Educator's Guide for:
Arrested development: when cells make mistakes
"Requirement for p53 and p21 to sustain G_2 arrest after DNA Damage"

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NOTE: We recommend HHMI's 2013 Holiday Lectures on Science "Medicine in the Genomic Era" as supplement to the annotated research article. In this lecture series, leading medical researchers explain how advances in genomics are revolutionizing their work, leading to a better understanding of disease and to improved treatments.

Content Overview of Research Article

Connections to the nature of science

This article illustrates how scientists can use human cell lines to study important molecular pathways. Cell culture works very well for studying the basics of proteins and genes. They're also small and inexpensive, and they divide rapidly, helping generate data quickly. But when scientists want to investigate cancer at a tissue or organism level, they need to use a more complex, expensive, and time-consuming model, such as the mouse.

The importance of this scientific research

Mutations in *p53* are very common in cancer cells. This paper was one of several in the 1980s and 1990s that illustrated its crucial role and explained why the loss of *p53* is so problematic for the cell. It also contributed to our understanding of the cell cycle and its checkpoints.

The actual science involved

The authors used several cell lines (both normal and cancerous) to investigate the checkpoint functions of *p53* and *p21*. They used homologous recombination to disrupt these genes and observe how the altered cells respond to DNA damage.

Topics covered

- Cell cycle regulation and cell division
- DNA damage
- Cancer cells

Why this research is important

Prior to this paper, p53 and p21 were known to function at the G_1-S checkpoint, but their roles at the G_2-M checkpoint, if any, were unclear. This paper showed that these proteins are required for sustaining arrest at the G_2-M checkpoint following DNA damage.

Methods used in the research

- Fluorescence microscopy
- Flow cytometry
- Western blotting
- Southern blotting
- γ irradiation

Conclusions

The p53 and p21 proteins play a crucial role at the G_2-M checkpoint of the cell cycle, preventing cells with DNA damage from entering mitosis.

Areas of further study

p53 has been the subject of a great deal of research for decades, and we now know that it plays a critical role in regulating the cell cycle and is often mutated in tumor cells. Because many "broad spectrum" chemotherapeutic drugs either induce DNA damage in cancer cell or disrupt

their cell cycle, understanding how *p53* and other genes regulate these pathways gives us a better chance of developing successful, targeted treatments. Efforts to specifically target mutant *p53* in cancer treatment are ongoing.

Discussion Questions

1. Figure 5, panels A and B show cells with defective p21 going through mitosis in the absence (A) or presence (B) of DNA damage. What would you expect these two panels to look like if normal cells were used instead?

   A: Panel A should look roughly the same, because the absence of p21 doesn't have much of an effect on nondamaged cells. However, in panel B, a normal cell subjected to radiation should arrest prior to entering mitosis. Therefore, if you observed the normal cell over the same time period, it would not begin to divide until after the cell recovered.

2. Vincristine is a drug used in chemotherapy to treat cancer. It functions similarly to nocodazole. Why do you think this could be an effective cancer treatment? Check the annotations to remind yourself what nocodazole does.

   A: Vincristine and nocodazole prevent microtubules from growing, thereby keeping a cell from dividing. Prolonged exposure to these drugs will kill cells. Because only dividing cells are affected by these drugs, they preferentially kill cancer cells, whereas normal cells are relatively unharmed.

3. Many of our genes have important functions in different signaling pathways. In the cell cycle, for example, some genes drive the cell cycle forward (called oncogenes), whereas others slow it down (called tumor suppressors). The two forces keep each other in check. Now, imagine a cell divides in the presence of DNA damage and the chromosomes don't separate properly, leaving the daughter cells with unequal amounts of DNA. Why is it problematic for a daughter cell to receive extra copies (or no copies) of a single chromosome?

   A: If an extra copy of an oncogene or a missing copy of a tumor suppressor gene upsets that balance, the daughter cell might divide inappropriately, leading to cancer.

4. *p53* is sometimes called "the guardian of the genome," and many cancer cells harbor mutations in this gene. Why do you think this is the case? You can also use this interactive to help you answer this question:
   http://www.hhmi.org/biointeractive/eukaryotic-cell-cycle- and-cancer (Click on "Cell Cycle Regulators" in the center and then click on the stop sign at the G1 and G2 checkpoint.)

   A: The p53 protein (encoded by the p53 gene) plays a critical role at cell cycle checkpoints, as demonstrated in this paper. We know a little more about how p53 functions today. The protein is activated by DNA damage, and binds to the promoters of specific target genes. These target genes are often involved in regulating cell cycle progression or DNA repair. When p53 binds to the promoters of these genes, their expression increases, allowing the cell to pause and fix the damage. If the damage is too overwhelming, p53 can also induce programmed cell death (apoptosis). This is a last resort for the cell because it will die, but it prevents the development of cancer, which could potentially kill the organism. So, in many ways, p53 protects the genome and ensures the cell recovers from damage. When it is mutated or deleted, the checkpoints that prevent a cell from becoming cancerous are no longer in place.

Multimedia Resources from HHMI's BioInteractive (www.BioInteractive.org)

Lectures

Cancer as a Genetic Disease, Charles Sawyers, MD, 2013 Holiday Lectures on Science (http://www.hhmi.org/biointeractive/cancer-genetic-disease). Understanding that cancer is caused by mutations in genes that regulate cell proliferation has led to the development of targeted drug therapies.

From Cancer Genomics to Cancer Drugs, Charles Sawyers, MD, 2013 Holiday Lectures on Science (http://www.hhmi.org/biointeractive/cancer-genomics-cancer-drugs). Genetic data from a large number of tumor types reveal commonly mutated genes and uncover connections between different types of cancer.

Research Mechanics: Putting the Brakes on Cancer, Bert Vogelstein, MD, 2003 Holiday Lectures on Science (www.hhmi.org/biointeractive/research-mechanics-putting-brakes-cancer). Although there are numerous kinds of cancer, all stem from alterations that allow cell division to outstrip cell demise.

Chaos to Cure: Bringing Basic Research to Patients, Bert Vogelstein, MD, 2003 Holiday Lectures on Science (http://www.hhmi.org/biointeractive/chaos-cure-bringing-basic-research-patients). The identification of hundreds of genes involved in the formation and spread of cancer is leading to promising new methods for diagnosis, prevention, and treatment.

Videos

Cancer as a Genetic Disease: Video Highlights, Charles Sawyers, MD, 2013 Holiday Lectures on Science (http://www.hhmi.org/biointeractive/cancer-genetic-disease-video- highlights).

Animations

p53 (http://www.hhmi.org/biointeractive/p53). A 3D animation showing the molecule p53 binds to DNA and initiates the transcription of mRNA.

Damage to DNA Leads to Mutation (http://www.hhmi.org/biointeractive/damage-dna-leads- mutation). Reactive molecules, such as free radicals, and solar ultraviolet radiation can lead to mutations in DNA. Most mutations are corrected, but in rare cases mutations can accumulate and cause diseases such as cancer.

Mismatch Repair (http://www.hhmi.org/biointeractive/mismatch-repair). During DNA replication mistakes can occur as DNA polymerase copies the two strands. The wrong nucleotide can be incorporated into one of the strands causing a mismatch. Fortunately cells have repair mechanisms.

Cancer and Cell Fate in the Intestinal Epithelium (http://www.hhmi.org/biointeractive/cancer- and-cell-fate-intestinal-epithelium). Disrupting the normal processes of differentiation and maturation of the intestinal epithelial cells can lead to cancer.

Classroom Activities

Cancer Discovery Activities (http://www.hhmi.org/biointeractive/cancer-discovery-activities). In Activity 1, students identify the locations on chromosomes of genes involved in cancer, using a set of 139 "Cancer Gene Cards" and associated posters. In Activity 2, students explore the genetic basis of cancer by examining cards that list genetic mutations found in the DNA of actual cancer patients.

Interactive Tutorials ("Click and Learns")

Cell Cycle and Cancer (http://www.hhmi.org/biointeractive/eukaryotic-cell-cycle-and-cancer). Explore the phases, checkpoints, and protein regulators of the cell cycle and find out how mutated versions of these proteins can lead to the development of cancer.

Teacher Guides

Gene Expression (http://www.hhmi.org/biointeractive/teacher-guide-gene-expression). This curriculum guide assists in filtering through the available resources from BioInteractive and HHMI on topics related to gene expression, including RNA structure and function, transcription, RNA processing, translation, and post-translational events.

DNA (http://www.hhmi.org/biointeractive/teacher-guide-dna). This curriculum guide assists in filtering through the available resources from BioInteractive and HHMI on topics related to DNA, including DNA structure and function, DNA replication, damage to DNA, and eukaryotic chromosomal structure.

Image of the Week

Cancer Evolution (https://www.hhmi.org/biointeractive/cancer-evolution). A computer simulation of cancer growth, in which cell colors correspond to different mutations, reveals that a tumor mass is a mixture of genetically similar cells.

Synchronized Division (http://www.hhmi.org/biointeractive/synchronized-division). The early embryonic cells of the sand dollar are caught in the act of synchronized cell division.

Collections

Biology of Cells (http://www.hhmi.org/biointeractive/biology-cells). Resources for teaching cell biology, including short films, animations, Click & Learn interactives, and posters.

DNA (http://www.hhmi.org/biointeractive/dna-collection). A variety of engaging animations, lecture clips, virtual labs, and other classroom resources teach key concepts related to DNA's structure and function.

Genetics (http://www.hhmi.org/biointeractive/genetics). Resources for teaching genetics, including short films, animations, Click & Learn interactives, and classroom activities.

Chemistry of Life http://www.hhmi.org/biointeractive/chemistry-life Resources related to chemistry, biochemistry, and biological macromolecules such as DNA, RNA, proteins, carbohydrates, and lipids.