Cancer genes

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Cancer is a genetic disorder involving dynamic changes in the genome leading to uncontrolled cell growth, ability to invade and metastatize. The genes implicated in cancer include those involved in cell cycle control, apoptosis, DNA repair, ageing and immortalization, angiogenesis and metastasis. The targets of these genes and their role in manifesting the characteristics of the malignant cell are summarized. Literature indicates that nearly all tumours have genetic alterations in multiple cancer genes. Several of the cancer genes and their products are proving to be useful 'tumour markers' and some as targets for cancer therapy. The post-genome era is now directed towards generating molecular portraits of different cancers in individuals so as to be able to provide individualistic treatment options.

CANCER is a genetic disorder involving dynamic changes in the genome leading to uncontrolled cell growth, ability to invade and metastatize. Experimental carcinogenesis studies in animals have shown that cancer involves an initiation process which is irreversible and not recognizable as a pathological entity. This is followed by promotion, a process which facilitates the expression of the initiated phenotype and progression which represents further phenotypic alterations in the initiated cells. All these involve genetic alterations varying from subtle point mutations to obvious changes in the chromosomal complement.

Genes responsible for the cancer phenotype have been termed as 'oncogenes (growth promoting) and tumour-suppressor genes' (growth suppressing). The conceptual foundations of the genetic basis of cancer have been revealed from the contributions of viral carcinogenesis in animals[1]. It has been known that transforming retroviruses carry genes with oncogenic capabilities. The viral oncogenes (v-onc) are derived from the cell they infect and are usually mutated during the acquisition process. Once incorporated into the viral genome, an oncogene is freed from normal cellular regulatory controls and is expressed constitutively in the transduced cells. The progenitor cellular genes, referred to as proto-oncogenes, (c-onc) have been identified as genes coding for components of the mitogenic signalling cascades and growth control. Many non-transforming retroviruses that do not possess viral oncogenes induce tumours in animals by integrating a retro virus near normal cellular proto-oncogenes and activating their expression by a mechanism termed 'proviral insertional mutagenesis'.

In 1982, Parada *et al.*[2] and Der *et al.*[3], showed that cellular-transforming genes from human bladder and lung tumour cell lines, were homologous to the *ras* genes coding G proteins and earlier identified as oncogenes carried by transforming murine sarcoma viruses. Sequence analysis showed that a point mutation distinguished the bladder carcinoma oncogene from its normal cellular counterpart and that the mutation occurred in the same codon that harbours the activating mutation in the retrovirus *v-ras* oncogene. It therefore became apparent that the same cellular proto-oncogene was a common target for viruses and chemical carcinogens. Several oncogenes which were previously identified in retroviruses were then found to be mutated in human tumours. It is now known that cellular proto-oncogenes can be activated to oncogenes not only by point mutations rendering a signalling molecule constitutively active, but also by amplification, as seen for *c-myc* in neuroblastoma[4], and by chromosomal translocations[5]. The effect of these alterations is dominant at the cellular level.

The existence of tumour suppressor genes (TSG) was first reported by Boveri[6] in 1914, and an indirect proof was provided by Harris *et al.*[7] in 1969. This was followed by the two-hit mutation theory by Knudson to support the recessive nature of TSG[8] and finally the first TSG, the retinoblastoma (Rb) gene was isolated in 1986 (ref. 9). DNA tumour virology identified tumour suppressor proteins as targets for inactivation by the oncogenes of DNA tumour viruses[1]. One of the criteria for TSG is the demonstration of loss-of-function or inactivating mutations in both copies of the gene. The mechanisms which either abolish or reduce the functions of the TSG are loss of heterozygosity, methylation, cytogenetic aberrations, genetic mutations, gain of autoinhibitory function and polymorphisms[10].

Oncogenes and TSG, are a major focus of human cancer studies today and additions to both classes of cancer genes that have no cognates among the tumour viruses have been identified. Over 100 oncogenes and about 30 TSG are now known and a listing of these is available at www.ncbi.nlm.nih.gov/ncicgap/.

Cancers develop through a stepwise process with the acquisition of activating mutations in dominant acting growth enhancing oncogenes and inactivating recessive

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mutations in growth inhibitory TSG. In addition, epigenetic abnormalities in the expression of these genes also play an important role in carcinogenesis. These widespread changes reflect the deleterious effects of mutagens, both exogenous and endogenous, germline mutations as well as various types of genomic instabilities acquired during tumour development.

Detailed analysis of the diverse functions of the known oncogenes and TSG shows that they code for components of the signal transduction cascade, i.e. growth factors, growth factor receptors, adapter molecules, protein kinases, G-proteins, nuclear transcription factors, molecules that repair DNA, regulate the cell cycle and the various check points, those mediating apoptosis, metastasis and invasion. As succinctly described by Hanahan and Weinberg[11], this catalogue of genes manifest six essential alterations in physiology that collectively dictate malignant growth, i.e. *self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, tissue invasion and metastasis*. These six capabilities are shared in common by most and perhaps all types of human tumours.

Normal cells require mitogenic growth signals to move from a quiescent G_0 state into an active proliferative state. These signals are either diffusible growth factors, extra cellular matrix (ECM) components or cell-to-cell adhesion molecules. Some cancer cells over express oncogenes which code for growth factors (e.g. sis, PDGFβ) to which they are responsive. These signals bind to specific cell surface receptors that transduce growth stimulatory signals into the cell interior, thereby promoting autonomous cell proliferation. Some of the oncogenes code for growth factor receptors, which when overexpressed lead to cancer, as reported for EGF-R/erbB-2 in breast cancer. Alterations in the structures of growth factor receptors, such as mutations in c-fms, can also elicit ligand independent signalling and cancer. In contrast to cell cycle control genes, many of which are expressed ubiquitously, growth factors and growth factor receptor genes appear to become cancer genes in a smaller more specific set of cancers, which is perhaps a reflection of the limited cell and tissue-specific expression of these genes. Cancer cells can also switch the types of extracellular receptors (integrins) they express, favouring ones that transmit pro-growth signals.

The complex mechanisms which lead to *growth signal autonomy* arise from alterations in components of the downstream signal transduction pathways initiated by ligand activated growth factor receptors and integrins which regulate the cell-cycle. The orderly progression of the dividing mammalian cells through the phases of the cell cycle is governed by a series of proteins called cyclins which exert their effects by binding to and activating a series of specific cyclin-dependent kinases (CDKs). This process is further modulated by the phosphorylation and dephosphorylation of CDK proteins by protein kinases and phosphatases and by a series of CDK inhibitor proteins (CDIs). Impairments in the functioning of the components involved in this regulation lead to over proliferation of cells – the basis of tumour formation. The protein products of many oncogenes and TSG regulate the activity of CDKs responsible for the initial steps of the presynthetic phase G1 (complexes of cyclins D1-D3 with Cdk4 or Cdk6 depending on cell type) and transition of G1 into S phase of DNA synthesis (cyclinE-Cdk2). Some proto-oncogenes and TSG regulate activity of complexes of cyclin A-Cdk2 (required for DNA replication) and cyclin B-Cdk1 (responsible for the transition of G2 phase to mitosis).

During mitogenic stimulation, growth factors bind to their receptors and promote their dimerization and autophosphorylation. This leads to the activation of SH2 domain containing proteins such as, PLCγ, PI3K, the oncoproteins Ras and Src and, in turn, the MAP kinase cascade. End products of these cascades, MAPK, p38, and JNK are translocated to the nucleus where they phosphorylate and activate many substrates including transcriptional factors such as Jun, Ets1, Ets2, Tcf, etc. This causes activation of other transcription factors. Similar reactions are also observed on binding of integrins with ECM proteins which promote the autophosphorylation of FAK. This results in the binding of FAK to the SH2 domain of the Src proto-oncoprotein followed by recruitment of the adapter Grb2 protein, and activation of Ras and MAP kinase cascades. The major consequence of the MAP kinase activated transcription factors is the increase in expression of the oncoprotein cyclin D1 and Myc, which increase the activity of cyclin-dependent kinases operating in G1 phase (cyclin D–Cdk4 and cyclin E–Cdk2). These phosphorylate the tumour suppressor protein pRb. Phosphorylation of pRb and its binding to a number of viral oncoproteins induces release and activation of transcription complexes E2F/DP, which increase expression of genes whose products are necessary for passage of the S phase[12].

Another pathway that converges on the cell cycle is that mediated by the cell surface molecule, E-Cadherin, which is bound to β-catenin essential for its cellular adhesion functions[13]. Unbound β-catenin acts as a transcription factor and binds another, i.e. Tcf4. The complex is then translocated to the nucleus where they activate several genes, among them are cyclin *D1* and *MYC*. This results in the activation of cyclin-dependent kinases responsible for the proceeding of the G1 phase and entrance into the S phase. Tumour suppressor APC (its mutations cause the development of adenomatous polyposis of the intestine) binds free cytoplasmic β-catenin. This is accompanied by β-catenin degradation. Thus APC inactivation stimulates the formation of the catenin-Tcf complex, increasing transcription of cyclin *D1* and *MYC* genes and in turn permitting entry of the cell into the S phase. Mutations of

β-catenin (found in patients with familial adenomatous polyposis, FAP) increase its stability and result in the same consequences without mutation of APC.

Insensitivity to *antigrowth signals* and uncontrolled proliferation also involves the Cdk–Rb–E2f signalling pathway. This is controlled not only by pRb, but also by many other suppressor proteins, some of which are inhibitors of Cdks, promoting arrest of the cell cycle at the G0/G1 phase, so that the S phase does not begin in response to various signals. These proteins are $p15^{INK4b}$ and $p16^{INK4a}$, $p21^{WAF1/CIP1}$, $p27^{KIP1}$ and $p57^{KIP2}$ (ref. 14). Proteins $p27^{KIP1}$ as well as $p15^{INK4b}$ are activated by the inhibitory signal transduction cascade induced by TGFβ binding to its receptors[15–17]. The protein $p21^{WAF1}$ is one of the main targets for the transactivating effect of p53 and consequently for suppressors involved in the stability/activity of p53, i.e. $p19^{ARF}$, ATM, WTI[18–21] or its transcriptional activity (BRCA1 and $p33^{ING1}$ (refs 22–23). The antigrowth circuit converging on Rb and the cell division cycle is disrupted in a majority of human cancers thereby defining the concept of tumour suppressor loss in cancer.

In conclusion, most known proto-oncogenes and TSG somehow regulate activity of cyclin-dependent kinases responsible for entrance to the S-phase. Impairment in the signalling pathways-regulating-Cdk 2,4,6-pRb-E2F/DP is therefore a necessary precondition for the appearance of constantly proliferating neoplastic cells. It is apparent from the above that the current paradigm in carcinogenesis is associated with activation of oncogenes and decreased expression of TSG. In several types of human cancers there is an increase in the expression of the products of the TSG, *p27*, *p21*, *p16* or *Rb*. This may reflect at least, in part, the existence of homeostatic feedback loops in cell circuitary that maintain an appropriate balance between growth-promoting and growth-inhibitory factors leading to the biologic effects of oncogenes and TSG that are highly context-dependent[24]. Some of the oncogenes, TSG, and their genetic alterations in representative cancers are given in Table 1.

The ability of the tumour cells to expand in number is determined not only by the rate of cell proliferation but also by *resistance* to *programmed cell death*. At least two different pathways seem to promote apoptosis by activation of aspartate proteases–caspases which act on key substrates leading to cell death[25,26]. Binding of death factors, Fas ligand and TNFα to their specific receptors generates caspases 3, 6 and 7, which are pivotal for apoptosis. An alternative mechanism involves the generation of caspases 3, 6 and 7 via caspase 9 which is activated via a pathway involving the TS *p53*. Activation of *p53* by several apoptotic signals such as DNA damage, activation of oncogenes, survival factor insufficiency or hypoxia, loss of cell contacts with other cells or the ECM leads to the regulation of the Bcl2 family of proteins[21], in particular increase in expression of *BAX* gene and repression of the *BCL2* gene. The Bax protein promotes release of cytochrome c and/or AIF (apoptosis-inducing factor) from the mitochondria. The Bcl2 and Bclx proteins inhibit this release. Bax in turn can bind to Bcl2 and Bclx and negate their effects. Cytochrome c and the protease AIF are instrumental in activating caspase 9 which in turn generates caspases 3, 6 and 7 leading to apoptosis. Resistance to apoptosis can be acquired by cancer cells through mutation in the tumour-suppressor *p53* gene, upregulation of *Bcl2* oncogene via chromosomal translocations as in follicular lymphoma[27] and also by other strategies. Apoptosis is also initiated by insufficiency of survival factors such as IGF-1/IGF-2, and IL-3 and their receptors[28,29]. Alterations in the PI-3 kinase-AKT/PKB pathway, which transmit antiapoptotic survival signals[30] or by loss of the *PTEN*, TSG which attenuates the AKT survival signal also lead to cancer cells evading apoptosis[31].

Cell proliferation can be regulated by arrest of the cell cycle which is a precondition for *differentiation*. The role of oncogenes and tumour suppressors in the impairment of the differention process has received much less attention and is not as clear[32].

The process of *ageing* and *immortalization* is as important as the complex information that regulates life and death. Cells in culture have a finite replicative potential. After a finite number of doublings they stop growing—a process termed senescence. This process can be circumvented by disabling their pRb and p53 proteins, which permit the cells to continue doubling till they reach a crisis state. This is characterized by massive cell death, karyotypic disarray associated with end-to-end fusion of chromosomes and the occasional emergence of a variant cell that has acquired the ability to multiply without limit, the trait termed immortalization. Most types of tumour cells are immortalized. The immortality of the cancer cells is being identified in the ends of the chromosomes, the telomeres which are composed of several thousand repeats of a short 6 bp sequence TTAGGG element. The normal shortening process seen in the telomeres during successive cellular divisions eventually causes ageing and cell death. This process is reverted by the stabilization of the telomere through the action of a complex ribonucleoprotein enzyme known as telomerase[33]. This enzyme is found in embryonic and germinal cells, but in undetectable levels in normal eukaryotic cells, except in tissues which are turning over. Virtually all types of malignant cells maintain their telomeres by upregulating expression of the telomerase enzyme. The activity of this enzyme is controlled by the Myc oncoprotein, which increases the transcription of the gene encoding the TERT subunit whose level determines telomerase activity[34].

Nutrients and oxygen required for tumour growth are supplied by the formation of new capillary networks from endothelial cells lining small venules. This process which is known as *neoangiogenesis*, is regulated by the termination of secretion of inhibitors and increase in growth factors, such as VEGF, FGF, EGF and TGFα, which are

required for the proliferation and migration of endothelial cells. Increase in growth factors is accompanied by increase in secretion and/or activity of proteases, leading to the proteolysis of the extra-cellular matrix and endotheliocyte invasion of the neoplastic tissue. The TS p53 activates production of the inhibitor thrombospondin[35] and suppresses the transcription of *VEGF* gene[36]. Inactivation of *p53* therefore plays a key role in the formation of the angiogenic phenotype of neoplastic cells. The *Ras* oncogene family induces activation of transcriptional complex AP-1 and increases VEGF secretion and production of MMP9 and 1 (refs 37, 38). The oncogene proteins Myc and Vhl are also reported to play a role in angiogenesis[39,40].

As the cancer progresses, there is further loss of control whereby the *tumour cells escape* from the primary foci and move to distant sites where they lodge and form new foci. One of the better studied cell surface molecules is E-cadherin, which is also a product of a TSG, mediating cell to cell interactions resulting in transmission of antigrowth and other signals via β-catenin and terminating in the activation of the Lef/Tcf transcription factors. E-cadherin function is lost in a majority of epithelial cancers, by mutational inactivation of the E-cadherin or β-catenin genes and transcriptional repression or proteolysis of the extracellular cadherin domain[41]. The proteins p53, Ras and Src are of major importance because changes in their activity cause simultaneous appearance of a few compo-

Table 1.

Type of gene	Gene	TS/ONC	Alterations in cancer	Associated cancers
Growth factors				
	SIS (PDGF)	ONC	Overexpression	Astrocytoma, osteosarcoma
	INT-2, HST-1	ONC	Overexpression	Melanoma, breast, urinary bladder, stomach
			Amplification	Head and neck cancer
	IGF-2	ONC	Methylation	Wilm's tumour
Growth factor receptor				
EGF-receptor family	*ERB-B2*	ONC	Amplification or overexpression	Breast, ovary, stomach, NSCLC, Head and neck
	EGF-R	ONC	Amplification or overexpression	Glioma, breast, NSCLC, Head and neck
	ERB-B1	ONC	Overexpression	Esophageal squamous cell carcinoma
Colony stimulating factor receptor	*CSF1R (fms)*	ONC	Overexpression/amplification/mutation	SCLC
Transforming growth factor βR2	*TGF βRII*	TS	Deletion	NSCLC, SCLC
Platelet derived growth factor receptor	*PDGF-Rβ*	ONC	Chromosomal translocation forming chimeric genes TEL/PDGF-Rβ, CVEb/PDGF-Rβ encoding permanently activated receptors	Chronic myelomonocyte leukemia, acute myeloblastic leukemia
Non-receptor tyrosine kinase	*C-ABL*	ONC	Translocation	Chronic myelocytic leukemia
	SRC	ONC	Mutation	Large-intestinal tumours
GTPase	*RAS*	ONC	Mutation	Pancreas, NSCLC, colon
GTPase activating protein	*NF1*	TS	Mutation or deletion	Neurofibrosarcoma
Transcription factor	*DPC4*	TS	Mutation or deletion	Colon, neuroblastoma
Transcription factor	*CTNNB1, (β-catenin)*	ONC	Mutation	Colon
Transcription factor	*MYC*	ONC	Chromosomal translocation	Burkitts lymphoma
			Gene amplification	SCLC, neuroblastoma
Cell cycle genes				
Transcription factor	*p53*	TS	Mutation or deletion	Many cancers
CDK inhibitor	*p16/p15*	TS	Methylation, mutation or deletion	Melanomas, NSCLC
	RB	TS	Mutation or deletion	Retinoblastoma, SCLC
	CyclinD1	ONC	Overexpression	Esophagus, breast
Transcriptional regulator	*BRCA1*	TS	Mutation or deletion	Breast, ovary (familial)
	BRCA2	TS	Deletion or mutation	Breast, ovarian (sporadic)
	VHL	TS	Mutation or deletion	Renal cell, pancreas
Others				
Dual-specificity phosphatase	*PTEN*	TS	Mutation	Glioblastoma, prostate, endometrium
Cell surface molecule p80	*CD44*	TS/ONC	Alternative splicing post-translational modifications	Breast, bladder, colon
DNA repair	*hMLH1*		Mutation or deletion	Colon (HNPCC), endometrium
	hMSH2		Mutation or deletion	
	APC	TS	Mutation or deletion	Colorectum (familial and sporadic)

NSCLC, Non-small cell lung carcinoma; SCLC, Small cell lung carcinoma; HNPCC, Heriditary non-polyposis colon carcinoma.

nents of the metastatic phenotype and genetic instability that promotes the appearance of additional alterations required for metastasis[42–44].

In response to DNA damage, normal cells undergo cell death to avert transmission of the genetic defects. In cancer cells however, there is suppression of induction of apoptosis, which increases the probability of preserving the genetic alterations. Tumour progression is therefore driven by acquisition of more mutations. In the past two years genetic instability and the mutator phenotype of cancer cells have received much attention[45]. The emerging concept is that genomes of cancer cells are unstable and this instability results in a cascade of mutations and besides apoptosis, there are more specialized systems which control genome integrity. These systems are those which control DNA repair (nucleotide and base excision repair) and those which regulate changes in the structure or number of chromosomes. Impairments in these systems also lead to cancer.

Deficits in repair systems are typical of a small proportion of tumours. For example, the nucleotide exchange repair (NER) system is affected in patients with xeroderma pigmentosa who are prone to skin cancers[46]. Inborn defects of another repair system involved in mismatch repair (MMR) during DNA replication causes HNPCC and/or ovarian tumours[47]. Four genes, *hMSH2*, *hMSH3*, *hMSH6* and *hMLHI*, have been identified, in which inactivating genetic alterations cause this syndrome. The *MMR* genes may be inactivated in three ways: somatic mutation or loss, silencing of the promoter region (of hMLH1) by hypermethylation and through inheritance of a germline mutation. The latter mechanism accounts for the autosomal dominant condition HNPCC[48]. Specific cancer genes with repeat sequences in their exons are targeted by this mechanism, including TGFβRII, IGFIIR and BAX. Easily detectable instability of micro-satellite DNA sequences as seen in these genes is a marker of inactivation of any of them. Restoring the function of the DNA repair genes cannot reverse the tumorigenesis process in cells that already contain mutations. Therefore they are different from the classical TSG.

Impairment in double strand break repair is suggested to result in the development of certain tumours with germinal mutations of suppressor proteins BRCA1 and BRCA2 (ref. 49). Repair of double-strand DNA breaks occurs at certain periods of the cell cycle and arrest at these periods sharply increases the efficiency of the repair process. Mutations in BRCA1 affect its ability to arrest cell cycle in damaged cells[50]. Checkpoints at G1, S and G2 phases of the cell cycle ensure intactness of DNA before the cell enters the S phase and before replication of damaged DNA will lead to transmission of genetic abnormalities to offspring. Mutations in G1/S checkpoint genes allow DNA replication in the presence of unrepaired lesions and result in enhanced mutagenesis. *p53* is known to be involved in the regulation of the G1, G2 and S checkpoints[21], the tumour suppressors $p21^{WAF1}$ and pRB regulate the G1 checkpoint, and $p21^{WAF1}$ the G2 checkpoint. The proto-oncogenes *Ras* and *Myc* are involved in the regulation of G1 and G2 checkpoints[12]. At the spindle assembly checkpoint, cells stop at metaphase until all kinetochores are attached to microtubules, so as to ensure correct chromosome distribution. Changes of interactions between kinetochore associated proteins, BUB1, BUBR1, MAD1 and MAD2 play a certain role in induction of this stop in metaphase[4]. Impairment in the functions of MAD1 or MAD2 is observed in some cases of breast cancer and T-cell leukemias. Mutations of genes *BUB1* and *BUBR1* have been recognized in some cases of large intestine cancers. Impairments of repair systems and related 'nucleotide instability' appear to be involved in the development of a relatively small number of tumours, while 'chromosome instability' involving gains and losses of whole chromosomes is typical for the overwhelming majority of solid tumours.

Impaired functions of *p53* typical for most human cancers, significantly attenuate controlling functions of the cell cycle checkpoints and simultaneously inhibit induction of apoptosis. Together with some other consequence of *p53* dysfunction, these impairments sharply increase the probability of appearance of proliferating cells with spontaneous or induced genetic abnormalities, changes of chromosome number, breaks and recombinations of chromosomes, and amplification of certain genes. Although appearance of genome instability is not required for transformation, it is ultimately required for acquisition of sufficient number of mutations to promote malignant growth of solid tumours.

Nearly all tumours have mutations in multiple oncogenes and TSG, indicating that cells employ multiple parallel mechanisms to regulate cell growth, differentiation, DNA damage control and death, and alterations in these lead to transformation. There is cross-talk between oncogenes and TSG and the biological effects of some of these genes are dependent upon the context of the specific cell type in which it is expressed. Although a large number of cancer genes have been discovered, many more remain to be identified. Large scale genome analysis, such as chromosome painting, comparative genomic hybridization, representation difference analysis, restriction landmark genome scanning and high-throughput analysis of loss of heterozygosity are now accelerating the localization of genetic aberrations. Identification of genes that are expressed differently in normal tissues and the cancers that originate in these tissues is another approach towards the identification of cancer-related genes. Differential display, nucleic acid substraction, serial analysis of gene expression (SAGE) and microarray analysis techniques permit very large scale quantitative analysis of gene expression. Cytogenetic and genome analysis techniques have revealed numerous regions that are frequently abnormal in tumours. Discovery and functional charac-

terization of genes in these regions and the order, if any in which they occur, should lead to improved understanding of the process of carcinogenesis and progression. Several of the cancer genes and their products are already proving to be useful as 'tumour markers'[51] and some of them as targets for cancer therapy[52–56].

After two decades of research on cancer, it is still not yet very clear as to why and when a cancer develops after a series of genetic and epigenetic changes in certain cells. Although many recurrent abnormalities have been identified, the exact spectrum of aberrations appears to vary according to tumour histology, genetic and ethnic background. The sequence of events leading to cancers in different tissue types has however started emerging[57–62]. Among these, the molecular genetic events in colorectal tumorigenesis are by far the best understood[47,62].

Analysis of the genetic alterations in the cancer-associated genes, raises several queries such as:

- Is there a causal relation between the gene altered, the tissue type and the type of cancer?
- Is there a relation between the type of genetic aberration in a gene and the resulting cancer?
- Does the type of genetic damage in a gene decide the progression of the disease?
- How is it possible for a cell to accumulate multiple genetic changes of the right type in the right order?
- Do different mutations within specific genes carry different risks for specific cancers?
- Is there a relation between an observed mutation and ethnic origin of an individual?
- What determines the interaction of a cancer gene product with a viral gene protein to generate a specific cancer?

Such queries can be addressed in this post-genome era. Cancer research is now at a point wherein genotype–phenotype relationships need to be pursued with intensity to understand the relationship between the genetic alterations, the type of cancer, susceptibility of individuals to cancer, diagnosis and evaluation of prognosis in cancer patients. The present focus is on generating molecular portraits of different cancers in individuals at the level of the expressed genome (genomics)[64] and the proteins (proteomics)[65] with the goal of identifying drug targets, improving the precision to predict responses to specific therapies at start of treatment or to detect conditions of resistance if applied during the course of treatment. It is the beginning of another exciting phase in cancer research.

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