

# Cancer Genes. The Molecular Militants

M S S Murthy

'What actually seems to have happened when a cancer appears in an organism is that the biological behavior of some of the cells which make up that organism have changed. Whereas these cells had hitherto behaved in a manner consistent with the life of the organism, obeying the 'rules' of growth and differentiation, they quite suddenly cease to respond to such restraints and set off on a 'lawless' course of increased division, invasion of adjacent normal tissues, and even migration (metastasis) via the blood stream and lymph channels to other parts of the body.' R J C Harris in *Cancer*, 1962.

## Introduction

Cell division is a fundamental property of all living organisms. In multicellular organisms life starts from a single cell and evolves to adulthood through cell division and differentiation. An elaborate system of controls tightly regulates cell division to the requirements of the organism. Any aberrations in this system may lead to autonomous cell division, which is the hallmark of cancer. Hence, to understand cancer, one has to understand the molecular process in the cell division cycle (mitogenesis).

The complex cell division cycle is orchestrated by five groups of proteins:

1. Extracellular growth factors.
2. Growth factor receptors.
3. A signal transducing system consisting of a chain of proteins, which transmits the signal for cell division from the cytoplasm to the nucleus.
4. A transcription system which activates the genes involved in cell division.
5. A cell cycle clock, which ensures that the various stages of the division precisely follow one another in a clock-like manner.



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## Extracellular Growth Factors

Proteins called growth factors initiate growth of normal cells. Generally, they are secreted by one group of cells and acted upon by another group of cells. However, some cells secrete their own growth factors. Hormones were the first proteins to be identified as growth factors. They are secreted by the endocrine glands and released to the circulatory system from where they migrate to the target cells. Some of the other growth factors are: i) nerve growth factors (affect differentiation and survival of sensory and sympathetic neurons), ii) epidermal growth factors (promote growth of epidermal cells), iii) platelet derived growth factors (secreted by platelets to promote the growth of fibroblasts and smooth muscle cells involved in wound healing), iv) vascular epidermal growth factors (stimulate the growth of endothelial cells which play a major role in angiogenesis – formation of new capillaries in developing tissues), v) peptide growth hormones (stimulate the growth of a variety of cells).

## Growth Factor Receptors

Growth factor receptors are proteins themselves. As there are various types of growth factors, there are also various types of growth factor receptors (GFRs). They span the plasma membrane of the cell. We will discuss one type of GFR known as protein tyrosine kinases (PTKs). These receptors have two domains – an external domain on the surface of the cell to which the growth factor binds and an internal domain, which triggers biochemical events inside the cell. The first signal for cell division is given when a growth factor (a ligand) binds to its receptor in a lock and key arrangement. The process by which the signal for cell division is transmitted from the extracellular domain to the nucleus is called signal transduction.

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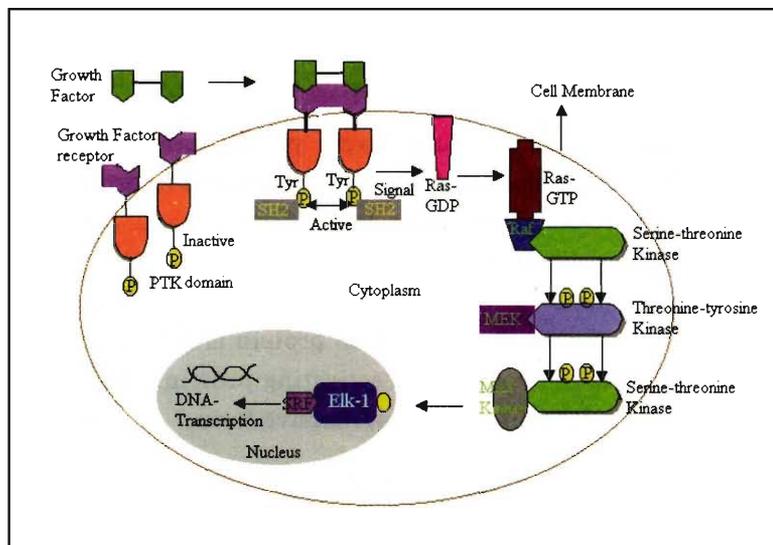
## Signal Transduction System

In unstimulated cells the growth factor receptors exist as monomers. One growth factor molecule can simultaneously bind to two receptors on the cell surface, thus dimerising them. As a

consequence of this, the PTK domains of the two receptors activate each other. Activation involves adding a phosphate group (phosphorylation) to the amino acid tyrosine present in them. This leads to conformational changes in the peptide chain making certain parts of it accessible for interaction with other proteins. The activated receptor complexes with a group of proteins among which is an SH2 protein. This complex activates the membrane associated RAS protein. Generally, RAS protein exists in an inactive state bound to guanosine diphosphate (GDP). The PTK-SH2 complex induces RAS to release the GDP and bind to guanosine triphosphate (GTP) which is available at a higher concentration in the cytoplasm. The activated RAS protein can interact with several targets downstream. One of these is a protein kinase called RAF

Activation involves adding a phosphate group.

(*Figure 1*) The activated RAF protein initiates a cascade of phosphorylations. Important among them is the phosphorylation of amino acid serine on MEK protein, which in turn phosphorylates another protein called MAP kinase. Activated MAP kinase can phosphorylate a wide range of proteins including transcription factors and regulators of protein synthesis.



**Figure 1. Steps in mitogenic signal transduction.**

Transcription factor is a protein, which when activated by the mitotic signal through, say, the MAP kinase, can bind to genes involved in cell division and stimulate their transcription.

## Transcription Factors

The final target for the mitogenic signal in the transduction pathway is the transcription factor. A transcription factor is a protein, which when activated by the mitotic signal through, say, the MAP kinase, can bind to genes involved in cell division and stimulate their transcription. Following the activation of transcription factors, a series of coordinated events takes place to push the cell through the cell division cycle. Among them are the synthesis of cell mass and DNA, complexing of DNA and protein to form chromosomes, chromosome condensation, spindle formation, segregation of chromosomes and finally their equal distribution to both the daughter cells. These events are precisely timed by a system known as 'cell cycle clock'. Two types of proteins which complex with each other are of importance in this regulation. One is a regulatory sub-unit called cyclins (so called because their abundance varies through the cell cycle). The other is a catalytic sub-unit called cyclin dependent kinases (CDK). Nearly a dozen cyclins (A through I) and their corresponding CDKs have been identified. A specific cyclin-CDK complex facilitates progression through a given stage of division cycle. After transition through the stage, the complex is broken down and a new complex is formed as the cell moves to the next stage.

## Negative Regulation

Any regulatory system should have a provision for applying brakes when required. The proteins in the signal transduction pathway exist either in an active or an inactive state. Inactive states are generally more stable than active states. Several mechanisms exist to maintain them in the inactive state. For example, the active GTP bound RAS protein is rapidly hydrolyzed to GDP-RAS by a GTPase activating protein. This conversion restores RAS protein to an inactive form.

There are other proteins, which directly deliver a negative signal. An important protein with such a function is p53 protein. It regulates various steps in the division cycle, includ-

ing DNA replication. Thus cell proliferation is critically balanced by some proteins which deliver positive growth signals, and others which antagonize cell growth.

### **Repair of DNA Damage and Maintenance of Chromosomal Integrity**

One of the important requirements for normal tissue function is that the integrity of DNA is maintained from one division cycle to another. If DNA is damaged due to exposure to ionizing radiation, UV or chemicals, progression in cell cycle is arrested until DNA is repaired. This type of control is known as 'checkpoint control'. Repair of DNA itself is orchestrated by a group of enzymes consisting of nucleases, polymerases, ligases, etc. If the damage is too extensive the cell is pushed towards a programmed death known as apoptosis. This is to ensure that daughter cells do not inherit damaged DNA. Similar checkpoint controls monitor the fidelity of chromosome transfer to daughter cells at mitosis. Negative signaling proteins such as p53 play a significant role in the functioning of checkpoint controls. While the signaling events are discernible in seconds or minutes, it generally takes many hours for mammalian cells to pass through all checkpoints and complete one cell division cycle.

After cell division the two newly formed daughter cells of a healthy tissue enter one of the following phases, depending on the homeostatic requirements of the tissue:

1. They may die.
2. One of them may go into a quiescent state as in the case of stem cells or both may differentiate and mature to replace dying functional cells in the tissue.
3. Reenter the division cycle.

However, in cancer tissue, the cells reenter the division cycle autonomously to the detriment of the organism. What makes the cells take this militant path? The answer to this question lies in the genes that control cell division cycle.

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It can be envisaged that certain genetic events may deregulate the cell proliferation system.

## The Genetic Control

In order to understand the oncogenic process at the molecular level, researchers in the past two decades have focussed attention on three groups of genes namely:

- i) Those that code for positive regulators which turn on cell division cycle – ‘proto-oncogenes’,
- ii) Those that code for negative regulators which inhibit cell division cycle – ‘tumor suppressor genes’, and
- iii) Those that code for DNA repair enzymes – DNA repair genes.

It can be envisaged that genetic events which lead to ‘gain of function’ of proto-oncogenes or ‘loss of function’ of tumor suppressor genes and/or DNA repair genes can deregulate the cell proliferation system and drive a normal cell to a malignant state.

## Activation of Proto-oncogene

Some of the genetic events which activate the proto-oncogenes leading to their gain of function are:

1. Retroviral transduction
2. Insertion mutations
3. Translocations and inversions
4. Point mutations
5. Gene amplification

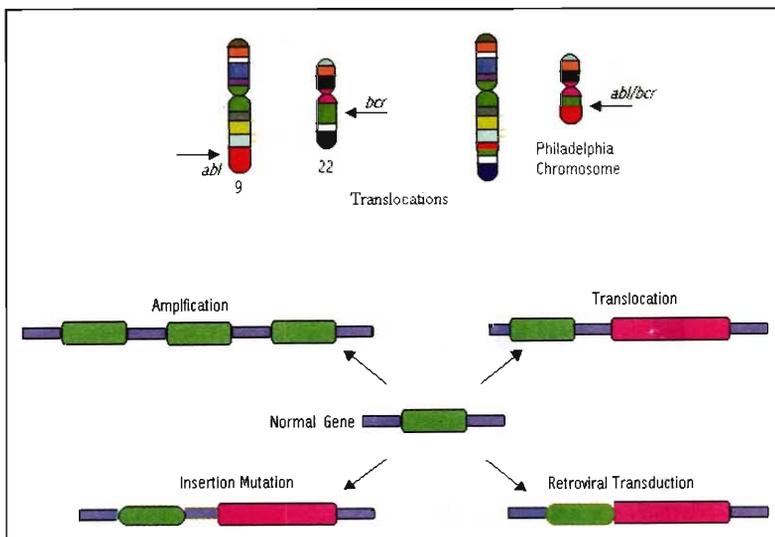
***Retroviral transduction:*** Many retroviruses pick up proto-oncogenes from host cells during infection. This process is known as transduction. A transduced proto-oncogene may be activated in two ways: a) by placing the gene under the control of a viral gene; the pirated gene may become tumorigenic because of sustained expression and mutations. b) By fusing the proto-oncogene with a structural gene whose product may stimulate cell division.

***Insertion mutations:*** Not all retroviruses possess pirated cellular



proto-oncogenes. Instead, some retroviruses may activate a proto-oncogene by a process known as insertion mutation. In this process a retrovirus may insert a DNA copy of its RNA genome into the chromosomal DNA of the host cell. If the proto-oncogene lies close to the site of insertion it may come under the powerful replicating influence of the viral genome. Such an event may increase the protein product of the proto-oncogene, or create an abnormal protein product, or turn on a silent proto-oncogene. Retroviral transduction and insertion mutations frequently occur in animal tumors, but have no role to play in human cancers except in the case of adult T cell leukemia.

**Translocations and inversions:** Translocations involve exchange of chromosome fragments between two chromosomes. In inversion, a broken chromosome may rejoin in a flip-flop manner. Such rearrangements may locate a proto-oncogene next to a strong promoter sequence leading to its over expression. Not all causes of translocations are external. Some arise from the normal metabolic processes; for example, bone marrow cells rearrange a special set of genes in preparation for mounting an immune response. On rare occasions this may go awry and bring about a bizarre rearrangement of genes. In the process a proto-oncogene may be activated (*Figure 2*).



*Figure 2. Mechanisms for activation of a proto-oncogene to oncogene. Translocation is further illustrated in the upper part.*

**Point mutations:** A single base pair in a gene may be altered, leading to the replacement of one amino acid with another in the protein product. Such replacements may lead to structural changes in the protein altering its properties.

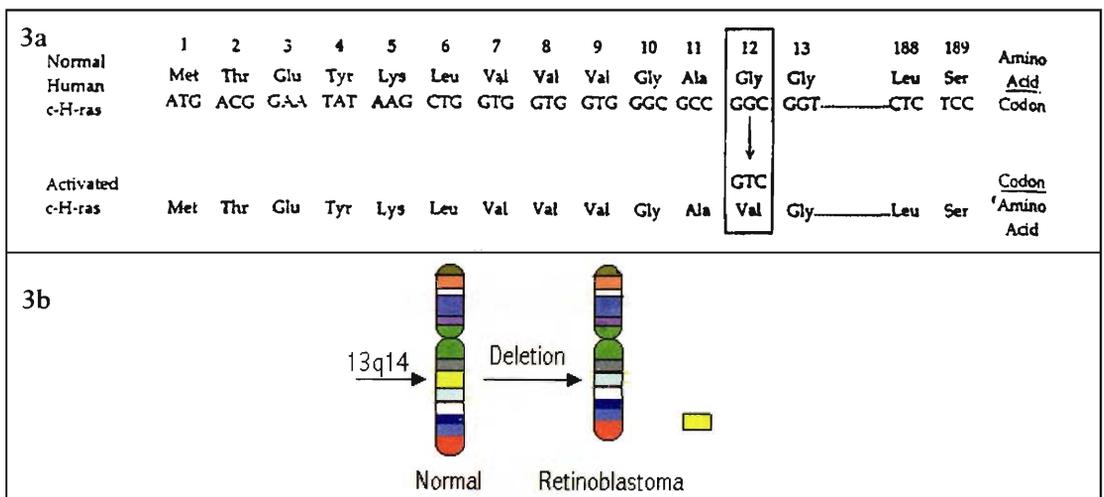
**Gene amplification:** Certain genes may be abnormally replicated in excess of 100 or even 1000 fold. This produces an excess of protein, which may have an oncogenic influence. For example, gene amplification may lead to an abundance of growth factor receptors.

**Figure 3. a) Point mutation.** *H-RAS proto-oncogene is activated to H-RAS oncogene by a point mutation at the 12th codon replacing glycine with valine in the RAS protein. Point mutations can also inactivate tumor suppressor genes and DNA repair genes. b) Deletion.* A segment of chromosome is lost. If the deleted segment contained vital information the gene may be inactivated. Tumor suppressor genes and DNA repair genes are inactivated by deletion.

Such activated proto-oncogenes are called ‘oncogenes’. More than 100 oncogenes have been identified. Oncogenes act dominantly. This means that even if one copy of the proto-oncogene is activated, it can exert oncogenic influence.

### Inactivation of Tumor Suppressor Genes and DNA Repair Genes

Deletions and point mutations bring about the inactivation of tumor suppressor genes and DNA repair genes. Deletion is a process in which a segment of DNA is lost due to strand breaks. If the lost portion contained important genetic information, the gene may become inactive (Figure 3). There are other ways of blocking the products of tumor suppressor genes. For example,



DNA viruses such as adenovirus and human papiloma virus produce protein products in the infected cell which bind to the products of tumor suppressor genes and render them inactive.

More than a dozen tumor suppressor genes and DNA repair genes have been identified. These genes are recessive in nature. This means that both the copies of the gene have to be inactivated to exert oncogenic influence. In familial cases such as cancer of breast and ovaries, susceptibility is inherited in the form of an inactivated copy of the tumor suppressor gene or DNA repair gene. During the lifetime of the individual the other copy may also suffer inactivation leading to loss of function of the gene. Homozygosity for loss of function may also be achieved by gene conversion with the inactivated gene as the template, chromosomal non-disjunction during cell division, or loss of wild type chromosome.

Inactivation of DNA repair genes, though it does not affect the normal process of cell division, results in an accumulation of mutations in proto-oncogenes and tumor suppressor genes leading to genomic instability. This can accelerate the process of tumor progression.

### The Aberrant Cell

Oncogenic mutations frequently involve one or more genes that code for growth factors, growth factor receptors, proteins of the signal transduction pathway including the negative signaling ones, the transcription factors and the components of the cell cycle clock. Such mutations produce proteins which are locked either in an active or an inactive state conferring growth autonomy to the cell. Mutations in the genes coding for growth factors may lead to their increased secretion to which the cells themselves may respond (autocrine signaling) or stimulate distal cells (paracrine signaling). An example of the latter is the case of stromal cells stimulated by carcinoma cells. Secretion of fibroblast growth factor and vascular endothelial growth factors by tumor cells lead to proliferation of endothelial cells and tumor angiogenesis, respectively. Mutant PTK receptors may

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Growth factor receptors dimerise even in the absence of growth factors, because of their close proximity to each other.

form dimers without the binding of growth factors and thus signal for uninterrupted cell division. For example, a deletion in the *ERB-B* gene produces an epidermal growth factor receptor that lacks an extracellular domain, but an intact PTK domain. It remains activated regardless of the presence of a growth factor. Such aberrant receptors are known to convert human glioblastoma to a more malignant state. A point mutation in the gene *ERB-B2* produces a PTK receptor, which is constitutively dimerised. It is found in rat neuroblastomas.

Similarly, gene amplification may lead to excess production of receptors which are normal. However, they dimerise even in the absence of growth factors because of their close proximity to each other. Amplification of *ERB-B* gene and over production of epidermal growth factor are commonly seen in carcinomas of breast, ovary, cervix, kidney, etc.

RAS protein occupies a coveted position in the signal transduction pathway. The *RAS* gene may undergo a point mutation that leads to the replacement of the code for amino acid glycine in the 12th position by that for valine. Similarly, mutations can occur at codons for amino acid residues 13,59,61 and 63 in the RAS protein. These altered RAS proteins may retain their capacity to get activated by binding to GTP, but cannot be hydrolyzed back to their inactive form. Thus, they remain locked in an active state, continuously sending the mitogenic signal. Alternatively, mutations at amino acid residues 116, 117, 119, 146 can increase the rate at which the RAS protein can exchange GTP to GDP from the cytoplasm. Activated *RAS* gene has been found in fifteen percent of all human cancers and fifty percent of colorectal cancers.

In normal cells, transcription factors are tightly balanced between active and inactive states. *MYC* protein is an important transcription factor. It can be over produced due to a translocation between chromosomes 8 and 14, which places the *MYC* gene under the control of strong promoters for gene expression. An elevated production of *MYC* protein shifts the balance



Loss of imprint (LOI) may result in the expression of a normally silent allele or vice-versa.

rity of DNA, *p53* gene is called ‘the guardian of the genome’. If *p53* is inactivated, neoplastic cells with damaged DNA can escape this control. *p53* gene is known to be one of the most frequently mutated genes in many human cancers. Indeed 75% to 80% of all colon cancers show abnormalities in both copies of the *p53* gene.

### DNA Repair Genes

It has been known since 1960 that germ line mutations and inactivation of genes involved in DNA repair predispose the carriers to inherited forms of cancers such as Xeroderma pigmentosum, Bloom’s syndrome, ataxia-telangiectasia and Franconi’s anemia. Breast cancer genes *BRCA1* and *BRCA2* are known to be deficient in the repair of DNA damage caused by ionizing radiation. Similarly, mutant alleles of at least four genes responsible for DNA mismatch repair – *MSH2*, *MLH1*, *PMS1*, and *PMS2* have been implicated in the hereditary non-polyposis colon cancer (HNPCC). Persons suffering from HNPCC reveal a high degree of genetic disorder throughout their genome.

Some representative mitogenic factors, the genes controlling them, their chromosomal locations, mechanism of activation, and the tumors caused are listed in *Box 1*.

### Other Genetic Events

In addition to the above genetic events there are others, which also participate in carcinogenesis. Mention can be made of two such events: genomic imprint and telomere length.

*Genomic Imprint:* Of the two copies of each gene we inherit, one comes from the father and the other from the mother. Genomic imprint is a normal process in which certain genes inherited from one parent are active while their copies coming from the other parent are inactive. It controls a number of cellular processes including signal transduction and cell division cycle. For example, in case of genes which code for insulin-like growth



**Box 1. Genes control the cell division (mitogenic) cycle. Aberrations in these genes result in loss of control on cell division and lead to cancer.**

Mitogenic Protein	Gene (chromosome)	Mechanism of Activation/ Inactivation	Some Major Human Cancers
<i>Growth factors</i>			
Platelet derived growth factor	<i>PDGFB/Sis</i> (22)	Autocrine stimulation.	Astrocytomas, fibrosarcomas and glioblastomas.
Fibroblast growth factor	<i>INT2</i> (11)	Gene amplification.	Breast cancer and glioblastomas.
<i>Growth factor receptors</i>			
Protein tyrosine kinase	<i>EPH</i> (7)	Gene amplification.	Carcinoma of kidney, testis, lung and liver.
Epidermal growth factor receptors	<i>RET</i> (10) <i>EGFR/ERBB</i> (7)	Point mutation. Gene amplification/rearrangement.	Thyroid cancer. Squamous cell carcinoma and glioblastomas.
<i>Membrane associated proteins</i>			
H-RAS	<i>H-RAS</i> (11)	Point mutation.	Bladder cancer.
K-RAS	<i>K-RAS</i> (12)	Point mutation.	Pancreatic, colorectal, lung cancer and leukemia.
N-RAS	<i>N-RAS</i> (1)	Point mutation.	Myeloid leukemia.
<i>Transcription factors</i>			
Nuclear proteins	<i>MYC</i> (8)	Gene amplification/translocation.	Breast cancer, cervical cancer. Burkitt's lymphoma.
	<i>FOS</i> (14)	Gene amplification.	Osteosarcoma.
<i>Cell cycle clock</i>			
Cyclin D1	<i>BCL1</i> (11)	Translocation (11:14)	Breast, head and neck cancers and leukemia.
Cyclin dependent kinase	<i>CDK4</i>	Gene amplification	Sarcomas.
<i>Negative regulators</i>			
p53 protein	<i>p53</i> (17)	Loss of heterozygosity (deletion/point mutation)	Many types of tumors.
Retinoblastoma protein	<i>Rb</i> (13)	-do-	Retinoblastoma, bone and bladder cancers.
	<i>APC</i> (5)	-do-	Colon cancer.
<i>DNA repair enzymes</i>			
Mis-match repair enzymes	<i>hMSH2</i> (2) <i>hMLH1</i> (3)	Loss of heterozygosity -do-	Colon cancer -do-

The beginning of cancer is in the end of the chromosome.

factor 2 (IFG 2) and a cyclin dependent kinase inhibitor, the paternal and maternal alleles respectively are expressed in normal situations. Any disruption in genomic imprint — loss of imprint (LOI) — may result in the expression of a normally silent allele or the vice-versa. In carcinogenesis this could mean activation of proto-oncogenes or inactivation of tumor suppressor genes. For example, in 70% of Willm's tumor, both maternal and paternal genes express IFG 2. LOI on chromosome 11 is known to be responsible for more than 12 human cancers.

**Telomere length:** The ends of each chromosome consist of a repeat sequence called telomere. Telomere prevents chromosomes from sticking to one another, interfering with their stability. During chromosome duplication the telomere is not generally copied. On the other hand, it is shortened keeping a count of the number of divisions a cell undergoes. When the telomere reaches a critical length the cell becomes terminally senescent, stops dividing and enters apoptosis. However, in germ cells and stem cells, which have to indefinitely maintain their capacity to divide, an enzyme called telomerase synthesizes the repeat sequences and adds them to the chromosome, maintaining its length. Immortal cells in culture and tumor cells are also known to exhibit a high level of telomerase activity, while it is absent in normal cells. Hence, it is speculated that a high level of telomerase activity may facilitate carcinogenesis. However, the exact role of telomerase in carcinogenesis and its genetic control are not known.

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### Gene Cooperation in Oncogenesis

The cell proliferation system is resistant to mutations. DNA repair and apoptosis provide a powerful defense against carcinogenesis. Oncogenic mutations must not only produce continuous growth signal, but also escape the negative control of tumor suppressor genes. Hence, development of neoplasm requires cooperation among several mutated genes. A classic example of this multigene, multistep process of carcinogenesis is provided by human colorectal cancer. Progression from normal epithel-

lium to hyperplastic epithelium, to dysplastic epithelium, to benign adenomatous polyps, to localized carcinoma, to metastatic carcinoma requires 5 to 6 genetic events. Similarly, cancers of breast, colon, rectum, lung, etc., exhibit multiple gene alterations.

What causes these genetic events that turn normal genes to mutants ones? Normal DNA metabolism itself produces significant amount of DNA damage. The DNA repair enzymes, however, repair most of these damages. The most important external causes of oncogenic events are the use of tobacco and high fat diet. Nearly 2/3 of all human cancers, which are wholly preventable, are caused by these two factors. About 10% of cancers are inherited. The remaining are due to environmental factors such as ionizing radiations, UV radiation, and chemicals including drugs.

Even though there has been an explosion of knowledge on the genetic basis of carcinogenesis in the past two decades, the last word on cancer genetics is not yet said. We are yet to understand the genetic control of genomic imprint and telomerase activity. The rapid progress being made in the Human Genome Project will unravel many more genes connected, directly or indirectly, with carcinogenesis. However, application of even the current knowledge to the clinical management of cancer is only modest. It is expected that in the coming decades all phases of cancer management from prevention to screening of high risk groups in the population, to diagnosis of cancer, to developing new strategies for treatment and to counseling cancer patients and their relatives, will draw heavily on this knowledge which, in the meanwhile, only keeps growing.

### Suggested Reading

- [1] *Scientific American Molecular Oncology*, Ed J M Bishop and R A Weinberg, Scientific American Inc., New York, 1996.
- [2] *Molecular Biology for Oncologists*, 2nd Edition, Ed. J R Yarnold, M Stratton and T J McMillan, Chapman and Hall, London, 1996.

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