CHAPTER 1

An Introduction to Cancer Cell Biology and Genetics

IN BRIEF

It is impossible to describe targeted cancer treatments without mentioning what it is they target. And when I try to explain what it is they target, I find myself going back to the beginning and explaining where cancers come from, what faults they contain, and why they behave as they do. And in order to explain that, I need explain concepts such as different types of DNA damage, oncogenes and tumor suppressor genes, and the hallmarks of cancer cells.

Hence, in this chapter, I provide an overview of the causes and consequences of DNA mutations in cells. And I describe how even just a handful of key mutations can force a healthy cell to become a cancer cell.

I also describe the cancer microenvironment – the cells and structures that cancer cells live among. Cancer cells have the ability to exploit their microenvironment and in many instances manipulate it. I explain what impact this has when doctors come to treat people with the disease.

In addition, I tackle topics such as genomic instability and intratumoral heterogeneity. Perhaps these are topics that right now don't mean anything to you, and you're unsure of why you need to know about them. But it's only through understanding these concepts that you can appreciate the limitations of targeted (and standard) cancer treatments and the promise of immunotherapy. It is also important to understand why cancer spreads and how cancers evolve and change over time.

Finally, I wrap up the chapter with a brief overview of why cancer is so difficult to treat successfully and why so many people currently cannot be cured.

1.1 INTRODUCTION

This book is all about the science behind targeted cancer treatments. And, almost without exception, **all targeted cancer treatments work by targeting proteins** that are either inside or on the surface of cancer cells or the cells around them. So in order to explain how targeted cancer treatments work, I need to describe the proteins found in cancer cells and how they differ from those in healthy cells. In order to do this, I need to explain the different types of DNA damage that cancer cells contain, **because a cell's DNA is its instruction**

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manual telling it how to make proteins. If we know what DNA damage a cell contains, this will tell us what faulty proteins it's making. And if we know what faulty proteins it's making, we will know which targeted treatments might work against it.

A general understanding of the DNA damage that cancer cells contain, and what impact this has on cancer cells, should help you make sense of why some treatments are applicable to some cancer patients and not others. It should also help you understand why it can be helpful to test a patient's cancer cells for the presence or absence of various DNA mutations.

So **this chapter is all about cancer cells, DNA, and proteins**. And, along with the chapter that follows (which is all about the two main groups of targeted cancer treatments: monoclonal antibodies and kinase inhibitors), this chapter will hopefully provide you with all the background information you need to make sense of the rest of the book.

However, even in this chapter, I've made some assumptions about what you do and don't know. For example, I've assumed that you have a rough idea of what DNA is and how cells use their DNA to make proteins. I'm also assuming that you know what proteins are and a bit about what some of them do. If you're not familiar with these concepts, I would recommend first of all taking a look at the Appendix, which contains a list of reading material about cells, DNA, chromosomes, genes, and proteins. When you've had a look at that, you'll be ready to read further.

1.2 DNA DAMAGE IS THE CAUSE OF EVERY CANCER

Our cells' DNA is essentially a huge instruction manual telling our cells what proteins to make, how to make them, when to make them, what to do with them, and when to destroy them. In turn, the proteins our cells make dictate their behavior. For this reason, **if you damage a cell's DNA, you also end up with damaged proteins**, leading to abnormal behavior.

A cancer starts to develop when a single cell accumulates DNA damage that causes it to make various faulty proteins that force it to behave abnormally. This normally doesn't happen. **A cell that finds its DNA is damaged usually either tries to repair the damage, or it self-destructs** through a process called apoptosis.[1] But, if a cell doesn't notice the damage and survives and later accumulates more damage, it might ultimately become a cancer cell.

Over the past 40 years or so, scientists have been gradually discovering what DNA damage cancer cells contain and how this affects their proteins. The scientists' primary focus has been to study the DNA that contains the instructions to make proteins – our cells' genes. This protein-coding DNA only takes up about 1% or so of our cells' total DNA [1]. (What exactly the other 99% of our cells' DNA is for is a matter of continued debate among scientists.)

Through initiatives such as The Cancer Genome Atlas [2] and the International Cancer Genome Consortium [3], hundreds of scientists have amassed an incredible catalog of information about the thousands of different DNA mutations cancer cells contain [4]. They've also discovered that **different types of cancer differ from one another in terms of the mutations they contain and the treatments they respond to**. And as well as the differences, we know that important similarities can exist between cancers that arise in different organs. For example, some breast cancer patients may have tumors that overproduce[2] a protein called HER2, as do the tumors of some patients with stomach cancer [5].

[1] Apoptosis is also referred to as "programmed cell death."
[2] Scientists generally talk about proteins being "over-expressed" rather than "overproduced," but they essentially mean the same thing.

Box 1.1 The names of genes and their proteins

As you read this book you might notice that protein names are written normally but that gene names are written in *italics*. For example, the *HER2* gene contains the instructions for making HER2 protein. You might also notice that sometimes the gene and protein have different names. An example of this is the *TP53* gene, which contains the instructions for making a protein called p53. It's also possible for a gene to contain the instructions for making more than one protein. For instance, the *CDKN2A* gene (sometimes referred to as the *CDKN2A* locus) contains the instructions for making several proteins, two of which are called $p16^{INK4a}$ and $p14^{ARF}$.

To add to the confusion, some genes and proteins have more than one name. For example, the HER2 gene is also called *ErbB2* and *NEU*. The reasons behind the various names often have a lot to do with what organism or group of cells the gene/protein was discovered in; if it's similar to another gene/protein that has already been discovered; what role the gene/protein is thought to play in the cells or organism it was found in; and whether or not abnormalities in the gene/protein cause disease. For example, HER2 stands for human epidermal growth factor receptor-2, because it's similar in structure to HER1 (although we usually refer to HER1 as the EGF-Receptor). *HER2* is also called *ErbB2* because a very similar gene, called *Erb-b*, was discovered in a disease-causing virus called the avian erythroblastosis virus. And *HER2* is also called *NEU* because a faulty version of it can cause a cancer called neuroblastoma in rodents.

A final point to note is that gene names are often written in capital letters, whereas protein names aren't. But this convention isn't always adhered to.

Because there is lots to say about the DNA mutations in cancer cells, I'm going to split it up into different topics. First, I'll talk about what **causes the DNA mutations** found in cancer cells (Section 1.2.1). Then I'll describe what **types of mutation** occur (see Section 1.2.2), how the **number of mutations** in cancer cells varies (see Section 1.2.3), and **which mutations have the greatest effect on cell behavior** (see Section 1.2.4). Then I'll talk about some of the most **common gene mutations** in cancer cells and what impact they have (Section 1.2.5).

Later in the chapter, we will look at the defining **characteristics of cancer cells** (Section 1.3), how cancer cells in a tumor can be **genetically different from one another** (Section 1.4); how they **interact with and influence the non-cancer cells** that live alongside them (Section 1.5), and how they **invade and spread** (Section 1.6).

All of this information is gradually helping scientists create new, more targeted cancer treatments, which are the subject of the rest of this book.

1.2.1 Causes of DNA Mutations

There are many different reasons why our cells' DNA gets damaged. Some of this damage is **natural and unavoidable**, whereas some of it is down to our **lifestyle, behaviors, exposures, geographical location,** and even **local customs**.[3] We can also **inherit** DNA damage from our parents. Depending on

[3] For example, in countries like Iran, people are used to drinking much hotter tea than people do in the United Kingdom, and this has been linked to a higher incidence of esophageal cancer.

what sort of data scientists look at (e.g., whether they examine individual cells or whole organs or tissues, or look at populations of people in different countries), they end up drawing very different conclusions about what proportion of cancers could be avoided [6]. So although I've listed some of the causes of DNA damage below, I haven't tried to pin down exactly how many cancers are caused by each one.[4]

Where DNA Damage Comes from – Lifestyle and Exposures

Cells that are exposed to high levels of **carcinogens** (anything that causes cancer is called a carcinogen) are particularly vulnerable to becoming cancer cells. This includes cells that line our lungs, skin, bowel, and stomach. Carcinogens include various constituents of cigarette smoke, alcohol, sunlight, radiation from X-rays, some viruses, asbestos, and food toxins [7, 8].

Our cancer risk is also linked to our **diet** (including our consumption of fruit and vegetables, red and processed meat, high salt intake, and low fiber), our level of **physical activity**, and our **weight**. This is a huge topic. If you would like to read more, I suggest looking at the Cancer Research UK [9, 10] and American Cancer Society [11] websites.

Where DNA Damage Comes from – Inherited Mutations

Some people are **born with DNA faults** that put them at higher risk of cancer than the people around them. Sometimes the fault has been passed down from generation to generation, with many family members affected. For example, actress and film director Angelina Jolie has inherited a fault in one copy of her *BRCA1* gene. Because this fault is shared by many of her relatives, she lost her mother, grandmother, and aunt to cancer [12]. Faults in high-risk genes such as *BRCA* genes are generally rare, but they can have an enormous impact on a person's cancer risk. More commonly, subtle variations in many genes will combine to affect our risk.

Faults can also arise in a mother's egg or a father's sperm. If the faulty egg or sperm goes on to create an embryo, this fault will be present in every cell. Or, the fault might occur later, as the growing embryo is developing. For example, faults that occur in an embryo's white blood cells as its immune system forms can cause infant or childhood leukemia [13].

Where DNA Damage Comes from – Chemical Reactions

Unfortunately for us, **our cells' DNA gets damaged every second of every day** – it is estimated that even without the influence of external factors like diet, smoking, or sunlight, each of our cells sustains damage to its DNA roughly 20,000 times each day [14].

Much of this damage is caused by the products of chemical reactions that are essential to keep us alive. For example, many of our cells' important chemical reactions produce oxygen free radicals[5] – high-energy oxygen atoms that essentially bash into and break DNA [15]. Our cells contain well over 100 different DNA repair proteins to fix this damage [16]. But sometimes they fail to spot all the damage, or they simply can't keep up.

Where DNA Damage Comes from – DNA Polymerase

Tissues that need to renew and replenish their cells often (such as the lining of our bowel, our

[4] If you do want to learn more about what you can do to reduce your risk, I would recommend looking at the Cancer Research UK website: http://www.cancerresearchuk.org/about-cancer/causes-of-cancer/can-cancer-be-prevented.

[5] These are also called reactive oxygen species – ROS.

skin, and immune system) are the most at risk of cancer [17–19].[6] This is because for a cell to multiply, it has to make a complete copy of all of its DNA – all 3,000 million base pairs of it [20]. The enzyme that copies DNA, called DNA polymerase, although spectacularly fast and accurate, does occasionally make mistakes [21]. Therefore, **cells that have to multiply often are at a greater risk of becoming cancer cells** than cells that rarely, if ever, multiply.

Where DNA Damage Comes from – APOBEC Enzymes

APOBEC[7] enzymes are a family of proteins that our cells use to help protect them from virus infections. **APOBEC enzymes attack viruses** by introducing mutations into their DNA. However, if an uninfected cell accidentally makes APOBEC enzymes, the enzymes will attack the cell's own DNA and introduce lots of mutations that could cause cancer [22]. Also, even when the cell has become a cancer cell, APOBEC enzymes continue to add more and more damage to the cell's genes [23].

Where DNA Damage Comes from – Cancer Treatments

Most **chemotherapies and radiotherapy work by causing so much DNA damage** that cancer cells die. However, not every cell is killed. Cells that sustain damage to their DNA and yet survive may later become cancer cells. Because of this, people treated for cancer sometimes develop second cancers months or even many years later.

The Influence of Sex Hormones

When discussing the causes of cancer, we shouldn't ignore the influence of sex hormones such as **estrogen, progesterone, and testosterone**. These tiny, fat-soluble chemicals encourage cells that contain receptors for them to survive, grow, and multiply (estrogen can also cause DNA damage [24]). Cancers that develop from hormone-sensitive tissues in the breast and prostate often retain their sensitivity to hormones. These cancers respond to treatments that block the production of hormones in the body or that block the impact of hormones on cancer cells.

In women, the risk of various cancers, including breast, ovarian, and endometrial cancer, is linked to their body's exposure to sex hormones such as estrogen. Reproductive factors (such as age of menarche[8] and menopause, along with the number of pregnancies and length of time they breast-fed) and bodyweight affect their lifetime exposure to estrogen and thus also influence their cancer risk.

The Influence of Inflammation

For many people, their cancer diagnosis was preceded by **years of inflammation, infection, or irritation** [25]. For example, people with chronic hepatitis B or hepatitis C are at high risk of liver cancer, whereas people with inflammatory bowel disease are at an increased risk of bowel cancer [25]. It seems that the presence of white blood cells in a tissue can increase the DNA mutation rate in the tissues' cells and encourage the cells to multiply, raising the risk of cancer [26].

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[6] If this seems like a simple and straightforward association, don't be fooled. There is huge controversy around the exact relationship between cancer risk and tissue renewal, number of stem cells, and DNA damage by environmental versus natural mechanisms. I've supplied a handful of references if you want to explore further.

[7] In case you're curious, APOBEC stands for apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like.

[8] The age at which a girl has her first period.

The Influence of Epigenetics

Epigenetics refers to chemical changes to the DNA double helix and to histone proteins that DNA wraps around [27, 28]. Epigenetic changes don't alter the sequence of the four bases in DNA. But **epigenetic changes do affect how compact and tightly coiled DNA is**. This in turn affects whether the information in genes is accessible to the cell's transcription factors and whether the genes can be used to make proteins. For example, if a stretch of DNA in a chromosome is very compact, it won't be transcribed. But if it's relaxed, it's available for transcription. The pattern of epigenetic changes in our DNA appears to be partly inherited from our parents, but it may also be affected by inflammation, exposure to some chemicals, nutrition, and our own and possibly even our parents' lifestyles. Epigenetics is also affected by many of the gene mutations found in cancer cells [29].

Causes of DNA Mutations – Summary

Our risk of cancer in any particular place in our body is therefore a combination of:

- Our age
- The natural rate that the cells multiply in that tissue
- The extent to which DNA polymerase, oxygen free radicals, and APOBEC enzymes have caused mutations in the tissue's cells (the amount of damage will gradually increase as we age)
- Our sex, our lifestyle, and behaviors (which will be hugely impacted by our cultural background, physical location, and personal choices and opportunities)
- Our cells' exposure to carcinogens, hormones, and factors that cause inflammation
- Our inherited genetic and epigenetic makeup

Cancer Research UK estimates that **around 42% of cancers are potentially preventable** through changes to lifestyle, behaviors, exposures, and weight [30, 31]. However, we cannot influence factors such as the activity of APOBEC enzymes or the accuracy of DNA polymerase. As I said before, estimating what proportion of cancers can be prevented is an incredibly contentious topic, and estimates vary widely depending on how the research was done.

1.2.2 Types of DNA Mutations

As we've seen, DNA damage is caused by a wide variety of different factors. Some causes of damage are natural and unavoidable, and others are potentially avoidable.

DNA mutations also come in many forms. For the sake of simplicity, I'm going to split them into two groups: (1) mutations affecting long stretches of DNA and whole chromosomes and (2) mutations affecting just a few DNA base pairs.

Mutations Affecting Long Stretches of DNA and Whole Chromosomes

For a start, **many cancers are aneuploid** – that is, the cells contain the wrong number (i.e., not the normal 23 pairs) of chromosomes [32]. However, although this is no doubt important, it's not always clear what impact this is having on the cell. Because the detection of extra chromosomes in cancer cells doesn't generally help doctors decide what treatment to use, I'm not going to talk about this further.

What can be more helpful is detecting chromosome faults such as translocations, inversions, insertions, deletions, and amplifications.

Chromosome Translocations and Rearrangements

A chromosome translocation is when two chromosomes break, and the cell accidentally sticks them back together incorrectly (see Figure 1.1). Chromosomal rearrangements are similar, but both breaks occur in a single chromosome. More often than not, the

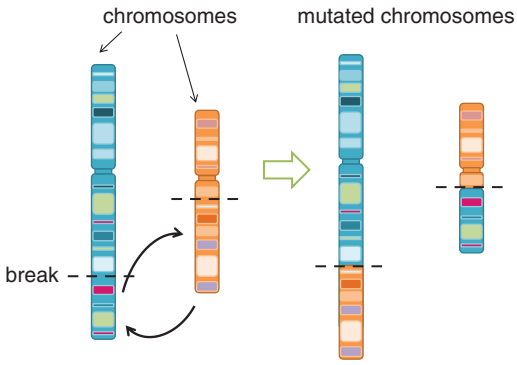

Figure 1.1 A chromosome translocation. Two chromosomes (colored turquoise and orange) break. The cell accidentally sticks them back together incorrectly. If the chromosomes have broken where genes are located, this may result in the creation of a gene fusion.

chromosomes break in regions that don't contain any genes (remember that the information to make proteins only takes up 1% or so of our chromosomes). However, **sometimes translocations and rearrangements do affect genes**, and this can have dire consequences. For example, the cancer cells of chronic myeloid leukemia (CML) almost always contain a translocation in which chromosome 9 and chromosome 22 have broken and been stitched back together incorrectly. This causes the *BCR* gene on chromosome 22 to become fused together with the *ABL* gene on chromosome 9 [33]. The fusion of these two genes forces the cell to make a Bcr-Abl **fusion protein** (a protein made from the information in the fusion gene), which forces the cells to grow and multiply.

In other cancers, you find translocations and rearrangements in which a control region from one gene (a promoter or enhancer[9]) has become fused to the protein-coding region[10] of a second gene. This has often happened during the development of prostate cancer and some forms of blood cancer such as non-Hodgkin lymphomas and multiple myeloma. In prostate cancer, the rearrangement often involves the *ERG* and *TMPRSS2* genes on chromosome 21. The rearrangement places the promoter from the *TMPRSS2* gene (a gene which is always active in prostate cells) next to the protein-coding region from a powerful, pro-growth protein called *ERG* [34] (see Figure 1.2). The consequence of this rearrangement is the massive overproduction of ERG protein, which forces the prostate cell to multiply.

Chromosome Insertions

This occurs when **part of one chromosome is inserted into another chromosome** (Figure 1.3). It can also occurs when part of a chromosome is re-inserted back into the chromosome it came from, but in the wrong place. An example is the "internal tandem duplications" affecting the *FLT3* gene that are found in the cancer cells of around a third of people with acute myeloid leukemia (AML) [35]. The insertion involves part of the FLT3 gene, which is copied and re-inserted back into the gene. This causes the cell to make an extra-large, overactive version of FLT3 protein. FLT3 inhibitors are in clinical trials. (See Chapter 7, Section 7.10.1 and Figure 7.21 for more about FLT3 mutations in AML.)

Chromosome Deletions

Not surprisingly, a chromosome deletion is when **part of a chromosome gets deleted** (Figure 1.4a). Examples include deletion of the part of chromosome 17 that contains the *TP53* gene, and deletion of the part of chromosome 9 containing the *CDKN2A* gene. Both *TP53*

[9] The Khan Academy website has a nice description of gene regulation: https://www.khanacademy.org/science/biology/gene-regulation/gene-regulation-in-eukaryotes/a/overview-of-eukaryotic-gene-regulation.
[10] That is, the part of the gene that contains the instructions to make a protein.

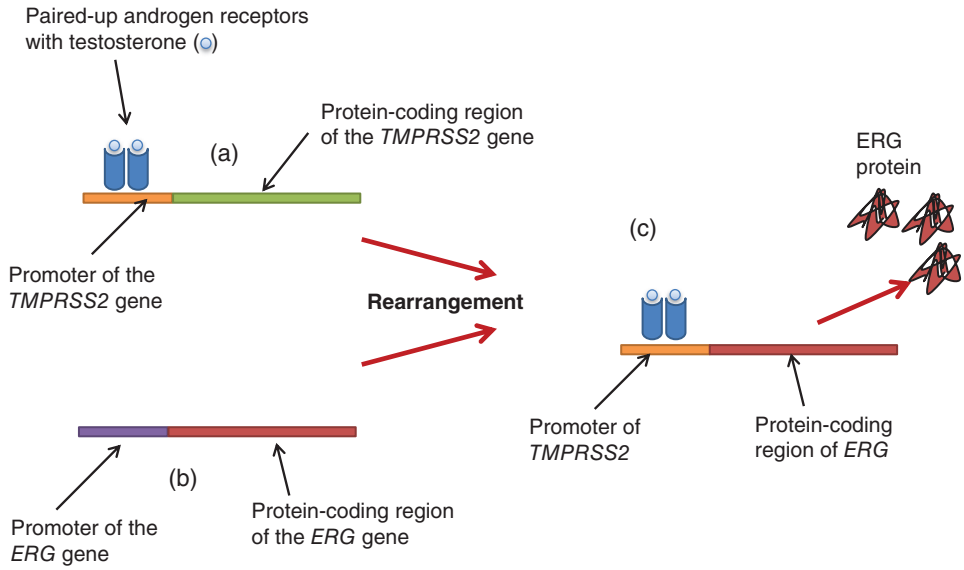

Figure 1.2 The TMPRSS2-ERG gene fusion found in prostate cancer cells. (a) In healthy prostate cells, androgen receptors pair up due to the presence of testosterone. Paired-up receptors then attach to the TMPRSS2 gene promoter and cause the cell to produce TMPRSS2 protein. **(b)** In contrast, prostate cells only rarely produce ERG, because the ERG gene does not contain attachment sites for androgen receptors. **(c)** 50% of prostate cancers contain a chromosome rearrangement which puts the protein-coding region of the ERG gene under the control of the promoter from the TMPRSS2 gene. This mutation causes the cell to produce ERG, which in turn forces the cell to multiply.

and *CDKN2A* are vital tumor suppressor genes that prevent our cells from becoming cancer cells (there is more about *TP53* and *CDKN2A* in Section 1.2.5). Their loss means that part of the cell's protection against cancer has gone.

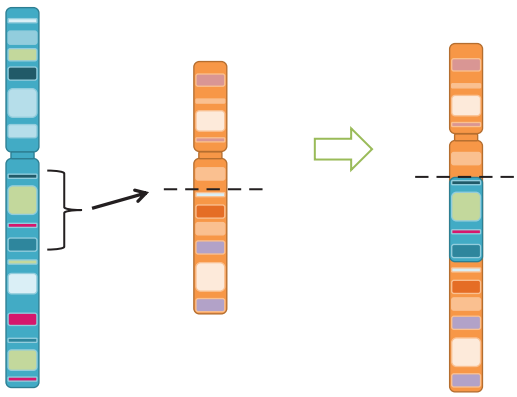

Figure 1.3 A chromosome insertion – part of one chromosome is inserted into another chromosome (as shown) or back into the same chromosome but in the wrong location.

Chromosome Inversions

Inversions (Figure 1.4b), in which part **of a chromosome is cut out, flipped over, and then re-inserted**, can also disrupt genes. For example, an inversion of part of chromosome 2 is found in about 4% of non-small cell lung cancers (this is the most common type of lung cancer). The inversion joins together the *ALK* gene with part of the *EML4* gene, creating an uncontrollable fusion protein that forces the cells to multiply [36]. Three ALK inhibitors are now licensed treatments for ALK-mutated lung cancers; they are crizotinib (Xalkori), alectinib (Alacensa), and ceritinib (Zykadia). (For more about ALK mutation in lung cancer, and ALK inhibitors, see Chapter 4, Section 4.2.4 and Chapter 6, Section 6.4.4.)

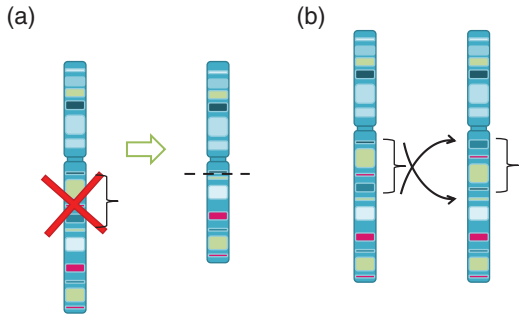

Figure 1.4 Chromosome deletions and inversions. (a) In a chromosome deletion, part of a chromosome is (not surprisingly) deleted. An example is the deletion of part of chromosome 17 containing the *TP53* gene in chronic lymphocytic leukemia, bowel cancer, and other cancers. **(b) Chromosome inversion** – a segment of the chromosome is cut out, flipped over, and inserted back into the chromosome; for example, inversions involving the *ALK* gene on chromosome 2 in lung cancer.

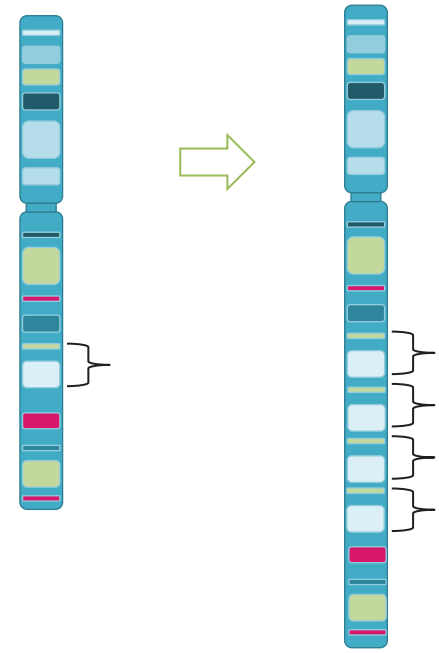

Figure 1.5 Gene amplification. The cell accidently makes extra copies of part of a chromosome. The duplicate segments are inserted into other chromosomes or back into the same chromosome; for example, amplification of a segment of chromosome 17 containing the *HER2* gene in breast cancer.

Gene Amplification

Lastly, one of the most commonly looked for types of chromosome damage is gene amplifications. Gene amplifications occur when a cell's DNA replication machinery **accidentally makes extra copies of a region of a chromosome** that contains one or more genes (see Figure 1.5). As a consequence, the cell overproduces (over-expresses) the proteins made from the amplified genes. A common amplification is that of the *HER2* gene (the HER2 gene is also commonly called *Neu* or *ErbB),* which is amplified in about 18%–20% of breast cancers [37].

Point Mutations

A point mutation is when **one DNA base is accidentally added, deleted, or swapped** for a different one. Most point mutations have no impact on the cell as they occur outside of genes. However, if a point mutation (such as a base substitution, addition, or deletion) occurs within a gene, it can have various consequences (see Figure 1.6).[11] Point mutations are classed as missense, nonsense, or silent, depending on what consequence the mutation has on protein production. They are also classified as "in-frame" or "frameshift" mutations.

Missense Mutations

If one DNA base is substituted for a different one, this might mean that the **protein made from that gene differs by one amino acid** from the normal protein (three DNA bases in a gene equate to one amino acid in the resulting

[11] If you need a refresher on gene transcription and translation at this point, I suggest taking a look at some of the resources suggested in the Appendix.

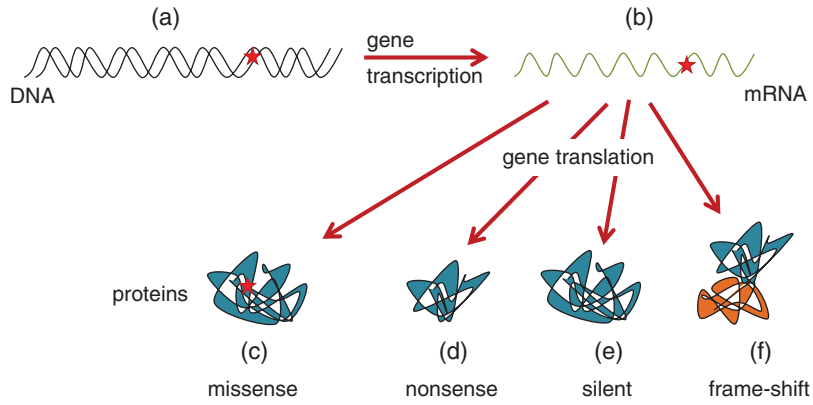

Figure 1.6 Point mutations. A point mutation (shown by a red star) is when one DNA base is added, deleted, or swapped for a different one in the cell's DNA **(a)**. If the mutation is in a gene, the mutation will be copied into the mRNA **(b)** and it may alter the resulting protein. The consequence might be that **(c)** due to a **missense mutation,** the protein made by the cell differs from the normal (the so-called "wild-type") version of the protein by one amino acid, **(d) a nonsense mutation in** the DNA introduces a stop signal into the mRNA, and the cell makes an extra-short (truncated) protein, **(e)** a **silent mutation** has no impact on the protein produced, **(f)** a **frameshift** mutation causes the cell to make a very different protein compared to the normal protein, one which is only partly the same as the original.

protein) (Figure 1.6c) [38]. Two examples are the faulty version of the B-Raf protein (called V600E), which is often found in the cancer cells of people with malignant melanoma, and some of the faulty versions of EGFR (epidermal growth factor receptor), which are found in the cancer cells of some people with lung cancer. In both cases, the faulty proteins (both of which contain hundreds of amino acids) are just one amino acid different from the healthy version of the protein. However, even changing that one amino acid is sufficient to create a massively overactive version of B-Raf or EGFR.

Nonsense Mutations

Nonsense mutations are those that cause the cell to make **a shortened (truncated) version of the protein** (Figure 1.6d). This happens because the change to the DNA sequence creates a "stop codon" in the resulting mRNA strand. As you might already know, proteins are made from 20 different amino acids, and each set of three bases (called a codon) in the mRNA strand tells the ribosome what amino acid to add next to the protein it's making.[12] But there are three codons (UAA, UAG, and UGA) which tell the ribosome to stop adding any more amino acids. If a DNA point mutation creates one of these stop codons part way through the mRNA from a gene, then the ribosome will stop part way through making the protein. For example, some of the inherited *BRCA* gene mutations that increase a woman's risk of breast and ovarian cancer cause her cells to produce a shortened version of a BRCA protein [39].

Silent Mutations

These point mutations **don't have any impact on the protein** the cell makes even if they occur within a gene (Figure 1.6e). For example, if a ribosome comes across the mRNA sequence CCC, this tells it to add a proline amino acid to the protein it's making. If a point mutation

[12] If you're struggling to make sense of this, I would suggest looking at the Appendix and learning a bit about gene transcription and translocation.

changes the mRNA from CCC to CCA, this has no impact because the sequence CCA also tells the ribosome to add a proline.

In-Frame and Frameshift Mutations

If one or two DNA bases are added or deleted to a gene's sequence, this can create a frameshift mutation that has an enormous impact on what protein is produced (Figure 1.6f). An example is if one DNA base is added to a gene that changes the mRNA from ….CGACGACGA…. to …CCGACGACGA…. Now, instead of reading the sequence as … CGA CGA CGA… adding three arginine amino acids to the protein, the ribosome reads …CCG ACG ACG A… and adds a proline followed by two threonines. The ribosome carries on going from there, adding a completely different selection of amino acids from the normal selection. As a result, **the protein the cell makes may bear very little resemblance to the normal protein**. Frameshift mutations also commonly introduce stop codons that create truncated proteins.

An "in-frame" mutation is opposite to a frameshift mutation – that is, it is a mutation that either swaps one base for a different one, or one in which bases are added in multiples of three, so they don't alter the rest of the protein made. For example, if three bases are added to ….CGACGACGA…. so that it becomes ….CGACCCCGACGA…, the ribosome will insert an extra proline in between the arginines, but it has no further impact on the rest of the protein.

1.2.3 Numbers and Patterns of DNA Mutations in Cancer Cells

In recent years, technologies have been developed that allow scientists to pinpoint the location of thousands of DNA mutations inside cancer cells. They have discovered that **different cancers contain different numbers, types, and patterns of mutations that arise due to different mutational processes**. For example, lung cancers from smokers contain lots of point mutations in which a C has been changed to an A. A different pattern of mutations – where there are lots of insertions and deletions of more than three DNA bases at a time – is common in people with cancers associated with inherited *BRCA* gene mutations. Other patterns are linked to overactive APOBEC enzymes. In all, scientists have so far discovered 30 different patterns of mutations, which they call "mutation signatures" [40]. And, of course, it's possible for one cancer cell to contain multiple patterns of mutations because the cancer has arisen due to a combination of causes.

The amount of damage in cancer cells' DNA varies greatly from cancer type to cancer type [41]. For example, cancers that have come about because of the effects of powerful carcinogens often contain a vast amount of DNA damage. Therefore, lung cancers in people who smoke or have smoked in the past contain ten times the number of mutations as lung cancers in people who have never smoked. Malignant melanoma skin cancers, which are almost always caused by UV light from the sun, also contain a vast number of mutations [41]. In general, cancers in older people contain more mutations than those in children and young adults. Older peoples' cells have simply had many more years in which to accumulate mutations.

Although cancer cells often contain hundreds or even thousands of mutations, the majority of these mutations have no discernible impact on the cell's behavior. They have occurred because the cancer cell is damaged and unstable and is picking up new mutations all the time. The mutations that are important in driving the cancer cells' abnormal behavior are referred to by scientists as **driver mutations**. Mutations that add little or nothing to the cells' behavior are called **passenger mutations**.

Perhaps not surprisingly, scientists are much more interested in finding a cancer's driver mutations than its passenger mutations. They want to know what's driving the cells' behavior so that they can do something about it.

1.2.4 Driver Mutations – Those That Affect Cancer Cell Behavior

In order for DNA damage to cause cancer, some of it must occur in genes that control the cell's behavior. These "driver mutations" affect cell processes and behaviors such as:

- How fast the cell grows
- How frequently it multiplies
- The way it communicates with neighboring cells
- How often and how well it checks its own health
- Its ability to survive in adverse conditions such as low oxygen levels
- Its ability to move through the body's tissues
- Whether it goes through all the normal checks and balances during the cell cycle[13]
- Whether it still has the ability to self-destruct
- The way it produces energy
- Whether it can hide from or suppress the person's immune system

The genes that have caused these changes in behavior are classed as oncogenes, tumor suppressor genes, and DNA repair genes.

Oncogenes

Many of the proteins made from these genes encourage our cells **to survive, grow, and multiply**. Others can make cells more **mobile and invasive** or help them to **hide from the immune system**. All these genes need to be tightly controlled to avoid cancer. In cancer cells, they're damaged in a way that they're overproduced and/or overactive. Examples of oncogenes include *EGFR*, *RAS*, *B-RAF*, *MYC*, *HER2*, and *SRC*.

Tumor Suppressor Genes

The proteins made from these genes **slow down or stop cell growth and proliferation and trigger cell death (apoptosis)**. In cancer cells, they're damaged in a way that causes their protection to be lost. Examples include *TP53*, *PTEN*, *RB1*, and *APC*.

DNA Repair Genes

The proteins made from these genes **sense and repair DNA damage**. In cancer cells, they're damaged in such a way that they can no longer do their job properly. Because of this, cancer cells pick up more and more DNA damage as time goes on. Examples of DNA repair genes include *BRCA1*, *BRCA2*, *ATM*, *ATR*, *RAD51*, and *ERCC1*.

In healthy cells, the proteins made from DNA repair genes keep the cell's DNA free from faults. There is also a balancing act between the oncogenes and the tumor suppressor genes. For example, a protein called Bcl-2 protects cells from death, whereas a protein called p53 triggers death. The gene for making Bcl-2 (called *BCL2*) is an oncogene; the gene for making p53 (called *TP53*) is a tumor suppressor gene. Healthy cells contain strict amounts of both proteins that balance each other out. But cancer cells often contain too much Bcl-2 and too little, or faulty, p53.

Multiple Driver Mutations Are Necessary for a Cell to Become a Cancer Cell

The sequence of events that leads to bowel cancer is often given as an example of how the gradual accumulation of mutations in several oncogenes, tumor suppressor genes, and DNA repair genes can ultimately cause someone to develop cancer.

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[13] The cell cycle is the normal, step-by-step process our cells go through when they multiply.

Our bowel is lined by orderly layers of cells known as epithelial cells. Because bowel cells are constantly getting scraped off by food passing through, our bowel cells have to multiply pretty often in order to keep the number of cells constant. Cells that multiply are prone to picking up mutations. So our bowel cells tend to contain more and more mutations as we get older. If a mutation affects a gene called *APC*, this is bad news as *APC* is an important tumor suppressor gene. But the situation isn't desperate as it's only one mutation, which isn't enough to cause cancer. However, if it's followed by a mutation in *KRAS*, then the situation becomes worse; *KRAS* is a powerful growth-promoting oncogene that forces the cell to multiply more rapidly. As the cells multiply, they pick up yet more mutations. The cell still isn't a cancer cell because other protective proteins are still doing their job. But if genes such as *PIK3CA* (an oncogene), *SMAD4* (a tumor suppressor gene), and *TP53* (a tumor suppressor gene) become faulty, then the cell will become a full-blown cancer cell [41] (see Figure 1.7 for an illustration of this process).

In other cancers, a similar **combination of mutations in a handful of important genes** is thought to drive their behavior [42].

1.2.5 The "Usual Suspects" – Genes Commonly Mutated in Many Cancers

Some gene mutations are common only in one or two types of cancer. These include the *VHL* mutations that are very common in kidney cancer and some of the translocations that are very common in hematological cancers (such as leukemias, lymphomas, and multiple myeloma). But **other gene mutations crop up time and time again** in many different cancer types. I'll be mentioning some of these gene mutations again and again in this book, so I've listed a handful of them in Table 1.1 to give you a rough idea of what they do.

One thing that might (or might not!) jump out at you from the table is that that many of the most commonly mutated genes in cancer cells are involved in **cell communication pathways**. These pathways are used by all our

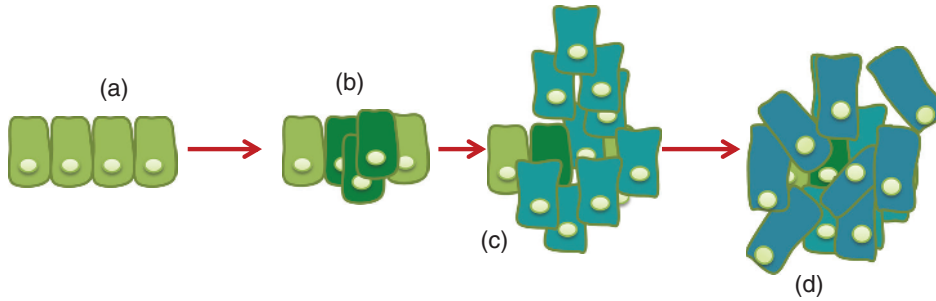

Figure 1.7 The series of mutations leading to many bowel cancers. (a) Orderly, well-connected cells line the bowel. **(b)** A random mutation in a bowel cell lead to loss of APC activity; this cell starts to multiply slightly faster than its neighbors, forming a little lump – an adenoma. The faulty cells are not yet cancer cells, but because they are multiplying more quickly than normal, they are prone to collecting more mutations. **(c)** Weeks, months, or years later, a mutation in the *KRAS* gene causes the K-Ras protein to become overactive; the cells now multiply rapidly and in a disorderly fashion. **(d)** Finally, genes like *TP53*, *PIK3CA*, and *SMAD4* are mutated. The faulty cells are now full-blown cancer cells, able to invade through local tissues and spread to other parts of the body.
Abbreviations: *APC* – adenomatous polyposis coli; *TP53* – tumor protein 53; *PIK3CA* -phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; *SMAD4* – SMAD family member 4

Table 1.1 A selection of some of the most commonly mutated oncogenes, tumor suppressor genes, and DNA repair genes in human cancers.

Gene name (protein name)	What protein is made from this gene?	What is the consequence for the cell if the gene is mutated?
Oncogenes		
***RAS* (Ras)**	The three Ras proteins (K-Ras, N-Ras, and H-Ras) are enzymes involved in cell communication. There are three versions of the gene (*KRAS*, *NRAS*, and *HRAS)*, which contain the instructions for making the three proteins [43].	All the proteins made from these genes are involved in cell communication pathways – the sequences of events triggered inside a cell when it receives a signal to grow and multiply from its neighbors. Therefore, all these proteins cause cells to grow and multiply. Overactive communication pathways also force cells to survive (even when damaged) and to become more mobile and invasive. For more on cell communication pathways and how they work, see Chapter 3, Section 3.2.
***PIK3CA* (p110-alpha)**	The PI3K protein is an enzyme involved in cell communication. It comes in many different forms and is made up of two component parts: an enzyme part and a regulatory part. The *PIK3CA* gene encodes an enzyme part called p110alpha (p110α) [44].	
***HER2/NEU/ ERBB2* (HER2)**	A receptor found on the cell surface; it activates cell communication pathways inside the cell [45].	
***MYC* (MYC)**	A transcription factor – it attaches to various gene promoters and triggers gene transcription. Many of the genes it controls are involved in cell growth and proliferation [46].	
***BRAF* (B-Raf)**	An enzyme involved in cell communication and activated by Ras proteins [47].	
***EGFR* (EGFR)**	A receptor found on the cell surface; it activates cell communication pathways inside the cell [45].	
Tumor suppressor genes		
***TP53* (p53)**	A transcription factor activated by DNA damage and other triggers – it attaches to various gene promoters and triggers gene transcription. The proteins produced as a result of p53 block cell proliferation and cause cell death [48].	If p53 is not working properly or is missing from a cell, the cell loses the ability to stop multiplying or die in response to DNA damage.
***PTEN* (PTEN)**	An enzyme involved in cell communication that blocks the activity of PI3K. PTEN also helps cells avoid DNA damage [49].	If PTEN is not working properly or is missing from a cell, the PI3K-controlled communication pathway becomes overactive.
***RB* (RB)**	It has a pocket in it that fits E2F proteins, which control entry into the cell cycle[a]. RB holds onto and blocks E2F proteins, and this prevents the cell from entering the cell cycle [50].	If RB is not working properly or is missing from a cell, E2F can force the cell into the cell cycle (for more about RB and E2F, see Chapter 4, Section 4.5).
***CDKN2A* (p16 INK4a)**	$p16^{INK4a}$ is a protein that blocks a set of enzymes called the CDKs. The CDKs force RB to let go of E2F proteins (see the description of RB above). Hence, p16 prevents cells from entering the cell cycle (see Chapter 4, Figures 4.18 and 4.19) [50].	If p16 ^{INK4a} is not working properly or is missing from a cell, E2F can force the cell into the cell cycle.
***NF1* (neuro-fibromin)**	A large protein that inactivates Ras proteins (see the description of Ras earlier in this table) [51].	If neurofibromin is not working properly or is missing from a cell, Ras proteins become overactive.
APC	The surface of the APC protein has various different regions through which it interacts with many different proteins involved in cell communication, mobility, adhesion to neighboring cells, and other processes [52].	If APC is not working properly or is missing from a cell, then levels of another protein, beta-catenin (β-catenin), rise. Beta-catenin causes cells to multiply.

Table 1.1 (Continued)

DNA repair genes		
***BRCA1* (BRCA1) & BRCA2 (BRCA2)**	BRCA1 and BRCA2 are both necessary for a DNA repair process called homologous recombination (HR). Our cells use HR to accurately repair double-strand breaks in their DNA (see Chapter 4, Section 4.3. for more information on BRCA proteins).	If either BRCA1 or BRCA2 is not working properly or is missing from a cell, the cell can no longer perform HR. It is therefore liable to pick up lots of DNA mutations.
ATM & ATR	These are enzymes whose activity is triggered when a cell detects that its DNA is damaged. They coordinate the cell's response to the damage [53].	If either ATM or ATR is damaged or missing from a cell, its ability to respond to DNA damage is compromised.

Abbreviations: *PIK3CA* – phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; HER2 – human epidermal growth factor receptor-2; EGFR – epidermal growth factor receptor; TP53 – tumor protein p53; PTEN – phosphatase and tensin homologue; RB – retinoblastoma protein; NF1 – neurofibromatosis type 1; APC – Adenomatous polyposis coli; BRCA – Breast cancer susceptibility gene; ATM – ataxia-telangiectasia mutated; ATR – ATM- and Rad3-Related

[a] The cell cycle is the very orderly and precise sequence of events that a cell goes through in order to multiply.

Source: Kandoth C et al. (2013). Mutational landscape and significance across 12 major cancer types. *Nature* **502**: 333–339.

body's cells to sense and respond to: changes in their environment, signals sent out by neighboring cells, the presence or absence of hormones, and signals sent out by white blood cells. A wide variety of communication pathways exist in our cells. And there are some proteins like Ras and PI3K that are involved in many different signaling pathways. These pathways are often the target of new cancer drugs, and the whole of Chapter 3 is dedicated to explaining them and the cancer drugs that block them.

1.3 THE DEFINING FEATURES (HALLMARKS) OF CANCER CELLS

All cancers are presumed to begin with a single cell that has sustained damage to its DNA and has multiplied out of control. As an adult, it's probably true that every cell in our body contains some sort of damage to its DNA. However, what sets a cancer cell apart from a non-cancer cell is:

- The **amount** and **type** of DNA damage the cells contain
- The **location** of this damage in oncogenes, tumor suppressor genes, and DNA repair genes
- The **changes in behavior** that the damage causes

The behavioral changes that set a cancer cell apart from a healthy cell are collectively known as "**the hallmarks of cancer**." Six hallmarks were listed and described by two scientists called Douglas Hanahan and Robert Weinberg back in 2000 [54], and they added two more in 2011 [55]. I've described all eight below.

1.3.1 The Eight Hallmarks of Cancer

1. **They can tell themselves to multiply**. A normal cell only multiplies when it receives an instruction[14] to do so. A cancer cell can generate those instructions itself.

[14] This instruction is usually in the form of small proteins known as "growth factors" released by the cells' near neighbors – see Chapter 3, Section 3.2.1 for more on this.

2. **They are insensitive to negative feedback**, because proteins that would normally tell them to stop multiplying and die (like p53) have been lost or don't work properly.
3. **They resist death**. Every day, millions of cells in our body self-destruct because they have worn out or become damaged. Cancer cells have defects that make it almost impossible for them to do this.
4. **Cancer cells can multiply forever** because they contain a protein called telomerase. Healthy cells lack this protein and eventually stop multiplying.
5. **They gain a blood supply**. Cancer cells release a tiny protein called VEGF that tells nearby blood vessels to sprout and grow (a process called **angiogenesis**). New blood vessels supply the growing cancer with oxygen and nutrients.
6. **They can invade and spread**. Most of our body's cells are connected to each other in orderly arrangements. Cancer cells have lost connective proteins from their surface, and they are independent and mobile.
7. **They have changed the way they produce energy**. Healthy cells use sugars from our food to make energy using a highly efficient, oxygen-dependent process. Cancer cells use an inefficient process that requires less oxygen but helps them multiply more quickly.
8. **They can avoid destruction by the immune system**. White blood cells constantly patrol our body looking for defective cells. Cancer cells hide from white blood cells; suppress cancer-fighting white blood cells; co-opt white blood cells for their own purposes (there is lots more about this in Chapter 5 on immunotherapy).

1.4 GENETIC VARIATION AMONG CANCER CELLS IN A SINGLE TUMOR

A major reason why many tumors fail to respond to treatment or become resistant later is **intratumoral heterogeneity** – the fact that inside a tumor there are various populations of cancer cells that are genetically different from one another (see Figure 1.8).

In fact, scientists analyzing multiple biopsies from a single tumor have found huge variations in the number, type, and chromosome location of genetic mutations in the cancer cells. One of the first and most

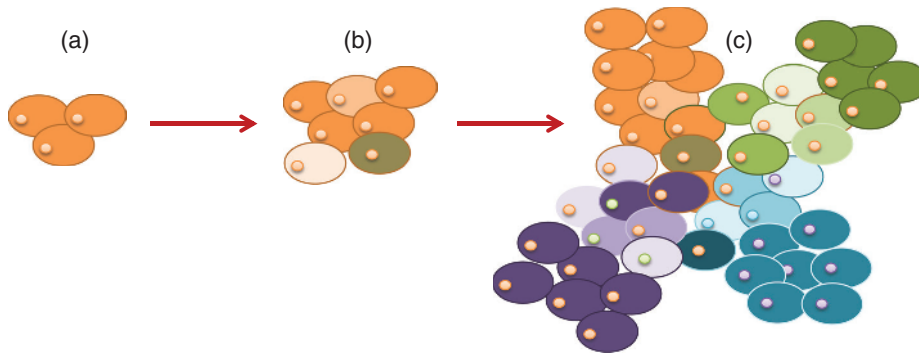

Figure 1.8 Genome instability drives intratumoral heterogeneity. (a) In a microscopic cluster of cancer cells, all the cells are likely to contain the same genetic faults. However, the cells are **genomically unstable** and likely to pick up more mutations. **(b)** The cells start to evolve and become different from one another. **(c)** As time goes on, the cells diverge from each other more and more, creating distinct populations of cells driven by different sets of mutations.

comprehensive analyses of this phenomenon was conducted by a group of British scientists who studied tumor biopsy samples from people with kidney cancer [56]. When investigating 12 samples from one patient, they found that only a third of the 128 DNA mutations they discovered were present in all 12 samples. A similar study investigating tumor samples from eight people with esophageal cancer has revealed a similar story [57, 58].

It seems that as a cancer grows, the cells within it evolve and change. This is because cancer cells are **genomically unstable** – they accumulate DNA damage at a faster rate than healthy cells. There are various reasons for this instability, some of the most important of which are the following [59, 60]:

- Cancer cells contain faults in DNA repair genes that compromise their ability to detect and repair DNA damage.
- Cancer cells' apoptosis machinery is faulty, which means they stay alive despite containing lots of DNA damage.
- The normal mechanisms that ensure the cell has the correct number of chromosomes and help to avoid chromosome breakages and fusions are lost.
- The cells' ability to replicate their DNA accurately is compromised.
- Some cancer cells are continually exposed to mutagens such as cigarette smoke or UV light from the sun.
- The cancer cells contain mutations in powerful oncogenes that destabilize the cell and lead to further mutations.

Because of genomic instability, over the weeks, months, and years that go by before a cancer is diagnosed (and in the weeks, months, and years afterward), cancer cells emerge that have different combinations of mutations compared to their predecessors. And, as time goes on, the cancer cells within a tumor become more and more diverse.

1.5 THE CANCER MICROENVIRONMENT

Tumors are not lumps of tissue made from millions of identical cancer cells. Instead, they **contain a variety of non-cancer cells** (collectively known as stromal cells) such as fibroblasts (these are common, structural cells found in many locations around the body), white blood cells, cells that make up the blood vessels (endothelial cells and pericytes), fat cells (adipoctyes), nerve cells, and other cell types (see Figure 1.9) [61]. In fact, in some tumors there are more non-cancer cells than there are cancer cells [61].

The cells in a tumor are also embedded in a network of proteins and complicated sugar molecules known as the ECM – the extracellular matrix[15]. This intricate web surrounds the cells in all our tissues and organs, and its makeup and role differs from place to place around the body. When a cancer develops, cancer cells and non-cancer cells (which are now under the cancer cells' influence) cause the makeup and density of the ECM to change. For example, in breast cancer, the ECM becomes stiffer, and this seems to help cancer cells to move and escape into the lymph vessels and bloodstream [62].

1.5.1 The Role of White Blood Cells

The number, type, and actions of white blood cells in a cancer can vary enormously. Solid tumors are often **host to millions of white**

[15] Examples of ECM proteins include collagen, fibronectin, laminin, and elastin. The complex sugar molecules (called glycoseaminoglycans) are generally chemically linked to proteins to form protein-sugar hybrids called proteoglycans. These proteoglycans form a jelly-like substance in which the fibrous proteins are embedded.

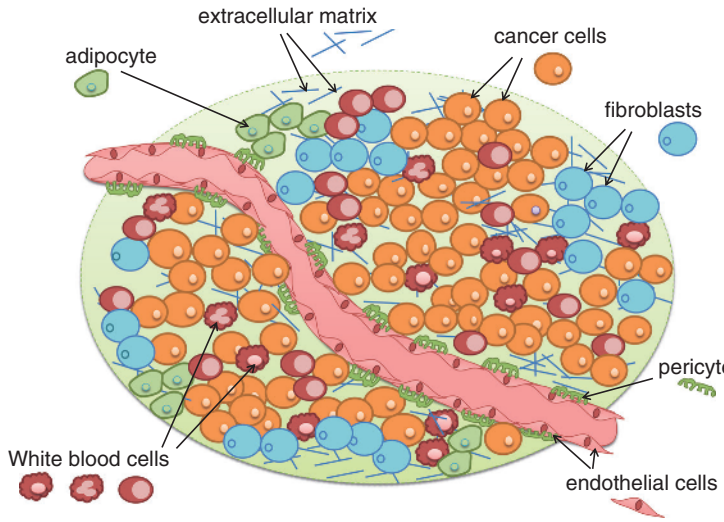

Figure 1.9 The cancer microenvironment contains many different types of cells. Tumors contain cancer cells, many different types of white blood cells, fibroblasts, fat cells (adipocytes) and other cell types (not shown). Winding their way through them are blood vessels, which are made up of endothelial cells and pericytes. Lymph vessels might also be present (not shown). All of these proteins are embedded in a protein scaffold called the extracellular matrix (ECM).

blood cells such as macrophages, mast cells, lymphocytes (B and T cells), and neutrophils. These cells can supply [63]:

- Small proteins known as growth factors that sustain the cancer cells' proliferation[16]
- Small proteins and chemicals collectively called "survival factors" that help cancer cells stay alive despite being in a hostile and toxic environment[17]
- Small proteins and chemicals that promote cancer cell migration, invasion, and metastasis
- Pro-angiogenic growth factors (see Section 1.5.3 below) and enzymes that destroy the extracellular matrix (ECM), providing an escape route for cancer cells
- Toxic molecules that cause cancer cells to mutate further

Each person's tumor will have a different collection of white blood cells inside it and at its outer fringes. The type of cells present, their number, and their behavior have a huge impact on how quickly or slowly the tumor grows and whether the person can be cured [64]. As yet, we're not in a position to use information about the number and type of white blood cells in a cancer before deciding what treatment to give someone. But it's likely that this will happen in the next few years [65].

There's more about the interactions between white blood cells and cancer cells in Chapter 5 on immunotherapy.

[16] We return to the topic of growth factors in Chapter 3 as many cancer treatments work by blocking growth factor receptors.

[17] This might not seem obvious, but because cancer cells grow in a haphazard manner and there aren't enough decent blood vessels around to supply them with everything they want and to take toxins away, their environment is toxic.

1.5.2 The Role of Other Cell Types

Fibroblasts sit in our tissues, and they normally produce structural proteins (such as collagen, elastin, fibronectin, and laminin) that form the ECM [66]. In tumors, the fibroblasts change in response to chemicals and signals sent out by cancer cells. They become perpetually activated and behave as though they are in a damaged tissue. For example, they release vast quantities of ECM proteins – much more than normal – and they produce growth factors and chemicals that encourage cancer cells to multiply [61].

Also found in some tumors are fat-storing cells called adipocytes. Again, the adipocytes found within tumors aren't normal; they've been altered by signals sent out by cancer cells. And, like the fibroblasts in tumors, the adipocytes also encourage and help cancer cells to grow and multiply [61].

1.5.3 Angiogenesis

Angiogenesis (**the sprouting and growth of blood vessels**) is almost always necessary for a cancer to become life-threatening. A tumor can grow to around 1 mm^3 without angiogenesis,[18] but to get beyond this it must have a blood supply [67]. (A 1 mm^3 tumor will typically have around 1 million cells, and it could have taken several years for it to get to this size.) By the time a cancer has reached 1 mm^3, the cells will be experiencing a drop in oxygen levels (hypoxia). To gain a blood supply and the necessary supplies of oxygen and nutrients, the cancer cells trigger angiogenesis.

The most important trigger for angiogenesis is a tiny protein called **VEGF** (vascular endothelial growth factor), which is released by cancer cells (and other cells) when oxygen levels drop. VEGF attaches to receptor proteins on the surface of endothelial cells – the cells that line our small blood vessels. Once VEGF has attached to its receptors, the endothelial cells multiply and move into place to form a new blood vessel.

When properly controlled, angiogenesis is an important and entirely healthy process. It happens normally: during the healing of cuts and wounds, during a woman's menstrual cycle, during the formation of the placenta in pregnancy, and in a growing embryo. However, when angiogenesis happens in cancer, it helps the cancer to grow and spread by supplying the cells with oxygen and nutrients and providing access to the bloodstream (see Figure 1.10).

1.6 CANCER SPREAD/METASTASIS

As soon as a cancer spreads (metastasizes) to another part of the body, treatment becomes more complicated, and the person's likelihood of being cured of their disease drops dramatically [68, 69]. **Scientists estimate that metastasis is responsible for around 90% of cancer deaths** [70]. Sadly, once a cancer has metastasized, the various new cancer growths quickly become resistant to treatment and eventually disrupt and destroy vital tissues and organs.

And, even when a cancer doesn't *appear* to have spread, there can be individual cancer cells, or microscopic clumps of cells that are circulating in the person's blood or lodged in distant organs or tissues [69]. These initially dormant cells can later cause metastasis and relapse.

[18] This is roughly equivalent to the size of a pin head or a grain of Demerara sugar.

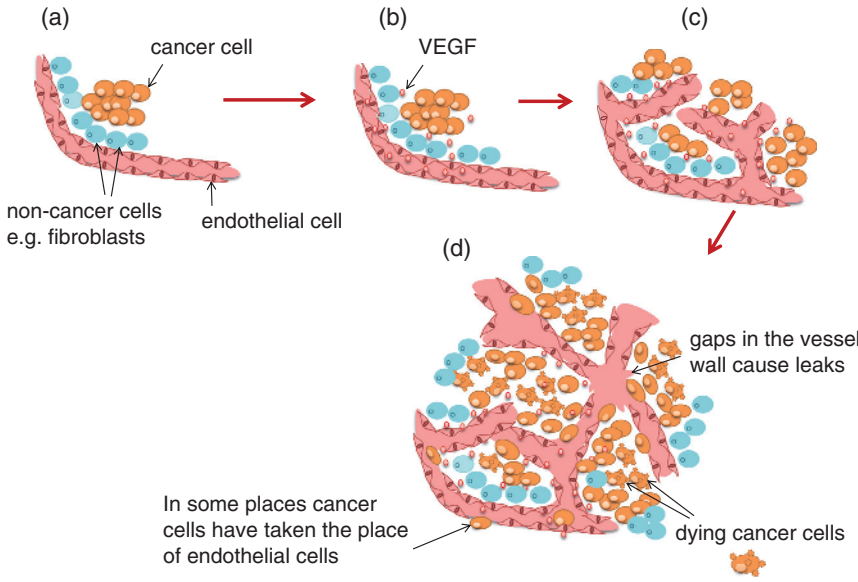

Figure 1.10 **Cancer angiogenesis. (a)** A cluster of cancer cells is too far away from the nearest blood vessel to receive an adequate blood supply. **(b)** The drop in oxygen levels triggers the cancer cells to release VEGF into their surroundings. **(c)** VEGF attaches to VEGF receptors on the surface endothelial cells, causing the blood vessel to sprout and grow. **(d)** The tumor contains a convoluted, lumpy, leaky network of blood vessels; many cancer cells now have sufficient blood supply, but many others do not. **Abbreviations:** VEGF – vascular endothelial growth factor

There are numerous reasons why cancers metastasize. For example:

- DNA mutations that some cancer cells contain might force them into behaviors that cause metastasis.
- Cancer cells that are on the move might enter a blood or lymph vessel and get carried along by the blood/lymph to distant sites.
- The cells, proteins, and structures in the cancer cells' environment, and the cancer cells' limited access to oxygen, can encourage cancer cells to become more mobile or to move in specific directions.

One important thing to realize is that cancer cells that metastasize might contain lots of mutations and display behaviors that aren't present in cancer cells that stay put. As a result, a patient's metastases might behave differently and respond to different treatments than the primary tumor.

1.6.1 Routes through Which Cancers Spread

There are five main routes through which a cancer can spread [71]:

- Local invasion
- Lymph vessels
- Blood vessels
- Nerves
- Fluid in the abdomen

Routes of Cancer Spread – via Local Invasion

"Local invasion" describes the process whereby cancer cells digest the ECM proteins that surround them and gradually move into, infiltrate, and destroy nearby tissues. Local

invasion is often the first step toward metastasis to distant organs.

Routes of Cancer Spread – via Lymph Vessels (Lymphatic)

The fluid around our cells drains into lymphatic vessels and from there into lymph nodes (also called lymph glands) and finally back into the bloodstream.[19] Cancer cells that have become detached from the cells around them are often caught up in this flow and carried to nearby lymph nodes.

Routes of Cancer Spread – via Blood Vessels (Vascular)

Individual cancer cells (and small clusters) are sometimes able to squeeze their way into small blood vessels. The red and white blood cells in the vessel then sweep the cancer cells along until they get stuck somewhere else. Cancer cells that have found their way into the bloodstream are called **circulating cancer cells** or **circulating tumor cells** (CTCs).

Routes of Cancer Spread – via Nerves (Perineural)

This is a relatively rare but dangerous route of cancer spread in which cancer cells spread along the course of nerve bundles. This type of spread is often very painful because cancer cells produce chemicals that trigger nerve activity.

Via Fluid in the Abdomen or (Transcoelomic)

Cancers that arise in the abdomen, particularly ovarian cancers, are liable to spread via the fluid that circulates within the abdomen. Cancer cells on the surface of the tumor break away and float in the abdominal fluid (this fluid bathes our internal organs). Cancer cells are carried along in the fluid and then adhere to tissues and organs in the abdomen such as the omentum[20] or bowel.

Once a cancer cell has reached a new location in the body, it won't necessarily cause a new cancer to grow. In fact, the vast majority of breakaway cancer cells die in the lymph or blood, are killed by white blood cells, or simply remain dormant (see Figure 1.11). In order for the cell to cause metastasis, it must survive and thrive in its new environment. And only a tiny proportion of breakaway cancer cells are ultimately able to go through this process.

1.6.2 Locations to Which Cancers Spread

Some cancers have particular routes of spread that are more likely than others (e.g., breast cancer commonly spreads via the lymph system). And each type of cancer is also more likely to spread to some locations than others [72]. For example:

- Breast cancers often spread to the bones, brain, liver, and lungs.
- Prostate cancers often spread to bones.
- Bowel cancers often spread to the liver, lungs, and the lining of the abdominal cavity (peritoneum).
- Lung cancers often spread to the adrenal glands, bone, brain, liver, and/or into the other lung.
- Melanoma skin cancers often spread to the lungs, brain, skin, and liver.

The preference that cancers have to spread to some locations rather than others is often due to the anatomical layout of lymph and blood vessels. For example, the blood supply to the bowel goes from there to the liver, hence the liver is where bowel cancers often spread to first [73].

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[19] For a colorful illustration of the lymph system, see the Cancer Research UK website: http://www.cancerresearchuk.org/what-is-cancer/body-systems-and-cancer/the-lymphatic-system-and-cancer [accessed April 4, 2017].

[20] The omentum is a fold of fatty tissue that hangs down from the stomach and covers our intestines and other organs.

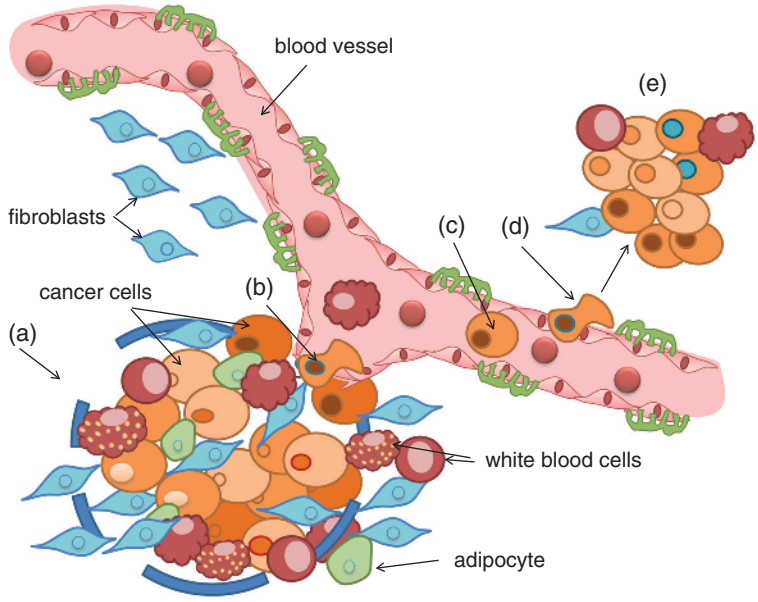

Figure 1.11 The path to metastasis. (a) A primary tumor containing many different cell types. **(b)** A cancer cell that is particularly mobile might invade locally and squeeze its way into blood vessels. **(c)** A cancer cell circulating in the blood. **(d)** The cancer cell squeezes out of the blood vessel into a new environment. **(e)** In its new location, the cancer cell may die or remain dormant for weeks or even years, kept in check by its new environment. However, eventually a change in its environment or the gain of new mutations might enable it to multiply and create a metastasis.

1.6.3 Reasons Why Cancers Spread

Many of the cells in a cancer seem to be relatively inert and dormant, perhaps because of low oxygen levels or in response to signals sent out by their cancer and non-cancer neighbors. However, other cancer cells can be highly mobile and likely to cause metastasis. Scientists believe that these mobile cells have gone through a change in appearance and behavior called **the epithelial-to-mesenchymal transition (EMT)** [74] (see Figure 1.12).

The EMT is a change that some healthy cells undergo in a developing embryo or in an adult when a tissue is damaged. It's when a stationary, well-connected epithelial cell becomes more like a mobile, independent mesenchymal cell. During the EMT, the cell produces more ECM proteins, becomes more resilient, and changes shape [74].

The EMT is thus a natural process that is hijacked and reactivated by cancer cells [74]. Understandably, if a cancer cell goes through this change, it's more likely to cause metastasis than other cancer cells.

Triggers that encourage cancer cells to go through the EMT include growth factors and other chemicals released by neighboring cells, low oxygen levels, and contact with various ECM proteins [75].

The EMT appears to be very important and poses huge problems for doctors. For example, cancers that contain a high proportion

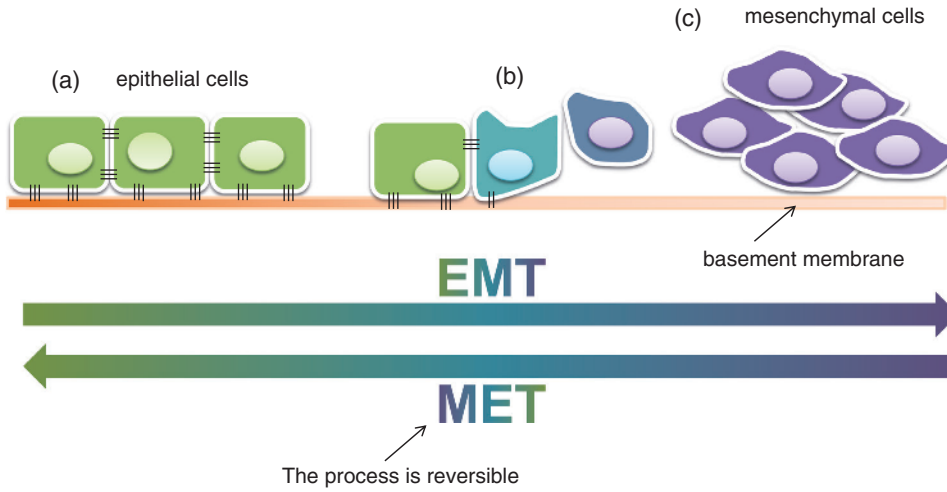

Figure 1.12 The epithelial-to-mesenchymal transition (EMT). (a) All our body's organs and tissues are lined with epithelial cells. Epithelial cells tend to be lined-up and well connected to one another. They are also physically attached to the basement membrane. **(b)** During the EMT, cells gradually lose epithelial proteins and gain mesenchymal proteins. **(c)** Mesenchymal cells are mobile and resilient and less well connected to one another and the basement membrane.
Abbreviations: EMT – epithelial-to-mesenchymal transition; MET – mesenchymal to epithelial transition

of mesenchymal cells are more likely to resist treatment and spread quickly [76]. Also, some treatments seem to cause cancer cells to go through the EMT, helping them survive the effects of treatment and causing metastasis [77].

1.7 CANCER STEM CELLS

Over the past 20 years or so, scientists have increasingly become convinced that a proportion of cancer cells behave somewhat like our body's stem cells[21] and can be classed as **cancer stem cells**. That is, they not only have the ability to multiply to generate further cancer stem cells, but they can also produce cancer cells with other properties. Therefore, **if you kill all the other cells in a tumor but leave the stem cells behind, they will cause the cancer to return**. Evidence suggests that cancer stem cells are relatively rare, slow-growing, drug-resistant cancer cells that can survive many cancer treatments [78]. The strength of evidence for their existence varies from cancer type to cancer type [78].

The precise properties of cancer stem cells and where they come from are hotly debated by scientists. Some scientists suggest that they could start out life as healthy adult stem cells that, due to DNA mutations, start behaving like cancer cells. Other scientists point to the

[21] Adult stem cells are slow growing, versatile cells found in small numbers in our organs and tissues. When they multiply they create mature, specialized cells that replenish, repair, and renew the tissue and keep it healthy. The number of stem cells differs from organ to organ and tissue to tissue around the body, depending on the turnover of cells in that tissue. For example, there are many stem cells in the lining of the bowel because cells are continually being scraped off as food passes through, and the scraped-off cells need to be replaced.

similarities between cancer stem cells and cancer cells that have gone through the EMT. And they suggest that cancer stem cells are derived from cancer cells that have gone through the EMT and that have later undergone further changes [79].

Two of the problems scientists face when trying to study cancer stem cells are that (1) they are highly changeable and adaptable cells and that (2) what constitutes a cancer stem cell varies from cancer to cancer and even from patient to patient [80]. So it's best not to get too worked up about the label "cancer stem cell." Instead, we will simply acknowledge that there are often cells in a cancer that are not easily destroyed by treatments and that can cause a cancer to return weeks, months, or years later.

1.8 OBSTACLES THAT PREVENT US FROM CURING CANCER

In this chapter, I've explained some of what we now know about how cancers come about and why cancer cells behave as they do. I've also described some of the behaviors that cancer cells exhibit. And I've described the diversity that often exists within tumors in terms of the types of cells found in them and the genetic diversity among cancer cells. Armed with all this knowledge about cancer, it's tempting to believe that we might know enough to cure everyone affected by the disease. However, as I'm sure you know, this isn't the case.

So what is it that still thwarts us? What features of cancer cells and cancer behavior are responsible for our inability to cure it, particularly when it has metastasized?

As a conclusion to this introductory chapter, I'm going to go through some of the chief obstacles to curing more cancer patients:

- The similarities between cancer cells and healthy cells
- The great dissimilarities between different types of cancer
- The fact that cancer spreads
- Genomic instability and intratumoral heterogeneity
- The tumor microenvironment

There are, of course, other obstacles to successfully curing a patient of cancer. Not least are the issues of late diagnosis, the difficulty of eliminating every single microscopic cancer cell, and the fact that many cancer patients are relatively elderly and frail and have other medical complaints that often preclude the use of aggressive treatments. However, these issues are beyond the scope of this book, so I'll stick to describing the five obstacles I've listed above.

1.8.1 The Similarity between Healthy Cells and Cancer Cells

All our cells **have the same repertoire of roughly 21,000 genes** that contain the instructions for making all the proteins our cells will ever need. And as you might have already gathered from the rest of this chapter, cancer cells never do anything completely new. Instead, they overproduce or produce faulty, overactive versions of proteins that help them grow, multiply, and stay alive. And they underproduce or produce dysfunctional versions of proteins that would normally limit their growth or encourage them to die.

So, although we might think that a cancer is an unnatural aberration that needs destroying, a patient's body doesn't necessarily think the same. And although it's true that our immune system is powerful enough to rid the body of cancer, it often doesn't do so (although it's impossible to say exactly how many of us have avoided cancer thanks to the vigilance of our immune system).

Because **cancer cells are very similar to healthy cells**, it's very difficult to create drugs that can kill one without the other. Newspapers

and web pages are often littered with stories about chemicals from many different sources that can kill cancer cells grown in a lab. But that isn't difficult. The difficulty is finding chemicals that can the kill cancer cells in a person **while leaving their healthy cells alone**. And this is virtually impossible. So every treatment, no matter how targeted we might think it is, will kill some healthy cells alongside killing cancer cells. And that means that every cancer treatment causes side effects. The severity of a treatment's side effects often limits how much of the treatment can be given to a patient safely, and that ultimately compromises the treatment's ability to cure them.

1.8.2 Differences between Different Cancer Types

I'm often asked whether there will ever be "a cure for cancer." And if all cancers shared the same DNA mutations and behaviors, my answer might perhaps be "yes." But as it is, there are many, many different types of cancer. And **each cancer has its own unique vulnerability to different treatments**. And not only is it possible to develop liver cancer, stomach cancer, bowel cancer, skin cancer, and so on, but there are also **many different types of cancer that can occur in each location**. For example, there are adenocarcinoma and squamous cell carcinoma versions of non-small cell lung cancer, estrogen receptor positive and estrogen receptor negative breast cancer, and various different types of skin cancer.

In recent years, scientists have uncovered more and more information about the various forms of cancer, what drives them, and what impacts their behavior. And this knowledge is gradually improving our ability to treat people more effectively. However, the complexity is mind-blowing. And even when two cancers appear to be driven by the same mutations, it's not necessarily the case that they will respond to the same treatments. It depends on precisely how the cells' internal proteins interact with one another, and how the cancer cells interact with the cells around them. For example, in 50% of people with melanoma skin cancer, the cancer cells contain a mutation in a gene called *BRAF*. Treatment with a B-Raf inhibitor shrinks 50%–80% of these cancers [81]. The same *BRAF* mutation is also found in the cancer cells of 8%–10% of people with bowel cancer. But in bowel cancer, a B-Raf inhibitor does not work, at least not unless it's combined with at least two other treatments [82, 83].

So, for every cancer, and for every subset of every cancer, we have to discover exactly how the cells are wired up – what's driving them and what's protecting them – before we can uncover how best to treat them. As a result, there will never be "one cure" for all cancers.

1.8.3 Cancer Spread

Cancer metastasis has important implications for treatment. For example, cancer cells in distant organs might contain different mutations compared to those in the original (primary) tumor. Consequently, they wouldn't be destroyed by a cancer treatment chosen by a doctor for its ability to target the primary tumor [69].

Also, there is often a lag between the cancer cells' arrival in a new location and their growth into a metastasis. During the lag period, the cancer cells are dormant and unlikely to be killed by chemotherapy or other cancer treatments [84]. The length of time the cancer cells remain dormant, and the likelihood that they will cause metastasis, varies from cancer to cancer. For example, relapses several years after surgery are common in people with breast, prostate, kidney, and melanoma skin cancer [84].

In addition, cancer cells that have traveled to locations like the brain or bone marrow will **receive protection and support from their new environment**. The brain, in particular, is difficult for drugs to penetrate, has a large nutrient

supply, and is relatively protected from the immune system [85]. Also, the bone marrow is full of white blood cells and other cells that churn out survival-promoting chemicals that can help cancer cells survive and multiply [86].

Lastly, scientists have made lots of progress in identifying the gene mutations that cause cancer and that drive its growth. They've also created many treatments that target the consequences of these mutations. However, a lot less progress has been made in identifying the mutations that drive metastasis. And they've developed very few treatments that specifically target metastatic cancer cells [69]. So once a cancer has metastasized and become resistant to treatments, doctors currently have very little to offer their patients.

1.8.4 Genomic Instability and Intratumoral Heterogeneity

Genomic instability and intratumoral heterogeneity[22] are huge obstacles for scientists and doctors.

One problem that intratumoral heterogeneity causes is **that a biopsy sample taken from a patient's cancer might not be representative** of their cancer as a whole. So if you analyze a biopsy sample for the presence of a particular protein or a particular mutation and then treat the patient accordingly, you may end up killing only a minority of the cancer cells [87, 88] (see Figure 1.13a). For example, it might have seemed from a biopsy that a cancer is driven by the high numbers of EGFR proteins on the cells' surface. However, in reality, these cells were in the minority, and the majority of cancer cells were driven by a different protein. Because of this, giving the patient an EGFR-targeted treatment would have little impact.

In addition, if you use a treatment that targets one particular protein (as is the case with many of the treatments mentioned in this book), it is inevitable that there will be cells in the tumor that have mutations that make them impervious to your treatment (see Figure 1.13b & c) [89, 90].

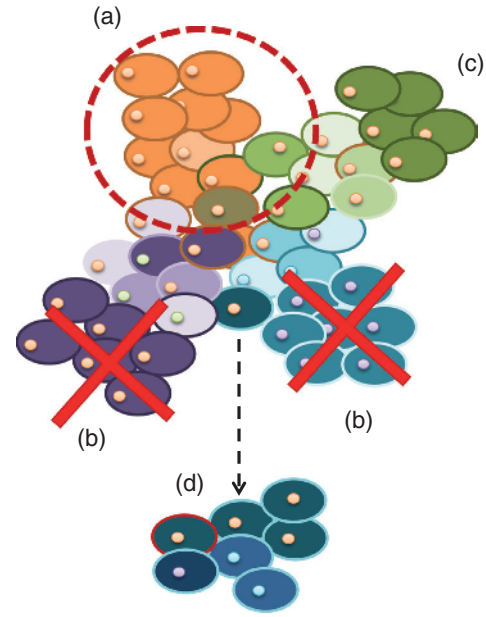

Figure 1.13 Intratumoral heterogeneity is an obstacle to effective cancer treatment. Due to the genomic instability of cancer cells, cancers often contain multiple populations of cancer cells driven by different combinations of mutations (represented by the different colors). **(a)** A biopsy sample (illustrated by the dotted red circle) does not contain representative cells from the whole cancer and may give scientists a skewed view of what mutations are driving the cells' behavior. **(b)** Some cancer cells are killed by a treatment (red crosses). **(c)** However, many other cells contain mutations that make them resistant and able to survive. **(d)** A cancer cell that leaves the original tumor and creates a metastasis elsewhere in the body may have very different properties from the original tumor.

[22] As you might remember from Section 1.4, intratumoral heterogeneity is the phrase scientists use to describe the fact that most cancers contain multiple populations of cancer cells driven by different combinations of gene mutations.

In fact, often there are multiple treatment-resistant cancer cells, each with a different resistance-causing mutation. And when the cancer returns, each new cancer growth may be driven by a different set of mutations [91].

In general, cancers that contain the most mutations (e.g., melanoma skin cancer and lung cancers in smokers) are also those that **evolve most quickly and contain the highest degree of intratumoral heterogeneity** [92, 93]. These cancers also have the shortest durations of response to treatment, and the patient's cancer quickly starts growing again [89].

A final problem caused by intratumoral heterogeneity is the way it enables cancers to change over time. Therefore, the cancer cells that drive recurrence and metastasis often contain different gene mutations and have different survival mechanisms than the cancer cells that were first present (Figure 1.13d) [88]. So when a cancer starts growing again, it's likely to be impervious to the treatments used previously (any cancer cell that was vulnerable to that treatment is already dead), hence **the cancer gets harder and harder to treat.** And referring back to an archived tumor sample might not tell you what is driving the cancer now, nor give you accurate information about what treatment to use [88].

Thus, intratumoral heterogeneity is a huge barrier to the successful treatment of cancer patients. Efforts to overcome this problem center on:

- Using logical combinations of drugs that target different faulty proteins and pathways and that synergize with one another to kill a more diverse range of cancer cells than any individual treatment used on its own
- Innovations in the analysis of cancer cells circulating in a patient's bloodstream, and using these cells to track the cancer cells' evolution and predict drug resistance-causing mechanisms
- Taking multiple biopsies from a tumor and its metastases to gain a fuller picture of the mutations driving the cancer
- Developing treatments such as immunotherapies that are less selective and may be able to kill a broad range of cancer cells driven by different mutations (see Chapter 5)

A final note: in the past, intratumoral heterogeneity was uniformly considered to be a bad thing because it causes rapid drug resistance. However, the creation of new immunotherapies (like checkpoint inhibitors – Chapter 5, Section 5.3) has led some scientists to think differently, as it seems that patients with **cancers with the most mutations are also the most likely to benefit from immunotherapy** (although it's not always that black and white) [94]. As a result the frustration of rapid resistance to targeted treatments is now balanced by optimism about the possibilities of immunotherapy.

1.8.5 The Cancer Microenvironment

The environment in which cancer cells live can have an enormous impact on whether a treatment given to a patient is effective. Even if a drug is highly targeted and (in theory) highly effective against a patient's cancer, it still might have no impact if the cancer cells' microenvironment is protecting them. Two main issues that affect a drug's effectiveness are (1) **the physical environment** in which the cancer cells live and whether the treatment can reach them, and (2) **the behavior of the non-cancer cells** that live alongside the cancer cells. For example [95]:

- Growth factors and other proteins released by non-cancer cells such as fibroblasts, white blood cells, endothelial cells, and adipoctyes (fat cells) can protect cancer cells from the effects of various treatments.
- In some cancers, the cancer cells' microenvironment contains a dense network of structural proteins (called **desmoplasia**) that compresses blood vessels and prevents cancer drugs from reaching the cancer cells.

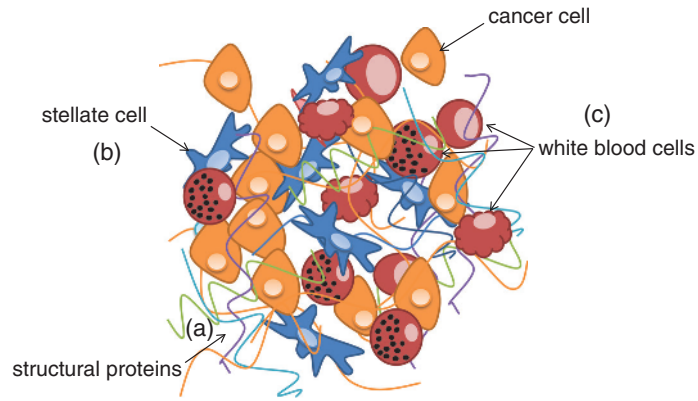

Figure 1.14 The pancreatic cancer microenvironment can protect cancer cells from the effects of treatment. (a) Pancreatic cancers often contain a dense, fibrous network of proteins that compresses any blood vessels that are present and prevents cancer drugs from penetrating the tumor. **(b)** Stellate cells (modified fibroblasts) produce fibrous proteins and release pro-survival proteins such as growth factors. **(c)** White blood cells secrete many small proteins and chemicals that protect cancer cells from treatments.

The classic example of the problems posed by the cancer microenvironment is pancreatic cancer. Many scientists have found combinations of chemotherapy and other treatments that can successfully kill pancreatic cancer cells grown in a lab, or grown in mice (called xenografts). However, these same treatments have failed to improve the survival times of most pancreatic cancer patients [96]. And one of the chief obstacles that stop treatments from working against pancreatic cancer is its microenvironment (Figure 1.14). It's not unusual for non-cancer cells to outnumber the cancer cells in these tumors, and the environment is awash with a diverse array of cells and proteins that together prevent drugs from penetrating and protect the cancer cells from death [96].

1.9 FINAL THOUGHTS

In this chapter, I have tried to give you a good idea of why cancers come about, what drives them, how they behave, and why we can't yet cure everyone who develops this disease. Do be aware, though, that this chapter covers just a small percentage of all that scientists have discovered about cancer cells. There are some big areas of science that I have missed out, such as epigenetics, micro-RNAs, the role of metabolic pathways and of viruses and infections, the similarities and differences between cancers in different organs, the difference between a benign tumor, a pre-cancerous lesion and an invasive cancer… Therefore, **this chapter is just a selection of information** that I have chosen because I think it might come in handy when you read later chapters.

Throughout the rest of this book, I'll be focusing on cancer treatments that target just one protein, or one cell process that is faulty in cancer cells. However, the proteins and processes that are targeted by these treatments represent just a small proportion of all the faulty proteins and processes that drive cancer cells and are responsible for the way they behave. I hope in this chapter I have given you a sense of this – that there are many genetic faults in cancer cells that aren't targeted by even the most recent cancer treatments.

Even so, the treatments described in the rest of this book target a range of different features

of cancer cells. These include treatments that target aspects of cell communication, the cell cycle, DNA repair, angiogenesis, and the interaction between cancer cells and the immune system. As well as mentioning them briefly in this introductory chapter, I will explain these processes in more detail when I come to describe the various treatments in later chapters.

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