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Invited Review

Tumor heterogeneity: morphological, molecular and clinical implications

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Summary. Malignant tumors are characterized by their great heterogeneity and variability. There are hundreds of different types of malignant tumors that harbour many oncogenic alterations. The tumor heterogeneity has important morphological, molecular and clinical implications. Except for some hematopoietic and lymphoproliferative processes and small cell infant tumors, there are not specific molecular alterations for most human tumors. In this review we summarize the most important aspects of carcinogenesis and chemo-radiosensitivity of malignant cells. In this regard, some oncogenes such as neu, ras and bcl-2 have been associated with cellular resistance to treatment with anticancer agents. The knowledge of oncogenic alterations involved in each tumor can be important to correlate the morphological features, the genetic background, the prognosis and the clinical response to treatment with anticancer agents. Based on the molecular background of the tumor there are new cancer gene therapy protocols. For example using adenovirus E1a in tumors with overexpression of neu oncogene, inhibitors of tirosine kinase specific for the PDGF receptor in glioma, inhibitors of farnesil transferase to prevent ras activity in tumors with mutations in the ras gene.

Key words: Review, Tumor, Heterogeneity, Radioresistance, Carcinogenesis

1. Introduction

Malignant tumors constitute the second greatest cause of mortality in western countries, with more than 500,000 deaths per year attributable to cancer in the US in 1991 (Mcginnis and Foege, 1993; Ames et al., 1995; Byers et al., 1999). Breast, colon, prostate, and lung tumors together were responsible for more than 50% of those deaths (Callahan et al., 1992; Aaltonen et al., 1993; Mcginnis and Foege, 1993).

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During the last 15 years the incidence of a number of different tumor types, especially prostate carcinoma, melanoma, hepatocellular carcinoma, and non-Hodgkins lymphoma, has risen. During the same period, an increase of around 15% in the rate of breast cancer has been observed, along with a steady rate of colon cancer. Correspondingly, the death-rate in the majority of the above-mentioned tumor groups has been on the rise.

It is estimated that in developed countries, approximately one third of the population will develop cancer at some time in their life. However the probability of contracting a specific type of cancer depends on many variables, among which should be mentioned: sex, age, geography, exposure to carcinogens and genetic predisposition.

Human tumor formation is a complex process which requires the accumulation of multiple oncogenic alterations. These alterations are associated with the development of tumors showing a histopathological progression from incipient lesions to carcinomas. Malignant cell transformation is related to the activation of several oncogenes and the inactivation of several tumor suppressor genes. In this process, cells fail to differentiate and regulate the cell cycle (Ramon y Cajal, 1997).

There is great heterogeneity and variability among human tumors (more than 250 types). The fact that some authors have encountered different spectrums of genetic alterations in primary tumors and their metastases is the result of intratumoral heterogeneity leading to dissemination in only some sub-clones (Zborovskaya et al., 1999). Also, evidence of intratumoral heterogeneity has been described by some authors in relation to DNA content (Ruiz-Cerda et al., 1999).

The development of molecular biology techniques has contributed considerably to a wider knowledge of oncogenes and tumor suppressor genes (Van der Luijt et al., 1994; Lleonart et al., 1997; Laken et al., 1998) Recently, the discovery of oncogenes has opened the way to a molecular approach to cancer, with more than 80 oncogenes described up to now, and more than 20 suppressor genes, whose deletion or mutation can be of great importance in tumor development (Bishop, 1991;

Cantley et al., 1991; Hunter, 1991; Bonetta, 1994; Vogelstein and Kinzler, 1994; Fearon, 1997). At present there is data on more than 25 inherited cancers genes (Table 1).

From a molecular point of view, morphological variability can be demonstrated as depending on the different oncogenes and oncogenic alterations existing in cells. Biological evolution, immune response and

Table 1. Summary of more notable inherited cander syndromes (Fearon, 1997).

SYNDROME	PRIMARY TUMOR	ASSOCIATED CANCERS	LOCATION	GENE	FUNCTION
Familial retinoblastoma	Retinoblastoma	Osteosarcoma.	13q14.3	Rb-1	Transcription regulation.
Ly-fraumeni (LFS)	Sarcomas, breast cancer	Brain tumors, leukemia.	17p13.1	p53	Transcription factor.
FAP	Colorectal cancer		5q21	APC	Regulation of β-catenin.
HNPCC	Colorectal cancer	Endometrial ovarian, Turcor syndrome	2p16, 3p21, 2q32, 7p22	MSH2, MLH1, PMS1, PMS2	DNA mismatch repair.
NF-1	Neurofibromas	Neurofibrosarcoma, brain tumors	17q11.2	NF-1	GAP for p21 proteins.
NF-2	Acoustic neuromas, meningiomas	Gliomas ependimomas	22q12.2	NF-2	Links membrane proteins to cytosketeton.
Wils tumor	Wils tumor	(WAGR) Wilms, aniridia, genitourinary, mental retardation.	11p13	WT-1	Transcriptional repressor.
Familial breast cancer 1	Breast cancer	Ovarian cancer.	17q21	BRCA1	Interacts with Rad-51, repairs breaks.
Familial breast cancer 2	Breast cancer	Male breast cancer, pancreatic cancer, ovarian.	13q12	BRCA2	Interacts with Rad-51, repairs breaks.
Familial melanoma	Melanoma		9p21	p16, CDKN2	Tumor suppressor gene.
Wiedmann-Beckwith syndrome	Wilms tumor	Organomegaly, hemi-hypertrophy, hepatoblastoma, adrenocortical cancer.	11p15	p57 ?, KIP2 ?	Cell-cycle regulator.
Nevoid basal cell carcinoma syndrome (NBCCS)	Basal cell, skin cancer	Jaw cysts, palmar and plantar pits, medulloblastomas, ovarian fibromas.	9q22.3	PTCH	Transmembrane receptor for hedgehog,signaling molecule.
von-Hippel Lindau syndrome (VHL)	Renal cancer	Pheochromocytomas, retinal angiomas, hemangioblastomas.	3p25	VHL	Regulates transcriptional elongation by RNA pol. II.
Hereditary papillary renal cancer	Renal cancer, papillary type	Other cancers.	7q31	MET	Transmembrane receptor for HGF.
Multiple endocrine neoplasia 1 (MEN 1)	Pancreatic islet cell	Parathyroid hyperplasia, pituitary adenomas.			
Multiple endocrine neoplasia 2 (MEN 2)	Medullary thyroid cancer	Pheocromocytomas, (type 2A, type 2B), parathyroid hyperplasia, mucosal hamartoma, familial medullary thyroid cancer	10q11.2	RET	Transmembrane receptor, tyrosine kinasa for GDNF.
Multiple exostoses	Exostoses	Chondrosarcoma.	8q24.1, 11p11-13, 19p	EXT1, EXT2, EXT3	?
Cowden disease.	Breast cancer, thyroid cancer	Intestinal hamartumor polyps, skin lesions.	10q23	PTEN	Dual specificity phosphatase
Hereditary prostate cancer	Prostate cancer	?	1q25, 17q25, others ?	?	?
Palmoplantar keratoderma	Esophageal cancer	Leukoplakia	17q25	?	?
Ataxia telangiectasia	Lymphoma	Cerebellar ataxia, immunodeficiency, breast cancer.	11q22	ATM	DNA repair, induction of p53?
Bloom's syndrome	Solid tumors	Immunodeficiency, small stature.	15q26.1	BLM	DNA helicase?
Xeroderma pigmentosum	Skin cancer	Pigmentation abnormalities, hypogonadism.	Multiple complementation groups	XPB, XDP, XPA	DNA repair helicases, nucleotide excision repair.
Falconi's anemia	AML	Pancytopenia, skeletal abnormalities.	9q22.3, 16q24.3	FACC, FACA	DNA repair?

resistance to radio/chemotherapeutic agents are also correlated with implicated genes in carcinogenesis. Depending on the number and type of molecular alterations in the tumors, we should predict the biological evolution of each type of cancer.

The wide variety of tumors, as well as the phenotypic and prognostic heterogeneity of many neoplastic processes, greatly complicates a single therapeutic and preventive approach to all tumor types. A model has been described in colon cancer where there is correlation between the progression of histopathological characteristics of a tumor and the accumulation of genetic alterations in each stage, from adenoma formation to the evolution of aggressive and metastatic cancer (Fearon and Vogelstein, 1989; Fearon and Jones, 1992).

The study of oncogenes can help rationalize the evaluation of different types of cancer, both by providing a molecular approach and by forming the basis for a more specific analysis of each tumor and patient, giving the biological characteristics of the tumor according to the oncogenes activated. Such information would reflect the individual features of each tumor and would permit the design of a therapeutic approach best suited to each patient (Barinaga, 1997).

Exhaustive surveys of the many chromosomal alterations known to date have been published (Weinberg, 1991; Fearon, 1997). Differences in capacities to repair reversible reactions produce errors in the DNA sequence by the DNA polymerase, (such as depurination, oxidation by free radicals generated in the cellular metabolism, and 5-methylcytosine deamination). These capacity differences can explain the differing susceptibilities of each cell type.

Today it is accepted that human tumor formation requires the joint activation of various oncogenes and the inactivation of at least one suppressor gene. The activation/inactivation sequence varies from one tumor to another, and one is tempted to establish a similarity between the different stages of carcinogenesis and previous histophatological phases of development in the same kind of malignant tumors. However, there is increasing evidence suggesting that the final malignant phenotype results from the accumulation of multiple oncogenic alterations and not from the order in which they appear. The number of neccesary alterations is unknown, although there seems to be a minimun of five steps in human as well as rodents (Fearon and Vogelstein, 1990). It is necesary to mention two different types of genes that can provoke tumoral development:

a) Gatekeeper genes, whose role is based on controlling the cell cycle directly. Patients who inherit a mutated gatekeeper gene need only a secondary hit to get an inactivated tumor suppressor gene, whose molecular alteration confers a clear cell-growth advantage. Subsequent activation of oncogenes or inactivation of other tumor suppressor genes drive cells to become fully transformed. Examples of this group are: Rb, NF-1, NF-2 or APC genes.

b) Caretaker genes, whose role is based on controling DNA replication; their role in the cell cycle is rather indirect, because its absence would produce genetic instability of the DNA. Examples of this group are: BRCA-1 and BRCA-2. Patients who inherit a mutated caretaker gene need at least three additional molecular events to develop neoplasia. One of them must be in the complementary allele of the cartaker gene and the two others in both alleles of a gatekeeper (Kinzler and Vogelstein, 1997). Interestingly, it is not known why hereditary-specific oncogenic alterations are associated with development of malignant tumors in specific locations.

Another important aspect is to show that except in the case of some haematological cancers or childhood tumors, there are no specific genetic alterations in tumors.

2. Generalities about tumors

2.1. Types

In accordance with morphological criteria, more than 250 different tumors have been described, each with multiple subtypes.

First of all, we must distinguish between 2 kinds of tumors or neoplasias: benign and malignant. Benign tumors are made up of cells which do not infiltrate neighboring tissues; once removed they do not reappear in any other parts of the body. Nevertheless, benign tumors do not always show this evolutionary or clinical behaviour; there have been some examples of tumors considered benign that transformed into malignant tumors.

The cells which form malignant tumors present nuclear and cytoplasmic alterations and various levels of non-differentiation and immaturity with respect to the original tissue. These cells infiltrate into organs, are able to destroy their structure and proliferate in a disorderly way, integrating into the general economy of the organism. One of the most important characteristics of malignant tumors is the marked heterogeneity displayed by their cells. This heterogeneity is not only morphological but includes other properties, such as drug resistance, metastatic capacity, proliferation index, chromosomic abnormalities and differentiation markers.

A second class division of tumors is made into 2 large groups: hematological and solid tumors. Hematological tumor study has greatly benefited from advances in molecular biology at the diagnostic and therapeutic levels (Faderl et al., 1999).

Solid tumors can be divided according to the type of cell from which they derive, so that they could be considered as epithelial, mesenchymal, neural, germinal, melanocytic or lymphoid tumors, among others. Given the pluripotential nature of germinal tumors, all the possible differentiations can occur.

Another possible way of classifying tumors is according to the organ of origin. Tumors from the same line acquire completely different morphological characteristics depending on the organ of origin, which implies entirely different therapy approaches. Each organ displays characteristic tumors, and the incidence of a particular type of tumor varies considerably depending on the origin.

2.2. Sex

Tumor incidence varies in men and women (Bastiaens et al., 1998). The most common cancer in men is lung and colon and in women is breast. The reasons for these differences are not always known. Some authors have proposed the possibility of a sex-determined risk linked to the AB-O blood group genes as in the case of several kinds of cancers, e.g. the acute leukemia that is more common in men that in women (Jackson et al., 1999)

2.3. Age

Children have an inferior tumor rate, estimated at 12.7% new cases per 100.000. In addition, their types are different. It is important to note the predisposition of certain organs with proliferative rates. Leukemias are the most frequent cancers in children, followed by lymphomas, central nervous system tumors, kidney, soft parts and Hodgkin's disease. In adults, lung, breast, colon, liver, bladder, esophagus and ovarian cancers generally occur, whereas leukemias and lymphomas are the most frequent in children. For some kind of cancers occurring in adults there are specific risks if other familial factors are involved (Gronberg et al., 1999).

2.4. Geography

In different parts of the world there are noteworthy differences regarding the incidence and prevalence of different tumors (Shiraishi et al., 1998). In this tumoral variability environmental factors are involved, specially those related to exposure to certain carcinogens related to specific infections such as in the cases of the Epstein-Barr virus in lymphomas or the human papilloma virus to cervical carcinoma (Muñoz and Bosch, 1997). As far as mutations are concerned, the current data point increasingly to the existence of a balance between endogenically and exogenically caused mutations, which can vary among different types of cancer in a given population, or for different populations with the same type of cancer.

2.5. Heredity

More than 20 different hereditary cancer syndromes have now been defined and attributed to specific germline mutations in various inherited cancer genes (Fearon, 1997). These syndromes affect about 1-5% of cancer patients. One of the most representative examples is the Hereditary non polyposis colorectal cancer (HNPCC), Lynch's syndrome, which involves 4% of all colorectal cancers. In these patients, tumors are proximal to the colon region and also in organs such ovary or uterus. These patients develop a specific carcinogenic mechanism similar to that in sporadic cancers with microsatellite instability. Morphologically these familial tumors are similar to the sporadic colon cancers mentioned above (Fushs et al., 1994; Lleonart et al., 1997). Another familial colon cancer is familial adenomatous polyposis (FAP) where the altered gene was identified as APC (Powell et al., 1993).

Moreover, 10% of all breast cancers are hereditary (Shattuck et al., 1995). Genes involved in this hereditary cancer are BRCA1 (breast cancer) and BRCA2 (breast and ovarian cancer), although at least 5 other genes have been associated. Moreover, according to cytogenetic analyses other genes seem to be involved in hereditary breast cancer.

3. Mechanisms of carcinogenesis

3.1. Alteration of critical genes involved in cell-cycle regulation

The activation mechanisms of different oncogenes, along with the inactivation of suppressor genes, are among the most important mechanisms of carcinogenesis involved in cell-cycle control. Proteins such as p53, p105 and pRb are relevant with respect to control and regulation of the cell cycle. Some of these proteins are regulated by phosphorylation produced by various cell cyclin-dependent kinases (cdk) which, in turn, are regulated by various proteins, such as p16 and p21/Waf (Bonetta, 1994). This complex puzzle of cyclin-dependent kinases and cdk inhibitors could be one of the crucial axes in the control of many malignant cell proliferations and those alterations in a lot of factors that are potentially oncogenic (Merlo and Hynes, 1994; Lee et al., 1999).

There are several mechanisms by which the oncogenic potentials of proto-oncogenes or normal genes are activated, converting them into malignant genes. Four activation mechanisms have been described:

3.1.1. Amplification

Increase in the number of copies of a single gene and in the quantity of the corresponding protein. The mechanisms through which amplifications are generated are unknown. However, gene amplifications have prognostic significance (Seeger et al., 1985). For the example, the HER2/neu gene in breast cancer (Pegram, 1998) and the N-myc gene in neuroblastoma (Kreipe et al., 1993). This mechanism affects only a few genes and occurs probably late in tumorigenesis.

3.1.2. Point mutation

Modification of the DNA nucleotide sequence, producing a protein with an altered activity. Point mutations that modify the function of the protein or produce an overexpression of the normal gene are generally associated with this mechanism.

Point mutations consist of subtle sequence changes that involve basic subsitutions, deletions or insertions of a few nucleotides. Cytogenetic analysis cannot give us information about these subtle changes. Some authors (Lengauer et al., 1998) have associated these changes with a kind of instability at the nucleotide level (NIN). These authors have speculated that the nucleotide-excision repair system could play an important role in this type of instability. When these changes are provoked, they could cause dramatic phenotypes, because in most cases the functions of the proteins codified by mutated genes are very far from their right functions. This type of subtle sequence change affects genes, suppressor genes, and proto-oncogenes that turn into oncogenes, as in the cases of p53 and K-ras, respectively.

3.1.2.a. c-K-Ras. The proto-oncogene c-K-ras acts as a signal transductor from the extracellular environment to the interior one.

There are three genes belonging to the same family but located in different chromosomes: H-ras, K-ras and N-ras, whose proteins are nearly 90% identical in sequence. The ras gene family encodes for a 21 Kda protein (p21-ras) with the capacity to synthesize and hydrolize GTP. The ras protein collaborates with some transcription factors, such as c-myc, and is involved in many cellular pathways (Sears et al., 1999).

Constitutive activation of ras-family genes occurs in 30% of human tumors, with a highly variable incidence according to tumor type and location. It is frequent in the colon, lung, stomach and breast (Somers et al., 1998).

K-ras mutations have been observed in over 90% of pancreatic adenocarcinomas, and 80% of colorectal carcinomas, whereas they are present in less than 5% of breast and thyroid cancers and lymphomas (Bouras et al., 1998).

The c-K-ras oncogene is usually activated by point mutations at codons 12, 13 and 61. These mutations are responsible for its transforming nature, given that they trigger a constitutively activated intracellular pathway (Al-Mulla et al., 1998). The results of several studies dealing with this oncogene imply that the number and type of point mutations in K-ras have a profound effect on the biological behaviour of the tumor, conferring a particularly aggressive nature on it (Kressner et al., 1998; Huncharek et al., 1999).

3.1.2.b. p53. The p53 protein was discovered in 1979 (Lane and Crowford, 1979) and the corresponding gene was isolated 3 years later. It was shown later that this protein has the ability to bind to several oncogenic viral proteins, such as E6 from the human papiloma virus, the nuclear antigen of the Epstein-Barr virus, or the 55kda from the E1b gene of adenovirus type 5 (Moran, 1993; Lowe and Ruley, 1993). This allows us to postulate the blockage of p53 gene function as one mechanism of viral carcinogenesis (Raycroft et al., 1990; Shaulsky et al., 1990). The average life span of this protein is very short, between 5 and 40 minutes, but in the presence of viral proteins an increase of its half-life is observed, although in the case of the E6 protein of VPH 16 and 18, the increase in time for the degradation of p53 is accompanied by a loss of functionality. Similarly, the presence of point mutations has been linked to an increase in life-span (Finaly et al., 1991; Harris, 1993; Iwabuchi et al., 1993; Landiny et al., 1993; Oliner et al., 1993; Zambetti and Levine, 1993).

The p53 protein acts as a cellular damage-control director (Yonish-Rouach et al., 1993; Lee et al., 1995). If a foreign agent damages the cellular DNA, the cell goes into a state of alert and p53 begins to accumulate in order to protect the genome's integrity and, if necessary, to initiate the process of copying DNA to repair it. This protein acts like a transcription factor, activating genes that directly inhibit cell growth (Truant et al., 1993; McDonald et al., 1996). When the number of errors becomes too great for p53 to control, the protein activates an apoptosis mechanism (Vogestein and Kinzler, 1992; Gottlieb et al., 1994; Hermeking and Eick, 1994).

Several syndromes in humans are related to p53 alterations (Santibañez-Koref et al., 1991; Kastan et al., 1992). Since p53 regulates genomic stability and prevents the continuation of the cell cycle after damage to the genome, one would expect the loss of function of p53 to be correlated to aneuploidy and high proliferative rates, characteristics associated with tumoral progression and poor prognosis (Greenblatt et al., 1994). The frequency of p53 alterations in human cancers is highly variable (Soussi et al., 1994), ranging from meningiomas or melanomas, where p53 alterations are practically nonexistent; lymphomas, where they are infrequent; breast, lung, liver, skin, prostate, bladder and cervical cancers, where the prevalence is 40% to 60%; and finally in colon cancer, where, depending on the series, incidences of up to 80% have been detected (Vogelstein et al., 1988; Vogelstein, 1990; Bennet et al., 1991; Hollstein et al., 1991; Moll et al., 1992; Eyfjörd et al., 1995). The p53 protein has been linked to more than 52 types of cancer and, in most cases, is activated by point mutations, although it can also be activated by other alterations at the DNA level or by interactions with other proteins (Jego et al., 1993).

The detection of p53 has diagnostic, prognostic and therapeutic implications (Baker et al., 1990; Thor et al., 1992; Pientenpol and Vogelstein, 1993; Kirsch and Kastan, 1998) The correlation between p53 mutations and highly advanced tumors, or advanced Duke's state, is frequent (Tortola et al., 1999) Loss of heterozygosity is not only increased by point mutations, but also by the loss of

various loci at which other tumor-suppressor genes besides p53 can possibly be located (Gerdes, 1991; Martin et al., 1992; Harris and Hollstein, 1993; Chang et al., 1995).

3.1.3. Deletion/Gains of DNA fragments

Loss or gains of genetic material give the cell a proliferative advantage.

Cancer cells frequently suffer gains and losses of genetic material. According to the quantity of genetic material lost or gained, the following classification can be made:

a) Loss or gain of small DNA fragments. Gain of genetic material from the arm of chromosome 17q is the most frequent cytogenetic abnormality in neuroblastoma cells (Brown et al., 1999), but the loss of small DNA fragments is a much more common process than their gain. This mechanism takes place in most tumors and the process is known as loss of heterozygosity. Loss of heterozygosity in most cases is detected in one allele accompanied by inactivating point mutations in the complementary allele, causing, as we have mentioned, the loss of function of some suppressor genes (Baker et al., 1989, 1990). Such is the case of the p53 gene, or the retinoblastoma gene (pRb), a key player in cell-cycle control (Bishop, 1991; Cantley, 1991; Hunter, 1991; Weinberg, 1991; Kuerbitz et al., 1992; Lowe et al., 1994)

A similar match takes place in Wilms tumor at 11p13 and in the hereditary cancer disease FAP (Familial Adenomatous Polyposis) at 5q21-22, with loss of heterozygosity and a mutation in the APC region in 57% of cases. Some 60-75% of colon cancer patients showed loss of heterozygosity at the 17p and 18q chromosomes, and 50% at the 5q chromosome (Kern et al., 1989; Khine et al., 1995). In tumors with loss of heterozygosity, the prognosis is less favourable compared to that of tumors without loss of heterozygosity.

Along with the discovery of tumor suppressor genes, these regions with a loss of genetic material have remained target regions for the localization of unknown genes which could play an important role in carcinogenesis.

b) Loss or gain of whole chromosomes. Structural alterations (translocations, inversions, deletions, insertions and amplifications) and numerical alterations (gain or loss of entire chromosomes) are included among the most frequent chromosomal alterations. In this group we are studying only numerical alterations; e.g., glioblastomas sometimes lose chromosome 10, reflecting the inactivation of the tumor suppressor gene PTEN, and in papillary renal carcinoma the gain of chromosome 7 reflects a duplication of the mutant MET oncogene. This process concerning deletions/gains of whole chromosomes represents a true chromosomal instability (CIN) in cancer cells, which persists throughout the tumor's lifetime (Lengauer et al., 1997).

3.1.4. Translocation

Chromosomal alterations in cancer can also be associated with the overexpression of certain gene products, most frequently involving growth factors, their receptors, and transcription factors. Alternatively, the resulting alterations may be the gain or loss of entire chromosomes, which causes the aneuploidy characteristic of most tumor cells.

The molecular mechanisms of translocations are not yet clear; however, we are here going to set out different types described, and speculate on their possible origins. Two basic types of translocations can be distinguished (Lengauer et al., 1998).

a) Rearragements of chromosomal segments in specific neoplastic diseases. The development of cytogenetics has enabled the detailed study of chromosomal alterations in an ever-increasing number of tumor cell lines (Rabbitts, 1994).

Generally, the translocated fragment or gene is either located near a strong promoter or fuses with another gene. Some of these translocations represent an aberration of the normal recombination process, like the one that is mediated by RAG proteins leading to rearrangment with inmunoglobin or T-cell-receptor genes (Hiom et al., 1998). The karyotypic presence of these kinds of translocations can be used to classify neoplasms and predict therapeutic responses.

One of the first clinical cytogenetic findings was the detection in 1960, of the Philadelphia chromosome in patients with myeloid chronic leukemia. The Philadelphia chromosome is the result of the 9;22 translocation, producing the chimeric gene bcr-abl, which expresses cellular proliferation and plays a central role in the pathogenesis of the chronic phase of myeloid chronic leukemia. Although the development of the process of this disease is not yet entirely clear, there could exist other genetic alterations, such as a trisomy of chromosome 8 and alterations in p53 or p16.

Moreover the t(9;22) translocation is also observed in acute lymphocytic leukemia (ALL) in 30% of adults and 3% of children, principally in those with type B, and its presence indicates a worse prognosis. Occasionally, this alteration is also detected in other hematological processes.

Another classic finding in the area of cytogenetics was the determination of the t(11;22) translocation, characteristic of Ewing/PNET sarcoma which has important therapeutic and prognostic connotations.

Burkitt's lymphoma, apart from its characteristic morphological pattern, is generally accompanied by the t(8;14) translocation in 75-85% of cases or, less frequently, by t(8;22) in 25% or t(2;8) in only 15%. These alterations lead to the overexpression of the c-myc gene in those cells where there has been a translocation. Finally, in endemic cases the breakage point is located on chromosome 14 and affects the heavy chain union region, thereby suggesting that the translocation occurs

in the early stages of B-cell development. In sporadic cases the translocation affects the heavy chain "switch" region.

b) Translocations observed in many solid tumors: This type of solid translocation appears almost at random and seems unrelated to translocations observed in other tumors. In some cases these translocations could be masked as a loss of heterozygosity, as in the case that the genomic fragment is presumably lost and has fused with another unknown gene or DNA sequence. A new mechanism for explaining this type of complex translocation has been described recently (Sanchez-Prieto et al., 1999).

3.2. Microsatellite instability (MIN)

Another mechanism initially described by Perucho and Vogelstein, is the demonstration of microsatellite instability in a significant number of human tumors (Ionov et al., 1993). Microsatellites are short sequences of DNA, of a few nucleotides which are repeated many times in tandem; they are specific to each microsatellite chromosomic region and display great genetic polymorphism. Some authors (Ionov et al., 1993), using non-specific primers for PCR amplification, showed a high incidence of alterations in colorectal adenocarcinomas, revealing the possibility of there being alterations in DNA repair-systems which would cause subsequent genetic alterations. Later, the genes responsible were identified as h-MSM2, h-MLH1, h-PMS1, h-PMS2, h-MSH3 and h-MSH6 which really play an important role in DNA repair mechanism (Leach et al., 1993; Malkhosyan et al., 1996). The most widespread way of studying the presence of instability is with DNA-marker analysis for these specific sequences (Lleonart et al., 1998). In addition to microsatellite instability, most human tumors show chromosomic instability that appears to be due to a lack/mutation of specific genes which control chromosomic segregation. The mechanism of microsatellite instability is present in almost all cases of patients with hereditary non-polyposis colorectal cancer, and this mechanism differs from those of sporadic cases (Losi et al., 1997). However, this mechanism is also present in a small percentage of sporadic cases of gastric or endometrial cancer (Chung et al., 1999)

3.3. Chromosomicinstability (CIN)

In most cases of cancer, instability is seen at the chromosome level, with frequent gains and losses of whole chromosomes (CIN). Chromosomic instability has a dominant quality, as has been shown by some authors after experiments such as cell fusion (Lengauer et al., 1997). Genes that are responsible for this kind of instability are hBUB1 and hBUBR1 components of the mitotic spindle checkpoint, and apparently only a single mutational "hit" of such a gene is required to produce the CIN phenotype (Cahill et al., 1998). This type of instability comprises most tumors and the result is an aneuploid karyotype.

3.4. Apoptosis

Finally, the study of programed cellular death, or apoptosis, is currently one of the most promising areas of research. Homeostasis is mantained through a balance between cell proliferation and cell death (Thompson., 1995). Apoptosis is controlled by multiple genes such as bcl-2, bcl-xL, bcl-w, bfl-1, brag-1, bax, bad, bid, bik, Hrk, ced-9, p53, myc, ras and A1 (Hockenbery et al., 1990; Oltvari et al., 1993; Merchant et al., 1996; Kroemer, 1997). In the last steps of this death program, a large cascade of caspases are involved (Caelles et al., 1994), some of them with a common role, not only in apoptosis but also in necrosis (Herceg and Wang, 1999). Alterations in the induction of apoptosis could condition the development of neoplasic processes and increase cell resistance to chemo- and radiotherapy.

Bcl-2 is an integral intracellular membrane protein (Jacobson et al., 1993) and belongs to a family of proteins of similar structure which have a 21% common homology. The bcl-2 gene was discovered in non-Hodgkin's lymphoma in B cells, where the molecular mechanism responsible for its activation is the t(14,18) translocation (Tsujimoto et al., 1984, 1985). Afterwards, however, high levels of bcl-2 protein were observed in solid tumors, including prostate adenocarcinomas, colon cancer, squamous lung carcinomas, breast cancer and nasopharyngeal carcinomas (Reed et al., 1990; Campos et al., 1993; Pezzella et al., 1993; Leek et al., 1994). The molecular mechanism that determines the deregulation of bcl-2 is different in solid tumors to that observed in lymphomas or leukemias (Kusenda, 1998).

Bcl-s plays an important role in the apoptosis prevention induced by a number of stimuli, such as radiation; therefore, it is considered an apoptosis inhibitor. Intracellular levels of bcl-2 protein have been linked to an increase in sensitivity to apoptosis induced by a wide range of chemotherapeutic drugs. The expression of a particular oncogene can be used to distinguish tumoral processes from normal ones, as in the case of the overexpression of bcl-2 used to differentiate a follicular lymphoma from a reactive hyperplasia.

4. Clinical and therapeutic implications

Tumor heterogeneity has biological and clinical significance, since the great phenotypic and biological variability of cancer leads to the possibility of tailoring specific and selective treatments to each type of tumor. Genetic and molecular alterations have parallel repercussions on the tumoral phenotype.

From an anatomopathological point of view, there are various steps in the formation of a malignant tumor,

from the initial hyperplasia/dysplasia of differentiated cells, to malignant in situ cells, infiltrating cells and metastases.

The most representative case of this sequential process in cancer has been clearly observed in colon tumors (Fearon and Vogelstein, 1990). These sequential alterations transform the normal epithelium via alterations/LOH at 5q of the Familial Adenomatous Polyposis (FAP) in early adenoma. Mutations at c-k-ras, together and LOH at the 12p site, are associated with intermediate adenoma. Finally, the loss of 17p, LOH at 18q (DCC) and mutations/deletions of p53 convert the benign adenoma into a carcinoma (Toribara and Sleinseger, 1995).

Sometimes the minimum number of necessary mutations are accompanied by the genetic predisposition of certain individuals to develop a tumor easily. Examples of such cases are the hereditary 5% of breast cancers, families with Li-Fraumeni syndrome, HNPCC (hereditary non-polyposis colorectal cancer) and FAP. Related syndromes are Gardner's Syndrome, Turcot's Syndrome, Peutz-Jeughr's Syndrome, Cowden's Syndrome, Cronkite-canada's Syndrome and Smooth Adenoma Syndrome (Hamilton et al., 1995; Syngal et al., 1999). Genetic and cytogenic studies are essential for the diagnosis of certain cancers and leukemias.

It is remarkable that Rb-1, p53 and p16 germline mutations predispose to retinoblastoma, osteosarcoma and melanoma, respectively (Table 1). However, somatic mutations in Rb, p53 and p16 are believed to be causally involved in a substantial fraction of many different sporadic cancers, including lung and colon cancers. A likely explanation is that the above-mentioned genes, function in intersecting or overlapping genetic pathways. These pathways may branch considerably, or the genes may have distinct functions, perhaps depending on the cell cycle (Fearon, 1997). The concept of branching pathways or alternative gene function in different tissues may help to explain the tissue-specific cancer spectrum in mutation carriers. In some cases, like in HNPCC, this explanation seems doubtful because repairing genes have the same function in all cell types, and they are able to provoke cancer only in specific organs.

The therapeutic possibilities of benign neoplasias are generally favorable. However, malignant neoplasias depend on the type of tumor, its location and, above all, the clinical stage of tumor dissemination. A timely diagnosis effectively enables intervention with therapeutic surgery or radical radiology when there is not yet a great diffusion of metastatic cells. In some cases, chemo and radiotherapy treatments are significant, but occasionally they have very aggressive secondary effects.

5. Tumoral heterogeneity and therapy

The activation of certain oncogenes can be related to the phenotypic tumoral heterogeneity observed in human pathology. The presumed activation of an oncogene/tumor suppressor gene could represent a new focus in the study of human tumors, grouping them according to specific oncogene activation or tumor suppressor repression. Correlations of characteristic pathologies, such as histological grade, proliferative rate, nuclear atypia, necrosis, tumor size, metastasis, previous adenomas (with molecular occurrences such as p53 alterations, or genes such as nm23, Rb, c-myc and c-K-ras) have already been shown in some types of cancer. These correlations promise to increase our knowledge of tumors and create new paradigms for risk evaluation, prevention, early detection, prognosis and therapy.

Tumor heterogeneity is an appealing aspect of the biological and pathological study of malignant tumors, because the variety of cellular populations can be a determining factor in treatment. Tumor heterogeneity presents, as we have seen, morphological and diagnostic implications. Heterogeneity may explain why tumors included in the same histological group have different therapeutic responses. All treatments present a margin of error so that there are tumors which in principle ought to respond to a certain type of treatment, such as chemo- and/or radiotherapy, but are resistant, whereas other tumors with identical morphological patterns respond. There are biochemical alterations which do not necessarily occur in all tumors of the same type, even within the same tumor.

Responses to treatment are unique and specific for cytogenic and molecular anomalies that are clearly identified, and this knowledge influences the choice of therapy in one or another individual. Detailed genetic analyses of leukemias and solid tumors, as described in the preceding section, offer the basis for diagnosis and prognosis, as well as giving an idea of the evolutive state of the illness.

The final objective of many clinical treatments is to destroy all tumoral cells without affecting (or affecting in the least possible way) normal cells. The genetic and histological differences between cells have always been studied with the ideas of identifying targets in order to attack tumoral cells only. In tumors of polyclonal origin, the search for these targets is more difficult, due to the great heterogeneity among them. The reason is that one must identify targets in each of the subpopulations that originated the tumor. Diversification of the capacities of tumors with polyclonal origins means that chemotherapy cannot be reduced to the use of a single chemotherapeutic agent.

Thus, there emerges a new therapeutic perspective, called gene therapy, which renders the destruction of cancerous cells unnecessary: the administration of a mutant gene to correct the physiological activity of cancer cells and, subsequently, revert them to the normal phenotype from which they derived.

Therefore, a possible therapeutic approach to treating tumors where the ras signaling pathway is affected is to identify inhibitors of critical downstream targets (for example, the raf/MEK/MAP kinase pathway) or inhibitors of farnesil transferase (Huang and

Heimbrook, 1997). In the case of the c-raf gene, some authors have used antisense c-raf oligonucleotides in human tumor cell lines (Monia et al., 1996).

In the case of a mutant p53 gene, the types of possible anti-cancer therapies are disruption of p53 tetrameres, disruption of protein-nucleic acid interactions, inhibition of gene expression by antisense molecules, or replacement of function by gene therapy using adenoviruses (Labrecque and Natlashewski, 1995; Yang et al., 1995), (Table 2).

Some authors have demonstrated that the activation of certain transcription factors, such as NF-kB, in response to chemotherapy is a principal mechanism for inducing tumor chemoresistance; the inhibition of NF-kB could be a new approach to adjuvant therapy in cancer treatment (Wang et al., 1999).

5.A. Chemoresistance mechanisms

Regarding chemotherapy, the following are some of the more classic mechanisms of chemoresistance (Hayes and Wolf, 1990):

A) Non-entry of the drug. This is the case of cerebral tumors, where the haematoencephalic barrier makes some tumors resistant to various chemotherapeutic agents. In the same way, the low level of vascularization of certain tumors blocks the drug's action.

B) Incorporation of the drug. Many treatments currently available require a transport system. For example, 5-Fu uses purinic and pyrimidic bases as a transport system.

C) Expulsion of the drug: This kind of mechanism, probably has the greatest clinical implication. The most studied model is the p170 glycoprotein, coded by the MDR gene (Hamada and Tsuruo, 1988). It was observed that this protein conferred resistance to Vinca derivatives (Vincristina, Vinblastina), colchicin and doxorrubicin among others (Ukeda et al., 1987). The appearance of multidrug-resistant (MDR) phenotypes is one of the most serious handicaps of chemotherapy (Fine et al., 1988; Chan et al., 1989).

The MDR protein has 12 transmembrane domains and has the shape of a pore, thus making it an ideal candidate for the expulsion of substances out of the cell. It is known that it presents ATPase activity, which implies the use of energy to expel the drug at the expense of the cell (Abraham et al., 1993). From the genetic point of view, it has been shown that cell lines with MDR-mediated resistance present a high number of copies of the gene, or the appearance of point mutations that could originate a considerably more effective p170 than the normal one (Choi et al., 1988). Moreover, there is evidence that demonstrates how genetic alterations in p53 or ras family genes could affect this protein's expression (Chin et al., 1992; Zastawny et al., 1993).

It has been described that the PKC may phosporylate the MDR protein and, in some cases, it could control its activity (Sato et al., 1990). This demonstrates that the use of staurosporine-like substances increases the accumulation of the drug in cells with multidrug-resistant phenotypes. Accordingly, studies using new derivatives of staurosporine have begun, such as that with Na-382, which is able to reverse multidrug-resistant phenotypes *in vivo* and *in vitro* (Miyamoto et al., 1993).

D) Drug metabolism. Many drugs need a mechanism prior to exerting their cytotoxic effect. Cyclophosphamide needs to be metabolized by cytochrome p450 in order to have a toxic effect. Thus, any change in metabolic enzymes could induce resistance.

E) Drug kidnapping. Tumoral cells could develop mechanisms that diminish the concentration of the active drug. This takes place with cisplatin and the metalothionins (group III).

F) Target modifications. This mechanism is mediated by changes in the drug's target. For example, mutations in microtubulins or in the tubulin itself, which would imply resistance to Vinca derivatives (Sten and Ziff, 1984).

H) Alterations in glutation metabolism. Glutation is a metabolite implicated in the elimination of free radicals, which are produced by diverse antitumoral agents (such as radiation, doxorrubicin and others). Thus, this process can be involved in the resistance to antitumoral treatments (Kramer et al., 1988). Moreover, a correlation between the presence of certain oncogenes and an increase in glutation metabolism has been demonstrated (Vizencini et al., 1993).

Table 2. New anticancer strategies.

DRUG TYPE	CELLULAR TARGET	CANCER
Antibody	Block HER2 growth-factor receptor, block EGF receptor.	Breast, kidney, prostate, breast, head and neck.
Tyrosine kinase inhibitor	Inhibit PDGF receptor.	Glioma, various tumors, various solid cancers.
Farnesyl transferase inhibitor	Prevent ras activity.	Various tumors.
CDK inhibitors	Block cell cycle.	Various tumors.
bcl-2 antisense	Restore apoptosis.	Lymphoma, various solid cancers.
Virus with p53	Restore lost p53 tumor suppressor.	Lung, head and neck, ovarian, liver.
Modified adenovirus	Selectively kill p53-lacking cells.	Head and neck, gastrointestinal, pancreatic, ovarian.

5.B. Chemo/radioresistance and cellular factors

Similar to the case of chemotherapy, there are several mechanisms by which a tumor could become radioresistant in different degrees (Tubiana et al., 1986). Among these are, the following:

A) Hypoxia. Cells submitted to conditions of hypoxia are 2 to 3 times more radioresistant than cells that are not.

B) Percentage of clonogenic cells. The smaller or greater number of cells capable of causing a tumor is a determining factor in the response to radiotherapy (Tubiana, 1986).

C) Intrinsic radioresistance. This concept originated in 1981 (Fertil and Malaise, 1981) and proposes that the differences observed in the initial phases of a radiation dose-response assay could conform to the cell type being studied. It has been observed that there exists a correlation between this parameter and the clinical response (Deacon et al., 1984; Isonishi et al., 1991).

D) Reparative capacity of DNA. It has been shown that the capacity for repairing potentially lethal damage varies among the different cell lines. This suggests that post-irradiation reparative processes could justify those differences in the response to gamma-irradiation (Iliakis et al., 1990). Reparative capacity is also applicable to chemotherapy; for example, in the case of cisplatin, how resistance is associated with a decrease in reparation of provoked lesions has been shown (Parker et al., 1991). Nevertheless, certain authors (Steel and Peacock, 1989) think that this concept should be considered as part of intrinsic radioresistance.

E) Genetic alterations. This concept has not been included in classic radiobiology texts, but how the presence of oncogenes such as *c-raf* from the ras family can alter radiosensitivity, at least *in vitro* has been shown (Kassid et al., 1989). The presence of mutations in genes that are directly implicated in DNA repair processes (e.g., MSH and BRCA genes) or genetic instability (Fishel et al., 1993) must play a role in the repair of damage done by physical or chemical agents (such as radiation). Nevertheless, the existence of specific genetic alterations could justify the appearance of phenotypes that are more or less resistant to chemo and radiotherapy.

5.C. Oncogenes and cellular resistance

As indicated above, tumor suppressor genes are implicated in chemo and radioresistance phenomena, as are oncogenes such as *c-myc* (Niimi et al., 1991; Sklar and Prochownik, 1991), but it is important to emphasize that the great majority of the results were obtained from experimental models. It seems clear that in the near future the detection of oncogenes will be decisive for therapeutic as well as for diagnostic treatment (Marchetti et al., 1994; Riva-Lavieille, 1994).

Thus, the *K-ras* oncogene has been related to antitumoral resistance phenomena (such as cisplatinum and Doxorrubicin) in various cell systems (Sklar, 1988a,b; Sanchez-Prieto et al.,1995a-c; White et al., 1996). The case of *raf* is similar because it could contribute to an alteration concerning radioresistancy, at least *in vitro* (Kasid et al., 1989). In the same way, we can see that there are other genes which present the opposite effect, such as the *E1a* gene of adenovirus, which has shown a chemo and radiosensitizing capacity in several murine and human models (Frisch et al., 1990; Frich and Dolter, 1995; Sanchez-Prieto et al., 1996).

Bcl-2 protein overexpression correlates with resistance to cisplatin in breast cancer (Silvestrini et al., 1994; Teixeira et al., 1995) instead of lung cancer (Wu et al., 1994). In the case of the bcl-2 gene, antisense bcl-2 oligonucleotides have been used to reduce bcl-2 mRNA and, in consequence, its protein expression. As expected, these antisense bcl-2 oligonucleotides increased sensitivity to therapeutic drugs, such as Ara-C and Meto (Kitada et al., 1994). In prostate cancer, it has been proven that the phosphorylated stage of the bcl-2 protein allows the apoptotic mechanism, accompanied with a treatment with taxol, to function (Haldar et al., 1996).

To conclude, the detection of oncogenes and tumor suppressor genes may play an important clinical role in developing more selective therapeutic protocols tailored to the particular alterations of each tumor.

5.C.1. Neu and chemoresistance

A new aspect which has emerged from the evidence implicating several oncogenes in tumor development is the possibility of therapies directed against them. Thus, in the case of the *K-ras* oncogene, it has been shown that farnesil transferase inhibitors have the capacity to cure murine tumors. In the case of the *HER2/neu* oncogene, it was shown that a viral gene, the adenovirus *E1A* can downregulate the transcription of the *neu* gene (Tsai et al., 1996). The *neu/HER-2* oncogene (*c-erbB-2*) is known to be overexpressed, or activated, by point mutation or deletions in many human cancers, including breast, ovarian, cervix, lung, gastric and oral cancers (Bargman and Weinberg, 1985; Slamon et al., 1989; Wilbur and Barrows, 1993; Disis et al., 1994; Mitra et al., 1994). HER-2/neu overexpression was shown to enhance malignancy and metastasis phenotypes. Furthermore, the *Neu* oncogene has been associated with agents such as tumor necrosis factor (Aggarvell et al., 1994).

Neu overexpression has been related to resistance to DNA damaging agents, such as cisplatin (Pietras et al.,1994). It has been shown in non-small-cell lung cancer (NSCLC) that the overexpression of the Neu causes the appearance of chemoresistance phenomena (Tsai et al., 1993). The same occurs in ovarian cancer with hormonal therapy (where it has been shown in the

SKOV3 cell), or in breast cancer with tamoxifen (Borg et al., 1994).

It seems logical to think about blocking this gene's expression in order to reverse the transformation associated with Neu overexpression. It has been shown that the use of the tyrosine kinase inhibitor Emodine is able to enhance the action of drugs such as Cisplatin in cells with HER2/neu expression (Stern et al., 1988; Piertras et al., 1994). In conclusion, the chemo-resistant phenotype associated with neu expression resides in its tyrosine kinase activity (Tsai et al., 1993, 1996; Zhang and Hung, 1996).

Some years ago, it was shown how the expression of E1a in NIH 3T3 cells, previously transformed by the expression of a mutated *neu* gene, reverted this transformation and that this phenomenon was accompanied by significant reductions in neu levels (Yu et al., 1991). It was later shown that the reexpression of neu in cells with E1a expression caused the loss of the antioncogenic effect of E1a, but not the metastatic effect associated with the expression of E1a (Yu et al., 1993, 1995).

Recently, it has been shown that E1a has a therapeutic effect in human tumors with neu overexpression, as well as in the ovary tumoral cell model, SK-OV3. The use of cationic liposomes or an adenovirus vector can efficiently deliver *E1a* genes into tumoral cells and inhibit transcription of the HER-2 promoter. This has resulted in suppression of tumor growth in a murine model (Hung et al., 1995; Zhang et al., 1995). HER-2 is proposed as an excellent target for developing anti-cancer agents.

Adenovirus mutants containing deletions of gene *E1b* are used as vectors to obtain E1a expression. The injection of these adenoviruses produces over 50% tumor repression in animals (Martin-Duque et al., 1999). The use of the *E1a* gene has chemo- and radiosensitivizing advantages, as previously described (Sanchez-Prieto et al., 1995a,b). The *E1a* gene induces sensitivity to drugs such as taxol in cells overexpressing the neu protein (Ueno et al., 1997).

However, some papers have shown that the E1a antitumoral effect in cells with neu overexpression does not exclude the existence of alternative mechanisms (Frisch, 1991; Frisch and Dolter, 1995). This shows that E1a is able to exert its antitumoral effect on diverse cellular systems with multiple genetic alterations without affecting the expression of the *Neu* gene.

5.C.2. Apoptosis, p53 and cellular resistance

The process of apoptosis has recently begun to gain importance in the world of oncology (Fisher, 1994; Milas et al., 1994). On one hand, it is a model for explaining the appearance of tumors, as well as being a mechanism for understanding the cellular response to radiation and chemotherapeutic agents. The existence of genes such as *Bcl-2* (an apoptosis inhibitor) has been associated with the appearance of chemo and radioresistant patterns. It has been proven that bcl-2 overexpression protects prostate cancer cells from apoptosis (Raffo et al., 1995). Likewise, genes of the same family, but with an opposite function, such as *Bcl-Xs*, have been associated with sensitivizing phenomena (Sinha et al., 1995; Sumantran et al., 1995).

In the case of antitumoral agents that provoke DNA damage, one of the genes that appears to be a key is the tumor suppressor gene *p53* (Zhan et al., 1993). The *p53* tumor-suppressor gene is the gene that clearly exemplifies the role of oncogenes and suppressor genes in response to chemo and radiotherapy. Among tumor-suppressor genes, probably *p53* along with *retinoblastoma*, are the most characterized genes (Hinds and Weinberg, 1994). The *p53* gene is a regulator of genes like as the bcl-2 family, *in vitro* and *in vivo* (Miyashita et al., 1994). In some cases, a down-regulation of bcl-2 by p53 (Haldar et al., 1994) and, in other cases, mechanisms of these genes acting independently (Clarke et al., 1993; Strasser et al., 1994), have been described.

The involvement of the p53 protein in chemo and radioresistance processes has emerged over the last years (Lu and Lane, 1993; Fujiwara et al., 1994). The vast majority of studies on the molecular mechanism by which p53 blocks cellular growth have been carried out in situations with DNA damage (Slichenmyer et al., 1993). Under such conditions, p53 accumulation has been observed (Fritsche et al., 1993) that could induce the expression of genes such as p21/WAF (Li et al., 1994), whose proteins bind to cyclin-dependent kinases, inhibiting their activity (Harper et al., 1993; El-Deiry et al., 1994). Recently, it has been demonstrated how p21/WAF is able to block the PCNA protein and its capacity to activate an important proteases (Waga et al., 1994).

The p53 protein and its relation to apoptosis was established in the work of Lowe and collaborators (Lowe et al., 1993a,b). Accordingly, there are studies which relate p53 status to the efficiency of the DOX response and R-gamma *in vivo* (Lowe et al., 1994). In order to observe this correlation, these authors relied on the fibrosarcoma model, originated by the fibroblasts transformed by E1a and activated ras from rats lacking p53 or having normal p53. The authors concluded that the presence of wild-type p53 implies a sensitivity to these treatments, so that tumors from p53-deficient fibroblasts present a resistant phenotype. Also, it was shown how sensitivity is accompanied by apoptotic phenomena.

This same work described how tumors, despite the apparent response of the normal p53 gene, recuperate developing chemo and radioresistant phenotypes. In some cases, this phenomenon was explained via the appearance of mutations in the p53 gene, but in others this alteration was not detected. These data, in the author's opinion, leads to the existence of other alternative mechanisms different to p53.

Even so, there is great controversy over the role of

p53 in chemo and radiotherapy. Moreover, it was shown that VPH E6 expression, which interrupted p53 functioning, did not affect the survival of cells treated with stimuli such as doxorrubicin, ionizing radiation or ultraviolet light. Nevertheless, in this last case there have been controversial results, such as with the RKO cell model (Smith et al., 1995) in which E6 expression provoked a decrease of viability (Hermens and Bentvelzen, 1992). Fibroblasts overexpressing p53 did show a greater sensitivity to cited treatments, accompanied by apoptosis. Previously, it has been described how in an ML1 cell line coming from lymphoblastic leukemia, the cell cycle was interrupted in G0/G1 after R-gamma treatment, and that this fact was dependent on a normal p53 gene presence (Kuerbitz, 1994). Similarly, after treatment with cisplatin, doxorubicin, or etoposid has been observed increases in p53 levels in several cell lines (Fritsche et al.,1993). In this way, the work of Lowe has shown the existence of p53-protein, dependent and independent, apoptosis mechanisms (Clarke et al., 1993; Lowe et al., 1993a-c) .

In this context, p53 gene is suggested as a guardian of the genome (Lane, 1992). The evidence supports the diagram proposed by Lowe, in which after treatment with DNA-damaging agents, the p53 protein can determine whether the cell cycle will continue with G0/G1 control (as with R-gamma) or if apoptosis will be initiated if the damage cannot be repaired. Thus, the p53 protein is important in the chemo and radioresistance response as has been confirmed by an author (Petty et al., 1994) whose study corroborate p53 levels with chemosensitivity.

A seemingly infinite number of studies, with a more practical focus, show the therapeutic potential of this gene, as in the cases of head and neck cancers, colon carcinomas (Benhattar et al., 1996), esophageal (Moreira et al., 1995), etc (Lavieille et al., 1998). In all of these studies the data obtained from various experimental laboratory systems is corroborated. Finally, it must be said that another interesting aspect of the p53 gene is its capacity as a tumoral growth suppressor. In this area there are studies which show that p53-mediated apoptosis is able to suppress *in vivo* progression of human tumoral cells (Liu et al., 1994) or murine tumors (Symons et al., 1994). These results have converted *p53* into one of the stars of gene therapy. The tumor suppressor gene p53, for all the above reasons, is one of the keys to understanding chemo- and radioresistant mechanisms.

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