure to bind to the ECM can lead to apoptosis, in this instance called 'anoikis' (Gk.'homeless'). That the pattern of organ distribution of metastases could not be explained by simple mechanical entrapment of tumour emboli in the first capillary bed they encountered was first noted by an English surgeon Stephen Paget, who in 1889 published his report 'Distribution of Secondary Growths in Cancer of the Breast'. He noted a discrepancy between the frequency of metastasis and the relative blood supply in several organs and proposed his 'seed and soil' hypothesis to explain patterns of metastatic spread, likening it to a plant going to seed, its seeds going in all directions, but they can only become established and grow on a congenial 'soil' (Langley and Fidler, 2011). While embolization *via*. lymphatic vessels is favoured by carcinomas, and sarcomas show a preponderance for the haematogenous route, other routes of spread are possible. For example, cancer cells can grow along under the protective connective tissue sheath surrounding bundles of nerve axons, so-called perineural invasion, often seen with prostate cancer, or can spread across body cavities, transcoelomic spread, classically a gastric carcinoma that has metastasized to the ovary giving rise to the so-called Krukenberg tumour. **See also**: **Molecular Genetics of Metastasis**; **Integrins: Signalling and Disease**

How do primary tumours grow?: after the angiogenic switch, experimental studies suggest that for a time, the doubling time of a tumour is constant, in other words, a plot of the number of tumour cells over time on a semi-log graph forms a straight line (**Figure 4**). However the growth rate may be slow at the beginning and end of the time period, so the resultant growth curve is sigmoid and can be fitted by the Gompertz mathematical model. The slowing of the growth rate has seemingly little to do with a decrease in the rate at which cells traverse the cell cycle and more to do with the fact that tumours outstrip their blood supply and suffer ischaemic cell death.

Cell death in tumours, particularly carcinomas, is therefore very common. Much of this death is a passive degradative reaction known as necrosis, most likely due to inadequate angiogenesis within the tumour. Apoptotic cell death, on the other hand, is controlled by a number of gene families, and to manipulate pro-apoptotic pathways specifically in tumours has been something of a holy grail for oncologists. Net tumour growth is due to the cell production rate through cell division exceeding the cell loss rate through cell death (**Figure 4**). Apoptosis was first recognized as a unique form of cell death in BCC, a common skin tumour where the paradox of a high mitotic rate, yet low overall growth rate was resolved by finding a high incidence of tumour cell death. By contrast with necrosis where the affected cells initially swell up with water, this cell death was characterized by the affected cells shrinking, often fragmenting into a number of membrane-bound bodies to be ultimately phagocytosed by neighbouring cells (Green, 2019). Originally called 'shrinkage necrosis' it was renamed 'apoptosis' (Gk. meaning 'dropping off', as leaves from trees) to imply (correctly) that it was a more physiological type of cell death. Indeed, apoptosis is a significant player in the fashioning of tissues during embryonic development. Importantly, apoptotic cell death does not elicit an inflammatory response. Apoptosis is often viewed as an altruistic cell suicide process: when DNA is damaged, signals go to both repair and apoptotic pathways, and if repair cannot be accomplished then the cell undergoes apoptosis - 'better dead than wrong'. Due to the disordered genomes in many tumours, potentially harmful genetic damage can often be tolerated because of uncoupling of these two pathways. In particular, cells harbouring mutant *TP53* will have a survival advantage over normal cells. In response to damage, normal cells upregulate TP53

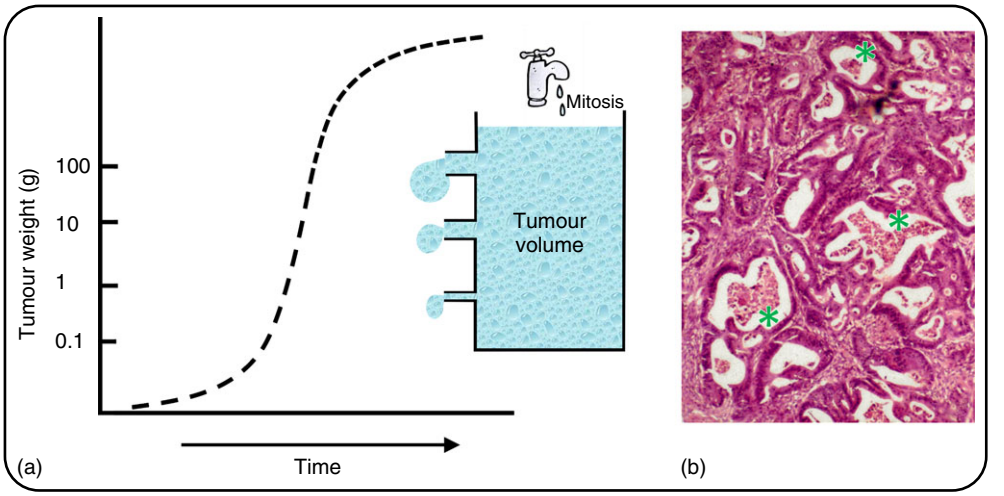

Figure 4 (a) Experimental studies suggest that tumour growth is initially described by a sigmoid growth curve on a semi-logarithmic plot, with a middle period of exponential growth. Net growth is determined by the balance between the cell production rate and the cell loss rate; at the time of clinical detection, most primary tumours are on this near-plateau phase of growth. As tumours grow, cell loss becomes an ever more significant factor by analogy with the container filling up with water. Based on a cartoon devised by G. Gordon Steel. Approaching the plateau phase, the cell production rate is almost matched by the cell loss rate. In a mouse weighing 25 g, exponential growth is curtailed once the tumour approaches 1 g in weight; in a human, the same phenomenon might occur after reaching a weight of ~100 g (b) An adenocarcinoma will typically present with lakes of necrosis (asterisks) in the glandular lumina, derived from sloughed-off cancer cells. Benign tumours rarely have necrotic areas.

which acts as a transcription factor for inducing cell cycle arrest and/or apoptosis, *TP53*-mutant cells cannot carry out this protective arrest and might survive with what otherwise would be lethal and potentially harmful genetic damage. This perhaps explains why *TP53* mutations are so common in human cancers, having a survival advantage (resisting cell death) is probably more crucial for tumour growth than having an accelerated proliferation rate. **See also**: **Death Receptors**; **Inhibitor of Apoptosis (IAP) and BIR-containing Proteins**; **The BCL-2 Family Proteins – Key Regulators and Effectors of Apoptosis**

While necrosis can be essentially a passive phenomenon associated with loss of plasma membrane permeability control, cell death with a necrotic morphology can be a regulated process, though this would not be recognized as such in tumours by simple observation alone. For example, 'pyroptosis' requires caspase-mediated cleavage of gasdermin D, promoting its oligomerization to form large pores in the plasma membrane, while 'ferroptosis' results from severe lipid peroxidation. The reader is referred to Galluzzi *et al*. (2018) for an extensive review of the myriad types of regulated cell death that result in a necrotic morphology.

Clonal expansion: most studies support the monoclonal origin (derivation from a single transformed cell) for the majority of tumours, but how then do tumours progress? **Figure 5** illustrates a possible scenario. The founder cell is likely to be a somatic stem cell and this cell multiplies to form a preinvasive lesion in which there could be a step-wise progression through the continued selection of ever-fitter clones (*The Selective Sweep Model*). Each 'driver' mutation induces a clone within the tumour, that if positively selected, sweeps to fixation, replacing much of the pre-existing population. Rounds of so-called 'public mutation' and selective sweeps can occur until the accrual of a sufficient mutational burden, including chromosome copy number alterations (aneuploidy) for malignancy to occur. IGH will largely be due to the remnants of evolutionary older clones before the new clone has swept to fixation (time '*t*'). In this model, major clonal populations will reflect the driver mutations that are selected. This is likely to be the situation in many pre-malignant lesions and these initial mutations are often referred to as truncal mutations (Levine *et al*., 2019). This is because tumour growth has been envisaged as a Darwinian tree, with the trunk representing the initial ubiquitous mutations and the branches representing the later subclonal 'private' mutations (Yap *et al*., 2012). At some point in time, before or after malignant potential is attained, a switch to 'punctuated or branched evolution' may occur under neutral evolutionary growth conditions, whereby subclones develop over time through the acquisition of new private genetic changes, thus creating substantial IGH. In this so-called 'Big Bang Model' clones arise continuously during tumour expansion and are not significantly constrained by clonal selection, selective sweeps are rare, both public and private mutations co-exist during tumour growth, and subclones can remain rare but may fuel drug resistance and relapse if only the clonally dominant truncal somatic changes are targeted by therapy. Most driver mutations are likely to be ancestral events and selection is minimal once cancer has been initiated; subclonal 'passenger' mutations might become drivers as a consequence of treatment. In the Big Bang Model, the frequency of a clone is a consequence of its time of appearance rather than its relative fitness (Sottoriva *et al*., 2015; Cross *et al*., 2016; McGranahan and Swanton, 2017).

Cancer stem cells: this term is used to describe the malignant cells within a tumour that perform stem-like functions. These cells are sometimes referred to as tumour propagating cells (TPCs) or tumour initiating cells (TICs) and should be not

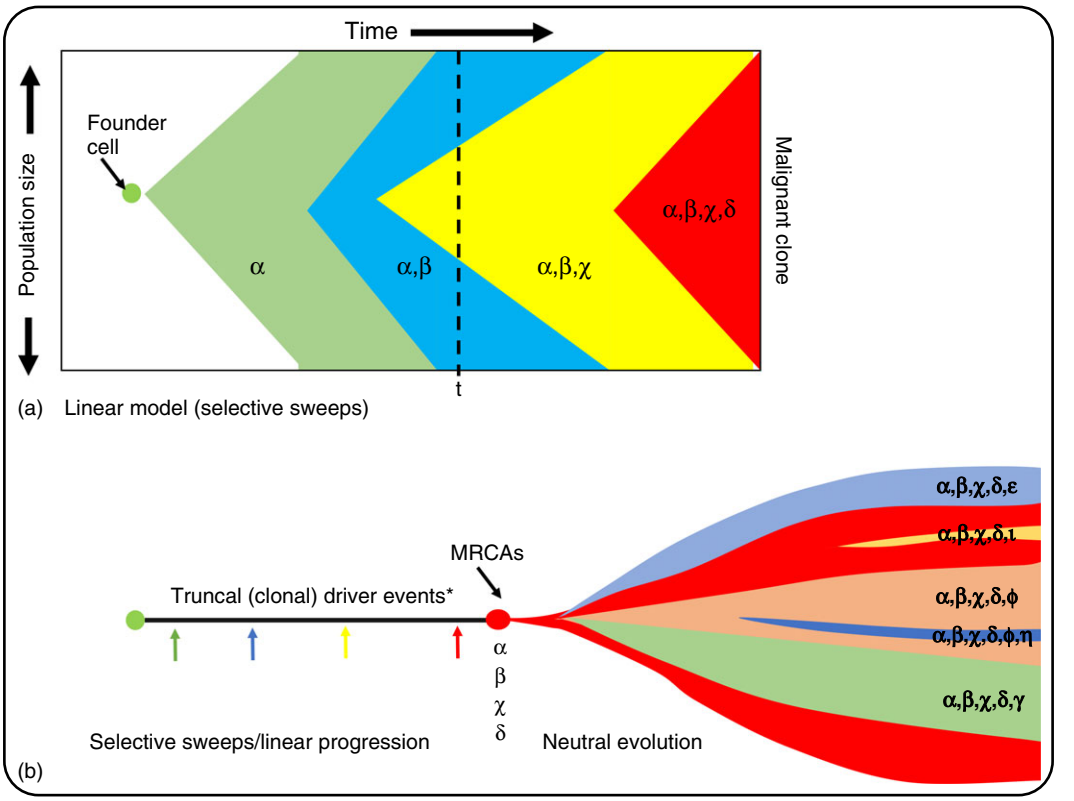

Figure 5 Models of somatic cell evolution in cancer. (a) Early growth is likely to conform to a selective sweep (linear progression) model, (b) but at some point in time branched evolution results in subpopulations with private (subclonal) mutations. Tumour evolution has been likened to tree growth, with the early ubiquitous mutations referred to as truncal mutations, branching evolution increasing the level of IGH. MRCAs: most recent clonal ancestors. *A driver event (α, β, …) can be any genetic change brought about by the likes of mutation, chromosomal loss/addition, epigenetic event.

confused with the founder cells of cancer. Some tumours conform to a stochastic model whereby all tumour cells are endowed with the potential to propagate the tumour and recapitulate the tumour's heterogeneity. On the other hand, it appears other tumours are sustained by a minority cell population of so-called cancer stem cells (CSCs) (Batlle and Clevers, 2017; Vessoni *et al*., 2020). CSCs express various cell markers, the most common being cell surface markers such as CD117, CD133, CD44 and EpCAM, enzymes of the aldehyde dehydrogenase family (ALDH) and the ability to efflux Hoechst dyes by virtue of high expression of multidrug-resistant efflux pumps of the ABC transporter family (Alison *et al*., 2010). While there has been considerable debate as to the best markers for particular cancers, there has been general agreement about some, notably leukaemic stem cells, like normal HSCs are $CD34^{+}$ $CD38^{-}$, brain CSCs are $CD133^{+}$, while breast CSCs are $CD44^{high}CD24^{low}$. Of course, marker expression is not a functional assay of stemness, and ideally, a single CSC should be able to form a large family of descendants (clonogenic) and be able to recapitulate the heterogeneity of the original tumour (tumourigenic). In practice, clonogenic assays are usually performed *in vitro*, while tumourigenic assays are carried out by xenografting selected human cells in immune-deprived mice (**Figure 6**). Critics of these assays suggest that the clonogenic assay simply demonstrates what is possible when cells are not constrained by the stem cell niche, while the tumourigenic assay simply shows which cells grow best in mice. Nevertheless, high expression of such markers is often correlated with aggressive tumour growth and a poorer prognosis. More recently, heritable genetic labelling in mouse tumours has identified cells with clonogenic capacity in their natural environment (Batlle and Clevers, 2017), thus supporting the CSC hypothesis. Of great significance is the fact that CSCs can be highly resistant to therapy (Alison *et al*., 2011, 2012) and maybe largely responsible for tumour recurrence after therapy. Moreover, the CSC state is not immutable and cells can show plasticity, moving in and out of a stem cell state, while EMT has been shown to be associated with the acquisition of stem cell features, important attributes for establishing a metastatic tumour. CSCs are often called 'the roots of cancer'; kill off the roots and the cancer will die by analogy with how to rid your garden of persistent weeds. While many plant-based substances such as sulforaphane found in broccoli and other cruciferous vegetables and curcumin from the spice turmeric appear to specifically inhibit the growth of CSCs *in vitro* (Alison *et al*., 2012), there has been little serious attempt to treat human cancer with these substances.

See also: **Cancer Stem Cells – Basic Biological Properties and Experimental Approaches**; **Cancer Stem Cells**; **Multidrug Resistance in Cancer: Genetics of ABC Transporters**

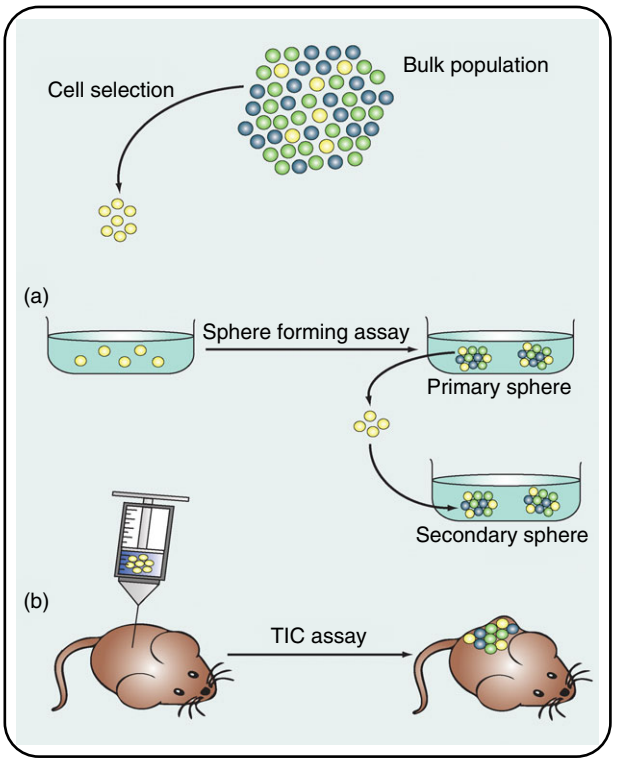

Figure 6 Assays for human CSCs. Selected cells (e.g. those being CD133$^+$, coloured yellow in this illustration) can be assessed either for their *in vitro* clonogenicity or for their ability to initiate new tumour growth after xenotransplantation into immunocompromised mice. (a) Cells can be seeded at low density in non-adherent conditions in a semi-liquid medium, and the percentage of cells generating 'spheres' (e.g. colonospheres) can be measured. Spheres should be composed of both CD133$^+$ and CD133$^-$ cells. The resulting CD133$^+$ cells can be serially passaged, generating secondary and tertiary spheres with a cellular composition resembling that of the primary spheres. (b) The 'gold standard' assay for CSCs is the tumour initiating cell (TIC) assay in which the selected cells are orthotopically (common for brain) or ectopically (e.g. subcutaneous site, commonly for colon) xenotransplanted into an immunocompromised mouse. The cellular composition of the xenografted tumour should resemble the primary tumour from which the selected cells were enriched. The suspected CSCs would be the most clonogenic (have the highest plating efficiency (PE), where PE = number of macroscopic colonies/number of cells plated × 100) and should be ones that can also form tumours in mice with the fewest number of cells transplanted. From Alison MR, Lim SM, Nicholson LJ. (2011) Cancer stem cells: problems for therapy? Journal of Pathology 223: 147–161. © 2011 John Wiley & Sons.

Prevention and Cure

The phrase 'prevention is better than cure' has been attributed to the Dutch philosopher Desiderius Erasmus in around 1500. This is now a fundamental principle of modern healthcare systems worldwide. While the adoption of a healthy lifestyle (limit consumption of red and processed meat, limit alcohol consumption, eat plenty of fresh vegetables and fruit, do not smoke etc.) might reduce the risk of cancer development, it will certainly not guarantee lifetime protection. However, there are many other preventative measures that can be implemented, including taking aspirin, an inhibitor of the enzyme cyclooxygenase (COX)-2 responsible for prostaglandin production. Where an infectious agent is known to be causative, then vaccination is a possibility. For example, cervical cancer is mostly caused by HPV, so in the U.K. young teenage girls are offered a vaccine (Gardasil) that protects against 4 types of HPV: 6, 11, 16 and 18, that account for >70% of all cervical cancers. Chronic HBV infection is a major cause of HCC and prophylactic vaccination to prevent infection has been hugely successful; however, the development of a 'therapeutic' vaccine to completely cure virus carriers is still awaited. Of course, the concept behind a vaccine is to identify an antigen or epitope that is specific to the disease and the vaccine will enhance immunity against it. Like infectious diseases, cancer cells display unique epitopes on their surface that can make them targets of the immune system, so the use of cancer-specific vaccines to treat cancer is now considered a very promising strategy (Lopes *et al.*, 2019). Tumour vaccination is just one of a growing list of immunotherapies that are finding their way into the clinic. **See also**: **Virus-Induced Human Oncogenesis**; **Papillomaviruses**; **Oncogenic Viruses**

Screening programs are, of course, vital for the detection of early cancers and premalignant lesions. Fortunately, if that is the right word, many cancers have a long lead-in time before overt malignancy. For example, oesophageal adenocarcinoma (OAC) may be preceded by intestinal metaplasia (Barrett oesophagus) from which dysplasia and OAC develop. A patient suffering heartburn (gastro-oesophageal reflux disease or GORD) would be screened by endoscopy for suspicious lesions and might be required to undergo regular check-ups (surveillance). Cervical

screening is advocated for women in the 25–64 age group and looks for suspicious undifferentiated cells on the surface of the uterine cervix. Once again, full-blown malignancy only develops after rounds of mutation and clonal expansion, probably according to a 'selective sweep' model through increasing grades of cervical intraepithelial neoplasia (CIN1-3), so affording a large window of opportunity for curative intervention. Perhaps the best known of all screening programs is the one for CRC, since the lead-time from the first early lesion (a small adenoma) to CRC development can often be measured in decades. Most adenomas are exophytic lesions, that is, they grow outwards into the bowel lumen, and so readily bleed with the passage of food. Thus, the most popular test has been the faecal blood test and, if positive, a colonoscopy would be performed to remove the offending adenomatous polyp(s). As noted, 'CRC is the most preventable yet least prevented form of cancer' (Nash *et al*., 2020). The breast screening program involving mammography and the prostate cancer PSA (prostate-specific antigen) test are further examples of widely adopted screening programs. Looking to the future, and for less invasive and more patient compliant tests, the use of the 'liquid biopsy' will become more widespread, analyzing the blood for the likes of circulating tumour cells (CTCs) and circulating tumour DNA (ctDNA) for early diagnosis and therapeutic monitoring (Keller and Pantel, 2019). **See also**: **Colorectal Cancer: Genetics**; **Colon Cancer**; **Barrett Esophagus**

Cancer treatment is usually a combination of a number of different modalities. If the tumour is amenable to surgery, then surgery is the single most effective tool in the anticancer armamentarium. Targeted radiotherapy is another option, as are combinations of anticancer drugs. Many conventional anticancer drugs have been designed with DNA synthesis and the rest of the cell cycle as their target. Therein lies the problem, in that tumour cells are not the only proliferating cells in the body; cells that line the alimentary tract, bone marrow cells that generate all blood cells including those cells that fight infection, and epidermal cells including those that generate hair are all highly proliferative. Therefore patients with cancer receiving chemotherapy commonly suffer unwanted hair loss, diarrhoea and are prone to potentially life-threatening infections due to pancytopenia, side-effects that limit treatment. Hence the need for such patients to shield in the COVID-19 era. Anticancer drugs that fall into this category include (1) agents that interfere directly with DNA synthesis (methotrexate, capecitabine, gemcitabine, 5-fluorouracil [5-FU]), (2) cause double strand breaks in DNA (irinotecan), (3) platinum-based alkylating agents that cause inter- and intra-strand DNA cross-links (oxaliplatin, cisplatin), (4) microtubule-acting compounds that cause cell death in mitosis (taxol, paclitaxel) and (5) anti-tumour antibiotics that can intercalate between DNA strands (doxorubicin). These drugs have been available for many years (so-called 'old drugs'), and while perfectly effective at cell killing, their use is limited by their side-effects. **See also**: **Taxol**

Commonly, several of these cytotoxic drugs are given together along with other agents that have a more supportive role. For example in treating advanced (stage III, see **Table 2**) bowel cancer, the drug regimen may be a combination of *Fol*inic acid (leucovorin), a vitamin D derivative that increases the cytotoxicity of 5-FU, 5-*F*U and *ox*aliplatin: abbreviated FOLFOX. *Iri*notecan can replace oxaliplatin (FOLFIRI). Such a regimen might be supplemented with an anti-angiogenic drug (e.g. bevacizumab) and an EGFR inhibitor such as cetuximab.

Much of new drug development has sought targets other than those directly concerned with killing cells in DNA synthesis and mitosis. This would include elements of the TME (Crusz and Balkwill, 2015), TAMs (Beltraminelli and De Palma, 2020), the metastatic process (Steeg, 2016), growth factor receptors and their downstream signaling pathways such as MAPK and PI3K (Clarke *et al*., 2019) and the developmentally significant pathways such as Wnt, Notch and Hedgehog. Since most growth factor receptors (e.g. EGFRs, FGFRs, PDGFR, Met, VEGFRs) are classed as receptor tyrosine kinases (RTKs), particularly important has been the development of tyrosine kinase inhibitors (TKIs) that can block signalling from these receptors, and include imatinib (Glivec®), erlotinib (Tarceva®), sunitinib (Sutent®) and trastuzumab (Herceptin®). **See also**: **The MERTK Signalling Pathway in Cancer**; **Oncogenic Kinases in Cancer**; **Fibroblast Growth Factors: Evolution**

Since a tumour's vasculature can be considered an Achilles heel, targeting the vasculature was an attractive proposition, though the early promise from mouse experiments has not really been fulfilled in the clinic. Therapeutic agents have direct access to the endothelium, and modes of action have ranged from blocking endothelial proliferation using TKIs such as sorafenib (Nexavar®), the use of VEGF-specific antibodies such as bevacizumab (Avastin®), VEGFR2-specific antibodies such as ramucirumab (Cyramza®), to the use of so-called 'angiostatics' such as angiostatin and endostatin that block integrin binding to the ECM, leading to reduced endothelial migration and viability. Interestingly, angiostatin is a natural 38 kDa proteolytic cleavage product of plasminogen and endostatin a 20 kDa cleavage product of collagen XVIII, both seemingly evolved to limit excessive sprouting of capillaries during normal wound healing.

Since the presence of abundant tumour infiltrating lymphocytes (TILs) has been recognized as a sign of a more favourable prognosis, much recent effort has been directed towards more cellular therapies. NK cells can be expanded and activated *ex vivo* to enhance their anti-tumour action (Shimasaki *et al*., 2020), while immune checkpoint blockade therapy can potentiate the body's natural immune response (Conway *et al*., 2018). There are two immune checkpoints, cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and PD-1, also known as CD279, and both are receptors on the T cell surface that negatively regulate T cell activity to prevent excessive inflammation. Nivolumab (Opdivo) and cemiplimab (Libtayo®) are recombinant human monoclonal antibodies that block PD-1 that normally prevents T cells from recognizing and killing cancer cells. Immunotherapy has come a long way from the first bone marrow transplants following irradiation therapy in the early 1960s, and more recently has been the development of chimeric antigen receptor (CAR) T-cell therapy (Singh and McGuirk, 2020). Two of the first examples of where T cells are redirected against tumour cells were engineered T cells that recognized and destroyed B cells that expressed the CD19 antigen, these FDA-approved medicines, axicabtagene ciloleucel (Yescarta®)

and tisagenlecleucel (Kymriah®) have been very effective against certain B cell lymphomas and leukaemias. The initial trials with CAR T cells used autologous T cells, but now efforts are being made to use 'off-the-shelf' allogeneic T cells with their obvious advantages (Depil *et al*., 2020). **See also**: **Tumour Immunology**; **Cancer Vaccines**

Apart from the unpleasant and potentially dangerous side-effects of many anti-cancer treatments, the other major issue has been the development of drug resistance. In the early phase of somatic neoplastic evolution it is likely that growth conforms to a selective sweep model (**Figure 5a**) in which all the early driver mutations are shared; targeting this relatively small set of genetic alterations is likely to be more successful than treating at a later stage when branched evolution has generated greater IGH, with many subpopulations having acquired subclonal ('private') mutations. It is likely that the selectivity of drugs exerts an evolutionary pressure on a cancer leading to the outgrowth of these minor (resistant) clones.

Glossary

Caretaker gene A gene encoding a protein involved in the maintenance of genomic integrity, many inherited cancer predisposition syndromes involve these genes.

CAR T cells Chimeric antigen receptor T cells are T cells redirected against a tumour antigen after engineered expression of the CAR.

Driver gene A gene whose altered expression has a demonstrable effect on a cell's phenotype, for example, accelerated cell proliferation.

Dysplasia Abnormal cell and tissue organization preceding malignant growth caused by underlying pathological genetic alteration.

EMT Epithelial–mesenchymal transition describes the conversion of cohesive epithelial cells to a more loosely organized arrangement that facilitates migration either as single or small groups of cells.

Kaplan Meier curve Can describe the survival, measured as a percentage, of a large cohort of patients with the same type of cancer from the time of diagnosis, recorded at small time intervals, usually over a 5-year period.

Liquid biopsy Sampling the blood for circulating tumour cells or tumour products (e.g. tumour DNA), useful as a minimally invasive, patient compliant procedure for diagnosis and the monitoring of treatment.

Metaplasia A major switch in tissue differentiation, most likely initiated at the stem cell level, invariably associated with chronic inflammation and often a forerunner of a dysplasia-carcinoma sequence.

Tumour grading A subjective/objective microscopic assessment of a tumour's differentiation status; poorly differentiated (high grade) tumours are the most aggressive.

Tumour staging An objective assessment of the primary tumour size and extent of regional and distant spread; the existence of distant spread assigns a patient to the most advanced stage irrespective of the other parameters.

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