1- Epidemiological studies can provide suggestive links between environmental factors and cancer. For example, as shown in Figure below the curve for deaths due to lung cancer in the US parallels the curve for per capita cigarette consumption. However, the curve for lung cancer is displaced by some 25 years from that for cigarette smoking. What do you suppose is the basis for this delay? What would you say to your uncle, who insists that people who smoke are inherently more cancer prone and that lung cancer really has nothing to do with cigarettes?

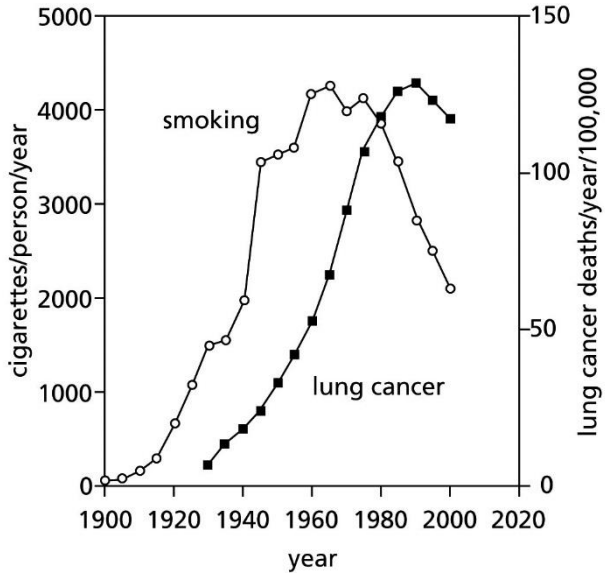


Figure 1 Lung cancer deaths and per capita cigarette consumption in the US from 1930 to 2000.

2- The Tasmanian devil, a carnivorous Australian marsupial, is threatened with extinction by the spread of a fatal disease in which a malignant oral-facial tumor interferes with the animal's ability to feed. You have been called in to analyze the source of this unusual cancer. It seems clear to you that the cancer is somehow spread from devil to devil, very likely by their frequent fighting, which is accompanied by biting around the face and mouth. To uncover the source of the cancer, you isolate tumors from 11 devils captured in widely separated regions and examine them. As might be expected, the karyotypes of the tumor cells are highly rearranged relative to that of the wild-type devil (Figure 2). Surprisingly, you find that the karyotypes from all 11 tumor samples are very similar. Moreover, one of the Tasmanian devils has an inversion on chromosome 5 that is not present in its facial tumor. How do you suppose this cancer is transmitted from devil to devil? Is it likely to arise as a consequence of an infection by a virus or microorganism?

Explain your reasoning.

Tasmanian devil (*Sarcophilus harrisii*)

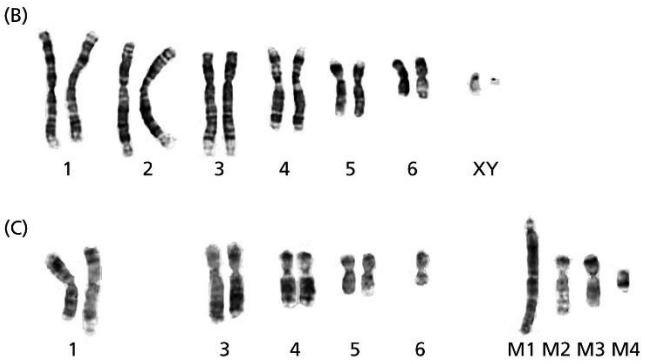


**Figure 2 Karyotypes of cells from Tasmanian devils.** (A) A Tasmanian devil. (B) Normal karyotype for a male Tasmanian devil .The karyotype has 14 chromosomes, including XY. (C) Karyotype of cancer cells found in each of the 11 facial tumors studied. The karyotype has 14 chromosomes, no sex chromosomes, no chromosome-2 pair, one chromosome 6, two chromosomes 1 with deleted long arms, and four highly rearranged chromosomes (M1 -M4).

3- Certain inbred strains of mice suffer tumors of the breast at a relatively high Frequency, whereas other inbred strains form breast tumors rarely or not at all. To investigate the basis for this hereditary difference, you set up a series of genetic crosses between the 'high' and 'low' tumor- forming strains of mice, as shown in Table below. You are amazed to find that high frequencies of tumors appear in F1 female mice only when their mothers were from the 'high'-frequency strains. When you cross the F1 progeny generated in an experiment to produce F2 mice, you find the same result: high frequencies of tumors appear in F2 female mice only when their grandmothers were from the 'high'-frequency strain.

A. Can you explain these results on the basis of inheritance of a chromosomal mutation: recessive, dominant, or X-linked?

B. In Experiment 4 one of your CBA (low) mothers died and you put her pups with an A (high) mother for foster care. Much to your surprise, the fostered female pups developed breast tumors. Moreover, pups from these fostered females passed on the tendency to form breast tumors to their daughters. What do you suppose might be the basis for these results?

| EXPERIMENT | FEMALE PARENT | MALE PARENT | TUMORS IN F1 FEMALES |
|---|---|---|---|
| 1. | D (high) | C57 (low) | 36.1% |
| 2. | C57 (low) | D (high) | 5.5% |
| 3. | A (high) | CBA (low) | 86.3% |
| 4. | CBA (low) | A (high) | 0.0% |
| 5. | Z (high) | I (low) | 90.0% |
| 6. | I (low) | Z (high) | 0.0% |

4- Retinoblastoma is an extremely rare cancer of the retina in the eye. The disease mainly affects children up to the age of 5 years because it can only occur while the nerve precursor cells are still dividing. In some cases tumors occur in only one eye, but in other cases tumors develop in both eyes. The bilateral cases all show a familial history of the disease; most of the cases affecting only one eye arise in families with no previous disease history.

An informative difference between unilateral and bilateral cases becomes apparent when the fraction of still undiagnosed cases is plotted against the age at which diagnosis is made (Figure 3). The regular decrease with time shown by the bilateral cases suggests that a single chance event is sufficient to trigger the onset of bilateral retinoblastoma. By contrast, the presence of a 'shoulder' on the unilateral curve suggests that multiple events in one cell are required to trigger unilateral retinoblastoma. (A shoulder arises because the events accumulate over time. For example, if two events are required, most affected cells at early times will have suffered only a single event and will not generate a tumor. With time the probability increases that a second event will occur in an already affected cell and therefore cause a tumor.)

One possible explanation for these observations is that tumors develop when both copies of the critical gene (the retinoblastoma, *Rb*, gene) are lost or mutated. In the inherited (bilateral) form of the disease, a child receives a defective *Rb* gene from one parent: tumors develop in an eye when the other copy of the gene is lost through somatic mutation. In fact, the loss of a copy of the gene is frequent enough that tumors usually occur in both eyes. If a person starts with two good copies of the *Rb* gene, tumors arise in an eye only if both copies are lost *in the same cell*. Since such double loss is very rare, it is usually confined to one eye.

To test this hypothesis, you use a cDNA clone of the *Rb* gene to probe the structure of the gene in cells from normal individuals and from patients with unilateral or bilateral retinoblastoma. As illustrated in Figure 4, normal individuals have four restriction fragments that hybridize to the cDNA probe (which means each of these restriction fragments contains at least one exon). Fibroblasts (nontumor cells) from the two patients also show the same four fragments, although three of the fragments from the child with bilateral retinoblastoma are present in only half the normal amount. Tumor cells from the two patients are missing some of the restriction fragments.

A. Explain why fibroblasts and tumor cells from the same patient show different band patterns.
B. What are the structures of the *Rb* genes in the fibroblasts from the two patients? What are their structures in the tumor cells from the two patients?

C. Are these results consistent with the hypothesis that retinoblastoma is due to the loss of the *Rb* gene?

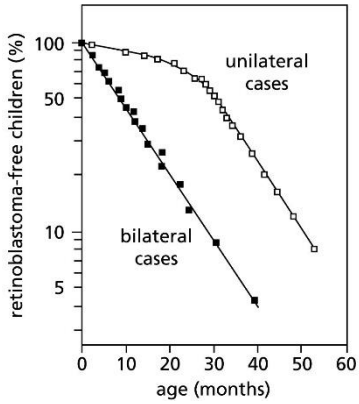


**Figure 3- Time of onset of unilateral and bilateral cases of retinoblastoma.** A population of children all of whom ultimately developed retinoblastoma, is represented in this graph. The fraction of the population that is still tumor free is plotted against the time after birth.

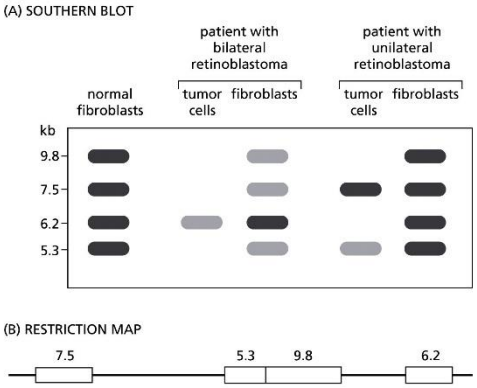


**Figure 4- Patterns of blot hybridization of restriction fragments from the retinoblastoma gene.**

(A) Southern blot for normal individuals and for patients with unilateral and bilateral retinoblastoma. *Lighter shading* of some bands indicates half the normal number of copies. (B) The order of the restriction fragments in the *Rb* gene. Fragments that contain exons (*rectangles*) hybridize to the cDNA clone that was used as a probe in these experiments.

5- Now that DNA sequencing is so inexpensive, reliable, and fast, your mentor has set up a consortium of investigators to pursue the ambitious goal of tracking down all the mutations in a set of human tumors. He has decided to focus on breast cancer and colorectal cancer because they cause 14% of all cancer deaths. For each of 11 breast cancers and 11 colorectal cancers, you design primers to amplify 120,839 exons in 14,661 transcripts from 13,023 genes. As controls, you amplify the same regions from DNA samples taken from two normal individuals. You sequence the PCR products and use analytical software to compare the 456 Mb of tumor sequence with the published human genome sequence. You are astounded to find 816,986 putative mutations. This represents more than 37,000 mutations per tumor! Surely that can't be right.

Once you think about it for a while, you realize the computer sometimes makes mistakes in calling bases. To test for that source of error, you visually inspect every sequencing read and find that you can exclude 353,738 changes, leaving you with 463,248, or about 21,000 mutations per tumor. Still a lot!

A. Can you suggest at least three other sources of apparent mutations that do not actually contribute to the tumor?
B. After applying a number of criteria to filter out irrelevant sequence changes, you find a total of 1307 mutations in the 22 breast and colorectal cancers, or about 59 mutations per tumor. How might you go about deciding which of these sequence changes are likely to be cancer-related mutations and which are probably 'passenger mutations that occurred in genes with nothing to do with cancer (but were found in the tumors because they happened to occur in the same cells with true cancer mutations)?
C. Will your comprehensive sequencing strategy detect all possible genetic changes that affect the targeted genes in the cancer cells?

6- You've just read about a really cool technique for high-throughput screens of protein kinase inhibitors. The trouble is, you don't understand it. It is clearly important since it allows one to rapidly screen a large number of potential kinase inhibitors against a large number of protein kinases. There are roughly 500 protein kinases, including about 100 tyrosine kinases, encoded in the human genome. Many of them are critical components in the signal transduction pathways that become misregulated in cancer. Chemicals that inhibit individual protein kinases could serve as important lead compounds for development of drugs that are useful in the fight against cancer (and other diseases). So you want to understand how this technique works.

There are several elements (Figure 5). First, individual protein kinase genes are fused to the major capsid (head) protein of T7 phage. When expressed in bacteria, the fusion proteins are assembled into the phage capsid, with the kinases displayed on the outer surface. Second, an analog of ATR which can bind to the ATP-binding pocket of the kinases, is attached to magnetic beads. Third, a bank of test compounds is prepared.

To measure the ability of a test compound to inhibit the kinase, phage displaying a specific kinase are mixed with the magnetic beads in several wells of a 96-well plate. Then the test compound is added to individual wells over a range of different concentrations. The mixtures are incubated with gentle shaking for 1 hour at 25°C, the beads are pulled to the bottom with a strong magnet, and all the free (unbound) components are washed away. Finally the remaining, attached phage are dissociated from the beads using an excess of the same ATP analog that is attached to the beads, and counted by measuring the number of plaques they form on a bacterial lawn on a Petri dish (Figure 5).

Although the assay is well described and the figure is clear, there are several things you just don't get. For example:

A. What is the point of the one-hour incubation?
B. How does the plaque count relate to the binding efficiency of the test compound? Will a test compound that binds a protein kinase strongly give more plaques or fewer plaques than one that binds the kinase weakly?
C. Do the test compounds compete for binding by the ATP analog? Or will a test compound that binds the kinase tightly someplace else also register in this assay?
D. Assuming that the test compounds bind to the ATP-binding site, how is it possible for them to bind one protein kinase, but not another? After all, every protein kinase has an ATP-binding site; that's how they bind to the ATP analog on the magnetic beads.

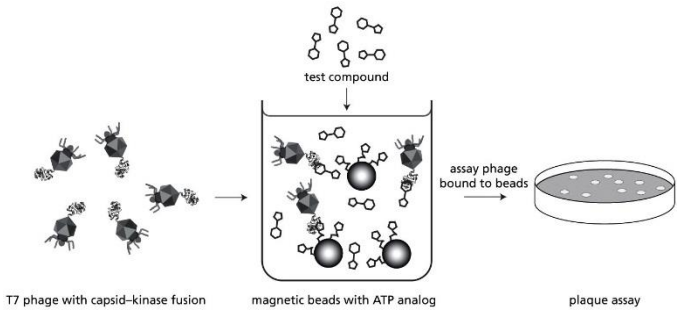


**Figure 5- Quantitative assay for screening potential inhibitors of protein kinases.** T7 phage carrying a kinase-capsid fusion are mixed with ATP-analog-coated magnetic beads in the presence of a test compound. After washing away unbound phage, attached phage are eluted and assayed for p