Can you spot a cancer mutation?

How does cancer develop, and how can geneticists tell that a cell is cancerous? This teaching activity developed by the **Communication and Public Engagement team** from the Wellcome Trust Sanger Institute, UK, answers these and other related questions.

All cancers result from changes to the DNA sequence in some of our cells. Because the genetic material within cells is exposed to mutagens such as UV radiation, it can accumulate mistakes during replication. Occasionally, one of these mutations alters the function of a critical gene, providing a growth advantage to the cell in which it has occurred and its offspring; these cells will divide at a faster rate than their neighbours. Gradually, the DNA acquires more mutations, which can lead to the disruption of other key genes, resulting in particularly fast-growing and invasive cells. The result is tumour formation, the invasion of the surrounding tissue and eventually metastasis – the spread of the cancer to other parts of the body.

Genes that lead to the development of cancer when mutated are known as 'cancer genes'.

Tumour suppressor genes (TSGs , Figure 1) encode the information for making proteins that normally slow down cell growth, preventing unnecessary division or promoting apoptosis (programmed cell death) if the cell's DNA is damaged. Both copies of a TSG would have to be inactivated by mutation before this control of the cell cycle is lost. If one functional copy remains, there is still a 'brake' on the cell's growth.

Proto-oncogenes (Figure 2), in contrast, encode proteins that promote cell division and differentiation (specialisation). When these genes acquire mutations that either make the proteins continually active or lead to the gene's activity not being regulated anymore, they become oncogenes, promoting uncontrolled cell growth and division. For proto-oncogenes, a mutation in one copy of the gene can be enough to drive cancer develop-

REVIEW

- Biology
- Medicine
- Mutation
- Ages 15+

The teaching activities described in this article aim to actively involve advanced secondary-school biology students in a search for mutations that could potentially lead to cancer development, using real genomic data. The procedure is not a truly experimental one, so no real laboratory instruments are needed. Instead, the search is a theoretical one based on authentic data. All materials required to run the activity along with detailed directions can be freely downloaded from the programme website.

Apart from describing the various steps of the activity, the article and the supporting website include important information on what cancers are, what causes them, how they develop and how genomic information can be useful in developing treatments. Furthermore, several discussion points are suggested to enhance students' understanding of cancer.

Michalis Hadjimarcou, Cyprus

Images courtesy of the Wellcome Trust Sanger Institute Communication and Public Engagement team

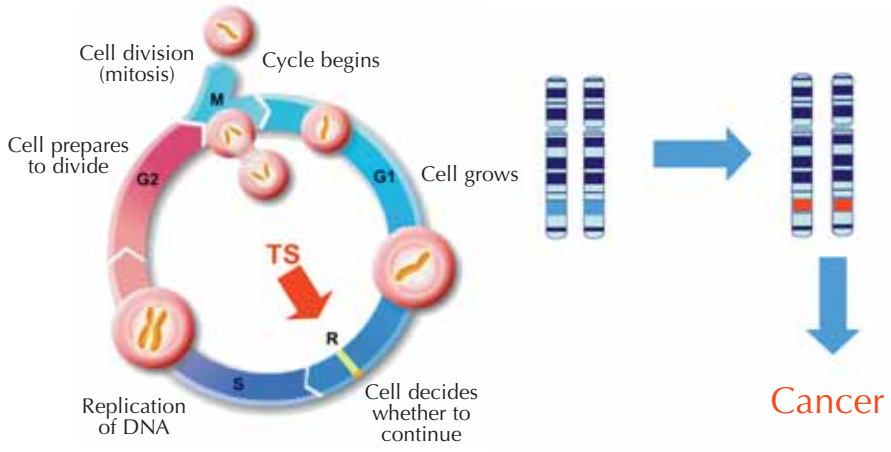

Figure 1: Tumour suppressor genes normally function to *prevent* cell growth and division. To lead to cancer, both copies of the gene would have to be mutated (marked in red)

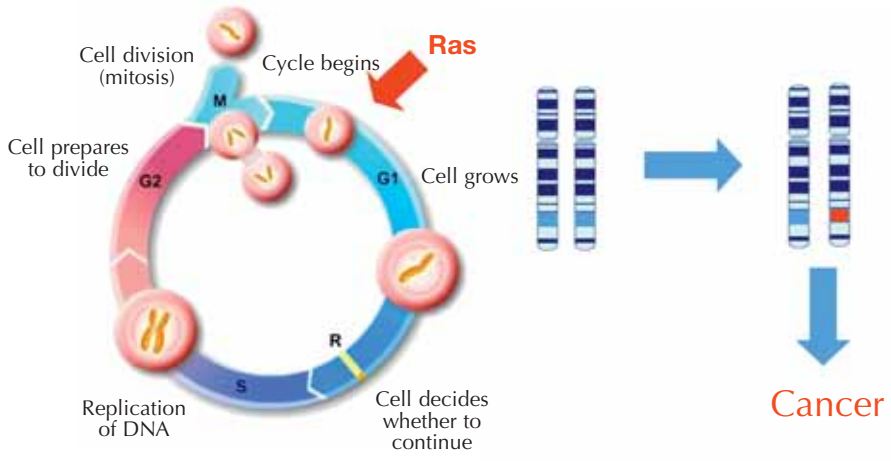

Figure 2: Proto-oncogenes normally function to *promote* cell growth and division in a controlled manner. A mutation in one copy of the gene (marked in red) can be enough to drive cancer development

Image courtesy of the Wellcome Trust Sanger Institute Communication and Public Engagement team

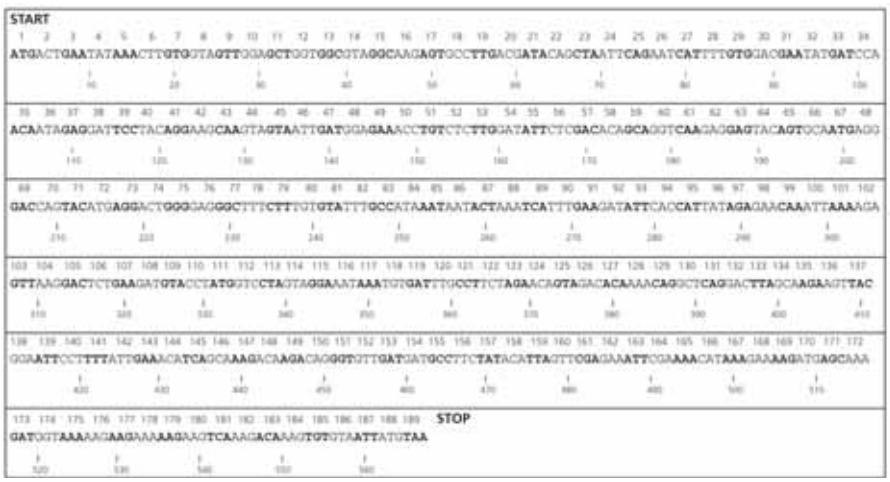

Figure 3: The *KRAS* gene sheet

ment.

Each individual case of cancer is caused by a unique set of mutations in proto-oncogenes and / or TSGs. Although the number is not yet known, it is thought that five or more mutations in cancer genes are needed for a cell (and its offspring) to become cancerous.

KRAS (pronounced kay-rass) is a proto-oncogene that encodes the protein KRAS, an intracellular signalling protein involved in promoting cell growth (to distinguish genes from proteins, gene names are conventionally written in italics). The following activity enables students to use real genomic data from the Cancer Genome Project[w1] to investigate common mutations in the *KRAS* gene that are associated with oncogenesis (cancer formation) and the development of pancreatic, colorectal, lung and other cancers. Originally developed for school visits to the Sanger Institute[w2], the activity was then made available through the Yourgenome.org[w3] website. It recently formed part of the first course on bioinformatics for European teachers run by ELLS[w4] at the European Bioinformatics Institute[w5] in Hinxton, UK. The full activity stimulates discussion about the causes of cancer, the function of gene mutations, protein structure and protein function.

The *KRAS* activity

Estimated duration: 45–60 minutes (with presentation and discussion)

Materials

All materials required to run the activity can be freely downloaded from the Yourgenome.org website either individually or as a compressed zip file[w6].

- One set of 11 worksheets (KRAS_student_wsheet.pdf) – one worksheet per pair or group of students. An alternative version is available for black and white printers or for use with colour-blind stu-

Amino acid code

A - Alanine
C - Cysteine
D - Aspartic acid
E - Glutamic acid
F - Phenylalanine
G - Glycine
H - Histidine
I - Isoleucine
K - Lysine
L - Leucine
M - Methionine
N - Asparagine
P - Proline
Q - Glutamine
R - Arginine
S - Serine
T - Threonine
V - Valine
W - Tryptophan
Y - Tyrosine

Image courtesy of C Brooksbank, European Bioinformatics Institute

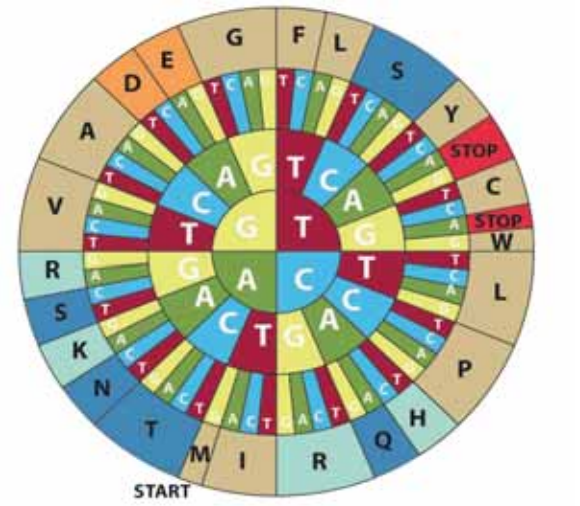

Figure 4: Use the codon wheel to translate DNA codons into amino acids. To decode a codon find the first letter of your sequence in the inner circle and work outwards to see the corresponding amino acid. For example, CAT codes for H (histidine). Note that this diagram uses the sense DNA codons (5′ to 3′)

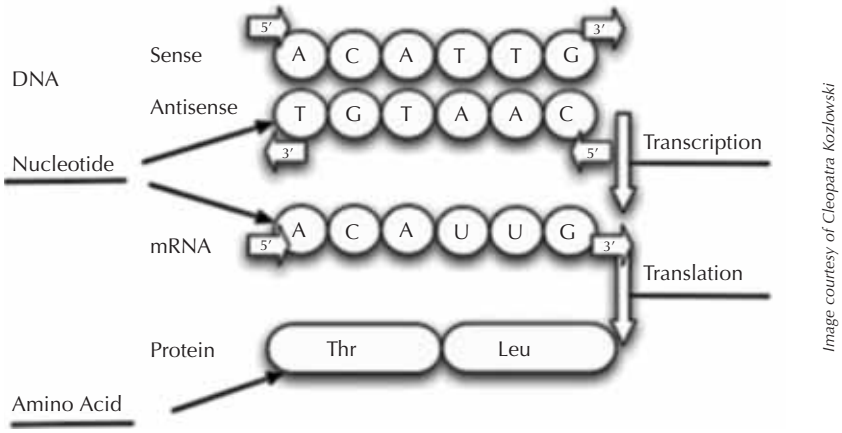

Image courtesy of Cleopatra Kozlowski

Figure 5: mRNA is synthesised from the antisense strand of DNA. The sense strand of DNA, used in this activity, has the same sequence as the corresponding mRNA strand, except that T is replaced by U

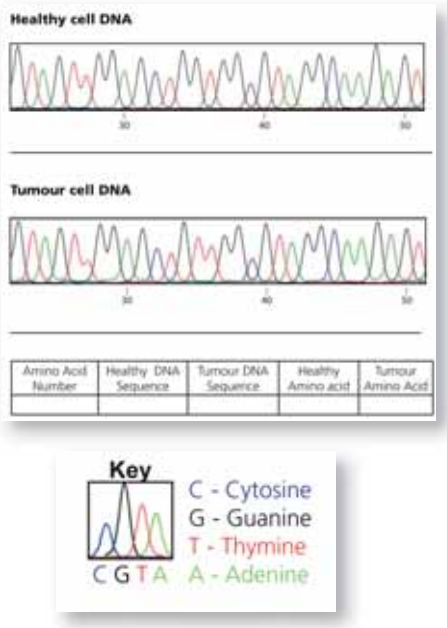

Figure 6: Example from a student worksheet

Image courtesy of the Wellcome Trust Sanger Institute Communication and Public Engagement team

dents. For large groups (20 or more), use two sets of worksheets, providing double coverage of the gene.

- One *KRAS* gene sequence banner (KRAS_gene_banner.pdf; Figure 3) for the whole class, and one *KRAS* gene sheet (KRAS_genesheet_yg.pdf; Figure 3) per group of students. The gene sheet (printed on A3 or A4) requires little preparation time. The KRAS banner, printed on several sheets of paper that are then stuck together, enables the results from the whole class to be displayed simultaneously.
- One codon wheel sheet (KRAS_codon_wheel.pdf or any other codon table for sense DNA, 5' to 3') per group, see Figures 4 and 5
- One summary sheet (KRAS_data_sheet.pdf) per group
- Pens

To use the banner, you will also need large arrows for marking mutations on the gene sequence, squares for marking regions which have been checked (KRAS_annotations.pdf), and reusable adhesive (e.g. Blu Tack®) for sticking arrows and squares to the gene sequence banner. Find out more about how to use this method in the downloadable teacher notes[w6].

In addition, you might find it helpful to have DNA, peptide and/or protein models to hand, and to use the Wellcome Trust Sanger Institute cancer animations (*Cancer: Rogue cells* and *Role of cancer genes*) on the KRAS activity website[w6].

Introduction to the activity

The *Investigating Cancer* presentation (available online[w6]) provides students with an overview of cancer. It introduces the concept that cancer arises due to abnormalities in DNA sequence, explains the various causes of these mutations and introduces the worksheets and activity. Several sections of the presentation encourage student discussions (see the presentation notes[w6]).

In the first part of the activity, students identify differences between *KRAS* gene sequences in healthy and

tumour cells on their worksheets, and mark these on the *KRAS* banner or gene sheet.

The worksheets have raw *KRAS* DNA sequencing traces from healthy and cancerous cell samples, represented as coloured line plots – one for each region of the gene. The four bases are represented on these plots by four different colours. Each coloured peak represents an individual DNA base:

Red: T

Green: A

Blue: C

Black: G (normally these peaks are yellow but this is not easy to read on paper)

There are 11 numbered worksheets in total, each showing two different regions of the *KRAS* gene. The six mutations in the *KRAS* gene are on worksheets one to six, so be sure to mix the sheets up before distributing them to the class. All must be completed to ensure full coverage of the gene. It is important to point out to the students that mutations are (relatively) rare, so not everyone will find one; this can be used to explore the importance of negative data and comprehensive coverage in scientific studies.

Identifying the mutations

Using the worksheets, the students will compare a section of DNA sequence from a healthy cell and a tumour cell from the same patient. The easiest way to identify whether a mutation has occurred is to write the DNA sequence below the coloured peaks (there is a colour key on the sheet to help) and to compare the written sequences.

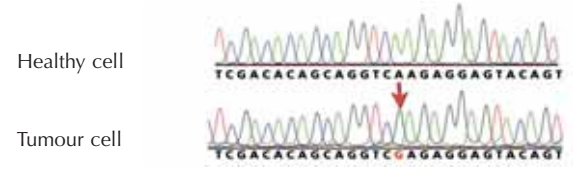

Figure 7: Identifying sequence mutations

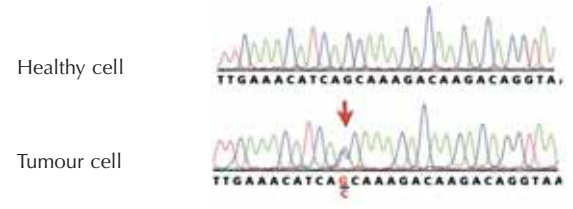

Figure 8: A heterozygous mutation

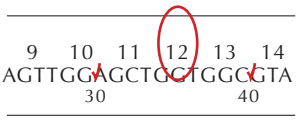

Figure 9: Ticking off the gene regions that have been checked, and marking any mutations

If one of the letters is different (a peak has changed colour), this indicates a mutation in the sequence. In Figure 7, the A in the DNA sequence from the healthy cell has been replaced by G in the tumour cell.

If the students find a double peak at one base position, this should be recorded with the two alternative bases at that position, one above the other. In the example below, the healthy DNA sequence has a G, whereas the tumour sequence has both G and C. This is not an insertion: it represents a heterozygous mutation where only one copy of the gene has substituted a C for a G. In this case the tumour sequence has replaced G with a C.

All students should indicate the gene regions they have checked by ticking off the relevant region on the gene sheet (see Figure 9).

Students who find a mutation should indicate the specific base by circling it on the gene sheet (see Figure 9) and make a note of which codon this lies in (in this example, codon 12).

They should also fill in the table at the base of the worksheet, using the codon wheel to translate the DNA sequence into the amino acid, as shown in Table 1:

Amino acid number	Healthy cell DNA sequence	Tumour cell DNA sequence	Healthy cell amino acid	Tumour cell amino acid
12	GGT	GTT	G (glycine)	V (valine)

Table 1: Mutations as listed on the individual worksheets

When all mutations have been found, record them on the summary data sheet (see Table 2).

Amino acid number	Healthy cell cell DNA sequence	Tumour cell DNA sequence	Healthy cell amino acid	Tumour cell amino acid
12	GGT	GTT	G (glycine)	V (valine)
13	GGC	GAC	G (glycine)	D (aspartic acid)
30	GAC	GAT	D (aspartic acid)	D (aspartic acid)
61	CAA	CGA	Q (glutamine)	R (arginine)
146	GCA	CCA	A (alanine)	P (proline)
173	GAT	GAC	D (aspartic acid)	D (aspartic acid)

Table 2: Mutations as recorded on the summary data sheet

Discussing the results

The results above are all single base substitutions. These mutations within the protein-coding region of the *KRAS* gene may be classified into one of three types, depending on the information encoded by the altered codon.

- Silent mutations code for the same amino acid.
- Missense mutations code for a different amino acid.
- Nonsense mutations code for a stop and can truncate the protein.

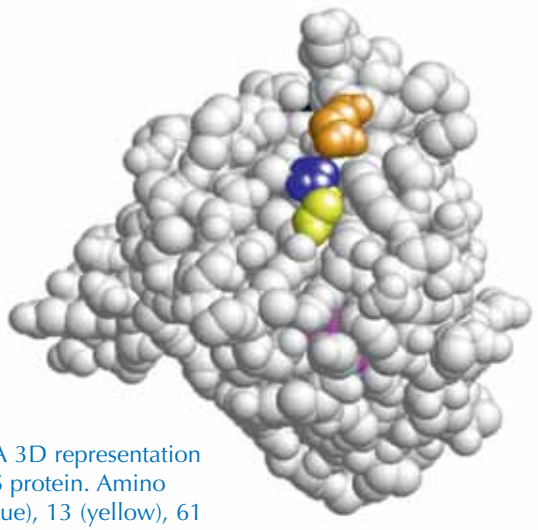

Figure 10: A 3D representation of the KRAS protein. Amino acids 12 (blue), 13 (yellow), 61 (orange) and 146 (pink) are those which carry mutations

Discuss whether the mutations are significant – will they have an impact on protein function or are they 'silent'? In this activity, codons 30 and 173 are silent and therefore do not have a functional impact.

The presentation has a 3D space-fill image of the KRAS protein (Figure 10); slides 26–30 show where on the protein the significant mutations are, and you will notice they are all in the same region. Codons 12, 13 and 61 were the first mutations to be associated with oncogenic transformation in the KRAS protein; mutation 146 was only discovered in 2005. Use these slides to discuss the impact that the mutations could have on protein structure and KRAS's function in growth signalling.

As an optional activity, the students can use RasMol, the molecular modelling software used to create the images on slides 26–30, to highlight the mutated amino acids in the protein structure. See the teacher notes[w6] for details.

How does information like this influence our approach to cancer?

The teacher notes[w6] contain a wealth of background information, using *KRAS* as an example, to stimulate discussion on how genomic information can be used to further our understanding of cancer and develop cancer treatments. Discussion points for students include:

- What experiments or approaches could be used to establish which cancers involve *KRAS* mutations?
- What could be the advantages of knowing this information?
- Cancer is a genetic disease: it is a result of changes in the DNA sequence. This is why many people believe that significant funding of research into cancer genetics is the best way of developing new cancer treatments and thus dealing with the disease. Cancer treatment and care for patients also requires large amounts of money (the UK National Health Service spent more than £2 billion on cancer care alone in 2000). Where and how do students think money should be spent?

Web references

w1 – Learn more about the Cancer Genome Project at the Wellcome Trust Sanger Institute here: www.sanger.ac.uk/genetics/CGP

w2 – To learn more about the Wellcome Trust Sanger Institute in Hinxton, UK, a leader in the Human Genome Project, see: www.sanger.ac.uk

The institute offers visits for school classes, teachers and the general public, as well as teacher support and further opportunities to get involved. See: www.sanger.ac.uk/about/engagement

w3 – The Yourgenome.org website was launched by the Sanger Institute to stimulate interest in and discussion on genetic research. It includes a section of varied and well developed resources for teachers, including the activity presented in this article. See: www.yourgenome.org

w4 – The European Learning Laboratory for the Life Sciences (ELLS) at the European Molecular Biology Laboratory provides continuing professional development courses (LearningLABs) in molecular biology for European secondary-school science teachers. In March

Amino acid number	Healthy cell DNA sequence	Tumour cell DNA sequence	Healthy cell amino acid	Tumour cell amino acid	Type of mutation	Significant yes / no
12	GGT	GTT	G (glycine)	V (valine)	Point (missense)	yes
13	GGC	GAC	G (glycine)	D (aspartic acid)	Point (missense)	yes
30	GAC	GAT	D (aspartic acid)	D (aspartic acid)	Point (silent)	no
61	CAA	CGA	Q (glutamine)	R (arginine)	Point (missense)	yes
146	GCA	CCA	A (alanine)	P (proline)	Point (missense)	yes
173	GAT	GAC	D (aspartic acid)	D (aspartic acid)	Point (silent)	no

Table 3: Type of mutation, as recorded on the summary data sheet

2010, ELLS ran the first bioinformatics LearningLAB for teachers at the European Bioinformatics Institute in the UK. For information on ELLS courses, please go to: www.embl.org/ells

w5 – To find out more about the European Bioinformatics Institute, see: www.ebi.ac.uk

w6 – To download all materials for the *KRAS* activity and for more background information, see: www.yourgenome.org/teachers/kras.shtml

Resources

Websites for student reference and discussion

The Cancer Research UK website offers accessible information on all the major cancers and current research. See: http://info.cancerresearchuk.org/cancerandresearch

The *New Scientist* website has an area focusing on cancer, featuring the latest articles on cancer research developments and interactive animations demonstrating targeted cancer drug functions. See: www.newscientist.com/topic/cancer

Nature Milestones in Cancer offers a collection of selected review-type articles and an online library of recent research papers from Nature Publishing Group, available for download as PDFs. It also has a cancer timeline showing major milestones in cancer research. See: www.nature.com/milestones/milecancer

The Inside Cancer multimedia website created by the DNA Learning Center offers a multimedia guide to cancer biology, diagnosis and treatment. See: www.insidecancer.org

Recent news articles

The BBC News website has published an interesting article on how newly discovered genetic hotspots for bowel cancer might help doctors to treat the disease better. See: http://news.bbc.co.uk or use the direct link: http://tinyurl.com/28o7zgf

Sample I (2009) First cancer genome sequences reveal how mutations lead to disease. *The Guardian*. See www.guardian.co.uk or use the direct link: http://tinyurl.com/yeknj5x

Roberts M (2009) Scientists crack 'entire genetic code' of cancer. *BBC News*. See http://news.bbc.co.uk or use the direct link: http://tinyurl.com/yb59qcz This article includes a video interview with Professor Mike Stratton, leader of the Cancer Genome Project.

Further reading

Friday BB, Adjei AA (2005) K-ras as a target for cancer therapy. *Biochimica et Biophysica Acta – Reviews on Cancer* **1756(2)**: 127-144. doi: 10.1016/j.bbcan.2005.08.001

Futreal A et al. (2004) A census of human cancer genes. *Nature Reviews Cancer* **4**: 177-183. doi: 10.1038/nrc1299

The author version of this paper can be freely viewed online. See: www.ncbi.nlm.nih.gov/pmc or use the direct link: http://tinyurl.com/3x5hah6

For a full catalogue of somatic cancer genes (COSMIC) described in the above paper and created by the Cancer Genome Project, see: www.sanger.ac.uk

Stratton MR, Campbell PJ, Futreal AP (2009) The cancer genome. *Nature* **458**: 719-724. doi: 10.1038/nature07943

Download the article free of charge from the *Science in School* website (www.scienceinschool.org/2010/issue15/cancer#resources), or subscribe to *Nature* today: www.nature.com/subscribe.

For more information on how genetic mutations cause diseases, see:

Patterson L (2009) Getting a grip on genetic diseases. *Science in School* **13**: 53-58. www.scienceinschool.org/2009/issue13/insight

For an interview with cancer researcher Joan Massagué, see:

Sherwood S (2008) On the trail of a cure of cancer. *Science in School* **8**: 56-59. www.scienceinschool.org/2008/issue8/joanmassague

For a classroom activity to discuss the ethics of knowing what your genes have in store for you, including the possibility of cancer, see:

Strieth L et al. (2008) Meet the Gene Machine: stimulating bioethical discussions at school. *Science in School* **9**: 34-38. www.scienceinschool.org/2008/issue9/genemachine

If you enjoyed this article, why not take a look at other medicine-related articles previously published in *Science in School*? See: www.scienceinschool.org/medicine

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