Genetic Basis Of Cancer

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Cancers arise from normal tissues as a result of sequential accumulation of somatic gene mutation. Mutations generally arise in several key genes before malignant change occurs. These mutations as well as the order in which they occur constitute the genetic pathway of tumour development. Although this pathway is unique for individual tumour, there is a great deal of overlap in tumours of similar origins. Cancers arise when changes in the DNA cause anomalous accumulation of cells.
Neoplasms therefore are proliferation of cells that do not differentiate normally, suggesting that cancer is a genetic disease.
At least one of the events in tumour development is mutation affecting growth control mechanisms of the normal cell.

Seven types of proteins participate in controlling cell growth
1. Growth factor
2. Growth factor receptor
3. Intracellular transducers (signal transduction proteins)
4. Transcription factors
5. Pro and anti-apoptotic proteins
6. Cell cycle control proteins
7. DNA repair proteins (genome stability).

Malignant tumours can result from expression of mutant forms of these proteins
Mutations that change the structure or expression of proteins in classes 1-4 generally give rise to oncogenes. Class 6 proteins mainly act as tumour suppressors. Mutations in the genes encoding these proteins act to release cells from control and surveillance, increasing the probability that the mutant cells will become tumour cell. Class 7 mutations greatly increase the probability of mutations in the other classes.

Oncogenic mutation can affect **genes encoding growth factors**. An example is the sis oncogene, which encodes the B- chain of PDGF. The sis oncogene causes cells in G_0 to enter into the cell cycle. The ras oncogene can also increase the expression of growth factor genes leading to cellular proliferation. Excessive cellular proliferation increases the possibility of error (mutation) in cells.

Mutations affecting growth factor receptors can lead to transmission of growth signals in the absence of normal ligands. That is, the abnormal (mutated) receptors may not require growth factor stimulation before transmitting signals.
Over-expression of a normal receptor can also be oncogenic. An example is the normal Her-2 receptor over-expressed by many breast cancers. The result is that cells are stimulated to proliferate in the presence of very low concentrations of growth factor and related hormones, at concentrations too low to stimulate proliferation in normal cells.

A large number of oncogenes are derived from proto-oncogenes which act as signal transducers e.g the *ras* oncogene and the *src* oncogene. Substitution of glycine by an amino acid (other than proline) at position 12 of the sequence transforms ras into an oncogene. Ras oncoproteins are expressed by tumours of the bladder, colon, breast skin, lung, leukaemias, neuroblastomas.
Ras is a key component in transducing signals from activated RTKs to a cascade of protein kinases, eventually leading to alterations in cell growth.
Increased levels of signal transduction can occur by over expression, by point mutation or by structural alteration of tyrosine kinase or guanosine triphosphatase (GTPase) binding proteins.

The nucleus is the final site of action for messages by growth factors. At this level, oncogene products bind DNA and control the transcription of genes Many oncogenes encode **nuclear transcription factors**, inappropriate expression of which can induce transformation. Examples are *c-fos* and *c-myc*, which normally stimulate transcription of genes encoding proteins that promote progression through G1 phase of the cell cycle and G1 to S transition.
Oncogenic forms are expressed at high and unregulated levels.

Over expression of the **anti-apoptotic gene** Bcl-2 is frequently found in human leukaemias and lymphomas. A cell can respond to carcinogen-induced DNA damage by:
- By delaying cell division until the damage is repaired.
- By undergoing apoptosis
- It can progress uninterrupted through the cell cycle.

Apoptosis is an efficient method of preventing malignant transformation because it removes cells with genetic lesions. Abnormal apoptosis can promote cancer development both by allowing accumulation of dividing cells and by obstructing removal of genetic variants with enhanced malignant potential. Bcl-2 is dis-regulated n follicular B cell lymphoma. The tumour cells have high concentrations of bcl-2 protein due to a t (14:18) translocation that places the bcl-2 gene under the control of a strong immunoglobulin heavy chain gene promoter. Cells lacking PTEN, a tumour suppressor, have elevated levels of PKB, which inhibits apoptosis. Loss of PTEN is common in many cancers.

Mutations causing loss of cell cycle control

Once a cell progresses beyond a certain point in late G1, called the restriction point, it becomes irreversibly committed to entering the S phase and replicating its DNA. Cyclins, Cdks and the Rb proteins are all part of the control systems that regulate passage through the restriction point. Over-expression of the oncogene encoding cyclin D1 by gene amplification or translocation can cause inappropriate, unregulated passage through the restriction point.

Mutations affecting genome stability

The p53 gene encodes a phosphorylated protein with a molecular weight of 53kDa. P53 plays a variety of roles in the cell, which has been summarized as the "guardian of the genome". One of its guardian functions is to stop cells from replicating damaged DNA. It is essential for the checkpoint control that arrests human cells with damaged DNA in G1-S cell cycle checkpoint until the damage is repaired. Mutations in p53 abolish G1 checkpoint control. Replication of damaged DNA presumably leads to random genetic changes, some of which are oncogenic. In response to oncogenic changes, cells undergo apoptosis. Tumour cells lacking p53 do not undergo apoptosis.P53 may be knocked out by deletion, by mutation or by the action of inhibitors such as Mdm2 and Mdm4 (Mdmx) gene products, which bind p53, leading to its degradation. Increased levels of the gene products contribute to the development of human cancers. Mutations in p53 gene occur in more than 50% of human cancers. MDM2 is a protein that normally inhibits the ability of p53 to restrain the cell cycle or kill the cell. Proteins encoded by DNA tumour viruses can also inhibit p53 activity e.g. HPV E6 and E7 genes.

Defects in DNA repair systems perpetuate mutations and are associated with malignancies such as ataxia telangiectasia, Bloom's syndrome, Cockayne's syndrome, Fanconi's anaemia, hereditary non polyposis colorectal carcinoma, xeroderma pigmentosum.

The key players in the upstream pathways that positively regulate p53 include: ATM, Chk2 and CKII that, phosphorylate p53 and the tumour suppressor $p14^{ARF}$.

Agents that cause cancer may be divided into 3 main groups: chemicals, radiation and viruses. These agents affect the functions of 3 sets of genes: proto-oncogenes, tumour suppressor genes and apoptotic genes.

Direct-acting carcinogens are activation-independent and are generally weak carcinogens. Examples are alkylating agents and acylating agents used in the therapy of malignant diseases and, sometimes, immunologic diseases. Cyclophosphamide and chlorambucil are notable examples. Indirect-acting carcinogens (procarcinogens) are potent carcinogens. They require metabolic activation to produce the ultimate carcinogen. Examples of indirect-acting carcinogens are: polycyclic aromatic hydrocarbons as well as aromatic amines and azo dyes. Direct-acting and ultimate carcinogens are highly reactive electrophiles and are capable of combining with electron-rich sites in atoms such as RNA, DNA and proteins. These non-enzymatic reactions lead to the formation of DNA adducts. Metabolic activation of the indirect-acting carcinogens is mainly by the cytochrome p450-dependent oxygenases. Environmental and genetic factors affect the level and activity of the enzyme. Other Carcinogens include: aflatoxin B1 an etiologic factor in hepatocellular carcinoma; asbestos occupational exposure to which is associated with pleural mesothelioma, bronchogenic carcinoma and GIT cancers; Vinyl chloride used in the manufacture of PVC is also implicated in hemangioblastoma of the liver; arsenic, nitrosamines, some insecticides etc.

Chemical carcinogenesis follows a series of steps known as initiation and promotion. Although DNA changes result from initiation by chemical carcinogens (CC).CC may also activate proto-oncogenes. Among the proteins encoded by proto-oncogenes are growth factors, growth factor receptors, signal transduction proteins, transcription factors and cell cycle control proteins. Activation of a proto-oncogene into an oncogene can occur by:

- Translocation
- Point mutation
- Gene amplification

Translocation removes a gene from normal regulatory sequences and places it next to a strong promoter or enhancer sequence. An example is the t(8,14) of the c-myc oncogene in Burkitt's lymphoma.

Point mutation leads to the formation of abnormal proteins. For example the *ras* oncogene, in which, substitution of glycine by an amino acid (other than proline) in the 12 th codon leads to transforming ability of the oncogene.

Gene amplification means formation of multiple copies of a gene, leading to increase in activity of that gene. An example is the *n-myc* gene in neuroblastoma. Increased

expression may also result from translocation.

Promoters induce clonal proliferation of initiated cells and alter their differentiation programmes by activating enzymes that are part of the normal signal transduction pathway.

SUMMARY

Carcinogen----Electrophile intermediate-----DNA adducts-----Initiation (permanent DNA lesion)-----Clonal proliferation and altered differentiation (promotion)-----Additional mutations------Malignant tumours.

TUMOUR SUPPRESSOR GENES

Not all human tumours contain activated oncogenes. This implies there must be other genes that play a vital role in tumour formation. One of such is the tumour suppressor (TS) gene. TS gene products inhibit events leading towards cancer. Mutant versions, which have lost their functions, are found in tumour cells. Some TS gene products normally prevent cell cycle progression; some steer deviant cells into apoptosis while some ensure accurate replication, repair and segregation of the cell DNA. TS gene may be silenced by deletion (leading to loss of heterozygosity or reduction to homozygosity) or by point mutation. TS genes could also be silenced epigenetically by DNA methylation. Both alleles of a gene pair must be inactivated in order to produce the transformed phenotype.

Knudson's two-hit hypothesis

This hypothesis is used to explain the molecular basis of familial and sporadic cases of retinoblastoma. Retinoblastoma is a childhood tumour 60% of which is sporadic. Familial cases with AD mode of inheritance account for 40% of cases.

A.G. Knudson in 1971 proposed that two successive mutations were required to turn a normal cell into a tumour cell, and that in familial cancers one of the hits was inherited and already present in all the somatic cells. The second mutation (hit) occurs in one of the many retinoblasts. Hereditary cases occur earlier than sporadic cases and are often bilateral.

Examples of familial cancers caused by tumour suppressor gene mutations

FAP coli 5q21	APC gene
Breast-ovarian cancer 17q21	BRCA-1
Breast cancer (early onset) 13q12 q13	BRCA-2
Li-Fraumeni 17p13	TP53
Ataxia telangiectasia 11q22-q23	ATM
Retinoblastoma 13q14	RB1
Neurofibromatosis type 1 17q12-q22 (Von Reklinghausen)	NF1
Neurofibromatosis type 2 22q12.2 (Vestibular schwannomas)	NF2

Telomerase expression contribute to immortalization of malignant cells

The ends of human chromosomes are protected by a repeat sequence (TTAGGG) that is maintained by a special RNA-containing enzyme, telomerase. Because most human somatic cells lack telomerase, telomeres shorten with each cell cycle. Complete loss of telomeres lead to end to end chromosome fusion and cell death. This phenomenon may contribute to the "mitotic clock" that limits the number of divisions a cell can go through.

Telomerase expression and cancer cell immortality

90% of human primary tumours possess telomerase activity. It is believed that telomerase expression is essential for a tumour cell to become immortal.

Therefore, specific inhibitors of telomerase have been suggested as possible therapeutic agents for tumours.

CONCLUSION

Cancer is a genetic disease. It is a multi-step process involving mutation of multiple types of genes. Damage to the genome, however does not always lead to malignancy due to the action of genes (such as p53) that function in the pathway for maintaining genome integrity and stability during cellular stress induced by DNA damage, hypoxia activated oncogenes etc. Mutations affecting these sets of genes by various mechanisms as well as by epigenetic mechanisms underlie the genetic instability that drives clonal evolution towards more malignant tumour phenotype, ultimately leading to malignancy.

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