The molecular biology of cancer

Although long suspected, it is now well established that most cancers have genetic origins and arise either as direct result of a mutation in a growth regulating gene or from the overproduction (in some cases underproduction) of a gene product involved in cellular growth control. This conclusion is based on the molecular analysis of such mutated genes, called oncogenes, or their promoters and will be discussed in this section. However, before we can discuss the possible defects of cellular growth control that must occur in cancer cells, we have to describe what is known about normal growth control.

The control of the cell cycle

The cell cycle has two main phases, interphase and mitosis. During interphase, which includes G1, S, and G2, the cell accumulates components, grows in size, and replicates its DNA. In mitosis, first the nuclear envelope breaks down, then the duplicated chromosomes become attached to the mitotic spindle which segregates them into two identical sets of chromosomes, and finally nuclear envelopes reform around each set of chromosomes allowing the cell to divide into two identical daughter cells. The entire process must be controlled precisely so that cells that have not completed their DNA replication cannot enter mitosis. This is important because the lack of a chromosome can kill the cell or cause it to become a cancer cell.

Mitosis is initiated by the maturation promoting factor (MPF), a protein factor consisting of at least two subunits, the cdc2 subunit and cyclin. MPF is a protein kinase that, among other functions, phosphorylates the proteins of the nuclear envelope, called lamins, thereby initiating the breakdown of the nuclear envelope. During most of the cell cycle, the cdc2 protein is inactive. For activation it must combine with cyclin which accumulates during interphase. The resulting pre-MPF is then converted to active MPF by several other enzymes, one of them being cdc25. Active MPF then initiates mitosis which includes the formation of the spindle apparatus. In addition, it activates enzymes that degrade cyclin. This degradation of cyclin is necessary for mitosis to proceed to completion.

A deletion mutant of cyclin, capable of cdc2 activation but resistant to degradation, has been shown to cause the cell to remain arrested in mitosis. As MPF becomes inactive, phosphatases inactivate the enzymes that MPF had turned on by phosphorylating them. This inactivation also includes the enzymes involved in cyclin degradation so that cyclin can begin to accumulate again to initiate a new cell cycle. The cell cycle has a second control point, called START, at the entry of S phase. This control also involves the cdc2 protein which complexes with a second type of cyclin that accumulates in G1. The accumulation of this second cyclin is influenced by nutrients, hormones, and growth factors. Cells that lack nutrients cannot pass through START and begin DNA replication.

The incidence of human cancer

One distinguishes two types of tumor, the benign tumor which grows into a large mass of cells but stays within its normal tissue boundaries, and the malignant tumor, whose cells can invade other tissues and form secondary tumors, called metastases, at sites removed from the site of the original tumor. Although human cancers can occur in almost any tissue, they are generally divided into three types:

1. Carcinomas are cancers derived from epithelial tissues, i.e. skin, gut, lung, glands.
2. Sarcomas arise from cells of bone, cartilage, muscle, fibroblasts.
3. Leukemias and lymphomas originate from blood cells.

Over 90% of all human cancers are carcinomas. Most likely this is because epithelial cells, whether in the skin, the gut, or the lungs are exposed to environmental influences, such as carcinogens or radiation, agents that cause mutations.

Epidemiological studies suggest that some forms of cancer are clearly caused by environmental agents, such as diet, life style, or pollution of the air, although the nature of the carcinogenic agent is often not yet known. For example, colon and breast cancer are most prevalent in the United States but occur much less frequently in Japan. On the other hand, cancer of the stomach is very common in Japan but much less frequent in the United States. The evidence that cigarette smoking causes most lung cancers is now overwhelming, and the role of exposure to excessive sun light is known to increase the frequency of skin cancer.

In addition to carcinogens, certain viruses are suspected of causing cancer as well. Hepatitis B virus, for example, is believed to be at the root of many liver cancers. The DNA viruses polyoma and SV40 produce cancers in hamsters. Retroviruses have been shown to cause cancers in many different species.

Most human tumors consist of a clone of cells that are all derived from a single mutated cell. This has been concluded from the fact that in females, that are heterozygous for the X-linked enzyme glucose-6-phosphate dehydrogenase, all tumor cells express the same isozyme, while in the rest of the body different cells have different X-chromosomes inactivated and thus express different isozymes.

While the initial event in the formation of a cancer cell usually is a mutation, the mutated cell often remains in a precancerous state for long periods of time. It requires the action of a tumor promoter for the expression of the cancer phenotype. Tumor promoters are typically agents that stimulate quiescent cells to divide, a necessary step in carcinogenesis. For example, when the skin of a mouse is treated with benzopyrene, a potent carcinogen, tumors rarely develop. However, if the skin is later painted with a phorbol ester, such as 12-O-tetradecanoyl phorbol-13-acetate (TPA), tumors will develop. TPA by itself is not a carcinogen, instead it is an activator of protein kinase C. The action of protein kinase C causes the mutagenized cell to proliferate and thereby establish its mutated phenotype. (Remember that you need DNA replication to establish the mutation.) Usually, a single mutation is not sufficient to convert a normal cell into a cancer cell. In some cancers, up to 7 individual mutations are required, in others only 2 suffice. Thus, the incidence of cancer increases as the nth power of age, n being the number of mutations required for expression of the cancer phenotype.

Isolation of human cancer-causing gene

When normal mammalian cells are grown in tissue culture, the cells divide until they form a monolayer, a single sheet of cells that covers the culture dish. At that point cell growth ceases. The culture is said to be confluent. In order to make the cells grow again, they must be dislodged from their support surface and from each other with the proteolytic enzyme trypsin and replated at a lower concentration in a new culture dish. For human cells, this process of propagation can be repeated until the cells have divided about 60 times; then all cells die. Presumably, a biological clock ticks inside each cell that makes the cell age and eventually die. Only germ cells are immune from aging. The nature of this biological clock is not known.

Sometimes, however, a cell in a primary culture acquires a mutation that stops the biological clock and thus makes it immortal. Cells derived from such a mutant are called a cell line; they can be propagated indefinitely but still form a monolayer in culture that must be trypsinized for growth to resume. Tumor cells behave very differently; they are immortal like cell lines, but instead of forming monolayers they continue to grow, causing the cells to pile up on top of each other. One cell line that has been most useful in the isolation of human cancer-causing genes is the mouse fibroblast 3T3 line. Normally, 3T3 cells grow in a monolayer. However, when DNA isolated from a human tumor is sheared to fragments of about 50 kilobase and added in the form of a calcium phosphate precipitate onto the 3T3 monolayer, some 3T3 cells incorporate the gene that was responsible for the cancer phenotype of the tumor cells and, as a consequence, assume the properties of the tumor cell. Such transformed 3T3 cells form foci, clusters of cells piled up on top of one another, that are readily recognized within the 3T3 monolayer (Fig. 7). DNA isolated from these primary 3T3 transfectants can be used to transfect new 3T3 cultures, producing foci which are called secondary transfectants.

The human gene that was incorporated into the mouse genome of 3T3 cells can be identified by the presence of the highly reiterated human Alu sequence, which is present on average about once in every 5 kb segment of the human genome . (Mouse DNA does not contain this sequence.) This is done by making a genomic library from a secondary 3T3 transfectant and screening it with a radioactive Alu probe. The first human oncogene isolated this way was the H-ras-1 gene found in a bladder carcinoma. It was soon discovered that this gene was identical to the H-ras transforming gene of the Harvey sarcoma virus, a retrovirus causing tumors in rodents.

By using the Harvey sarcoma virus H-ras gene as a hybridization probe, an H-ras homolog was also found in normal human cells. When this H-ras-1 gene was cloned and used to transfect 3T3 cells, no foci formed. Sequence analysis soon revealed that the H-ras-1 oncogene isolated from the bladder carcinoma contained a single base pair change as compared to the normal cellular H-ras-1 gene. Apparently, the product of the H-ras-1 gene is somehow involved in cellular growth regulation, and the mutation that occurred in this gene caused the cells to proliferate producing a bladder carcinoma. The H-ras gene of the Harvey sarcoma virus was found to have a mutation at the same site in addition to further mutations.

If the development of cancer requires more than one mutation, why then is a single mutated H-ras-1 gene capable of transforming 3T3 cells? The answer probably is that 3T3 cells are not really normal cells, even though they do not cause tumors when injected into animals. It is possible that the immortalizing mutation, responsible for making mouse fibroblasts a cell line, together with the mutated H-ras-1 gene is sufficient for transformation. When primary mouse cells are transfected with the mutated H-ras-1 gene, they do not become transformed.

ONCOGENES

As the example with the H-ras gene showed, cancer-causing genes, oncogenes, arise by mutation from normal cellular genes, called proto-oncogenes. Some RNA tumor viruses also carry oncogenes which are derivatives of normal cellular protooncogenes. Since the genome of tumor viruses integrates into its host's chromosomal DNA during part of the virus's life cycle, it is likely that a cellular protooncogene was picked up when the provirus genome was transcribed into viral RNA for incorporation into virus particles. There the protooncogene mutated into an oncogene, and when the virus now infects a normal cell, it expresses the oncogene and thereby transforms the cell into a tumor cell. Many different viral oncogenes, called v-*onc*, have been isolated and characterized from retroviruses. Corresponding cellular protooncogenes, called *c-onc*, have been found in every case. One might ask what the normal cellular function of these genes is. In several cases, the properties of their gene product or their cellular function is known. In other cases, only the subcellular location and time of expression of the protooncogene product is known. Table 1 lists some of the oncogenes which can be grouped into five distinct classes.

Table 1. Properties of some oncogenes.

Oncogene	Retrovirus or tumor	Location	Function
Class1:Protein kinases			
src	Rous sarcoma virus (RSV)	Cell membrane	Protein kinase (tyr-specific
yes	Avian sarcoma virus (AVS)	Cell membrane	Protein kinase (tyr-specific
abl	Abelson murine leukemia virus (MLV)	Cell membrane	Protein kinase (tyr-specific
fes	Feline sarcoma virus(FeSV)	Cell membrane	Protein kinase (tyr-specific
*erb*B	Avian erythroblastosis virus (AEV)	Cell membrane	EGF-receptor
fms	Feline sarcoma virus	Cell membrane	CSF-1 receptor
mos	Moloney sarcoma virus	Cytoplasm	Protein kinase (ser/thr-specific)
Class 2: GTP-binding proteins			
H-*ras*	Harvey mouse sarcoma virus (Ha-MSV)	Cell membrane	G-protein with GTPase activity
K-*ras*	Kirsten MSV	Cell membrane	G-protein with GTPase activity
N-*ras*	Human neuroblastoma	Cell membrane	G-protein with GTPase activity
Class 3: Cellular growth factors			
sis	Simian sarcoma virus(SSV)	secreted	PDGF
Class 4: Hormone and growth factor receptor			
*erb*A	Avian erythroblastosis virus (AEV)	Cell membrane	Thyroid hormone receptor
Class 5: Nuclear proteins			
myc	Burkitt' s lymphoma	Cell nucleus	Transcription factor
fos	Murine osteosarcoma virus	Cell nucleus	Transcription factor

(EGF = epidermal growth factor; CSF = colony stimulating; PDGF = platelet-derived growth factor). Most oncogenes discovered by the 3T3 focus assay were found to be of the *ras* type. This may be because 3T3 cells are especially sensitive to transformation by ras. The human genome contains 5 ras proto-oncogenes, two of which (H-*ras*-2 and K-*ras*-1) are pseudogenes that are not expressed. H-*ras*-1 and K-*ras*-2 have been found mutated, and thus activated, in bladder, lung and colon carcinomas. The proteins of these genes (p21) are highly homologous and resemble the guanine nucleotide-binding elongation factor EF-Tu. The third ras protooncogene, N-ras, found in mutated form in human neuroblastoma, is less homologous. Like other cellular protooncogenes the c-ras genes contain several