

Investigating Cancer

KRAS Activity

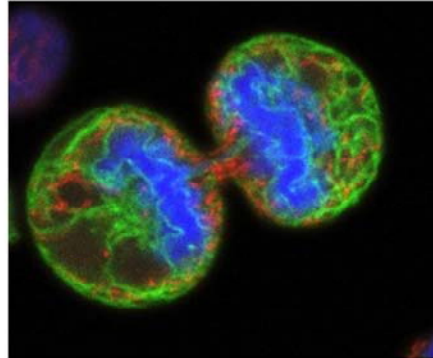


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What is cancer?

- All cancers derive from single cells that have acquired the characteristics of continually dividing in an unrestrained manner and invading surrounding tissues.
- Cancer cells behave in this abnormal manner because of changes in the DNA sequence of key genes, which are known as cancer genes. Therefore all cancers are genetic diseases.



Human melanoma cell undergoing cell division
Credit: Paul Smith & Rachel Errington, Wellcome Images



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All cancers result from changes in the DNA sequence of our genome. These changes occur throughout life because the genome within our cells is exposed to mutagens like UV radiation and accumulates mistakes during replication. These changes result in a progressive, subtle divergence of the DNA sequence from the original copy from the fertilised egg.

Occasionally, one of these mutations alters the function of a critical gene, providing a growth advantage to the cell in which it has occurred. This means that this cell and its offspring divide at a faster rate than that of their neighbours. The result is tumour formation, invasion of surrounding tissue and eventually 'metastasis', or spread of the cancer to other parts of the body.

The image in the slide shows a human melanoma cell undergoing cell division. The chromosomes (blue) have separated and the two daughter cells have almost split apart – only a small bridge of cytoplasm remains. The green staining labels the endoplasmic reticulum and the red labels the mitochondria.

Cancer information



- One in three people in the Western world develop cancer and one in five die of the disease
- There are approximately 200 types of cancer, each with different causes, symptoms and treatments
- In 2007, 297,991 people were newly diagnosed with cancer in the UK
- An individual's risk of developing cancer depends on many factors, including age, lifestyle and genetic make-up

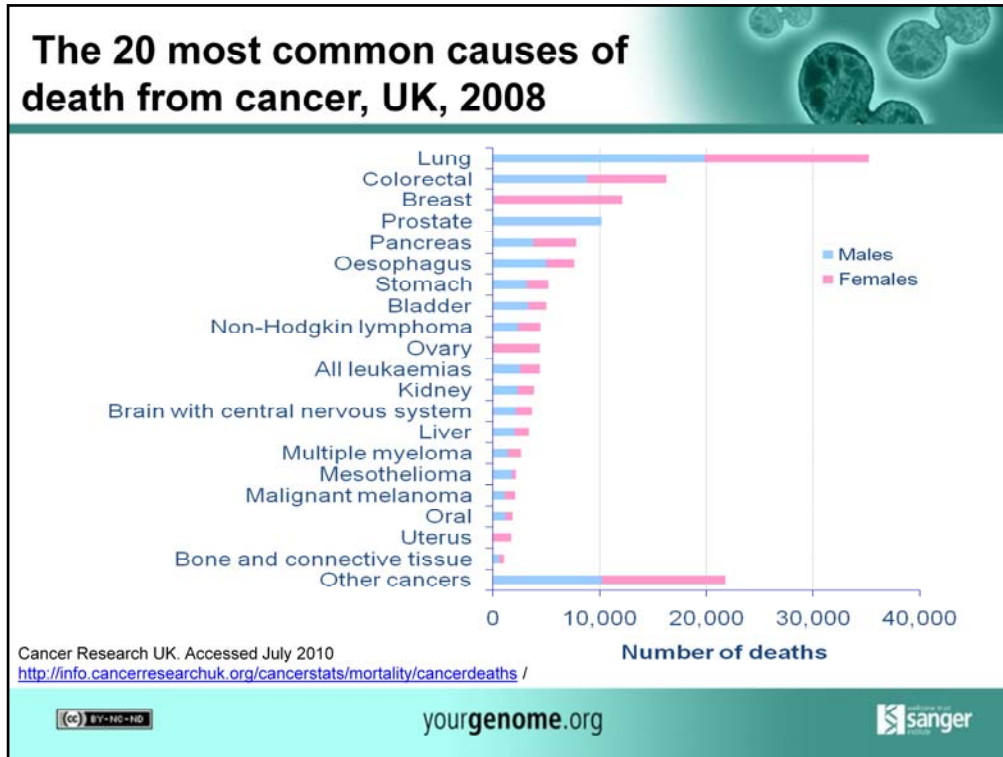
Cancer Research UK
<http://info.cancerresearchuk.org/cancerstats/incidence/?a=5441>



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Some important information about cancer.



This graph displays the major causes of death from cancer in the UK, compiled by Cancer Research UK. You can see that the commonest causes of death are lung cancers, colorectal cancer, breast and prostate cancers. These together account for over half (54%) of all new cancers each year.

It may be worth highlighting the incidence of malignant melanoma, a form of skin cancer associated with UV damage. This is the most common cancer in young adults aged 15 – 34 years old.

You can at this point encourage the class to think about why certain cancers are more common and what their potential causes may be.

Cancer cells have altered genomes

Karyotype illustrating structural abnormalities in cancer



Credit : Mira Grigороva and Paul Edwards, Department of Pathology, University of Cambridge, unpublished
Source: www.path.cam.ac.uk/~pawefish/BreastCellLineDescriptions/HCC38.html



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Ask the students how many chromosomes a healthy cell should have (46) and compare that with the number of chromosomes they can see in the karyotype (78).

Explain that this is a karyotype from a breast cancer tumour and demonstrates the accumulation of genetic changes arising from uncontrolled growth of cells.

In the cancer cell shown, several of the chromosomes are present in more than two copies. On some of these chromosomes, there are specific genes that are giving the cell a growth advantage as a result of being present in multiple copies. Each numbered chromosome should be a pair and just one uniform colour. For example, the Chromosome 1 box should show two yellow chromosomes, instead it shows six multicoloured chromosomes.

Draw attention to Chromosomes 1, 8 and 17 which have multiple colours, representing regions from other chromosomes. This is an example of interchromosomal rearrangement where chunks of the genome have been broken and then joined to other parts of the genome.

What is a mutation?



- **Germline mutation**
 - A change in the DNA sequence that can be inherited from either parent
- **Somatic mutation**
 - A change in the DNA sequence in cells other than sperm or egg
 - The mutation is present in the cancer cell and its offspring, but not in the patient's healthy cells



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Two key terms that students will encounter are presented on this slide. They may have come across the terms 'somatic' and 'germline' in discussions of gene therapy previously.

Mutations & cancer genes

- Cancer genes are causally implicated in *oncogenesis*
- Mutations in cancer genes can occur somatically or can be inherited.
- Mutations in some cancer genes can be inherited from parents, in which case they are present in every cell of the body. Such people are at a higher risk of developing cancer.
- Somatic mutations can occur in any of the cells of the body except the germ cells (sperm and egg) and therefore are not passed on to children.



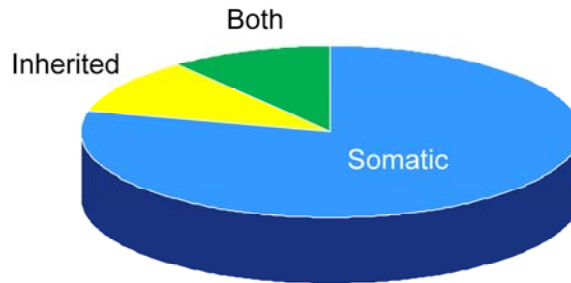
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Cancer genes are causally implicated in *oncogenesis* – the cellular changes that result in malignant tumour formation

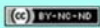
Somatic mutations are defined as mutations or other alterations in DNA that occur **after** conception. Somatic mutations can occur in any of the cells of the body except the germ cells (sperm and egg) and therefore are not passed on to children. These alterations can (but do not always) cause cancer.

Importance of somatic DNA changes in human cancer



Only 5 –10% of cancer cases have a clear hereditary component, e.g. *BRCA1* and *BRCA2* in breast cancer

Even in those cases where susceptibility is clearly inherited, somatic changes are required for cancer to develop



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An example of germline cancer mutations inherited from parents are mutations in the *BRCA1* and *BRCA2* genes. These are breast cancer susceptibility cancer genes. They are rare and the risk of cancer is high.

Women who have inherited a mutation in one of these genes from a parent will have a life time risk of breast cancer of approximately 70% , compared to 10% in the rest of the population. However, even in those cases where someone has inherited a susceptibility, additional somatic mutations are required.

Cancer genes

- There are two types of cancer genes:
 - **Tumour suppressor genes**
 - **Oncogenes**
- To date, we know of approximately 400 somatic “cancer genes” * but there are almost certainly more to be found
- COSMIC is a catalogue of somatic mutations found in cancer genes in human tumours and is available at:
<http://www.sanger.ac.uk/genetics/CGP/cosmic/>

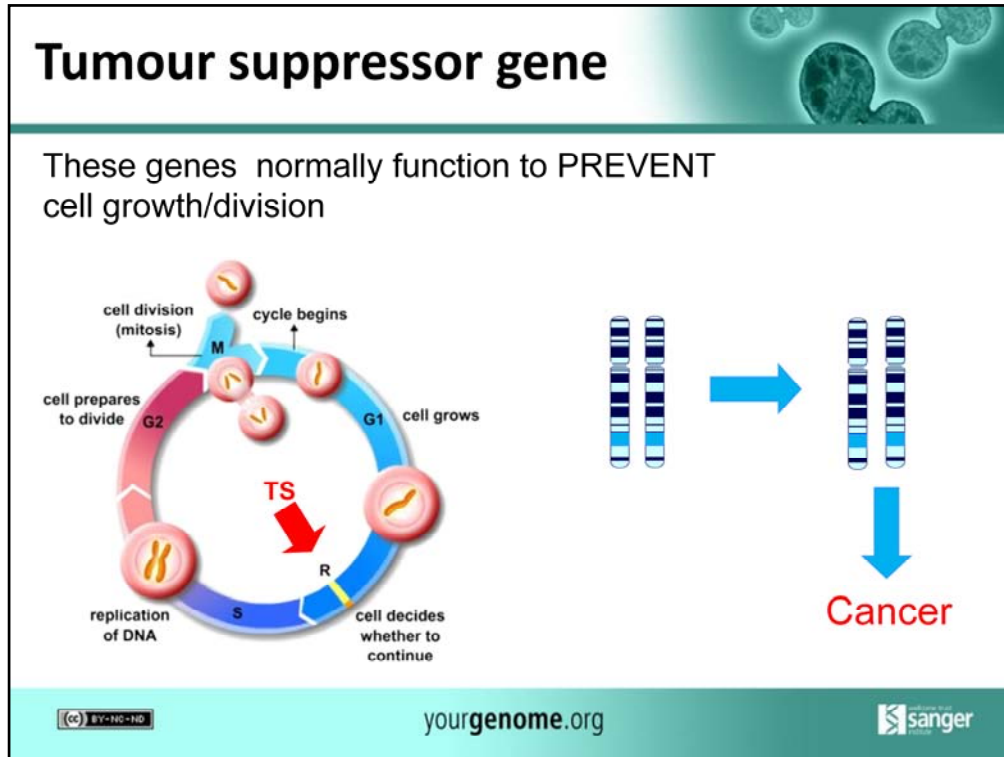
*(COSMIC v47 release. July 2010)



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COSMIC: Catalogue Of Somatic Mutations In Cancer was created by the Cancer Genome Project at the Wellcome Trust Sanger Institute.



Tumour suppressor genes (TSG) code for proteins that slow down cell growth. They can halt the cell growth cycle to stop unnecessary division or promote apoptosis (cell death) if the cell's DNA is damaged.

Different tumour suppressor proteins carry out the following functions:

- Repression of genes which are essential to the cell cycle, therefore inhibiting cell division.
- Linking the cell cycle to DNA damage; if there is damage to the cell it will not allow it to divide.
- Identifying where the DNA damage is irreparable and initiating apoptosis (cell suicide).

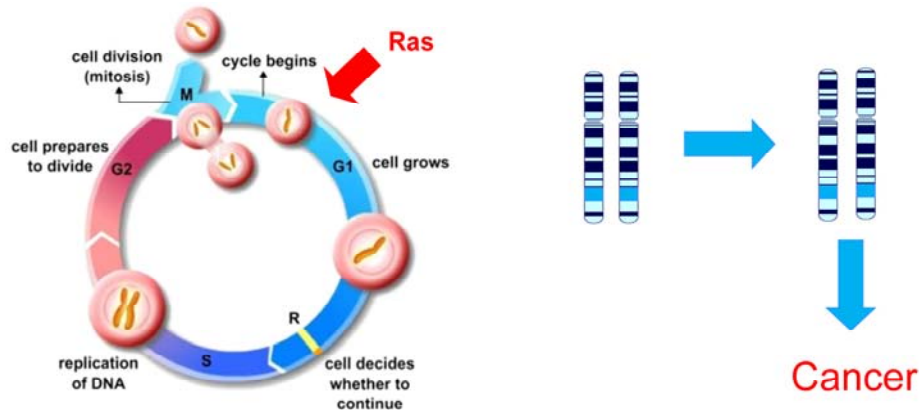
The animated chromosome diagram illustrates that both copies of the TSG have to be inactivated by mutation or other alteration for there to be a loss of cell cycle control. If one functional copy remains, there is still a "brake" on the cell's growth.

The "cell as a car" analogy:

One way to think about TSGs is to see them as the brakes of a car. There is a gene on both chromosomes so in a sense there are two brakes. If one gene is mutated and its protein loses its function, the cell can still halt and prevent unregulated cell growth as the other copy of the gene (or brake) is still functioning. However if that back up copy also changes and no longer codes for a functioning protein, the cell cycle is no longer under control and this can lead to cancer.

Oncogene

Genes which normally function to PROMOTE cell growth/division in a controlled manner



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sanger

Proto-oncogenes code for proteins that drive cell division. When these genes acquire mutations that result in continually active proteins they become **oncogenes** and cause uncontrolled cell growth and division.

The “cell as a car” analogy:

If you are using the car analogy, oncogenes can be seen as the accelerator. When one healthy copy of the proto-oncogene is altered it is the equivalent of the accelerator pedal being stuck – speeding up cell growth and division. Notice in the case of oncogenes that it only takes one copy of the gene to undergo changes to lead to cancer, rather than both copies as is the case with TSGs.

Examples of mutations

Sequence 1	Sequence 2	Type
ACTCGTTAGGCA	ACTCCTTAGGCA	Substitution
ACTCGTTAGGCA	ACTCGGCA	Deletion
ACTCGTTAGGCA	ACTCGTTATCAGGCA	Insertion
ACTCGTTAGGCA	ACTTTGCAGGCA	Inversion
ACTCGTTAGGCA	ACTCGTTAGTTAGGCA	Duplication



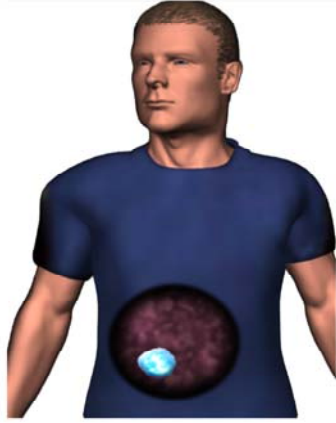
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Encourage the students to spot the differences between the sequences and identify the type of mutation.

Cancer progression

Mutations in multiple cancer genes are required for the development and progression of a single cancer



- Benign Tumour
- In situ* cancer
- Invasive cancer
- Metastatic cancer

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Cancer development is a gradual process where genetic changes are accumulated over a period of time. The different stages of cancer progression are shown:

Benign tumour: A cell begins to divide more rapidly than it's neighbours and a localised mass of cells forms.

***In situ* cancer:** A cell begins to divide in an uncontrolled way.

Invasive cancer: The tumour gets bigger and cells begin to invade surrounding tissue. In this case the illustration is showing the tumour arising from a cell within a villi of the large intestine, expanding outwards into the surrounding area.

Metastatic cancer: Tumour cells travel to other parts of the body through the bloodstream and deposit in other organs leading to secondary tumours, known as metastasis.

Before you click to the next slide, you can ask students what kinds of factors can trigger the formation of a cancerous cell.

External causes of cancer: ultraviolet radiation



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Natural radiation, such as ultraviolet (UV) rays from the sun, is known to be the primary cause of skin cancer. Through prolonged, unprotected exposure to sunlight and UV light (in the form of sun beds) we increase our risk of developing skin cancer.

Malignant melanomas are the least common type of skin cancer, but the most serious. There are around 10,000 new cases each year in the UK, around 60,000 in Europe and around 54,000 in the USA. Malignant melanoma is responsible for three out of four skin cancer deaths. Most forms of skin cancer, however, are relatively treatable, especially if detected early.

Recent research* has shown that a malignant melanoma genome contains more than 33,000 somatic mutations, many of which bear the imprint of the most common cause of melanoma - exposure to ultraviolet (UV) light. UV-light-induced mutations leave a typical signature of C>T or G>A mutations. These form the vast majority of the mutations. These specific changes occur as the DNA damage from UV radiation leads to the formation of covalent bonds between two adjacent pyrimidines (C and T) in the DNA molecule. If these DNA changes occur in critical genes such as *BRAF*, this can lead to inappropriate and sometimes aggressive cell growth and therefore the development of malignant melanoma.

*Plesance ED, Cheetham RK *et al.* A comprehensive catalogue of somatic mutations from a human cancer genome. *Nature* 2010; 463 (7278):191–196.

Available online at doi: [10.1038/nature08658](https://doi.org/10.1038/nature08658)

External causes of cancer: tobacco smoke



The chemicals in cigarette smoke have been causally linked to the development of lung cancer. Lung cancer causes around one million deaths worldwide every year, almost all of which are associated with smoking.

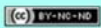
Tobacco smoke contains more than 60 mutagens that bind and chemically modify DNA. These brand the lung cancer genome with characteristic mutational patterns. For example research* has show that the tobacco smoke carcinogens polycyclic aromatic hydrocarbons (PAH) and the nicotine-derived nitrosamines cause G – T mutations.

Globally, lung cancer claims more than one in six cancer deaths each year. Of the two major types, small cell lung cancer is found in 15% of cases, but has much the worse prognosis with only one in 20 patients surviving for five years. Cancer of the respiratory tract (lung, trachea, bronchus) is the most common cause of death from cancer in Europe and the USA.

*Pfeifer GP *et al.* Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. *Oncogene* 2002; 21, 7435 –7451.

Pleasance ED *et al.* A small-cell lung cancer genome with complex signatures of tobacco exposure. *Nature* 2010; 463 (7278) :184 –190.
Available online at doi: [10.1038/nature08629](https://doi.org/10.1038/nature08629)

Lifestyle factor: diet



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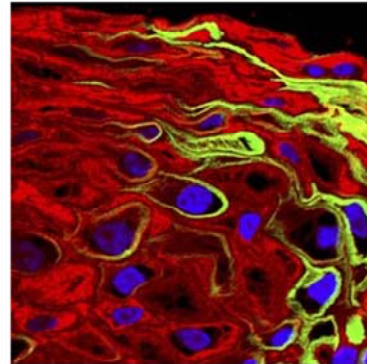


Overeating leads to obesity. Obesity is known to be associated with bowel and stomach cancers.

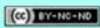
If obesity can be reduced then the number of cancer cases will be reduced.

Biological factor: virus

- HPV is a cause of cervical cancer
- Proteins from the virus activate and deactivate cancer genes
- The role of HPV in cervical cancer has led to the development of vaccines



HPV in cervical epithelium
Credit: MRC NIMR, Wellcome Images



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Biological factors can also be linked with cancer. For example, Human papilloma virus (HPV) is recognised as a cause of cervical cancer.

HPV is a sexually transmitted virus which has more than 100 different strains. Thirty of these can cause cervical cancer and genital warts.

When HPV enters cells of the cervix proteins made by the virus activate and inactivate oncogenes and tumour suppressor genes respectively. It is like a machine for turning on lots of cancer genes. The image shows a lesion in a human cervical epithelium infected with human papilloma virus (HPV16). Early viral proteins (green) bind to and re-organise the keratin filaments (red) towards the edge of the cell. Cell nuclei are stained with Dapi (blue).

In 2007, there were more than 550,000 new cases of cervical cancer, and approximately 260,000 deaths from cervical cancer worldwide. The overwhelming majority of these women were in developing countries, where cervical cancer screening programmes are often unavailable and healthcare infrastructure weak.

The role of HPV in cervical cancer has led to the development of vaccines against HPV. Vaccination against HPV dramatically reduces cases of cervical cancer. The development of the vaccine is regarded by cancer experts as a stunning advance in the prevention of cancer. A HPV vaccine is available and vaccination and screening programmes are being introduced in the UK. Since 2008, the vaccine has been offered to 12- to 13-year-old girls (school year 8). A three-year catch-up programme was started in 2008 and offers the vaccine to older girls aged 13-18 years old.

If time: you could discuss the students' thoughts on this.

Activity

- The *KRAS* gene codes for a signalling molecule
- Mutations in *KRAS* are present in many cancers, including pancreatic cancer
- You have to look for the mutations by comparing healthy DNA sequence with tumour DNA sequence
- Not all of you will find a mutation



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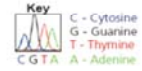


This is your opportunity to hand out the worksheets. It is important to stress that not everyone will find a mutation.

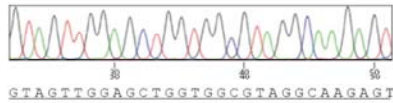
Your Worksheets

Can you spot a cancer mutation?

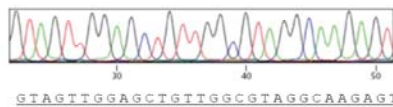
Below are traces of sequenced DNA displaying different regions of the KRAS gene. DNA sequence from a healthy cell is shown above that of a tumour cell. Using the key provided, write out the DNA sequence for each trace. Compare the healthy and tumour sequences. If you find a difference, circle the letter(s) that have changed and complete the table below using the banner and codon wheel provided.



Healthy cell DNA

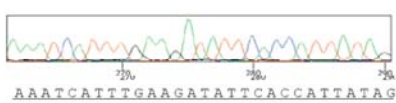


Tumour cell DNA

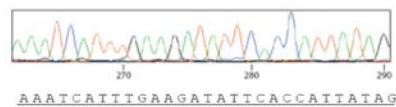


Amino Acid Number	Healthy DNA Sequence	Tumour DNA Sequence	Healthy Amino acid	Tumour Amino Acid

Healthy cell DNA



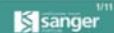
Tumour cell DNA



Amino Acid Number	Healthy DNA Sequence	Tumour DNA Sequence	Healthy Amino acid	Tumour Amino Acid



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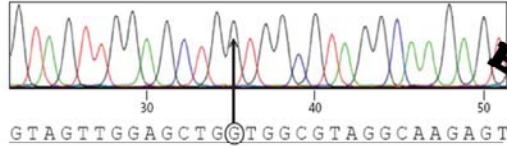


You will need to introduce which areas need to be completed on the worksheet.

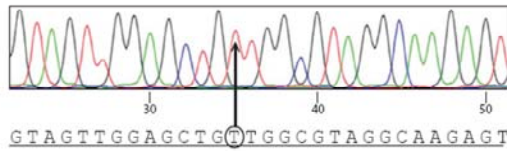
The process is simpler, and the results tend to be more accurate, if the students write out the whole sequence rather than just circle the mutation and translate the peaks around it.

If you find a mutation

Healthy cell DNA

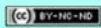


Tumour cell DNA



EXAMPLE ONLY

Amino Acid Number	Healthy DNA Sequence	Tumour DNA Sequence	Healthy Amino acid	Tumour Amino Acid
12	GGT	GTT	Glycine (G)	Valine (V)



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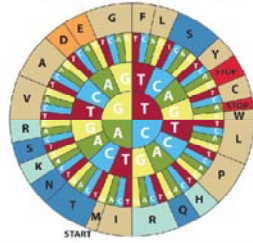


This shows how to complete the table if the students find a mutation.

How to use the codon wheel

Codon Wheel Decoding DNA

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 use the corresponding amino acid. For example: CAT codes for H (Histidine).



START

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

Start from the centre
and move outwards



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A series of three bases is called a codon, which translates into an amino acid. Amino acids are the building blocks of proteins.

Use the wheel from the inside out. Start in the quadrant that matches the first letter of the sequence and move outwards from there. The outermost circle gives you the first letter of the amino acid that matches your codon.

For example, a DNA sequence of CAT = H (Histidine), ACG = T (Threonine).

Mark up your sequence



START	
Amino acid No.	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Sequence	ATG ACT GAA TAT AAA CTT GTG GTA GTT GGA GCT GGT GGC GTA GGC
	10 20 30 40
Region Checked	<input checked="" type="checkbox"/> <input type="checkbox"/>

Example only

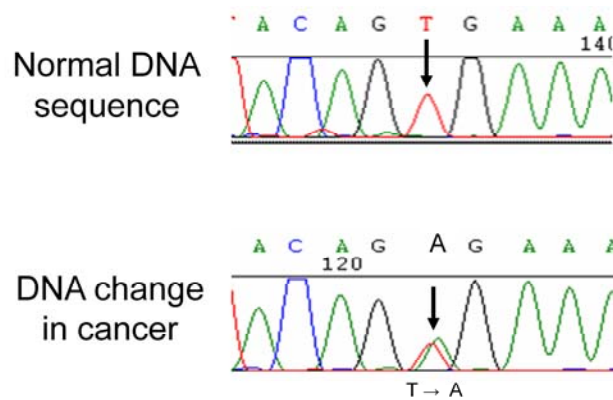


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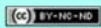
This slide demonstrates how to use the banner.

Heterozygous mutations



A double peak indicates a mutation on one chromosome and not the other i.e. a heterozygous mutation

BRAF gene mutation Nature 417, 906-7 (June 2002)



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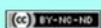


This shows you a heterozygous mutation where the mutation is present on only one chromosome.

The sequencing method cannot decipher which chromosome the mutation is on so shows two peaks of equal height.

Results

Amino Acid Number	Healthy DNA Sequence	Tumour DNA Sequence	Healthy Amino Acid	Tumour 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)



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Base substitution mutations that occur within the protein coding region of a gene may be classified into three kinds, depending upon what the erroneous codon encodes. These are:

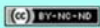
- Silent mutations that code for the same amino acid
- Missense mutations that code for a different amino acid
- Nonsense mutations that code for a stop and can truncate (shorten) the protein.

The results show silent and missense mutations.

When the students are ready you can reveal the correct answers. Encourage the students to read out their results and discuss whether the mutations are significant. All the results are point mutations.

Significant mutations

Amino Acid Number	Healthy DNA Sequence	Tumour DNA Sequence	Healthy Amino Acid	Tumour Amino Acid
12	GGT	GTT	G (glycine)	V (valine)
13	GGC	GAC	G (glycine)	D (asparatic acid)
61	CAA	CGA	Q (glutamine)	R (arginine)
146	GCA	CCA	A (alanine)	P (proline)

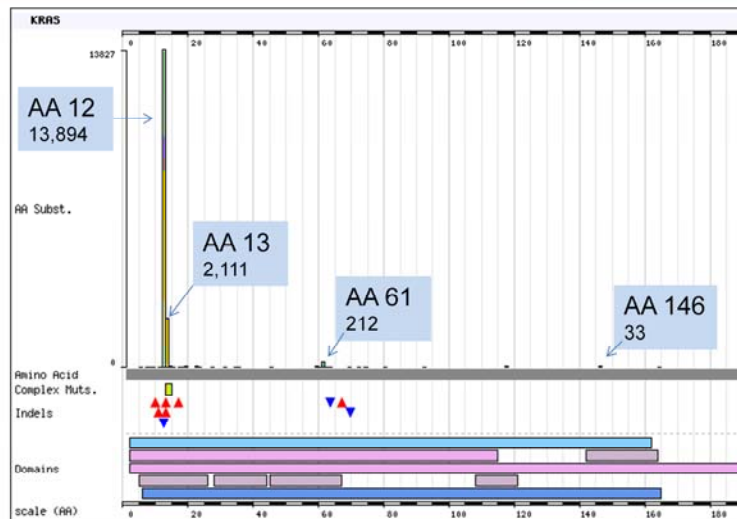


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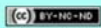


The results show silent and missense mutations. The silent mutations have no impact, so for the purposes of this activity, they are not significant. However, they do illustrate the concept of continual accumulation of mutations during a person's lifetime, only some of which may have an effect.

How common?



Source: COSMIC July 2010



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This slide shows a histogram representing the frequency of mutations in the KRAS gene in cancer samples on the COSMIC database.

The most common mutation is found at amino acid 12 with over 13,894 samples having this particular mutation.

Amino acid 13 is the second most common with 2,111 samples featuring this mutation.

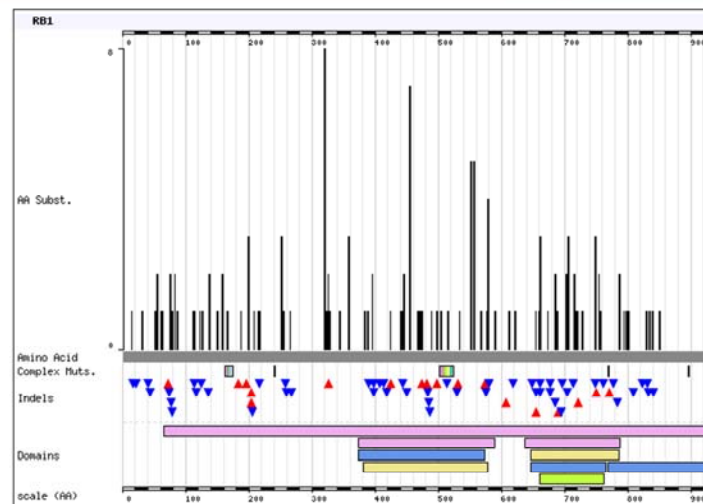
Amino acid 61 is mutated in 212 samples.

Amino acid 146 is mutated in 33 samples.

You can see there are other regions with mutations but these are the four most common in this gene.

This pattern of mutations across the gene is similar to that of other members of the *Ras* family and has the signature of an **oncogene** – clusters of mutations which only occur in specific regions of the gene. This means the protein will still function but in this case will encourage inappropriate cell growth as the protein is permanently “switched on”, or activated.

***RB1*: tumour suppressor gene**



Source: COSMIC July 2010



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This slide shows the frequency of mutations in a different gene: *RB1*.

Red triangles indicate insertions. Blue triangles indicate deletions.

Question to students: What is the difference between this and the previous histogram?

The key difference between *KRAS* and *RB1* is the range and frequency of mutations.

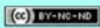
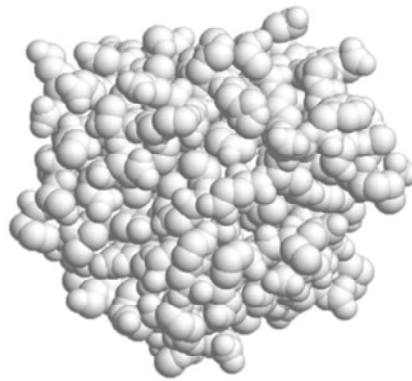
RB1 has 194 different mutations many of which truncate the encoded protein. For example, 88 (28%) are nonsense substitutions which result in the protein being truncated. 53 (17%) are deletions that cause a frameshift and also truncate the protein.

From earlier slides we know that tumour suppressor genes and the proteins they encode normally prevent inappropriate cell growth. However, the mutations shown “break” the gene, disrupting the sequence so that it can no longer encode a functional protein product, therefore the tumour cell continues to grow unchecked.

Revisit the car analogy

If we imagine the TSG as a brake – to stop it working you can cut the brake cable anywhere and it will stop functioning. In the case of an oncogene or accelerator, there are only a limited number of ways for it to become stuck “on”. This is why the oncogene *KRAS* has a limited number of gene regions which can acquire mutations (and still function albeit inappropriately) and why a TSG such as *RB1* has so many different regions of acquired mutations.

How does this affect the KRAS protein?



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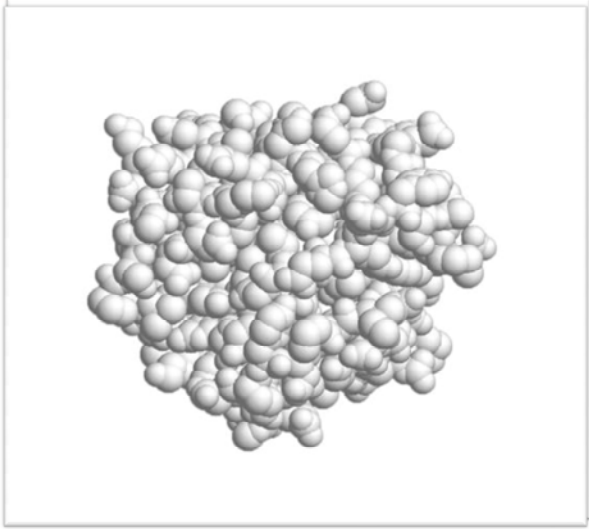


The 3D model shows the KRAS protein.



Here you can ask the students how they think the mutations could affect the protein. The next slides will show where they occur on the molecule.

The teachers notes provide more information to start a discussion around the significance of the mutations, including the role of the protein.

Amino acid 12

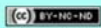
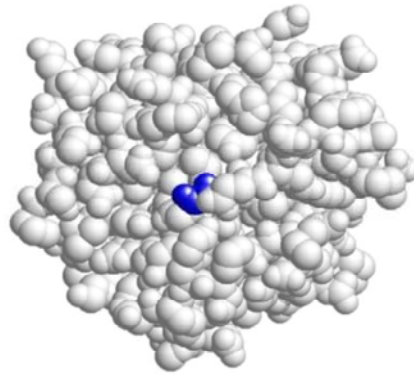


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Click to reveal amino acid 12 (blue).

Amino acid 13

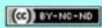
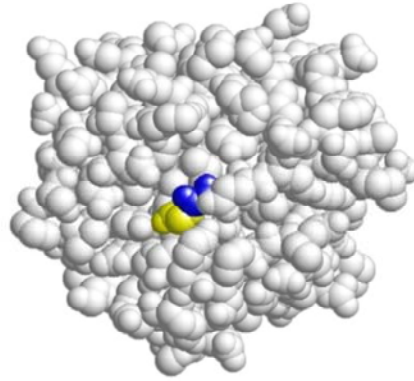


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Click to reveal amino acid 13 (yellow).

Amino acid 61

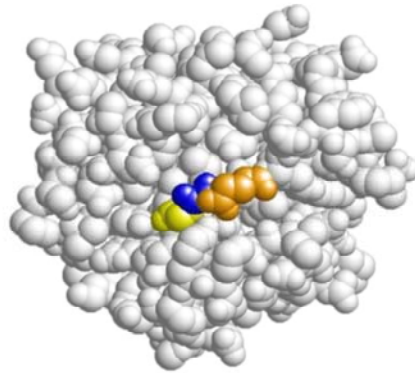


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Click to reveal amino acid 61 (orange).

Amino acid 146



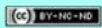
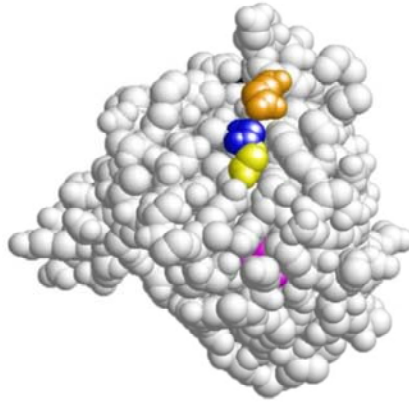
yourgenome.org



Click to reveal amino acid 146. You will notice that you can't see this amino acid but if we rotate the molecule, you can get a better view.

Click to rotate.

Amino acid 146



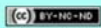
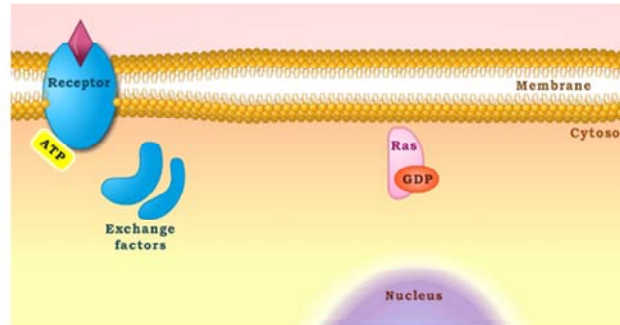
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Amino acid 146 is tucked into the centre and is coloured magenta.

What's the impact?

- KRAS helps to transmit external growth signals to the cell nucleus, driving normal cell growth. It is:
 - Activated when it binds GTP
 - Inactivated or “switched off” when GTP is hydrolysed to GDP



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This slide is animated to summarise the series of cellular events that occur to activate and deactivate KRAS.

KRAS is a guanine nucleotide (GDP/GTP) binding protein that acts as a self-inactivating signalling molecule.

“Normal” or wildtype KRAS can be activated by signals from cell surface receptors that promote the exchange of bound GDP for GTP.

KRAS is “switched off” when GTP is hydrolysed.

Ask the students what they think the mutations will do to the function of the protein and how that could lead to the development of cancer?

The *KRAS* oncogene has mutations that result in amino acid substitutions in key regions (AA 12, 13, 61 & 146). The mutated gene encodes proteins with reduced GTPase activity. These proteins are locked into a GTP active state – continuously sending cell growth signals. This along with other mutations can lead to the development of cancer.