Do Plants Get Cancer? The Effects of Infecting Sunflower Seedlings with *Agrobacterium tumefaciens*

Overview

Students infect sunflowers with *Agrobacterium tumefaciens*, record the growth of any tumors, and section the stem in several locations to observe changes in cell number, shape, and size.

Introduction

Cancer develops when cells in a part of the body begin to grow out of control, usually due to a mutation in the DNA. Although there are many kinds of cancer, they all appear to start because of out-of-control growth of abnormal cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide more rapidly until the person becomes an adult. After that, cells in most parts of the body divide only to replace worn-out or dying cells and to repair injuries. Because cancer cells continue to grow and divide, they are different from normal cells. Instead of dying, they outlive normal cells and continue to form new abnormal cells.

Cancer cells can sometimes travel to other parts of the body where they begin to grow and replace normal tissue. This process, called metastasis, occurs as the cancer cells get into the bloodstream or lymph vessels of our body. When cells from a cancer, like colorectal cancer, spread to another organ like the liver, the cancer is still called colorectal cancer, not liver cancer.

Cancer cells develop because of damage to DNA. DNA is in every cell and directs all activities. Most of the time when DNA becomes damaged, the body is able to repair it. In cancer cells, the damaged DNA is not repaired. People can inherit damaged DNA, which accounts for inherited cancers. More often though, a person's DNA becomes damaged by exposure to hazardous chemicals, like cigarette smoke.

Cancer usually forms as a tumor. Some cancers, like leukemia, do not form tumors. Instead, these cancer cells involve the blood and blood-forming organs and circulate through other tissues where they grow. Remember that not all tumors are cancerous. Benign (non-cancerous) tumors do not spread to other parts of the body (metastasize) and, with rare exceptions, are not life threatening.

Different types of cancer can behave very differently. For example, lung cancer and breast cancer are very different diseases. They grow at different rates and respond to different treatments. That is why people with cancer need treatment that is aimed at their particular kind of cancer.

Cancer is a leading cause of death in the United States. Nearly half of all men and a little over one third of all women in the United States will develop cancer during their lifetimes. Today, millions of people are living with cancer or have had cancer. The risk of developing most types of cancer can be reduced by changes in a person's lifestyle -- for example, by quitting smoking, eating a better diet, and increasing physical activity. The sooner a cancer is found and treatment begins, the better are the chances for living for many years.

Why does cancer kill? The way in which cancer leads to early death is varied, and there is no single answer to this question. The answer really depends on the parts of the body that are affected. Generally speaking, if cancer spreads to take over a part of the body that performs an essential function, it can lead to death. For example, if the cancer is growing in part of the

digestive system, it can prevent the digestion and absorption of food. If cancer is affecting the lungs, then eventually there is too little effective lung tissue to allow enough oxygen to be absorbed into the body to sustain life. A person with an advanced cancer does not always have the strength to fight off such an infection, even with the help of antibiotics, and so the infection can lead to death. If the cancer has spread to the liver or the bones, this can upset the body's delicate chemical balance. The human body operates within very fine limits of certain body salts and chemicals. For example, there has to be a certain amount of calcium in the circulating blood. Too much or too little calcium can upset the whole system. If the cancer is affecting many of the bones in the body, then a lot of calcium is released into the blood stream. Normally the body has mechanisms to right this sort of imbalance. But when the balance goes out of control, then the mechanisms to correct the imbalance can become overwhelmed.

When cancer is growing in the bone marrow, then eventually there will not be enough healthy bone marrow to make blood cells. This will cause anemia (not enough red blood cells) and not enough oxygen will be carried around the body. It will also cause a drop in white blood cells. As these fight infection, it becomes more and more difficult for the body to keep bacteria and viruses under control. Many treatments can control cancer for a long time even if it cannot be cured. But if a cancer continues to grow, then unfortunately it can become too much for the body to cope with and ultimately the treatment can no longer keep it at bay.

Can plants get tumors? In short, yes, plants can get a type of tumor. However, in general it is not as harmful to plants as to animals. This is because plant cells have a cell wall and this cell wall inhibits the cells from moving. So while a plant can get uncontrolled division of cells and make a tumor, without cell movement, the cells can't metastasize. The tumors tend to remain localized. By and large, plants just live with their mutations and with tumors.

Additionally in animal cells, the mobility of cells requires mechanisms that prevent inappropriate cell movement and the disruption of patterning. This is usually accomplished by killing the cell using a mechanism called apoptosis, or programmed cell death. Animal cells require survival factors from neighboring cells to stay alive. With no such signal, the cells undergo apoptosis. While plants have capacity for apoptosis (for instance apoptosis is used during the formation of leaf lobes and the abscision of leaves in the fall), they also have the ability to police cell fate by de-differentating and reassigning the cell's fate. In culture, individual cells can not only survive but are totipotent, able to regenerate the entire plant. Instead of killing a cell off if in the wrong place, the plant will try to "reform" it to the appropriate fate.

One bacterial disease of plants, called crown gall, is caused by tumorigenic strains of the Gram-negative bacterium *Agrobacterium tumefaciens*. The bacterium overwinters in infested soils. When host plants are growing in infested soils, the bacterium enters the roots or stems near the ground through wounds caused by factors such as freeze damage, grafting, or mechanical injury. The bacterium finds plants by detecting phenolic substances produced by wounded plant cells. Once inside the plant tissue, the bacterium moves from cell to cell, stimulating surrounding host cells to divide at a rapid rate. The bacterium does this by transferring a piece of its own DNA into the plant cell. This piece of genetic information does not come from the chromosome of the bacterium, but from a separate piece of DNA called a plasmid. The *A. tumefaciens* plasmid is called the tumor-inducing or Ti-plasmid, and the piece of DNA that is transferred to the plant is called the T-DNA. Following transfer to the plant, the T-DNA becomes integrated into the chromosomes of the plant cell. Genes on the T-DNA cause the plant cell to divide repeatedly, forming the gall or mass of undifferentiated tissue, and to produce chemicals called opines, which are used by the bacterium as food. The bacterium itself lives and multiplies in the intercellular spaces of the gall. These galls are one instance of tumors in plants.

Motivation

How many of you know someone who has had or has cancer? Can anyone tell me what cancer is? Do animals get cancer? What about plants?

Objectives

Upon completion of this lab, students should be able to

1. Describe the mechanism of action of the bacteria *A. tumefaciens.*
2. Discuss how cancer behaves in humans and in plants.
3. Explain why the plant cell wall makes a crucial difference in the pathophysiology of cancer.
4. Draw and label plant tumor tissue under magnification.
5. Explain how cells divide and the role of cell division in tumor formation.

Materials

- Sunflower seeds
- Planting pots (4" square ones work well)
- Potting soil
- Grow lights
- Inoculating needle
- Slides
- Five gallon bucket and bleach
- 70% ethanol
- Coverslips
- Razors
- Thread
- Bunsen burner or alcohol burner
- Compound microscope
- Matches

Associated California State Biology Standards

1a. Students know cells are enclosed by semipermeable membranes that regulate their interactions with their surroundings.

1c. Students know how prokaryotic cells, eukaryotic cells (including those from plants and animals), and viruses differ in complexity and general structure.

1j. Students know how eukaryotic cells are given shape and internal organization by a cytoskeleton or cell wall or both.

5e. Students know exogenous DNA can be inserted into bacterial cells to alter their genetic makeup and support the expression of new protein products.

Pre-lab preparation

1. Decide where you will be purchasing your *A. tumefaciens* strain. Carolina Biological Supply sells cultures of this bacterium.
2. Fill out the USDA forms for procuring infectious plant agents. You may download this form from Carolina's website. Make sure that you have a way to dispose of infected plants and soil. A bucket of dilute bleach works quite well. You can cut the plants into small pieces and soak in bleach for at least 12 hours.
3. Allow several months to process this application and get your permit to purchase the bacterium—fax the application in for faster response time. This permit is good for three years.

Procedure

Planting

1. Fill the bucket part way with a good general potting soil mix. Add water until this soil is moist, but not really wet. You can test the moisture content of the soil by squeezing a handful and seeing how much water is evacuated. You should be able to squeeze out a couple drops of water, but not much more.
2. Have students fill their planting pots (make sure pots have holes in the bottom) with this soil and press down lightly on the soil to remove large air pockets. Warn them not to compact the soil. Have students level the surface with their hands or a ruler.
3. Have groups place 4-5 sunflower seeds in their pots. The seeds should be spread out in the pot and pushed down in the soil approximately ½". Students should lightly cover the seeds with soil.
4. Place the pots into a tray in which water may be added.

5. Keep the soil moist, but not overly wet (bugs will gather in wet soils). When soil feels like it needs more moisture, have a student add water to the tray(s) that is holding all the pots.
6. Germinate and grow the plants under light banks that are on 24 hours a day. Try to avoid exposing the plant to temperature extremes (20-25° C is ideal).
7. When the seedlings are 2-3 inches tall, students should remove all but two of their seedlings. The two seedlings that remain should be the healthiest looking of the lot. You may transplant seedlings from one group to another at this time if one group was unsuccessful at germinating their seeds.
8. Plants are ready to inoculate when they are 7-10" tall and have two sets of true leaves (do not count the cotyledons). This is typically about 2 weeks after planting.

Inoculating the Plants

1. Wash your hands well and disinfect the work surface with 70% ethanol or other commercially available disinfectant.
2. Each group should have a control pot. With these plants, students will "mock inoculate" in that they will follow the inoculation procedure (which includes wounding the plant) but will not use bacteria.
3. Each group should follow this step one-at-a-time: A student should remove the cap from the bacteria tube and flame (with the Bunsen burner) the top of the culture tube. Then flame the inoculating needle and pick up a small amount of bacteria with the end of the needle. The student should then puncture each stem of their test plants several times at the node (the area where the stem meets the leaves) that is as close as possible to the top of the plant. Flame the needle again. For extra sterilization, the needle may be dipped into 70% ethanol before flaming.
4. For each site of infection, mark the area of the plant with a piece of thread. This will help keep track of site of infection.
5. The students, with a sterile inoculating needle, should then puncture the control stem plants *without* the bacteria as a control.

Observing Tumor Development

1. Students should begin to notice changes in the appearance of their plants by around 7-10 days post inoculation. Optimal tumor development for dissection occurs around three weeks post infection.
2. Have students observe and record the development of the tumors. You should have the students record their observations at least once a week.
3. At about 5 weeks time, have students draw and observe their control and infected plants.
4. Using a sterile razor blade, have students cut out sections of stem where either the tumor or puncture occurred. Then have students cut extremely thin slices of tissue cross-sections through the tumor or infection site. Place the thin slice of stem onto a microscope slide for observation under the compound scope. Each group should have a control slide and at least one tumor cross section slide. Several cross sections of a plant can go on one slide. Razor blades should be sterilized with alcohol after use.
5. Have students make observations and comparisons of the control and the infected stem cross sections. How many cells are there (at least roughly)? What shape are the cells? Are there other differences?
6. Once observations have been made. Collect all plant tissue, soils, and the culture tube for disposal. It is best if they are autoclaved, however that is not always practical. In this case, the plant matter can be chopped up and place in a bucket with the soil. Add a disinfectant to this bucket so that all the matter is covered. Dilute bleach works well. Leave the material in this solution for 12 hours before disposing of it.

Evaluation

The following questions are listed under the Analysis section of the student handout and may be used as part of a report, class discussion or assessment.

1. What did you notice that was different between the cross-sections of the stems infected with *A. tumefaciens* and those which where not infected?
2. Explain the role the cell wall plays in tumor development and cancer in plants. Contrast that with cancer in humans, whose cells don't have cell walls.
3. Explain how the bacteria *A. tumefaciens* tricks cells into making food.
4. How do cells control what goes into and out of a cell?
5. Did you notice differences in the shape of the cells between your control and your test plants? Make a hypothesis about how and why the cells' shape is different between those cells infected with *A. tumefaciens* and those that are not.
6. Explain how scientists have manipulated these bacteria and used it to alter other organisms for economic or social changes.
7. Draw a schematic diagram of the cell cycle and label where cell division and DNA replication take place. What are the differences between animal and plants cell division?
8. Do tumor cells replicate through mitosis or meiosis?
9. Having seen that the formation of tumors depends on the ability of the cells to rapidly divide, can you think of possible therapeutic regimens that might help cure cancer?

Extension Activities

1. Go on a nature walk in your local school area and try to spot "tumors" on your local plants. Do some species seem more vulnerable that others?
2. Have the students investigate one treatment used in cancer of humans using the library or Internet sources. Students should explain how the drug or surgical procedure is used. What is the treatment targeting? How does the treatment work? Have the student design a poster presentation of this treatment. Have half the class visit the posters of their classmates while the other half presents and then switch roles. Have each student fill out an evaluation of the poster.

Test Preparation

1. Which of the following does not occur by mitosis?
   (A) Growth
   (B) Production of gametes
   (C) Repair
   (D) Development in the embryo
   (E) Cleavage
2. Which of the following is not found in plant cells?
   (A) Cell plate
   (B) Actin and myosin filaments
   (C) Microtuble organizing center
   (D) Cleavage furrow
   (E) Middle lamella
3. Which is TRUE of the cell cycle?
   (A) The timing of cell division is controlled by cyclins and CDKs
   (B) A characteristic of cancer cells is density-dependent inhibition
   (C) The cell cycle is controlled solely by signals external to the cell

Student Sheet: Do Plants Get Cancer? Name:_____

Procedure

Planting

1. Fill your 2 planting pots (make sure pots have holes in the bottom) with the soil provided and press down lightly on the soil to remove large air pockets. Do not compact the soil. You may level the surface with your hands or a ruler.
2. Place 4-5 sunflower seeds in each of your pots. The seeds should be spread out in the pot and pushed down in the soil approximately ½". Gently cover the seeds with a little more soil.
3. Place your pots under the grow lights in a tray in which water may be added. Make sure your pots are labeled with your names.
4. Keep the soil moist, but not overly wet (bugs will gather in wet soils). When soil feels like it needs more moisture, add water to the tray(s) that is holding all the pots.
5. When the seedlings are 2-3 inches tall, remove all but two of your seedlings. The two seedlings that remain should be the healthiest looking of the lot.
6. Plants are ready to inoculate when they are 7-10" tall and have two sets of true leaves. This is typically about 2 weeks after planting.

Inoculating the Plants

7. Wash your hands well and disinfect the work surface with 70% ethanol or other commercially available disinfectant.
8. When it is your turn and your area has been cleaned, remove the cap from the bacteria tube and flame (with the Bunsen burner) the top of the culture tube. Then flame the inoculating needle and pick up a small amount of bacteria with the end of the needle. Puncture each stem of the test plants several times at the node (the area where the stem meets the leaves) that is as near as possible to the top of the plant. After each inoculation, reflame and pick up more bacterium.
9. For each site of infection, mark the area of the plant with a piece of thread. This will help keep track of site of infection.
10. Then with a sterile inoculating needle, puncture the control stem plants *without* the bacteria.

Observing Tumor Development

11. You may begin to notice changes in the appearance of your plants by around 7-10 days post inoculation. Optimal tumor development for dissection occurs around three weeks post infection.
12. Record your observations every week as the tumor develops.
13. At three weeks time, draw and observe the control and infected plants.
14. Using a sterile razor blade, cut out sections of stem where either the tumor or puncture occurred. Cut extremely thin slices of tissue cross-sections through the tumor or infection site. Place the thin slice of stem onto a microscope slide for observation under the compound scope. Each group should have a control slide and at least one tumor cross section slide. Several cross sections of a plant can go on one slide. Razor blades should be sterilized with alcohol after use.
15. Write your observations and comparisons of the control and the infected stem cross sections. How many cells are there (at least roughly)? What shape are the cells? Other differences?
16. Collect all plant matter and soil and place in the appointed disposal area. Anything that has come in contact with the bacterium must be sterilized.

Analysis

On a separate sheet of paper please complete the following:

1. What did you notice that was different between the cross-sections of the stems infected with *A. tumefaciens* and those which where not infected?
2. Explain the role the cell wall plays in tumor development and cancer in plants. Contrast that with cancer in humans, whose cells don't have cell walls.
3. Explain how the bacteria *A. tumefaciens* tricks cells into making food.

4. How do cells control what goes into and out of a cell?
5. Did you notice differences in the shape of the cells between your control and your test plants? Make a hypothesis about how and why the cells' shape is different between those cells infected with *A. tumefaciens* and those that are not.
6. Explain how scientists have manipulated these bacteria and used it to alter other organisms for economic or social changes.
7. Draw a schematic diagram of the cell cycle and label where cell division and DNA replication take place. What are the differences between animal and plants cell division?
8. Do tumor cells replicate through mitosis or meiosis?
9. Having seen that the formation of tumors depends on the ability of the cells to rapidly divide, can you think of possible therapeutic regiments that might help cure cancer?