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Topic- Genetics and Cancer.

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Genetics and Cancer

Cancer is a large class of diverse diseases, all of which exhibit uncontrolled cell growth and divisions. In non-circulatory tissues, such uncontrolled cell growth produces cell masses called tumors. Cancerous or malignant tumors are those from which cells detach and migrate to other parts of the body giving rise to secondary tumors (a process called metastasis).

Non-cancerous or benign tumors do not metastasize. Cell divisions, like all other biological processes, are under genetic control. Certain genes must regulate the process of cell division in response to environmental signals.

These regulatory genes are undoubtedly subject to mutation like all other genes. Mutations that abolish the function of these regulatory genes would be expected to lead to abnormal cell division in the extreme, either the inability to divide at all or the inability to stop dividing.

Today, due to studies of viral genes, it has been found that some of the retroviruses contain a gene known as "oncogene" which can cause a loss of the normal control of cell divisions. This discovery have led to the identification of a set of homologous genes, called proto-oncogenes in the genomes of normal animals including humans (Table 16.1).

Table 16.1 : Location of potential recessive oncogenes and their association with specific tumors

Oncogenes Chromosome location	Associated tumors
2	Uveal melanoma
3 p	Renal cell carcinoma, small cell carcinoma of lung
5 q	Colon, Leukemia
10	Gliomas
11 p	Ductal breast carcinoma
	Wilms' tumor
11	Rhabdomyosarcoma
13 q, 14	Retinoblastoma, osteosarcoma, soft tissue sarcoma, small cell carcinoma of lung, bladder carcinoma, ductal breast carcinoma
17 p	Colon cancer, osteosarcoma, astrocytoma
18 q	Colon carcinoma
22	Meningioma, Bilateral acoustic neurofibromatosis

These normal cellular proto-oncogenes can be converted into tumor-causing cellular oncogenes by mutation or by becoming associated with new regulatory sequences through recombination processes. These observations indicate that the normal cellular functions of the proto- oncogenes involve some aspects of the control of cell division.

Recently, however, two different experimental approaches have provided evidence for the involvement of at least 15 different normal cellular genes – the proto-oncogenes in the occurrence of certain types of cancer in animals.

These two approaches are:

(1) Looking for cellular proto-oncogenes homologous to the oncogenes of animal viruses.

(2) The second involved looking directly for cancer-causing genes in the genomes of cancer cells by transfection experiment; experiment in which the DNA of tumor cells is isolated and added to normal tissue culture cells to see if it will convert any of them to the cancerous state. Both the

above approaches have been successful and both have resulted in the identification of some cellular oncogenes.

Cellular Oncogenes Contain Introns — their Viral Homologs Arc Single Exons:

When viral Oncogenes such as src are cloned by recombinant DNA techniques and are used as hybridization probes to search for homologous sequences in normal host cells, such sequences are almost always found.

These homologous sequences present in the chromosomes of normal cells of normal animals are not integrated viral oncogenes, because they differ from the viral oncogenes in having interrupted coding sequences, like most other eukaryotic genes.

That is, the cellular oncogenes and proto-oncogenes have multiple exons separated by introns, whereas the viral oncogenes are single exons. For example, the chicken cellular src proto-oncogene contains 11 introns separating 12,coding sequences, whereas the RSV v-src gene has a single, uninterrupted coding sequence (Fig. 16.2).

The v-src and c-src genes both code for protein kinases that phosphorylate tyrosine residues. Moreover, these two protein kinases are the same size and have very similar structures. In addition, both proteins react with antibodies prepared using the v-src protein as antigen. Comparison of the nucleotide sequences of the chicken c-src gene and the v- src gene of one strain (the Schmid-Ruppin strain) of RSV indicates that the two genes encode very similar proteins.

The c-src protein is 533 amino acids long, the v-src protein is 526 amino acids long. The major difference between these two proteins occurs at the COOH terminus, where the last 12 amino acids of the v-src protein are replaced by 19 completely different amino acids at the terminus of the c-src protein. In addition, there are 18 single niicleotide-pair differences between the coding sequences of v-src and c-src that result in 8 amino acid changes in the protein products.

These 8 amino acid changes in v-src protein of the Schmid-Ruppin strain of RSV do not appear to be involved in the oncogenicity of the v-SK protein since none of them is found in common in the v-src oncogenes that have been sequenced from other RSV strains. Clearly, the major difference between these two genes is the presence of the 11 introns In c-src and their absence in v- src (Fig. 16.1).

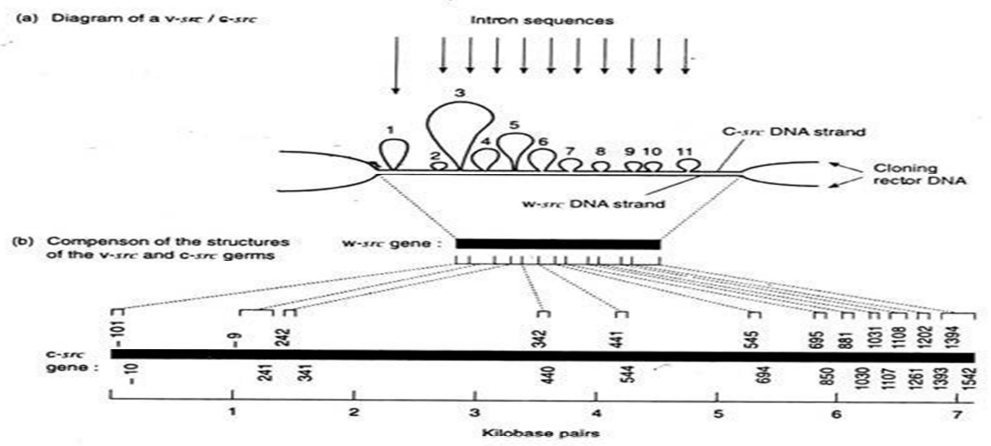

Fig. 16.1 Structures of the v*src* oncogene and the c-*src* protooncogene of the chicken. (a) Diagram of the predicted DNA heteroduplex formed by hybridization of one strand carrying the c-*src* gene (top strand) with a (partially) complementary strand carrying the v-*src* gene (bottom strand) as observed by electron microscopy. The intron segments of the c-*src* strand form single-stranded loops (numbered 1-11). The light blue lines represent the DNA strands of the plasmid in which these genes were cloned. (b) Schematic comparison of the organization of the coding sequences (exons) of the two genes : exon sequences are shown in blue. The v-*vrc* gene has a single, uninterrupted coding sequence. The c-*src* gene contains 12 exons separated by 11 introns (shown in red). The starting and ending nucleotide positions of the exons are shown above and below, respectively, the map of the c*src* gene, position 1 corresponds to the first nucleotide of the coding sequence. The first exon (position −101 to −10) is in the 5 leader region of the trans-cript, not in the coding sequence. Tbe dashed lines indicate the approximate correlations between the coding sequences of the two genes. (Based on the data of T. Takeya and H. Hanafusa, *Cell* 32 : 881-890, 1983)

We do not yet know how the protein kinases encoded by these viral oncogenes cause tumors, but the mechanism probably relates to the large quantities of these enzymes made in retrovirus-infected cells. There is 100 times as much v-src protein kinase per cell in chicken tumors induced with Rous sarcoma virus as there is csrc protein kinase in normal chicken cells.

Conservation of Proto-Oncogenes during Evolution:

One argument for the importance of the proto-oncogenes and the products that they encode in normal cell growth and/or cell division is that the proto-oncogenes have been highly conserved during evolution, c-src genes are found riot only in chickens, but in other birds, in mammals (including humans), in fish, and even in insects such as Drosophila melanogaster. Moreover, this picture of the conservation of proto-oncogenes across a wide range of species is observed for most of the other proto-oncogenes as well. All vertebrate animals contain proto-oncogenes that are homologous to essentially all the oncogenes listed in Table 16.1.

The fruit fly Drosophila melanogaster contains normal cellular genes that exhibit strong homology to the vertebrate cellular oncogenes c-abl, c-erbB. c-fps, c-raf, c-ras, and c-myb, in addition to the c- src homolog. In fact, the genome of D. melanogaster contains two genes with homology to src and three genes with homolog to ras, just like the genomes of vertebrates. In the case of the ras proto-oncogenes, the genome of the yeast Saccharomyces cerevisiae has even been found to contain two homologous sequences. Clearly, then, the various proto-oncogenes have been widely conserved during the course of evolution.

When the sequences of the homologous proto-oncogenes of different species are compared, the sequences are almost always very highly/ conserved, often differing by less than 15 percent in nucleotide-pair sequence.

Even in the case of the relatively distantly related yeast and vertebrate ras proto-oncogenes, the predicted amino acid sequences (predicated from the nucleotide sequences) of the conserved amino-terminal two-thirds of the protein products are identical at 75 percent of the amino acid positions. Presumably, the highly conserved domains of these homologues proto-oncogene products carry out the same or very similar functions in all the different species that contain these genes.

This high degree of conservation of proto-oncogene structure in all vertebrate animals and even in many invertebrate species argues strongly that these genes encode important products, and that the sequences of these genes have been conserved by natural selection acting to maintain the functional integrity of the important gene- products that are encoded by these genes. Although we do not yet understand the exact roles that these proto-oncogene products perform in normal cells, it seems certain that they are directly involved in the control of cell division.

Proto-Oncogene Products Key Regulators of Cell Division:

During the last few years, a wealth of information has accumulated regarding the structure and function of the various proto-oncogenes. It now seems clear that the only property that unites these genes as a group is that they all play central roles in the control of cell division.

When classified according to function, the different proto-oncogenes appear to fit into four groups:

(1) Those that encode growth factors (c-sis) or growth-factor receptors (c-firms and c-erbB);
(2) Those that encode GTP-binding proteins with GTPase activity (c- H-ras, c-K-ras, and N-nas);
(3) Those that encode protein kinases, either tyrosine-specific protein kinases (c-abl, c-fes, c-fgr, c-fps, c- ros, c-src, and c-yes) or serine/threonine- specific protein kinases (c-mi/, c-mos, and c-raf)\ and

(4) Those that encode transcriptional regulators (c-fos, c-jun, c-erbA, c-myc, and possibly c-myb and c-ets).

We probably know the most about the function of the proto-oncogene products that are growth factors or growth-factor receptors because they were studied long before we knew of the existence of proto-oncogenes. For example, consider the growth-factor receptors encoded by c-erb B and c-fms.

The prototype structure of such growth-factor receptors that have intracellular tyrosine-specific protein kinase activities is shown in Fig. 16.3. Although we still do not know exactly how these proteins function, it seems quite clear that they are involved in the transfer of signals from the cell surface to the cell nucleus.

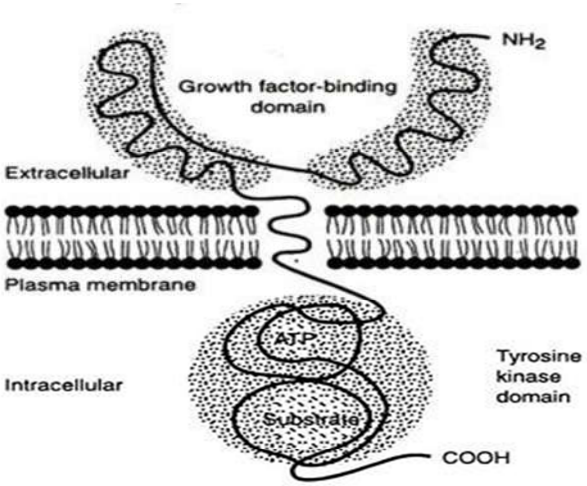

Figure 16.2 Schematic drawing of the prototype structure of transmembrane growth factor receptors with protein tyrosine kinase activities. These receptor proteins play key roles in signal transduction from the cell surface to the cell nucleus; details of the molecular mechanisms by which they transmit these signals are still unknown. (After Y. Yarden and A. Ullrich, *Annu, Rev. Biocbem.* 57 : 443-478, 1988)

They bind growth factors at their extracellular binding sites and transmit a signal, presumably via an allosteric transition, to the intracellular tyrosine kinase site. Thus, in turn, must activate the kinase and induce phosphorylation's of key intracellular proteins. Activation of the tyrosine kinase site may involve auto phosphorylation, because these receptor protein kinases have been shown to undergo reversible autophosphorylations of specific tyrosine residues near the intracellular COOH termini of the proteins.

The epidermal growth factor receptor is also known to undergo phosphorylation by other cellular protein kinases (e.g., protein kinase C) and to interact with other protein factors that modulate its activity. Thus, an accurate picture of the mode of action of these key regulatory proteins in signal transduction must await the results of further studies. When available, this picture will almost certainly depend on an understanding of the three-dimensional structure and function of the receptor molecule plus all the other macro- molecules with which it interacts.

The c-src protein and the products of several of the related proto-oncogenes also have tyrosine-specific protein kinase activities. However, these protein kinases are not trans membrane proteins, but rather are associated with the cytoplasmic face of the plasma membrane.

Presumably, these protein kinases also are involved in signal transduction, but we do not know what signal(s) they respond to or how this signal is transmitted. As a working model, it seems reasonable to assume that phosphorylation of key intracellular protein targets is the most likely mode of action of these proto-oncogene products.

Clearly, the mechanisms of action of the c-ras gene-products and the proto-oncogene products that function as transcription activators are totally distinct from those of the proto-oncogene products just discussed. The available information about the modes of action of the c-ras, c-fos, and c-jun gene- products is discussed in the next two sections of this article.

Comparison of Viral Oncogenes with Cellular Proto-Oncogenes:

Studies of different retroviruses have led to the identification of at least 15 different viral oncogenes and it has also been found that different retroviruses that induce similar types of cancers often carry the same oncogene. When viral oncogenes are cloned by recombinant DNA techniques and are used as hybridization probes to search for homologous sequences in normal host cells, such sequences are almost always found.

These homologous sequences present in the chromosomes of normal cells and normal animals are not integrated viral oncogenes, because they differ from the viral oncogenes in having interrupted coding sequences like most other eukaryotic genes. The cellular oncogenes and proto-oncogenes have multiple exons separated by introns whereas the viral oncogenes are single exons.

The protein coded by both the viral oncogenes and cellular oncogenes (known as protein kinase) have very similar size and structure. Even both proteins are complexed by antibodies prepared using the viral protein as antigen.

When the isolated cellular oncogenes were compared with the oncogenes of retroviruses by various procedures it has been found that some of them are quite homologous to the viral oncogenes.

Oncogene Products as Regulators of Cell Division:

Considering that oncogenes induce uncontrolled cell growth resulting in tumors, one might well anticipate that the products of these genes would act by stimulating cell division in some manner. Indeed, it is now clear that the products of these oncogenes play various roles in regulating cell division in one or more cell types.

For example, the product of the u-sis oncogene of simian sarcoma virus is closely related to a polypeptide growth hormone called platelet-derived growth factor (PDGF). PDGF is produced by platelet cells as the name indicates; it promotes wound healing by stimulating the growth of cells at the wound site.

Simian sarcoma viruses carrying the v-sis gene induce sarcomas when injected into woolly monkeys, also transform fibro-blasts growing in culture to a neoplastic or tumorous state. Presumably, this cellular transformation to the cancerous state occurs by a mechanism that is related to the effect of normal PDGF on cells at a wound site.

Other oncogenes encode products that are similar to growth-factor and hormone receptors. Oncogenes erbB and fms encode proteins that are closely related to the receptors for epidermal growth factor and CSF-1 growth factor, respectively.

CSF-1 is a growth factor that stimulates growth and differentiation of macrophages. Both of these growth- factor receptors are trans membrane proteins with growth-factor binding domains on the outside of the cell and protein kinase domains on the inside of the cell.

These receptors are key components in trans membrane signaling pathways. Finally, the erbA gene-product is an analog of the nuclear receptor for the thyroid hormone T_3. Thus, all of

these gene- products are undoubtedly involved in the intercellular communication circuitry that regulates cell division during the growth and development of highly differentiated tissues and organs in multicellular animals.

Because these trans membrane receptor protein tyrosine kinases are capable of transmitting a mitogenic signal (a signal telling a cell to divide), it is not surprising that alterations in the structure and function of these proteins will sometimes be oncogenic. If they malfunction and transmit a signal telling the cell to divide when it normally should not divide, the result will be tumor formation.

The largest group of the oncogenes (including src) encode protein kinases that phospborylate tyrosine residues. Some of these may well be analogous to the receptors for epidermal growth factor and CSF-1 growth factors, but contain receptors for mitogenic factors that have not yet been identified.

However, the src tyrosine kinase is not a trans membrane protein, but rather is tightly associated with the inner face of the plasma membrane. Although the src protein is a very active protein kinase that phosphorylates specific tyrosine residues in proteins, we still do not understand the molecular basis of its oncogenicity or what proteins are the important regulatory factors that are subject to its action.

The ras oncogenes encode proteins that bind GTP and exhibit GTPase activity. They may be analogous to proteins called G proteins that-have GTPase activity and play a role in the regulation of the enzyme adenylcyclase and, thus, the levels of cyclic AMP in cells.

The function(s) of the ras gene-products is (are) of particular interest because considerable evidence implicates the involvement of mutant ras products in several distinct types of human cancers.

Lastly, other oncogenes such as jun, fos, erbA, and myc encode nuclear transcription factors that activate the expression of specific genes. Undoubtedly, some of the genes that they activate will prove to encode products that function as positive regulators of cell division.

In summary, oncogene products are simply proteins that play central roles in stimulating cell division in one or more cell types. In some cases, these oncogene products are probably altered or “mutant” proteins that trigger the division of cells that should normally not divide under the existing conditions, in other cases, the oncogene products stimulate abnormal cell division by being overproduced—being synthesized in much larger amounts than in normal cells.

Proto-oncogenes and Cellular Oncogenes:

Genes with DNA sequences that are very similar to the retroviral oncogenes and that encode proteins with similar properties have been identified in the genomes of higher animals including humans by the use of two distinct experimental approaches:

(1) One approach involved looking for cellular DNA sequences that would cross-hybridize with the oncogenes of animal viruses.

(2) The second approach involved looking directly for cancer-causing genes in the genomes of cancer cells by transfection experiments, experiments in which the DNA of tumor cells is isolated and added to normal tissue culture cells to see if it will convert any of them to the cancerous state.

Both approaches have been successful and, in some cases, both have resulted in the identification of the same cellular oncogenes. Genes homologous to some of the viral oncogenes are even present in lower eukaryotes such as the yeasts.

Homology with Viral Oncogenes:

As was mentioned earlier, the src oncogene was first identified in the genome of Rous sarcoma viruses (RSV) isolated from chickens. When reverse transcriptase was used to convert the src oncogene of RSV to a cDNA form and this cDNA was labeled with ^{32}p and used as a probe in southern blot hybridization experiments with genomic DNA from normal chickens, the src cDNA hybridized with specific restriction fragments of genomic DNA in every experiment. This was true regardless of the source of the chicken genomic DNA. Moreover, similar genomic DNA sequences that hybridize with the viral src cDNA probe have been identified in all vertebrate animals and even in the fruit fly Drosophila melanogaster.

Subsequent experiments have demonstrated that genomic DNAs from normal (noncancerous) cells of all higher animals contain DNA sequences that hybridize with essentially all the retroviral oncogene sequences. In some cases, sequences homologous to retroviral oncogenes (eg., ras) are even found in lower eukaryotes such as Saccharomyces cerevisiae.

One might initially guess that these genomic DNA sequences that hybridize with oncogenes are simply present on integrated proviruses. However, this has proven not to be the case. Instead, when these sequences were isolated from genomic libraries and characterized, they were found to be normal cellular genes with structures that distinguish them from the homologous viral oncogenes.

The normal cellular genes with homology to oncogenes are now called proto-oncogenes. In some cases, these proto-oncogenes can mutate to forms that are capable of inducing oncogenesis— the ability to transform cells to a neoplastic or cancer like state (see the following section). In the latter form, they are called cellular oncogenes (abbreviated c-onc, e.g., c-src, c-myc) to distinguish them from their viral counterparts. This means that we must now denote the viral oncogenes more precisely as u-onc's, for example, u-src, u-sis, and u-myc.

Interestingly, some of the same cellular oncogenes that were identified by their cross-hybridization to viral oncogene sequences have also been identified on the basis of their ability to transform cells growing in culture to neoplastic or tumor like states in direct DNA transfer studies called transfection experiments.

Cancer Cells: Loss of Control of Cell Division:

Cancer is a large class of diverse diseases, all of which exhibit uncontrolled cell growth and division. In neocirculatory tissues, such uncontrolled cell growth produces cell masses called tumors. Cancerous or malignant tumors are those from which cells detach and migrate to other parts of the body, giving rise to secondary tumors (a process called metastasis). Non-cancerous or benign tumors do not metastasize.

Human cancers are responsible for an enormous amount of suffering. Thus, a large amount of money and effort have been directed to the study of these diseases. Although there has been great progress in the detection and treatment of cancers, there has been little progress towards under standing the molecular bases of cancers. However, there is now extensive evidence for the involvement of over 40 different oncogenes in the occurrence of various types of cancer in animals.

Probable Causes Regarding the Origin of Human Cancer:

Cancer in human can arise in many types of differentiated cells and they can be classified according to the cell type in which they arise, e. g.

Gliomas — cancer of glial cells

Hepatomas — cancer of liver cells, (hepatocytes)

Melanomas — cancer of pigment producing cells.

But we can differentiate the human cancer into three major groups which are:

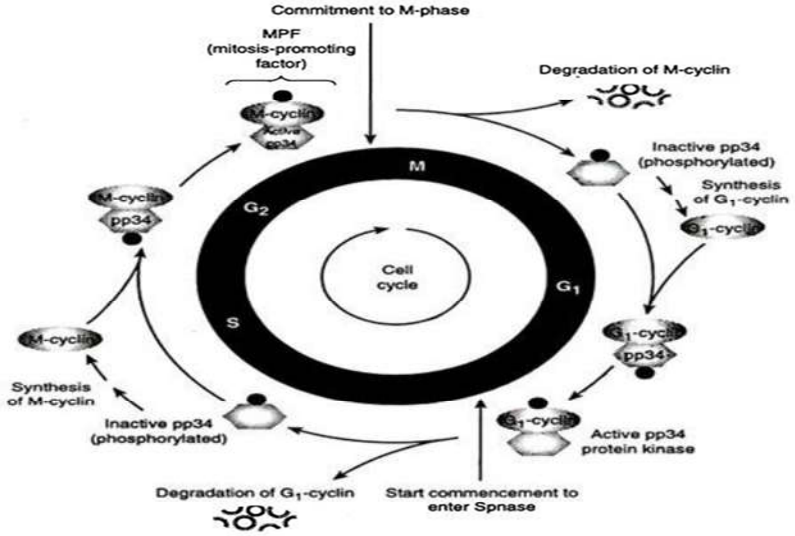

Fig. 16.3 Diagram showing some of the factors that regulate the progression of eukaryotic cells through the mitotic cell cycle. Cells make commitments to proceed through the ensuing stages at two points : (1) *start*, a point late in *G1* at which a commitment is made to subsequently initiate DNA synthesis (*S* phase), and (2) the beginning of *M* phase, at which time a commitment is made to proceed through the chromosomal condensation and chromatid separation phases of mitosis. Proteins called *cyclins* undergo cycles of synthesis and accumulation and then degradation as the cell passes through the cell cycle. The cyclins interact with a key regulatory protein designated pp34 (pp for phosphoprotein, 34 for 34,000 molecular weight). The phosphorylation/dephosphorylation of a single tyrosine residue of pp34 has been shown to determine the inactive/active states of this important regulator. P represents a phosphate group on the protein pp34 yielding the inactive state. (For additional details, see the review by A. W. Murray, *Nature* 342: 14-15, 1989)

Carcinomas:

Cancer that arise in cells of the sheets or epithelia that cover our surfaces (skin, gut etc.)

Sarcomas:

Cancers of the supporting tissues like bone, muscle, blood vessels, fibroblasts etc.

Leukemia's and Lymphomas:

Cancers of the cell that produce the circulating cells of the blood and the immune system. It is interesting to note that more than 90% of human cancers are carcinomas. Although a few forms of cancers are associated with childhood, most cancers incidence increase sharply with age.

According to Kundson et a I (1980) and Klein and Klein (1985) human cancers are the result of multiple mutations. Since mutation can occur at any time the probability that any particular cell will acquire a particular mutation increases in direct proportion to age. Most of the leukemia's are believed to result from the accumulation of 2-4 specific mutations within a single cell while carcinomas may require anywhere from two to six or seven.

Other evidence that cancer results from multiple mutations comes from studying certain individuals who inherit a very high probability of developing a specific form of cancer, e.g. retinoblastoma. These people appear to have inherited one of the mutations that can lead to the cancer. Hiatt et al (1977) advocated that environmental factors rather than intrinsic, inevitable ageing processes must play a large role in determining the probability of getting cancer.

But it is not very easy to identify the factors in the environment, diet or personal habits that can cause cancer, however, some clear-cut examples are cigarette smoking—can cause 85-90% of the lung cancer; asbestos which causes mesotheliomas; sunlight induces the skin cancer. Yunis (1983) pointed out that both chemical mutagen and certain viruses can cause human cancer (some viruses may cause cancer precisely because they can cause mutations in cellular DNA).