1

Pathogenesis of Cancer

OUTLINE

The Origin of Cancer

Cancers are often named after tissues or cell types they originate from. For example, *carcinomas* are cancers that originate from epithelial cells covering the skin surface and internal organs (see Figure 1.1). They are derived from the embryonic **ectoderm** and **endoderm**. *Sarcomas* are cancers that arise from bone, connective tissue, fat, or muscle cells derived from the embryonic **mesoderm**. *Gliomas* arise from glial cells (nerve cells) that have become altered. *Myeloma* is a type of blood cancer arising in the bone marrow, from altered plasma cells that produce many abnormal antibodies. *Leukemia* arises from malignant white blood cells (leukocytes). Since cells of various embryonic origins become differentiated in dissimilar ways, their growth and biochemistry are also different. Even though cancer consists of different diseases, all of them have similar neoplasia, anaplasia, and metastasis.

Cancer often originates from agents outside of the human body, illustrated by some cancers occurring in people with similar genetic backgrounds that develop different cancers and live in different countries. A good example is the high incidence of stomach cancer in Japan. If a Japanese person moves to the United States, where stomach cancer rates are low, he or she becomes much less likely to develop the disease. This shows that outside factors are involved, including diet and lifestyle. However, for all people in all countries, the likelihood of older people to develop cancer is higher than for younger people. A 65-year-old person has 10 times higher chances of developing cancer than an adult that is 40 years old. This shows that the development of cancer is a progressive process. Multiple, random changes accumulate with aging, and may be of two types: genetic or epigenetic.

Genetic changes are DNA nucleotide gene sequence mutations or alterations. This affects

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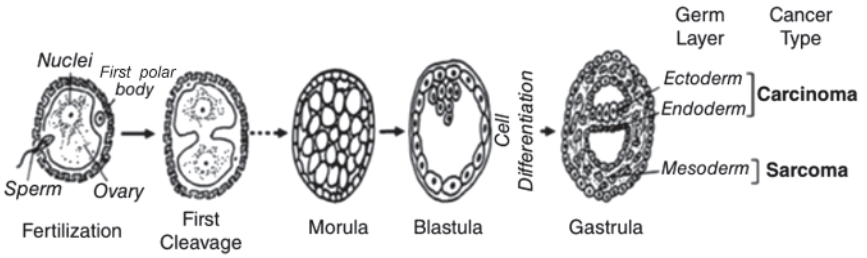

Figure 1.1 Early stages of embryo development and differentiation, leading to the formation of carcinomas and sarcomas. *Source*: Stein and Luebbers [36].

the structure and function of proteins encoded by the affected genes. Mutations may be only changes in one DNA base pair of a gene, known as a **point mutation**. They can also involve a large portion of a chromosome that contains multiple genes being deleted, inserted, duplicated, or translocated. Mutations are either hereditary (familial) or acquired (somatic). Hereditary mutations are also called **germline mutations**. They are inherited from the parents and exist in mostly every body cell. Acquired mutations occur during life, and can be seen only in *some* body cells.

Epigenetic changes influence gene activity and expression, but do not alter the DNA nucleotide sequence. This means that epigenetic changes are not mutations. The two main examples of epigenetic changes are as follows:

- Chemical modification of histones – this means the acetylation, deacetylation, and methylation of special proteins that join with genomic DNA to form chromatin
- Hypomethylation and hypermethylation of cytosine residues in DNA

These epigenetic changes affect the structure and function of **chromatin**, which in turn affects gene expression. If there is histone deacetylation or DNA methylation, chromatin can be condensed. It is then inaccessible for transcription factors required for gene expression. Histone hypoacetylation or DNA hypermethylation can stop the expression of tumor suppressor genes. These genes then cannot prevent the growth and proliferation of cells. This means that carcinogenesis is able to occur.

Every day, we are exposed to carcinogens in the environment that are able to cause both genetic and epigenetic changes (see Figure 1.2). There are three primary categories of carcinogens, as follows:

- Chemical carcinogens – tobacco, alcohol, poor quality foods, arsenic, benzene, beryllium, cadmium, chromium, nickel, radon, vinyl chloride, asbestos
- Biological carcinogens – bacterial or viral infections
- Physical carcinogens – ionizing radiation and ultraviolet radiation

Less often, age-related changes in the immune system and imbalances of hormones contribute to carcinogenesis, and familial genes are also linked to the origin of cancer.

Chemical carcinogens are estimated to cause 80% or more of all cancers. Smoking is a proven cause of lung, oral, pharyngeal, laryngeal, esophageal, colorectal, renal, bladder, hepatic, and cervical cancers, along with acute myeloid leukemia. Tobacco smoke has more than 50 different carcinogenic agents. These include acetaldehyde, benzopyrene, arsenic, and benzene. Chewing tobacco or other smokeless tobacco products increases risks for oral, esophageal, and pancreatic cancers.

The consumption of alcohol is linked to oral, pharyngeal, laryngeal, esophageal, colorectal, hepatic, and breast cancers. Alcohol is thought to increase cancer risks in a variety of ways, which include:

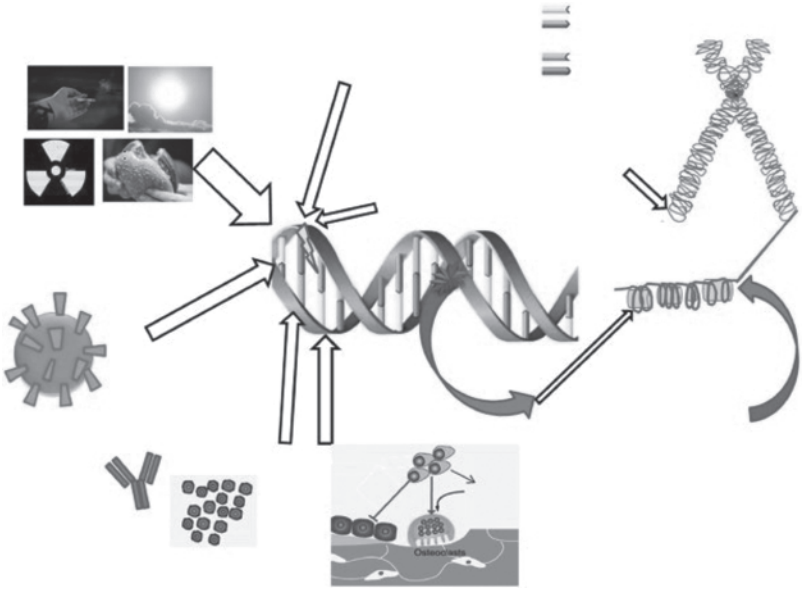

Figure 1.2 The causes of cancer, with mutations due to many different factors. DNA mutations result from the environment, lifestyle choices, epigenetic alterations, viruses, cell microenvironments, heredity, inflammation, and telomere shortening. *Source*: Stein and Luebbers [36].

- Metabolizing ethanol (ethyl alcohol) to acetaldehyde – this is a strong agent with high DNA-damaging characteristics
- Forming reactive oxygen species – via oxidation, these can damage DNA, lipids, and proteins
- Increasing blood levels of estrogen – which is linked to increased risks for breast cancer

Combining alcohol with tobacco smoking results in a much higher risk for a variety of cancers. Asbestos exposure is linked to higher risks for lung cancer and mesothelioma, which is cancer of the pleura lining the chest and abdomen. Asbestos contains six natural fibrous materials. If they enter the deeper lung tissues, they can cause irritation and fibrotic scarring, which has a proven connection with cancer. Therefore, exposure to asbestos eventually results in full-blown lung cancer. Asbestos is released as the brake linings of our cars wear out, and as tires wear down. Asbestos fibers that are inhaled by a smoker increase the risk of developing lung cancer.

About 30% of all types of cancer in the United States and other Westernized countries is related to poor diet. Breast, colorectal, and prostate cancers are highly linked with excessive meat consumption. Animal meat contains proteins, saturated fat, and sometimes, carcinogenic compounds. These include heterocyclic amines as well as polycyclic aromatic hydrocarbons that form as meat is being processed and cooked. Bile acids from the gall bladder chemically alter meat fats inside the intestine, so that the fats can be absorbed. However, bacteria convert the bile acids into *secondary bile acids*, which are carcinogen-promoting agents. High fat content in meat and other animal food sources increases the production of hormones such as estrogen and testosterone, increasing risks for breast and prostate cancers. Many chemicals must be changed inside cells to become carcinogenic. Certain foods and drugs change the enzyme systems that activate carcinogens. For example, phenobarbital stimulates the **P450 enzyme** system, which activates benzopyrene and other carcinogens. Therefore, cancer origination is linked to combinations of dietary and environmental agents.

Coal tar products such as benzopyrene and dibenzanthracene are carcinogens. Benzopyrene

is also a hydrocarbon that is converted via oxidation to extremely reactive compounds such as epoxides. The reactions mainly occur by enzyme-driven catalysis in the liver. The activated carcinogens can then affect other body cells.

Bacterial and viral infections are linked to about 15–20% of all cancers. Chronic infection of the stomach wall with *Helicobacter pylori* is implicated in stomach cancer, and *Chlamydia pneumonia*, in lung cancer. Chronic inflammation from bacterial infections is related to the origin of cancer. Inflammation due to parasites is also related. In developing countries, the parasitic flatworm *Schistosomiasis haematobium* is prevalent, and infestation is linked to bladder cancer. In Asia, *Schistosomiasis japonicum* is linked to colorectal cancer. In relation to viruses, two terms that should be understood are *tumor viruses* and *oncoviruses*. There are five DNA tumor viruses. These include Epstein–Barr virus (EBV), hepatitis B virus (HBV), human herpesvirus 8 (HHV8), human papillomavirus (HPV), and Merkel cell polyomavirus (MCV). There are also 2 RNA viruses linked to cancer, which include hepatitis C virus (HCV) and human T lymphotrophic virus type I (HTLV-1). These viruses cause cancer via the introduction of their genetic material into normal host cells.

In a DNA virus, the genetic material that is used is the DNA. In an RNA virus, the genetic material is the RNA that is reverse-transcribed into the DNA. Either way, the amount of DNA needed to cause cancer is only in one tiny part of the virus genome, and is enough to code for one or several proteins. A viral infection alone is usually not enough to cause cancer, so events such as immunosuppression, genetic disposition, exposure to carcinogens, or somatic mutations are also needed. With acquired immune deficiency syndrome (AIDS), the human immunodeficiency virus (HIV) cannot cause cancer directly. However, HIV damages cellular immunity so much that the individual is more likely to be infected with a tumor virus such as HHV8, causing cancer. Therefore, carcinogenesis requires multiple steps, of which a virus is only one.

Physical carcinogens, such as radiation of various types, are all around us. The X-rays and gamma rays that come from outer space, radioactive portions of the earth, or nuclear power plants are all strong carcinogens. These rays are examples of high-frequency ionizing radiation that can contact DNA molecules in cells and result in direct damage. Ionizing radiation can also produce free radicals that react with and damage DNA. When only low doses of this radiation are present, human cells are able to undergo repair, and will survive. All of us are continually exposed to low levels of ionizing radiation from natural sources. However, dosages of X-rays as used in diagnostic equipment, as well as in the body scanners that are used at airports, need to be kept as low as possible.

Ultraviolet radiation from the sun consists of UVA and UVB rays. The DNA of our skin absorbs UVB more easily than UVA. However, both types of UV rays are able to cause cancer. Melanoma is a common skin cancer caused by UV rays. When UVB is absorbed by DNA, it bonds with nearby thymine base pairs in genetic sequence, forming **pyrimidine dimers**. These dimers are normally removed when DNA is repaired, but if the process becomes defective, the cells survive but have been altered by radiation. They can now develop into cancers. The UV radiation from sunlamps and tanning booths is also carcinogenic.

Significant Point

Some of the earliest evidence of cancer was found in fossilized bone tumor, human mummies from ancient Egypt, and in very old manuscripts. The actual oldest description of cancer, though the term was not yet used, was found in Egypt and dated back to approximately 3000 BC. The *Edwin Smith Papyrus* described eight cases of tumors or ulcers of the breast removed by an early method of cauterization. The papyrus described the disease as being otherwise untreatable.

Cancer Biology

Cancer cell biology is controlled by signaling pathways and molecular components. The cells are able to proliferate, avoid attrition, and invade body tissues. Today we know more than ever about the alterations of cancer cells, and these alterations may help us develop better-targeted therapies against the disease. Each type of cancer is different in phenotypes and heterogeneity created from different types of cells, as well as genetic or epigenetic differences. The two common forms of cancer development include *clonal evolution* and the development of the *cancer stem cell* – both of which influence the ability of cancer to metastasize. Another proposed method of cancer development is *plasticity* between non-cancer stem cells and cancer stem cells (CSCs) – the possibility that non-CSCs are able to reacquire a CSC phenotype. Also, there is a theory about cancer development as a result of inflammation.

The clonal evolution of cancer begins with just one cell. The first mutations that occur result in an ability to proliferate spontaneously, without any need for external cytokines, growth factors, or hormones that are usually required for proliferation. The next generations of the cells are predisposed to acquire more mutations, which continually change cellular behavior. Tumor growth and progression occur because of selective growth of cells after multiple mutations (see Figure 1.3). The steps of cancer progression include *initiation* events during the early stages and *promotion* events that signify obvious neoplasia. There may be months to years in between initiation and promotion. Mutagens are agents that cause initiation. They include benzopyrene, found in tobacco smoke, that causes DNA damage. Once damaged, a cell must multiply to become cancerous, which is a process aided by an irritant that acts as a promoter. Examples of irritants include asbestos and **phorbol esters**, which are uncommon products that come from plants. These and other promoters cause DNA-damaged cells to rearrange their chromosomes during multiplication. This causes even more gene changes. Cancer may develop even when there is a lot of time between the exposures to initiators and promoters.

With the retinoblastoma (Rb) gene mutation, we can see that one genetic defect alone is not enough to cause the development of cancer. Additional genetic changes are required, even though the familial Rb mutation does predispose the individual to cancer. The further genetic or epigenetic changes, from continued exposure to chemicals, hormones, or viruses alter the regulating factors that normally control cell proliferation, differentiation, and cell death.

Since stem cells survive much longer than normal cells, there is an increased chance that they may accumulate genetic mutations. It could take just a few mutations for a stem cell to lose control, potentially becoming a source for cancer. CSCs are a rare subset that can self-renew, differentiate into defined daughter cells, and then initiate and sustain tumor growth in vivo. They have distinctive capacities for self-renewal, proliferation, and

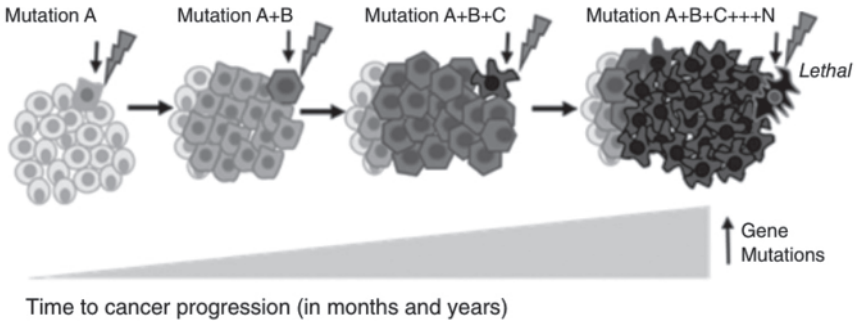

Figure 1.3 The clonal origin of cancer, from one cell, through multiple gene mutations. *Source*: Stein and Luebbers [36].

differentiation. The CSCs are believed to play an important role in cancer initiation, maintenance, progression, resistance to drugs, recurrence, and metastasis. These cells were first identified in leukemia patients during the experimental technique called *xenotransplantation*, and since have been identified in many other malignancies. Treatment failure may be partly due to the ability of CSCs to resist therapy. If they are intrinsically more resistant than other tumor cells, treatments are likely to *increase* the percentage of CSCs in comparison with the percentage before treatment. In a recent study of colon cancer xenografts, the *CD44 + ESA + cells*, which is the population containing colon CSCs, were much greater after treatment with cyclophosphamide.

During cancer progression, tumor cells experience molecular and phenotypic changes. These changes are known as **cellular plasticity**. They are caused by microenvironmental factors, genetic and epigenetic changes, and treatment-related factors. These causes contribute to tumor heterogeneity and resistance to therapy. The most common example of tumor cell plasticity is epithelial–mesenchymal plasticity. The condition of cellular plasticity influences premalignant progression, evolution of tumors, and adaptations to treatments. Changes in cell identity are common at various stages of tumor progression, and it is clear that cellular plasticity is a strong mediator of tumor progression as well as **chemoresistance**. When we understand the mechanisms of cellular plasticity, we may develop new methods of targeting the most lethal aspects – metastasis and resistance to treatment.

Chronic inflammatory processes can develop from infections that are not resolved, abnormal immune reactions to normal tissues, and even obesity. Chronic inflammation leads to DNA damage, and therefore, an increased likelihood of developing cancer. As far back as 1863, an observation was made that cancer often developed at the sites of chronic inflammation. People with chronic inflammatory bowel diseases such as ulcerative colitis and Crohn's disease have an increased risk of colon cancer. As many as 20% of all cancers may be caused or influenced by chronic inflammation. Cytokines are produced, which stimulate the growth of blood vessels that carry nutrients and oxygen to tumors. Free radicals may be generated, causing further DNA damage. Basically, anything that causes inflammation causes DNA to replicate more quickly. The higher the speed of replication, the higher chance of developing cancer. Inflammatory pancreatitis increases risks for pancreatic cancer, and hepatitis increases risks for liver cancer. Chronic inflammation from a *Helicobacter pylori* infection increases risks for stomach cancer. Chronic lung inflammation from asbestos exposure increases risks for mesothelioma. There are many studies investigating the use of anti-inflammatory medications, including aspirin and non-steroidal anti-inflammatory drugs, as a method of reducing the risks for cancer. Inflammation may be the initiator for cancer metastasis as well, by producing chemicals that help tumor cells infiltrate tissues. Table 1.1 summarizes the links between chronic inflammation and cancer.

Table 1.1 Chronic inflammation and various types of cancer.

Inflammatory condition	Causative agents	Types of cancer
Hashimoto thyroiditis, Sjögren syndrome	———————–	MALT lymphoma
Barrett esophagus, reflux esophagitis	Gastric acid	Esophageal carcinoma
Silicosis, asbestosis	Silica particles, asbestos fibers	Lung carcinoma, mesothelioma
Gastritis, ulcers	*Helicobacter pylori*	Gastric adenocarcinoma, MALT lymphoma

(Continued)

Table 1.1 (Continued)

Inflammatory condition	Causative agents	Types of cancer
Pancreatitis	Alcoholism, germ line mutations, such as of the trypsinogen gene	Pancreatic carcinoma
Chronic cholecystitis	Bacteria, bile acids, gallbladder stones	Gallbladder cancer
Hepatitis	Hepatitis B or C virus	Hepatocellular carcinoma
Cholangitis, opisthorchis	Liver flukes (*Opisthorchis viverrini*)	Cholangiocarcinoma, colon carcinoma
Inflammatory bowel disease	————	Colorectal carcinoma
Osteomyelitis	Bacterial infection	Carcinoma in draining sinuses
Chronic cystitis	Schistosomiasis	Bladder carcinoma
Lichen sclerosis	————	Vulvar squamous cell carcinoma
Chronic cervicitis	Human papillomavirus	Cervical carcinoma

Significant Point

Unlike normal cells, cancer cells grow out of control. They ignore signals that "tell" them to stop dividing, to differentiate, or to die. Cancer cells do not recognize their own boundaries and can spread to other sites in the body. Several genes mutate in cancer cells and their function becomes defective. Abnormal cell division occurs when active oncogenes are expressed, or when tumor suppressor genes are lost.

Genetics and Cancer

There are genetic conditions that make the development of cancer more likely, though they do not actually cause it. Familial polyposis is one example, in which there are polyps or other small growths in the intestinal tract. These can easily transform into cancer. When a person has such a genetic defect, the chance of developing cancer is higher because the DNA is not repaired very well. Conditions that involve inherited defects of the DNA repair system include **Bloom syndrome**, **Fanconi anemia**, and **Werner syndrome**. Mutations of the DNA repair genes *breast cancer 1 (BRCA1)*, *breast cancer 2 (BRCA2)*, *tumor protein p53*, and *retinoblastoma (Rb)* are linked to inherited breast, ovarian, pancreatic, and prostate cancers. These particular genes are also described as *tumor suppressor genes*. To avoid the development of cancer, it is crucial that damaged DNA can be correctly repaired. Even so, a large number of cancers are not inherited. Generally, inherited mutations are present in about 7.5% of all cancers. Today, there is ongoing study about hereditary genetic mutations that increase the likelihood of cancer development, to use this information for the surveillance and prevention of cancer. Table 1.2 illustrates specific cancer genes with inherited predisposition for specific cancerous conditions.

Genetic tests for hereditary cancer syndromes can detect if a person from a family that shows the signs of a syndrome has a suspected gene mutation. The tests also show if family members with no obvious signs of disease have the same gene mutation. Genetic testing is often recommended when there is a personal or family

Table 1.2 Specific cancer genes with inherited predispositions for cancer.

Cancer genes	Inherited predisposition
Autosomal dominant cancer syndromes	
APC	Familial adenomatous polyposis/colon cancer
BRCA1, BRCA2	Breast and ovarian tumors
CDKN2A	Melanoma
MEN1, RET	Multiple endocrine neoplasia 1 and 2
MLH1, MSH2, MSH6	Hereditary nonpolyposis colon cancer
NF1, NF2	Neurofibromatosis 1 and 2
PTCH1	Nevoid basal cell carcinoma syndrome
RB	Retinoblastoma
TP53	Li–Fraumeni syndrome, with various tumors
Autosomal recessive syndromes, with defective DNA repair	
ATM	Ataxia-telangiectasia
BLM	Bloom syndrome
Various genes involved in DNA cross-link repair	Fanconi anemia
Various genes involved in nucleotide excision repair	Xeroderma pigmentosum

history suggesting an inherited cancer risk condition. This type of testing is often very helpful in guiding future medical treatments. If a cancer susceptibility variant is present in a family, not everyone who inherits the variant automatically develops cancer. This is influenced by **penetrance**. When not everyone carrying the variant develops the disease related to the variant, it is said to have *incomplete* or *reduced* penetrance. Hereditary cancer syndromes also vary in expressivity. This means that those with the variant may be different in the extent that they exhibit signs and symptoms of the syndrome. Disease expression is also influenced by lifestyle and environment. An extended list of hereditary cancer syndromes includes the following:

- BRCA1 hereditary breast and ovarian cancer syndrome – also called familial susceptibility to breast/ovarian cancer 1 or BROVCA1
- BRCA2 hereditary breast and ovarian cancer syndrome – also called familial susceptibility to breast/ovarian cancer 2 or BROVCA2
- Cowden syndrome – also called Cowden disease
- Diaphyseal medullary stenosis with malignant fibrous histiocytoma – also called bone dysplasia with medullary fibrosarcoma
- Duodenal carcinoid syndrome
- Endolymphatic sac tumor
- Familial adenomatous polyposis
- Familial isolated pituitary adenoma
- Gardner syndrome
- Hereditary diffuse gastric cancer
- Hirschsprung disease ganglioneuroblastoma
- Li–Fraumeni syndrome
- Lynch syndrome
- Multiple endocrine neoplasia type 1 (MEN1)

- Multiple endocrine neoplasia type 2A (MEN2A)
- Multiple endocrine neoplasia type 2B (MEN2B), and formerly, multiple endocrine neoplasia type 3
- Perlman syndrome
- Pheochromocytoma
- Stewart Treves syndrome
- Von Hippel–Lindau disease

Significant Point

Though nearly every family has members that have had cancer, this does not generally mean they have a hereditary predisposition. Most cases of cancer originate from a combination of external influences such as environmental and lifestyle factors, along with a small genetic component. Only a genetic defect can be inherited – in other words, a genetic predisposition, but not cancer itself.

Cell Division

Complicated regulatory networks cause cells to proliferate and maintain homeostasis in tissues or organs that they originated in. The circuits within these networks are made up of enzymes and proteins. Their expression as well as activity is linked to mitogenic signals from cytokines, growth factors, and other extracellular molecules in the surrounding tissues. Random gene mutations encoding the enzymes and proteins disrupt activation and expression, allowing cells to change in function, increasing the likelihood of cancer. All types of cancer need the following six characteristics to progress to metastatic cancer:

- Proliferative autonomy
- Resistance to anti-proliferative signals
- Evasion of **apoptosis**
- Unlimited ability to replicate
- Tumor neovascularization
- Invasion and metastasis

Cell division occurs step by step, through four unique phases or periods (see Figure 1.4). The steps are described as follows:

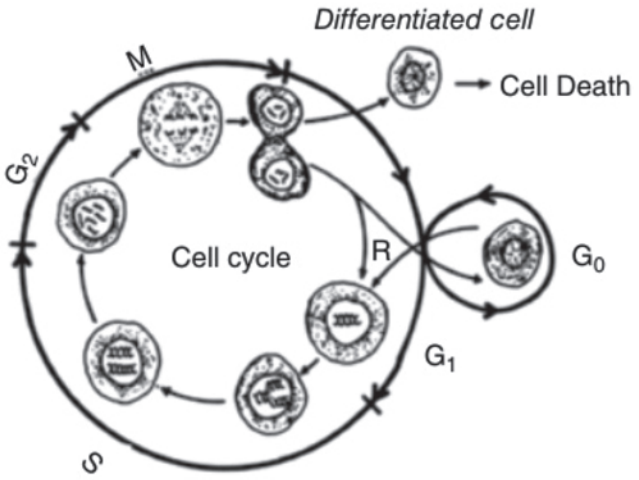

Figure 1.4 The cell cycle and differentiation, in which each division will result in two daughter cells being generated. Note that cancer cells stay in the cycle without differentiating or entering G0, regardless of any improper conditions. *Source*: Stein and Luebbers [36].

- Gap 1 (G1) – A biochemically active period involving synthesis of protein and RNA as the cells grow before dividing; this lasts until the start of DNA synthesis
- Synthesis (S) – The cells start to duplicate genetic material
- Gap 2 (G2) – Newly replicated chromatin is condensed, forming chromosomes
- Mitosis (M) – Duplicated chromosomes separate, and cell division begins; if there is a lack of enough nutrients or growth factors, cells exit their cycle to enter a resting state known as quiescence or G0; if conditions become favorable again, the cells can reenter the cell cycle; the "decision" to remain in the cycle or go into quiescence is made during G1

Also, after mitosis (M), cells can exit the cycle and differentiate, performing different variations of normal functions. For example, nerve cells can conduct impulses and muscle cells can cause contraction.

Significant Point

In 2020, it was reported that the protein LEM2 was discovered to have two important functions during cell division. The protein first creates "seals" in the protective coating of nuclei that are forming, keeping the two sets of DNA from being damaged. Next, LEM2 recruits factors that disassemble the "machinery" of fibers that are responsible for separating the DNA sets. Scientists devised a method that allowed them to actually view this process in the laboratory.

Cell Cycle

The events in the cell cycle are regulated by proteins, such as cyclins and cyclin-dependent kinases (CDKs). This means that the cells will only divide when necessary, when circumstances are adequate. Normal cells need the mitogenic signals from external growth factors. These growth factors include the following:

- Epidermal growth factor (EGF)
- Fibroblast growth factor (FGF)
- Platelet-derived growth factor (PDGF)
- Insulin-like growth factor (IGF)

These growth factors help the cells to move from the G0 state into active proliferation. Growth factors bind to similar transmembrane receptors. They transmit mitogenic signals required for G0 or G1 phase cells to grow and conduct proper DNA synthesis. When growth factors are not present, normal cells stop dividing. However, cancer cells are different, continuing to divide regardless of whether growth factors are present. This is proliferative autonomy, occurring because of genetic or epigenetic changes that may change the structure of growth factor receptors, or cause extreme overexpression of the growth factors. In some breast cancers, shortened EGF receptor (EGFR) continually sends signals that stimulate growth even when there is no EGF binding. In various other breast cancers, *human epidermal growth factor receptor 2 (HER2)/neural (neu)* receptor overexpression causes cells to be over-responsive to low levels of growth factor, which usually would be insufficient to cause cell division.

Cells also contain integrins, which are transmembrane receptors that can promote the active cell cycle. Integrin receptors are heterodimer proteins. They attach the cytoskeletons of cells to extracellular matrix, which makes up about 30% of the tissue in each organ. The extracellular matrix is noncellular material consisting of a "mesh" of glycoproteins, proteins, proteoglycans, and polysaccharides. These substances provide structure and biochemical activity, along with being needed for chemical signaling. There are 24 or more heterodimer integrin receptors. They are formed by 18 alpha-subunits combining with 8 beta-subunits, and have specific binding to certain protein portions on the extracellular matrix. Integrin receptors are activated to generate signals in cells that influence their proliferation, survival, migration, and invasion. In cancer, integrin heterodimers that favor growth and proliferation are significantly upregulated.

Proliferative autonomy is also due to changes in fundamentals of downstream effector proteins. These proteins receive and process signals from ligand-activated growth factor receptors as well as integrin receptors. Proliferative signaling in normal tissues is opposed by many anti-proliferative signals, which result in cellular quiescence. Tissue homeostasis requires this delicate balance. Anti-proliferative signals come from growth inhibitory molecules on the surface of contacted nearby cells, or within the extracellular matrix. The signals also come from soluble growth inhibitory factors.

Growth and proliferation of normal cells are regulated via interactions between growth factor signaling, nutrient availability, and cell density. Normal cells require a minimum area of space that they can spread in, for normal growth. Cells that are widely spaced grow and divide, eventually covering all available space. With increased cell density, the cells touch each other. Movements are restricted, and growth stops even though there are enough nutrients and growth factors. This is called *density dependent* or *contact inhibition* of cell growth and proliferation. This is needed to regulate normal tissue growth, differentiation, and development. Contact inhibition regulates the size of each organ as it develops. When tissues are injured, it also plays a role. If the liver is partially resected, the hepatocytes become mobilized for rapid division so that the organ can regenerate. Upon reaching its normal size, the liver stops growing – controlled by the hepatocytes to prevent overgrowth. Most cancer cells do not behave in this manner – continuing to grow and proliferate regardless.

Programmed cell death, or apoptosis, is a genetically controlled type of cell death that occurs naturally, often initiated by the Fas ligand and tumor necrosis factor (see Figure 1.5). Dysfunctional or unneeded cells within tissues self-destruct, known as *cellular suicide*. This occurs in what appear to be normal physiological conditions. Over 10 billion cells self-destruct every day in the human body, balancing the production of new cells from stem cells. Apoptosis is needed for tissue homeostasis, immune tolerance, and embryogenesis. It stops unwanted cell proliferation, which could lead to tumorigenesis. If apoptosis becomes dysregulated, there can be one of two conditions:

- Tissue outgrowth – cells die slower than they can divide, as in cancer
- Tissue atrophy – cells die faster than they can divide, as in neurodegenerative diseases

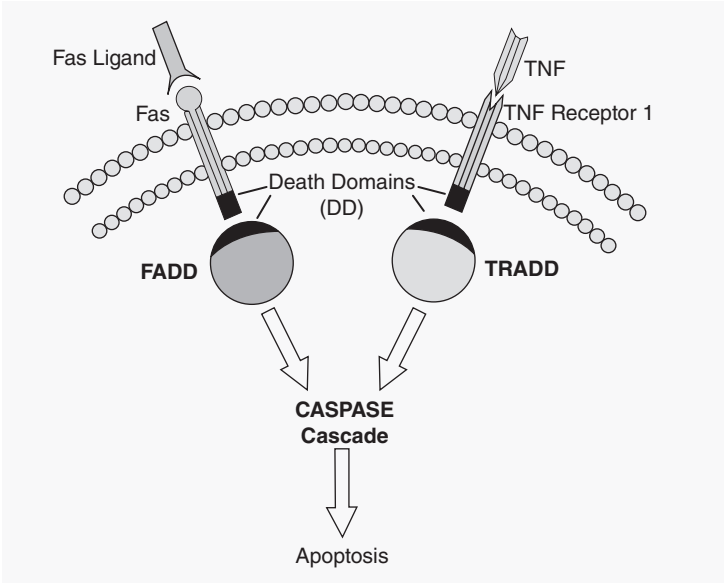

Figure 1.5 Apoptosis, as related to the effects of the Fas ligand and tumor necrosis factor (TNF). *Source*: Israels and Israels [18].

Significant Point

Apoptosis is initiated by two primary pathways: the mitochondrial (intrinsic) pathway and the death receptor (extrinsic) pathway. The intrinsic pathway is triggered by loss of survival signals, DNA damage, and the accumulation of abnormally folded proteins. The extrinsic pathway is triggered by involvement of death receptors, from the *TNF receptor family*, by ligands on nearby cells.

Carcinogenesis

Cancer cells, to continue proliferating, must acquire *replicative immortality*. For carcinogenesis, the cells must overcome *replicative senescence*. **Telomeres** are structures of DNA protein at the ends of chromosomes. They are the primary determinant of replicative potentials of cells. They protect the ends of linear chromosomes from fusing together, which maintains genome function, integrity, and stability. Telomeres have several **kilobase pairs** of double-stranded DNA. The pairs are made of hexanucleotides sequence repeats. The **end replication problem** is caused by a limitation of the DNA polymerases to completely replicate the ends of chromosome DNA duplexes. Therefore, the DNA polymerase that duplicates chromosomal DNA leaves 50–200 base pairs in the ends of telomeric DNA strands unreplicated. This causes telomere shortening after each round of DNA replication. Since most somatic cells do not express telomerase, their telomeres are progressively shortened, reaching a dangerously short length as telomeres become dysfunctional. Cells that do not enter senescence die from p53-controlled apoptosis. Senescence and apoptosis help prevent proliferation of cells that are at risk of genome instability, which may lead to neoplastic transformation. Just one in 10 million cells that survive experience genome instability, acquiring gene mutations. They can develop unlimited proliferative potential, known as **immortalization**, from additional genetic or epigenetic changes. The catalytic subunit of telomerase (*telomerase reverse transcriptase*) is expressed. It activates telomerase, stabilizing telomeres for continued proliferation. Nearly all (90%) of cancer cells need telomerase for stability of the telomeres. The other 10% experience *alternative lengthening of telomeres*. So, telomere dysfunction and genome instability, without normal senescence or an apoptotic response with telomere stabilization, allow cells to have unlimited proliferation.

The p53 protein is usually mutated late during tumorigenesis in the majority of organs, but primarily the bladder, colon, pancreas, and prostate. However, p53 mutation is also found in ductal cell carcinoma in situ, which is a *premalignant* lesion of the breast. Also, p53 is lost or mutated early in tumorigenesis of astrocytomas and esophageal adenocarcinomas. In liver cancer, p53 as well as pRb may be eliminated early during tumorigenesis. Germline p53 mutations are linked to carcinomas of the adrenal cortex, breast, gastrointestinal tract, lungs, sarcomas of soft tissues and bone, and lymphomas – but at much earlier ages than expected. Germline pRb mutations are linked to childhood retinoblastoma, plus a predisposition for osteosarcoma. There appears to be a strong relationship between loss of pRb and lack of functional p53 in different tumors. Loss of function of both p53 and pRb is needed for cancer cells to have replicative immortality and to become tumorigenic.

Tumor cells, like normal cells, need a good supply of nutrients and oxygen, plus ways to discharge carbon dioxide and metabolic waste. Primary sources for this are the blood vessels and lymphatic vessels. During pre-malignancy, tumors are separated from vascularized peritumoral tissue by a basal lamina. This does not allow blood vessels to infiltrate the tumors. Without vasculature, tumors stop growing past about 1–2 mm in diameter, becoming necrotic

or apoptotic. Tumors must have neovascularization that can deliver blood to intratumoral spaces for survival and transformation into a fully malignant form. Neovascularization is mostly achieved via angiogenesis, in which new blood vessels develop from preexisting vessels.

Angiogenesis starts as vascular endothelial cells being to proliferate, as a response to angiogenic stimuli. They then acquire an angiogenic phenotype. In adults, endothelial cells of mature blood vessels are mostly dormant and only divide once per every 1,000 days. Endothelial cell growth and proliferation initiates angiogenesis. Hypoxia or oncogene activation causes the cells of quickly growing tumors to secrete vascular endothelial growth factor (VEGF) and basic fibroblast growth factor. These factors diffuse through nearby tissues, binding to similar receptors on endothelial cells of preexisting blood vessels. The endothelial cells are stimulated to secrete proteases such as **matrix metalloproteases** (MMPs). These degrade the basement membrane and the peri-vascular extracellular matrix. Proliferating endothelial cells can then escape and migrate toward the source of the angiogenic stimulus. *Primary sprouts* are formed, and extending sprouts have two distinct forms of endothelial cells: *tip cells* and *stalk cells*.

Tip cells only proliferate slightly, but allow breakdown of the extracellular matrix around preexisting blood vessels. There is growth of new sprouts toward tumor cells secreting angiogenic factors. The stalk cells have active proliferation, extending sprouts and promoting the formation of the vascular lumen. The endothelial cells stabilize the vasculature via secretion of the platelet-derived growth factor. This attracts pericytes and smooth muscle cells, forming a basement membrane around new vesicles. Without the basement membrane, the new vessels are unstable and often regress. The formed blood vessels bring blood to the tumors and sustain the proliferating tumor cells.

Antiangiogenic molecules also regulate angiogenesis. Proangiogenic factors must be accompanied with downregulation of antiangiogenic molecules that restrict vascular growth. The most prevalent proangiogenic factor is VEGF. The levels of angiogenic factors and their receptors are prognostic about the state of cancer progression. Expression of the VEGF family of angiogenic factors is linked to the progression of breast, colon, head and neck, lung, stomach, and uterine cancers. The amount of neovascularization between these cancers is varied, however. Highly aggressive pancreatic ductal carcinomas are largely avascular, while renal and pancreatic neuroendocrine carcinomas are highly vascularized. This is because some tumors form and progress by using preexisting vessels without angiogenesis.

Natural antiangiogenic factors include **angiostatin**, thrombospondin-1 (TSP-1), endostatin, interferon, and metalloprotease inhibitors. They can cause apoptosis in tumor cells and endothelial cells. They also block migration and formation of lumens in sprouting endothelial cells. The factors are often downregulated in tumors, from loss of function of tumor suppressor genes. Synthetic inhibitors of angiogenesis include sorafenib, sunitinib, and temsirolimus. They are being studied in combination with chemotherapies to treat various aggressive cancers. Antiangiogenic drugs may improve the delivery of chemotherapy to tumors by normalizing tumor blood vessels. New vessels in tumors are both structurally and functionally abnormal. The blood vessels are immature, and leakage occurs. Such abnormalities affect the delivery of chemotherapies to tumors. Because of excessive proangiogenic stimuli, antiangiogenic drugs may normalize tumor vessels by neutralizing angiogenic stimuli. However, antiangiogenic drugs interfere with blood pressure, wound healing, clotting, and kidney function – increasing risks for heart attacks and strokes. Newer antiangiogenic drugs with selective effects on tumor vessels are needed to treat aggressive cancers.

Significant Point

The basics of carcinogenesis include oncogenes – the genes that are activated in tumors – acting at key stages of proliferation, differentiation, and programmed apoptosis, gene mutations that lead to cells no longer responding to external regulation, and combined disorders of several oncogenes that lead to malignant transformation.

Mutation and Cancer

Gene mutations related to cancer can be extremely varied. **Driver mutations** are those that change the function of cancer genes, directly contributing to the disease's development and progression. Driver mutations are usually acquired and less often inherited. **Passenger mutations** are acquired and do not affect cellular behavior. They occur randomly, throughout the entire genome, while driver mutations are usually clustered tightly in cancer genes. In cancers caused by carcinogen exposure, such as lung cancer and melanoma, there are many more passenger mutations than driver mutations. The carcinogens that a person is exposed to directly cause most genomic damage. Passenger mutations create genetic variants that may give tumor cells an advantage over normal cells. There may be mutations that result in drug resistance. It appears that the effects of treatment convert neutral passenger mutations *into* driver mutations, making the cancer more difficult to cure or treat.

Point mutations activate or inactivate protein production by affected genes, based on position and strength. These mutations, when converting proto-oncogenes into oncogenes usually produce a gain or function. They do this by changing amino acid residues in part of the microenvironment that usually keeps the protein's activity controlled. A good example is with the proteins that convert the RAS gene into a cancer gene. Oppositely, point mutations in tumor suppressor genes limit or stop functions of the encoded proteins. The TP53 gene is often affected by point mutations.

Gene rearrangements may be caused by chromosomal inversions or translocations. These occurrences are greatly linked with neoplasms that are derived from hematopoietic and other mesenchymal cells. There are two ways that gene rearrangements activate proto-oncogenes, as follows:

- Removing proto-oncogenes from normal regulatory elements – the rearrangements place them under the control of a highly active enhancer or promoter, as occurs with B cell lymphomas. In over 90% of patients with Burkitt lymphoma, there is a translocation, usually between chromosomes 8 and 14. This results in overexpression of the MYC gene on chromosome 8, via juxtaposition with regulatory elements of the immunoglobulin heavy chain gene on chromosome 14. With follicular lymphomas, there is a reciprocating translocation between chromosomes 14 and 18. This results in the overexpression of BCL2 on chromosome 18, and is also controlled by immunoglobulin gene regulatory elements.
- Creation of fusion genes that encode certain chimeric proteins – for example, with the Philadelphia chromosome in chronic myeloid leukemia, there is a balanced, reciprocal translocation between chromosomes 9 and 22. Therefore, the Philadelphia chromosome (22) appears smaller than usual. This change occurs in over 90% of chronic myeloid leukemia cases. It causes fusion of parts of the BCR gene on chromosome 22, and of the ABL gene on chromosome 9. The small amounts of Philadelphia chromosome-negative cases have a cytogenetically silent BCR-ABL fusion gene. Its presence is the essential component of chronic myeloid leukemia.

Chromosomal translocations have two modes of alteration. They can break initially, followed by more mobility of their broken ends, resulting in juxtaposition. The second mode is when they contact each other initially, suffering breaks, and then juxtaposition – see Figure 1.6.

Deletions are also common, in which specific chromosomal regions are removed, which can cause the loss of certain tumor suppressor genes. There is often an inactivating point mutation in one allele. This is then followed by the deletion of the other allele, even though it is not mutated. **Gene amplifications** convert proto-oncogenes to oncogenes, with resulting overexpression and hyperactivity of proteins that would otherwise be normal. There can be several hundred copies of the gene, with either multiple small, extrachromosomal structures known as **double minutes**, or *homogenously staining regions*. Important examples of gene amplifications include the NMYC gene (neuroblastoma) and the HER2 gene (breast cancer). **Aneuploidy** is the number of chromosomes that is not a multiple of 23, which is often linked to cancer.

Significant Point

Usually, several gene mutations are needed before a cell becomes malignant. Most cancers begin because of acquired gene mutations over time. Sometimes the mutations are caused by external factors such as tobacco use or exposure to sunlight, but they can also be random events that happen without any understood cause. Genetic testing is indicated if one or more family members already have a known gene mutation.

Metastasis

Metastasis is the deadliest occurrence related to cancer. Tumor cells invade nearby tissues and use the blood or lymphatic vessels to spread, when conditions for this are favorable. Whether a blood or lymphatic vessel is entered is based on the amount and easiness of accessibility of the

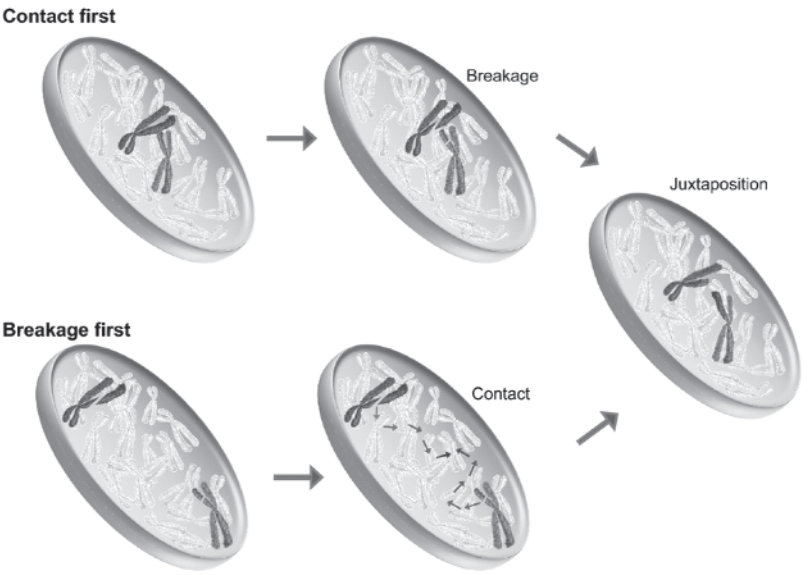

Figure 1.6 The two modes of alteration in chromosomal translocations. *Source*: Javadekar and Raghavan [19].

vessels. A vasculature network formed by angiogenesis means that cancer cells are more accessible to blood vessels than lymphatic vessels. Often, lymphatic vessels within tumors are not as highly functional as those formed outside of tumors. Tumor cells must then invade through nearby connective tissues to enter functional lymphatic vessels. So, most tumor cells utilize the bloodstream for invasion and metastasis. Those that enter the lymphatic vessels often eventually also enter the blood vessels, since the lymphatic vessels drain into the venous blood as they join the large thoracic duct. On the way to the thoracic duct, a series of lymph nodes can be encountered. They are often the first sites of tumor cell metastasis. The tumor cells can also invade localized lymph nodes directly via penetration through nearby connective tissue. This is why lymph nodes closer to a primary tumor are evaluated for tumor cells, as an early indicator of metastasis.

Metastasis involves migration (invasion) of tumor cells at the initial site into surrounding normal tissues to reach blood or lymphatic vessels. The cells must **intravasate** the endothelial barrier to enter the circulation. They must **extravasate** from a distant capillary bed so that they can colonize (grow) at a secondary location (see Figure 1.7).

Invasion is the first step in metastasis, involving migration of tumor cells toward the blood or lymphatic vessels. Solid tumor cells must cross the basement membrane, an extracellular matrix made of collagen and many other cell types. Metastasizing cells then migrate through the extracellular matrix to reach the blood or lymphatic vessels. For motility, cancer cells develop morphological and migratory characteristics; due to changes in the expression of oncogenes and tumor suppressor genes. These cells usually have increased contractility, migrating individually or in a stream of cells. However, cells with a mesenchymal phenotype have lower contractility, migrating in clusters of at least five cells – such as with breast and colorectal tumors.

Tumor cells must use genes involved in embryonic development to develop factors needed for them to invade and metastasize. Tumor cells acquire an epithelial–mesenchymal transition (EMT)-regulated mesenchymal phenotype during invasion. However, they later change to the epithelial phenotype in metastasized tumors at a secondary location. They are similar, both

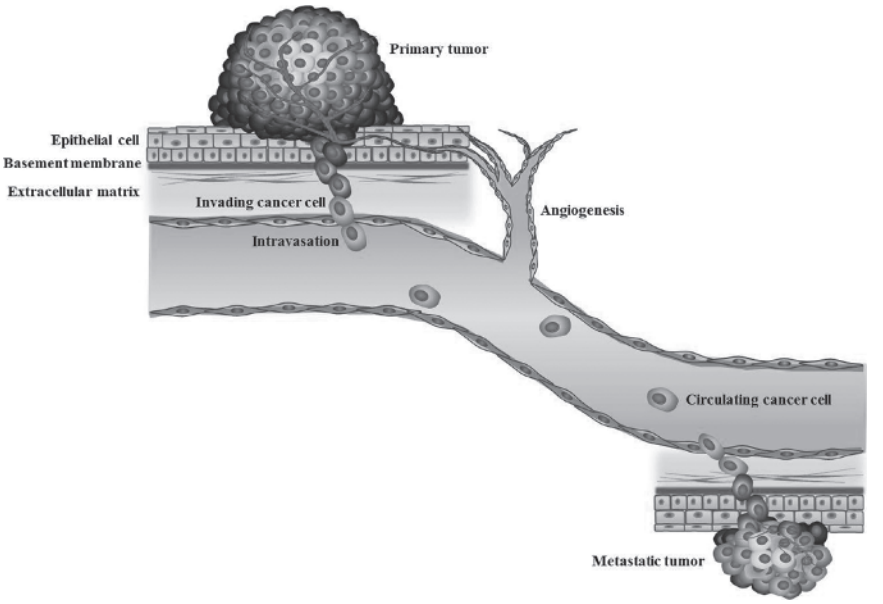

Figure 1.7 Biological processes linked to cancer spread from a primary site to other body organs. *Source*: Courtesy: "From Invasion to Metastasis".

histopathologically and morphologically, to cells in primary tumors before EMT. Therefore, the mesenchymal phenotype is only temporary, during invasion and metastasis. Returning to an epithelial phenotype requires a reversal of the EMT process, called *mesenchymal–epithelial transition* (MET). The metastasized cells become noninvasive so that they can grow and colonize at the secondary location. These transitions are still not fully understood.

Intravasation, the entry of cancer cells into the blood or lymphatic vessels, determines the amount of tumor cells that will metastasize, and the likelihood of tumor formation at another body site. Intravasation occurs naturally as part of embryonic development, wound healing, and to fight infections. The ability of cancer cells to intravasate the vessels is not as efficient as it is in these processes. Tumor cells can intravasate lymphatic vessels more easily, since cell–cell junctions of endothelial cells in peripheral lymphatic walls have spaces of about 3 μm – large enough for cancer cells to squeeze through. In the blood vessels, the junctions are much tighter, and endothelial cells are also supported by a basement membrane stabilized with **pericytes**. To cross, the cancer cells must experience cytoskeletal changes as well as upregulate integrins and other adhesion molecules. Also, macrophages exiting blood vessels produce the EGF, which guides cancer cells to them. Cancer cells with the mesenchymal phenotype may be able to move with endothelial cells to enter blood vessels at locations where the macrophages exit. Cancer cells can also passively enter the circulation via **tumor neovasculature**. Usually, the vasculature of tumors is disorganized and leaky. The looser vascular basement membrane plus endothelial cells and pericytes create holes that are about 2.5 μm in size, allowing cancer cells to move through. Growing tumors push cells against the blood vessels, forcing them through the holes. Entry of cancer cells in this way does not greatly disrupt the basement membrane.

In the bloodstream, there are damaging mechanical forces, including those from blood flow itself. **Shear stress** is so extreme that more than 99.9% of cancer cells die in a few minutes. Cytoskeletons are fragmented, and the cells become microparticles absorbed by myeloid cells. However, the cells can be shielded if they aggregate along with platelets and coagulation factors into **cell pellets**. The cancer cells in the center can survive. Also, single cancer cells with an amoeboid-like morphology usually survive by attaching to the substratum, and developing more contractility.

Circulating tumor cells also have to evade the immune system. Cells in solid tumors are protected by the strong immunosuppressive microenvironment within the tumors. Once a tumor cell is in the bloodstream, they are exposed to aggressive antitumor activity and destruction. Natural killer (NK) cells are the biggest threat. They contain *NK group 2d (NKG2D)* receptors able to recognize *major histocompatibility complex (MHC)* class 1 polypeptide-related sequences A and B, also known as *MICA* and *MICB*. These are expressed on malignant cell surfaces. *Cytolytic granzyme B* and **perforin** are secreted, which destroy tumor cells.

Colonization of tumor cells at secondary sites is the least efficient part of the metastatic process. Most tumor cells that infiltrate distant organs are unable to develop overt metastasis. They must colonize the tissues, which of course sometimes occurs. The most likely sites in which this occurs include the bones and liver. Tumor cells must *seed* or colonize, and some organs allow them to do this with much greater ease than other organs. Effective colonization is also based on tumor cells' intrinsic ability to survive hostile tissue environments. Infiltrated tumor cells express specific genes in various organs that encourage metastatic growth.

Transendothelial migration is better in the liver and bones, compared with the brain and lungs. In the liver and bones, there are sinusoidal capillaries consisting of discontinuous basal lamina and fenestrated endothelial cells. These allow extravasation and a higher incidence of metastasis. In the lungs, the tight endothelial junctions are not as conducive for extravasation. Brain capillary walls are also strengthened by **astrocytes** and pericytes that make up the blood–brain barrier.

Significant Point

When cancer metastasizes, there can be some signs and symptoms that may resemble those of a variety of other diseases. These include progressive headaches, weakness, seizures, balance problems, pain, bone fractures, dizziness, shortness of breath, jaundice, and inflammation in body areas related to the type of cancer. In some patients, metastatic cancer causes no symptoms, and is discovered incidentally.

Key Terms

Aneuploidy
Angiostatin
Apoptosis
Astrocytes
Bloom syndrome
Cell pellets
Cellular plasticity
Chemoresistance
Chromatin
Deletions
Double minutes
Driver mutations
Ectoderm
Endoderm
End replication problem
Epigenetic
Extravasate
Fanconi anemia
Gene amplifications
Gene rearrangements
Germline mutations
Immortalization
Intravasate
Intravasation
Invasion
Kilobase pairs
Matrix metalloproteases
Mesoderm
Metastasis
P450 enzyme
Passenger mutations
Penetrance
Perforin
Pericytes
Phorbol esters
Point mutation
Pyrimidine dimers
Shear stress
Telomeres
Tumor neovasculature
Werner syndrome

Bibliography

1 Ahmad, A. (2016). *Introduction to Cancer Metastasis*. Cambridge: Academic Press.
2 Armstrong, S. (2014). *P53: The Gene that Cracked the Cancer Code*. London: Bloomsbury Sigma.
3 Banfalvi, G. (2017). *Cell Cycle Synchronization: Methods and Protocols (Methods in Molecular Biology, 1524)*, 2nd Edition. Totowa: Humana Press.
4 Bunz, F. (2016). *Principles of Cancer Genetics*, 2nd Edition. New York: Springer.
5 Chen, G.G. and Lai, P.B.S. (2009). *Apoptosis in Carcinogenesis and Chemotherapy (Apoptosis in Cancer)*. New York: Springer.
6 Compton, C. (2020). *Cancer: The Enemy from Within: A Comprehensive Textbook of Cancer's Causes, Complexities and Consequences*. New York: Springer.

7 DeVita, V.T., Jr., Lawrence, T.S., and Rosenberg, S.A. (2020). *Cancer: Principles and Practice of Oncology: Primer of the Molecular Biology of Cancer*, 3rd Edition. Philadelphia: Lippincott, Williams, and Wilkins.

8 Evens, A.M. and Blum, K.A. (2016). *Non-Hodgkin Lymphoma: Pathology, Imaging, and Current Therapy, Indexed in PubMed/Medline (Cancer Treatment and Research)*. New York: Springer.

9 Fang, D. and Han, J. (2020). *Histone Mutations and Cancer (Advances in Experimental Medicine and Biology, 1283)*. New York: Springer.

10 Frank, D.A. (2012). *Signaling Pathways in Cancer Pathogenesis and Therapy*. New York: Springer.

11 Gerstman, B.S. (2020). *The Physics of Cancer: Research Advances*. Singapore: World Scientific Publishing Company Pte. Ltd.

12 Gilkes, D.M. (2019). *Hypoxia and Cancer Metastasis (Advances in Experimental Medicine and Biology, 1136)*. New York: Springer.

13 Giordano, A. and Galderisi, U. (2010). *Cell Cycle Regulation and Differentiation in Cardiovascular and Neural Systems*. New York: Springer.

14 Gotta, M. and Meraldi, P. (2017). *Cell Division Machinery and Disease (Advances in Experimental Medicine and Biology, 1002)*. New York: Springer.

15 Gregory, C.D. (2016). *Apoptosis in Cancer Pathogenesis and Anti-cancer Therapy: New Perspectives and Opportunities (Advances in Experimental Medicine and Biology, 930)*. New York: Springer.

16 Grundman, E. (2012). *Current Topics in Pathology, 67: Carcinogenesis*. New York: Springer.

17 Heim, S. and Mitelman, F. (2015). *Cancer Cytogenetic: Chromosomal and Molecular Genetic Aberrations of Tumor Cells*, 4th Edition. Hoboken: Wiley-Blackwell.

18 Israels, L.G. and Israels, E.D. (2009). *Stem Cells Journals Volume 17, Issue 5, P. 306–313 Special Feature: Apoptosis*. Durham: AlphaMed Press. Figure 4.

19 Javadekar, S.M. and Raghavan, S.C. (2015). *The FEBS Journal Volume 282, Issue 14, P. 2627–2645 Review Article: Snaps and Mends: DNA Breaks and Chromosomal Translocations*. Cambridge: Federation of European Biochemical Societies. Figure 6.

20 Juan, H.F. and Huang, H.C. (2018). *A Practical Guide to Cancer Systems Biology*. Singapore: World Scientific Publishing Company Pte. Ltd.

21 Kleinsmith, L. (2005). *Principles of Cancer Biology*. Upper Saddle River: Pearson.

22 Lyden, D., Welch, D.R., and Psaila, B. (2011). *Cancer Metastasis: Biologic Basis and Therapeutics*. Cambridge: Cambridge University Press.

23 Martin, T.A. and Jiang, W.G. (2013). *Tight Junctions in Cancer Metastasis (Cancer Metastasis – Biology and Treatment, 19)*. New York: Springer.

24 Maxwell, C. and Roskelley, C. (2015). *Genomic Instability and Cancer Metastasis: Mechanisms, Emerging Themes, and Novel Therapeutic Strategies (Cancer Metastasis – Biology and Treatment, 20)*. New York: Springer.

25 McIntosh, J.R. (2019). *Understanding Cancer: An Introduction to the Biology, Medicine, and Societal Implications of This Disease*. New York: Garland Science.

26 Mercier, I., Jasmin, J.F., and Lisanti, M.P. (2012). *Caveolins in Cancer Pathogenesis, Prevention and Therapy (Current Cancer Research)*. New York: Springer.

27 Morgan, D.O. (2006). *The Cell Cycle: Principles of Control (Primers in Biology)*. Sunderland: Sinauer Associates, Inc.

28 Nikiforov, Y.E., Biddinger, P.W., and Thompson, L.D.R. (2019). *Diagnostic Pathology and Molecular Genetics of the Thyroid: A Comprehensive Guide for Practicing Thyroid Pathology*, 3rd Edition. Philadelphia: Lippincott, Williams, and Wilkins.

29 Noguchi, E. and Gadaleta, M.C. (2014). *Cell Cycle Control: Mechanisms and Protocols (Methods in Molecular Biology, 1170)*, 2nd Edition. Totowa: Humana Press.

30 Pecorino, L. (2016). *Molecular Biology of Cancer: Mechanisms, Targets, and*

Therapeutics, 4th Edition. Oxford: Oxford University Press.

31 Pezzella, F., Tavassoli, M., and Kerr, D. (2019). *Oxford Textbook of Cancer Biology (Oxford Textbooks in Oncology)*. Oxford: Oxford University Press.

32 Poirier, M.C. (2018). *Carcinogens, DNA Damage and Cancer Risk: Mechanisms of Chemical Carcinogenesis*. Singapore: World Scientific Publishing Pte. Ltd.

33 Prodi, G., Liotta, L.A., Lollini, P.L., Garbisa, S., Gorini, S., and Hellmann, K. (2013). *Cancer Metastasis: Biological and Biochemical Mechanisms and Clinical Aspects (Advances in Experimental Medicine and Biology)*. *Volume*. 233, New York: Springer.

34 Seyfried, T. (2012). *Cancer as a Metabolic Disease: On the Origin, Management, and Prevention of Cancer*. Hoboken: Wiley.

35 Sompayrac, L. (2004). *How Cancer Works*. Burlington: Jones & Bartlett Learning.

36 Stein, G.S. and Luebbers, K.P. (2019). *Cancer: Prevention, Early Detection, Treatment and Recovery*, 2nd Edition. Hoboken: Wiley-Blackwell. Figure 2 from Page 17, Figure 1 from Page 54, Figure 3 from Page 23, Figure 4 from Page 25, Figure 10 from Page 41.

37 Tassan, J.P. and Kubiak, J.Z. (2017). *Asymmetric Cell Division in Development, Differentiation and Cancer (Results and Problems in Cell Differentiation, 61)*. New York: Springer.

38 Weinberg, R.A. (2013). *The Biology of Cancer*, 2nd Edition. New York: W.W. Norton & Company.