

Pathology Practical Class 25

Problem-Solving Exercise (Neoplasia)

The Molecular Basis of Cancer: Colorectal Adenocarcinoma

1.0. Introduction: point mutations

Cancer of the colon and rectum is probably the best understood cancer at the point-mutation level. Some of the point mutations that are present in a high proportion of colorectal cancers have been known for some time. Leading up to 1990, Vogelstein and others measured the frequency of the known mutations in adenomas and adenocarcinomas and found that: (1) mutations in the *APC* gene were almost equally frequent in all lesions; (2) the frequency of *K-RAS* mutations was low in early adenomas, but high in later adenomas and adenocarcinomas; and (3) mutations in p53 and deletions of chromosome 18 were uncommon except in late adenomas and adenocarcinomas. Based on these data, Vogelstein suggested a model for how a typical colorectal carcinoma might develop, shown in [Figure 1](#). This picture is oversimplified, and a recent update of the model¹, still oversimplified, is also shown.

We now have more accurate data on genes that are point-mutated in many (but by no means all) colorectal cancers. We will focus on *K-RAS*, *APC* and *PIK3CA*.

2.0. The *K-RAS* gene

The picture in [Figure 2](#) shows DNA sequence analysis of the *K-RAS* gene in a cancer, with normal tissue for comparison. In the cancer two bases, A and G, are detected at position 436.

Questions:

Q1. Why are two bases detected at this position in the cancer?

¹ Jones et al Comparative lesion sequencing provides insights into tumor evolution. Proc Natl Acad Sci U S A 2008;105(11):4283-8

Mutations in the *K-RAS* gene are well documented and Table 1 provides the results from a larger study in which 18,000 genes were screened for point mutations in a series of colorectal and breast carcinomas (Wood et al, 2007)². The cancer cells were purified and the exons of the genes were sequenced. The sequence analysis results are shown in Table 1.

Q2. Is K-RAS a tumour suppressor gene or an oncogene, and how can you tell from the data?

Look at which amino acids are mutated in the *K-RAS* gene. The mutations found in human colorectal tumours (indeed in any human cancer where they occur) are mostly at amino acids 12, 13, and less frequently at a few other residues (among 181 amino acids in the protein).

Q3. Why are these mutations only found at particular amino acids?

3.0. The APC gene

Mutations in the *APC* gene were also investigated (see Table 1).

Q4. How frequent are APC mutations in these tumours? 35 tumours were screened; note some tumours have 2 mutations.

Q5. What do these mutations do to the protein encoded by the gene, in particular, what would be the consequence of the INDEL (insertion or deletion of one base pair) mutations?

Q6. Compare the range and type of mutations found in APC with the range and type of mutations found in K-RAS. Is APC a tumour suppressor gene or an oncogene, and how can you tell from these data?

Figure 3a shows a Western blot of APC proteins from colon cancer cells, and other cancer cells. On these blots, the further the band migrates (downwards) into the gel by electrophoresis, prior to blotting, the smaller the protein.

Q7. Why are the proteins different sizes; why do the colonic tumours (usually) have only one band (one size of protein); why are the pancreatic tumour bands all the same size? Do you think this is typical for a tumour suppressor gene?

² Wood, LD et al. The genomic landscapes of human breast and colorectal cancers. *Science* 2007;318:1108-13.
<http://www.sciencemag.org/cgi/content/full/318/5853/1108>.

Q8. Why are APC mutations more variable than K-RAS mutations?

3.1. Adenomatous Polyposis Coli / Familial Adenomatous Polyposis

The *APC* gene is so named after the hereditary condition Adenomatous Polyposis Coli, which is also known as Familial Adenomatous Polyposis (FAP) or polyposis coli.

[Figure 4](#) is a photograph of the large bowel of an FAP patient.

Q9. Why would the APC gene be named after the disease?

Q10. What would we expect to find if we sequenced the APC gene in germ-line DNA of a patient with FAP, and a colonic adenoma or cancer from that patient? If we sequenced germ-line DNA in a more typical sporadic colon cancer patient, how would the result be different?

[Figure 3b](#) shows a Western blot of APC protein in lymphocytes from FAP patients. Compare these with the tumours.

Q11. What are the bands, why are there two bands in the lymphocytes but only one band in the tumour?

Q12. Suppose someone is discovered to have Familial Adenomatous Polyposis, and there is concern that some of their close relatives may also be affected. How would you identify the affected relatives?

Q13. Tricky question. Suppose a family has a clear history of inherited predisposition to colorectal cancer. Are they likely to have FAP, i.e. an APC mutation, or what other explanation would you consider first?

4.0. The PIK3CA gene

Table 1 also has sequence analysis results for *PIK3CA* (the catalytic subunit of phosphatidylinositol-3-kinase, which, rather like *K-RAS*, signals downstream of receptor tyrosine kinases). It was recently discovered that this kinase is quite often mutated in cancers, particularly breast (about 40% of cases).

Q14. Compare the range and type of mutations found in *PIK3CA* with the range and type of mutations found in *K-RAS* and *APC*. Is *PIK3CA* a tumour suppressor gene or an oncogene, and how can you tell from these data?

5.0. Larger-scale DNA alterations

Point mutations account for only some of the genetic changes in cancer. Larger-scale DNA alterations, such as extra copies of chromosomes, deletions (of part or whole chromosomes), chromosome translocations, insertions, inversions and gene amplifications (many extra copies of the gene), are also important, but have been studied less.

[Figure 5](#) shows the chromosomes of a cultured cell line derived from a colonic cancer (obtained in Dr Edwards' lab). Each chromosome is marked with a different fluorescence colour.

Q15. How many pieces of chromosome 5 are there (coloured brown).

Q16. Notice that several chromosomes are present in four copies and several of the abnormalities are present in two copies. Can you think of a way this could have happened?

Table 1: Mutations in K-RAS, APC, and PIK3CA, in colorectal and breast cancer samples, found by Wood et al, 2007.

Mutations are heterozygous unless stated to be homozygous. 35 colorectal cancers were scanned.

K-RAS mutations (only found in colorectal cancers, not found in breast cancers)

Sample ID	genomic DNA change*	Homozygous?*	Amino acid* change	type of mutation	consequence for protein
Co82	g.chr12:25269914A>T		p.K117N	Missense	altered a a
Co92	g.chr12:25289551G>A		p.G12D	Missense	altered a a
Co94	g.chr12:25289552G>A		p.G12S	Missense	altered a a
Hx189	g.chr12:25289551G>A		p.G12D	Missense	altered a a
Hx206	g.chr12:25289551G>T	+	p.G12V	Missense	altered a a

Hx218	g.chr12:25289551G>T		p.G12V	Missense	altered a a
Hx219	g.chr12:25289551G>T		p.G12V	Missense	altered a a
Hx5	g.chr12:25289548G>A		p.G13D	Missense	altered a a
Mx22	g.chr12:25289548G>A	+	p.G13D	Missense	altered a a
Mx26	g.chr12:25269829G>A	+	p.A146T	Missense	altered a a
Mx27	g.chr12:25271543A>G		p.Q61R	Missense	altered a a
Mx3	g.chr12:25269829G>A		p.A146T	Missense	altered a a
Mx30	g.chr12:25289551G>C		p.G12A	Missense	altered a a
Mx34	g.chr12:25289551G>A	+	p.G12D	Missense	altered a a
Mx35	g.chr12:25289548G>A		p.G13D	Missense	altered a a
Mx41	g.chr12:25289551G>A		p.G12D	Missense	altered a a
Mx43	g.chr12:25289548G>A		p.G13D	Missense	altered a a

APC mutations (only found in colorectal cancers, not found in breast cancers)

Sample ID	genomic DNA change	Homozygous?	Amino acid change	type of mutation	consequence
Co108	g.chr5:112203538C>T	+	p.R1450X	Nonsense	trunc'n
Co74	g.chr5:112201995C>A		p.Y935X	Nonsense	trunc'n
same	g.chr5:112203663delT		fs	INDEL	trunc'n
Co82	g.chr5:112203663delT	+	fs	INDEL	trunc'n
Co92	g.chr5:112203538C>T	+	p.R1450X	Nonsense	trunc'n
Hx169	g.chr5:112203351T>A		p.C1387X	Nonsense	trunc'n
Hx172	g.chr5:112203318T>A	+	p.Y1376X	Nonsense	trunc'n
Hx174	g.chr5:112203310G>T	+	p.E1374X	Nonsense	trunc'n
Hx185	g.chr5:112182891G>A	+	p.W421X	Nonsense	trunc'n
Hx188	g.chr5:112202269_ 112202270insT	+	fs	INDEL	trunc'n
Hx189	g.chr5:112203134C>G	+	p.S1315X	Nonsense	trunc'n

Hx219	g.chr5:112203494delG	+	fs	INDEL	trunc'n
Mx22	g.chr5:112203502delA	+	fs	INDEL	trunc'n
Mx26	g.chr5:112203146delC	+	fs	INDEL	trunc'n
Mx27	g.chr5:112179160C>T		p.R302X	Nonsense	trunc'n
same	g.chr5:112203651_ 112203652dupTT		fs	INDEL	trunc'n
Mx29	g.chr5:112190795T>A		p.Y500X	Nonsense	trunc'n
same	g.chr5:112203663delT		fs	INDEL	trunc'n
Mx3	g.chr5:112203389C>A	+	p.S1400X	Nonsense	trunc'n
Mx31	g.chr5:112202323C>T	+	p.Q1045X	Nonsense	trunc'n
Mx32	g.chr5:112203227C>G	+	p.S1346X	Nonsense	trunc'n
Mx34	g.chr5:112144491C>T		p.R213X	Nonsense	trunc'n
same	g.chr5:112203686_ 112203687insG		fs	INDEL	trunc'n
Mx35	g.chr5:112191596_ 112191597insA		fs	INDEL	trunc'n
same	g.chr5:112203423delT		fs	INDEL	trunc'n
Mx38	g.chr5:112202948_ 112202949insG	+	fs	INDEL	trunc'n
same	g.chr5:112202950A>T	+	p.I1254F	Missense	altered a a
Mx40	g.chr5:112182876C>G		p.Y416X	Nonsense	trunc'n
same	g.chr5:112201945A>T		p.R919X	Nonsense	trunc'n
Mx42	g.chr5:112203651delT		fs	INDEL	trunc'n
Mx43	g.chr5:112190790C>T		p.R499X	Nonsense	trunc'n
Mx8	g.chr5:112202623G>T		p.E1145X	Nonsense	trunc'n
same	g.chr5:112203146delC		fs	INDEL	trunc'n

PIK3CA (catalytic subunit of Phosphatidyl-inositol-3-kinase) mutations

Sample ID	Colon or breast	genomic DNA change	Homozygous?	Amino acid change	type of mutation	consequence
B3C	B	g.chr3:180434787A>G		p.H1047R	Missense	altered a a
BB12T	B	g.chr3:180418784G>A		p.E542K	Missense	altered a a
BB18T	B	g.chr3:180418793G>A		p.E545K	Missense	altered a a
BB33T	B	g.chr3:180434787A>T		p.H1047L	Missense	altered a a
BB5T	B	g.chr3:180418793G>A		p.E545K	Missense	altered a a
Hx174	C	g.chr3:180430529G>T	+	p.C901F	Missense	altered a a
Hx206	C	g.chr3:180430552T>C		p.F909L	Missense	altered a a
Mx30	C	g.chr3:180434787A>G		p.H1047R	Missense	altered a a
Mx38	C	g.chr3:180434787A>G		p.H1047R	Missense	altered a a
Mx41	C	g.chr3:180418784G>A		p.E542K	Missense	altered a a

***Mutation Nomenclature:**

Genomic DNA change e.g. g.chr3:180418784G>A means **g**enomic DNA at 180418784 base pairs on chromosome 3 has a change G>A, i.e. guanine to adenine. ins means insertion, del means deletion.

Homozygous? : Mutations were heterozygous (both mutant and normal copies present) unless noted by a + in this column, which indicates they were homozygous (only the mutant form present).

amino acid change: e.g. p.E542K means protein encoded is changed at codon/amino acid 542 from E (single letter code for amino acid glutamine) to K (single letter code for lysine).

Missense mutations lead to a change of amino acid, so the consequence is an altered amino acid (a a) at that codon. Nonsense means a change from an amino acid codon to a STOP codon, so the consequence is a truncation (trunc'n) of the protein sequence at that codon. INDEL means insertion or deletion, which usually results in a frame shift (fs). Note that frame shifts usually result in truncated proteins because the out-of-frame reading frames have a lot of STOP codons, so the protein sequence stops shortly after the frame shift mutation when the ribosome reaches the first STOP codon.