LESSON 1.3 WORKBOOK

What do we know about how cancer is caused?

As our understanding of cancer as a disease has evolved we have increasingly turned our attention to questions of what causes cancer in the first place. This lesson shows how three competing theories about the cause of cancer - infectious agents, environmental toxins, and genetics were reconciled. How? The answer, as you will see, lies in our DNA.

What causes cancer? — carcinogens

In that last lesson, we discussed how our understanding of cancer as a disease shifted as our understanding of physiology evolved. Until we progressed beyond belief in the influence of supernatural forces, such as gods or spirits, the question ‘What causes cancer?’ would not get a rational answer. It was the rise of empiricism - the view that natural phenomena occur because of natural forces – in the 16th century that set the stage for rational studies into the natural causes of cancer.

One of the first approaches was simply to determine what different people suffering from a similar cancer had in common. Percival Pott, an English physician working in the 16th century employed this approach very successfully to discover the cause of scrotal cancer. Pott noticed that young chimney sweeps were particularly susceptible to scrotal cancer. Chimney sweeps were usually poor, indented orphans, small enough to fit up chimney flues. They were often sent up chimneys naked and lubricated in oil.

Figure 1: Percival Pott (left) was the pioneer of the field of epidemiology, the study of patterns of disease within a population. He identified the cause of scrotal cancer among young chimney sweeps (right) in London.

MC Questions:

1. What would be the first step in identifying the cause of a specific cancer?
   a. Determine how people with that cancer are similar.
   b. Inject people with the agent you think might cause the cancer.
   c. Do studies to see if the agent can cause the cancer in rats.
   d. All of the above would be a good first step to identify a cause of cancer.
LESSON READINGS

Careful observation (coupled with poor hygiene) allowed him to detect the “lodgement of soot in the rugae [ridges] of the scrotum”. Pott’s work identified exposure to chimney soot as the culprit in scrotum cancer, leading the British government to improve working conditions for children that reduced their exposure to chimney soot. Potts was one of the first pioneers of epidemiology, the study of how patterns of disease are found in a population.

More than a century after Pott’s work, two Japanese scientists, Katsusaburo Yamagiwa and Koichi Ichakawa, confirmed Pott’s finding by painting the tar found in chimney soot onto rabbits’ skins. They hypothesized that the tumors that developed where the tar was applied were caused by chemicals in the tar. They then identified which chemicals in the tar were responsible, and coined a new term for these chemicals - carcinogens.

But it soon became clear that cancer could be caused by other agents, not just carcinogens, and the race was on to identify what these agents are, and how they work.

What causes cancer? - pathogens

Before Louis Pasteur, the popular notion was that illness is caused by “bad air”. After Pasteur proposed the germ theory of infectious diseases, the idea that disease is caused by pathogens became very attractive. Could pathogens also cause cancer? To many scientists the idea that pathogens could be carcinogenic was very plausible, and they set about identifying them.

By 1911, viruses were being isolated, and Peyton Rous had the idea that viruses might also be able to cause tumors. He started off with chicken sarcomas (a type of solid tumor), isolated the tumors, ground them up and passed the mixture through a very small filter that would permit a virus to pass through but would hold back other types of pathogens. Then he injected the filtrate into healthy chickens. Sure enough the healthy chickens developed sarcomas too. Rous went one step further. He made filtrates from the newly formed sarcomas and injected them into more healthy chickens. These filtrates too caused sarcomas. Rous identified the infectious agent that caused the tumors as a new virus and called it Rous sarcoma virus after himself and the type of tumors it caused.

MC Questions:

2. True or False: A carcinogen is any chemical agent that causes cancer
   a. True.
   b. False.

3. Why were pathogens so popular as a model to explain the cause of cancer? (Circle all correct.)
   a. Louis Pasteur had developed the germ theory, so people thought diseases should have a specific cause.
   b. The experimental evidence linking carcinogens to cancer was indirect.
   c. The pathogen model was favored by strong personalities.

Figure 2: Rous showed that he was able to transfer cancer (red arrow) in chickens, suggesting cancer was an infectious disease.
After Rous’ discovery, many more viruses capable of causing tumors in animals like mice, rats and cats were identified including viruses that caused leukemia and lymphoma (cancers of the immune system) as well as breast cancer. Puzzlingly though, none of these cancer-causing viruses were found in humans. Nor were there any other viruses associated with human cancers. If viruses cause cancer, why couldn’t anyone find viruses in human cancers?

What causes cancer? - our genes

At the same time cancer virologists were isolating the viruses that caused cancers in animals, yet other scientists had turned their attention to genes. Even as far back as the 19th century physicians had noticed that some types of cancers, particularly of the breast and ovaries, ran in families, but what this observation meant was not yet clear. Then, in 1872, a Brazilian ophthalmologist, Hilério de Gouvea, successfully treated a young boy with a rare eye cancer called retinoblastoma by removing the affected eye. His patient grew up and married a woman with no family history of cancer. The couple had several children, and two of their daughters developed the same type of retinoblastoma that their father had suffered from. Both died. de Gouvea believed this provided evidence that retinoblastoma is inherited, not caused by environmental carcinogens or pathogens.

Around the same time, Theodor Boveri, an assistant of Rudolph Virchow (who we read about in the last workbook) was studying mitosis by treating sea urchin eggs with a dye that stained chromosomes. He observed that if the eggs were fertilized by multiple sperm their progeny would have aberrant chromosomes. In a burst of creativity, he likened this chromosomal chaos to cancer. In his 1914 manuscript, “Concerning the Origin of Malignant Tumors”, he argued that cancer too was caused by disrupting chromosomal order and structure.

In retrospect, given how little we understood at the time about what chromosomes actually do, this logical leap was extraordinary. However because so little was known about how cells worked internally, it did not answer the question: “How could external agents like carcinogens and viruses as well as internal structures like chromosomes all be responsible for causing cancer?” With little to reconcile these opposing views, the cancer field remained divided into 3 camps until well into the 20th century.

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**MC Questions:**

4. Which of the following were a proposed cause of cancer? (Circle all correct.)
   a. Environmental factors.
   b. Viruses.
   c. Genetic inheritance.
   d. Animal bites.

5. Which of the following cancers were studied through observation, not direct experimentation? (Circle all correct.)
   a. Scrotal cancer in chimney sweeps.
   b. Familial retinoblastoma.
   c. Lymphoma.
   d. Leukemia.
A unified theory of cancer emerges

Eventually a number of discoveries began to build bridges between these seemingly irreconcilable theories: One of the first, in 1915, was by Thomas Hunt Morgan, who showed that chromosomes are like necklaces, the beads on the necklace being traits or genes. Morgan suggested that the aberrant chromosomes Boveri had seen in the hyper-fertilized sea urchin eggs were caused because the genes on the chromosomes had been disrupted. Morgan’s work was followed by Oswald Avery’s discovery in the 1940s that genes are encoded by DNA, and Watson and Crick in the 1950s of the code DNA uses to store information about what each gene does.

The logical interpretation was that if DNA in normal cells becomes damaged, the cells will form tumors. But how could external agents like carcinogens, or infectious agents, like viruses all damage DNA? In the 1970s, Bruce Ames used bacteria, which divide, and therefore mutate, very rapidly, to show that how carcinogens cause DNA to mutate. Importantly, how well a potential carcinogen mutagenizes DNA in bacteria directly relates to its ability to induce a tumor in rats.

The Ames test uses a specific bacterium that already has a mutation in a gene involved in making the amino acid histidine, which the bacteria needs for growth and survival. As a result these defective bacteria grow very poorly. There are two ways we could overcome the defect in histidine: We could give the bacteria a source of histidine so they wouldn’t have to make it themselves – by incorporating histidine into the agar in the petri dish for example. Alternatively we could treat the bacteria with a chemical that would mutate the mutation, making the bacteria normal again! This is the basis for the Ames test: The defective bacteria are treated with a possible mutagen and then their ability to grow is monitored. If the mutagen fixes the existing mutation, the bacteria will be able to make their own histidine again, and will grow normally on agar, even without a histidine supplement.

**DEFINITIONS OF TERMS**

**Mutagen** – any chemical or agent that is capable of mutating DNA sequence.

**Oncogene** – a mutated gene that, when expressed, promotes cancer in cells.

**Proto-oncogene** – a gene that when expressed acts as a growth factor, and that, when mutated, will become an oncogene and promote cancer in cells.

**MC Questions:**

6. Whose work explained how carcinogens cause cancer by mutating a gene’s DNA?
   - a. Theodor Boveri.
   - b. Bruce Ames.
   - c. Varmus and Bishop.
   - d. All contributed in explaining this model.

7. True or False: The Ames test can show that a chemical is a carcinogen.
   - a. True.
   - b. False.
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So now we can see how carcinogens turn normal cells into tumors by mutating their DNA, but how do viruses cause tumors to form? Carrying the gene idea forward, a group of scientists identified that the Rous sarcoma virus needs a certain gene in order to cause tumors. They called this gene src (pronounced ‘sarc’, a diminutive of sarcoma). Because of its ability to cause cancer they called src an oncogene. The virus camp was excited because they finally had a model – when viruses infect cells they cause cancer via oncogenes.

Then disaster seemed to strike! Another group, led by Harold Varmus and Michael Bishop made the surprising discovery that src is present in cells of most species, including humans. But if src is already present in normal cells how could it cause cancer? It didn't make any sense. Finally Varmus and Bishop figured out that in normal cells the src gene is important to promote growth and survival, but in viruses it has been mutated so it can cause cancer. Viruses must have originally picked up src when they infected normal cells and then mutated it as they replicated. They called the host cell src genes, proto-oncogenes to emphasize that they came before the viral oncogenes (protos is Greek for “first”). Soon many other proto-oncogenes and oncogenes were identified.

A unified theory of cancer

With this last piece of the puzzle a unifying theory of cancer became could be built:

- Proto-oncogenes normally promote cell growth and survival and become mutated to cause cancer.
- The mutations can either occur randomly or be induced by carcinogens.
- Or they can be introduced into cells by viruses that contain the mutated oncogenes.
- If the mutations are found in germ cells (like eggs and sperm) they will be inherited.

It is important to emphasize that in order for a carcinogen to cause cancer it must mutate a proto-oncogene in just the right way. But mutations are random, so not all mutations of a proto-oncogene will be cancer-causing and in fact most mutations won’t even affect proto-oncogenes at all. So we simply can’t

DEFINITIONS OF TERMS

Synergistic – phenomena where two factors increase the risk of cancer in combination than each factor by itself.

MC Questions:

8. True or False: Carcinogens randomly mutate DNA to cause cancer.
   a. True.
   b. False.

9. Why is it so difficult to establish that a substance is a carcinogen? (Circle all correct.)
   a. Exposure doesn’t always cause cancer.
   b. Carcinogens randomly mutate DNA and not all mutations will lead to cancer.
   c. We don’t know all the proto-oncogenes involved in cancer.
   d. Few cancers are caused by pathogens.
predict whether a specific exposure to a carcinogen will cause cancer. By the same token though, the more exposure occurs, the more likely the critical mutation will happen.

But this is not the full story either: As we saw with RSV, a mutation in a single proto-oncogene can cause a tumor to form, but will it be sufficient to cause actual cancer? We will see in Chapter 3 that more than one proto-oncogene needs to be mutated for a tumor to turn into cancer. In fact there is a whole other class of genes (called tumor suppressors) that can also be mutated to lead to cancer - we will talk about them too in Chapter 3. In fact the reason why cancer symptoms often appear later in life, is because it takes a while for mutations in different proto-oncogenes to accumulate.

It should be clear by now that cancer may have many different causes: For instance, lung cancer can occur through random mutation of proto-oncogenes, or there may be a genetic predisposition, or it may be caused by environmental carcinogens such as the coal tar compounds in cigarette smoke or asbestos. The effects can be synergistic - a genetic predisposition for lung cancer because of a proto-oncogene mutation coupled to prolonged exposure to carcinogens in smoke asbestos will increase the chances of lung cancer developing.

MC Questions:

10. Which of the following might increase your risk of developing cancer?
   a. Heavy metals.
   b. Smoking.
   c. Excessive tanning.
   d. All of the above.
Describe the link between how (a) a carcinogen, (b) a pathogen, and (c) an inherited mutation, all promote cancer.
**TERMS**

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<thead>
<tr>
<th>TERM</th>
<th>DEFINITION</th>
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<tbody>
<tr>
<td>Carcinogen</td>
<td>An agent that is responsible for, or associated with cancer.</td>
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<tr>
<td>Empiricism</td>
<td>The view that things occur because of natural, observable causes.</td>
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<tr>
<td>Epidemiology</td>
<td>The study of how patterns of disease are found in a population.</td>
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<tr>
<td>Leukemia</td>
<td>A cancer of cells of the immune system.</td>
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<td>Lymphoma</td>
<td>A cancer of lymph nodes, or other cells of the immune system.</td>
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<td>Retinoblastoma</td>
<td>A specific type of cancer affecting retina cells of the eye that is commonly inherited as a disease.</td>
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<td>Sarcoma</td>
<td>A specific type of cancer that is caused by Rous Sarcoma Virus in chickens.</td>
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<tr>
<td>Synergistic</td>
<td>The phenomena where two factors increase the risk of cancer in combination than each factor by itself.</td>
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