The Tenth Annual Pezcoller Symposium was held in Trento, Italy from June 29 to July 1, 1998 and focused on issues concerning the genetic predisposition to cancer and genetic modifiers of this predisposition. The main topics discussed were: (a) genes and genetic pathways involved in the development and control of cancer and their alterations; (b) animal models useful in studies of the genetics of cancer and in the elucidation of mechanisms to be searched for in humans; (c) the relationship of gene penetrance to gene expression and their practical significance in carcinogenesis; and (d) the consequences of the development of mutator phenotypes and population studies validating experimental findings and providing leads on the functional relationships of gene action to environmental factors in the etiology of cancer.

Richard Klausner (National Cancer Institute, Bethesda, MD) opened the meeting stressing the importance of genetically determined pathways in the etiology of cancer and pointing out the intricacies of the genetic determinants of cancer and the significance of genetic instability. He also emphasized the importance of carrying out population genetic studies that include identification and measurement of relevant genetic markers.

**Genes and Genetic Pathways**

E. Harlow (Massachusetts General Hospital Cancer Center, Charlestown, MA) reviewed the biochemical activities of pRB and the signaling pathway in which it functions. pRB acts as a negative regulator of transcription by binding to transcription factors such as E2F. During the mid-to-late stage of the $G_1$ cell cycle phase, phosphorylation of pRB changes its conformation, transcription factors are released from interaction, and key target genes are activated. The phosphorylation of pRB is directed by cell cycle-regulated kinases, primarily from the cyclin D/CDK4 subfamily. In turn, the cyclin D/CDK4 kinase activity is controlled by several mechanisms including the binding of p16, a CDK inhibitor. In most human tumors, normal pRB function is lost by mutations that can involve either RB, cyclin D, CDK4, or p16 genes; mutations are seldom found in more than one member of the pRB signaling pathway in the same tumor. This suggests that any method of losing pRB negative regulation is sufficient to promote tumor development. To understand the functional role of the pRB signaling pathway, the phenotype of mutations in the RB pathway were examined in mice. The transcription factor E2F-1 has been inactivated. E2F-1 can act as an oncogene in standard tissue culture experimental models. In the mouse, loss of E2F-1 leads to tumor development, arguing that the E2F-1 is also a tumor suppressor gene. This is the first example of a gene that can act both as an oncogene and as a tumor suppressor gene. Cells with mutations in the RB pathway are being used to examine the role of these proteins in differentiation. For instance, using pRB, p107 and p130 knockout cells, and adding back the genes of interest, it was found that p107 and p130 inhibit differentiation, whereas pRB favors it; this indicated that distinct roles for various proteins in the same pathway can be discerned by this approach.

J.Y.J. Wang (University of California, San Diego, CA) discussed defects in DNA damage-induced regulatory pathways that compromise the integrity of the genome. Genes involved in these pathways are frequently mutated in human cancer. The $Atm$ gene is mutated in the human autosomal recessive disorder AT. Human AT patients are hypersensitive to X-ray and IR, which induce strand breaks in DNA. A high degree of homology exists between ATM and the Schizosaccharomyces pombe Rad3; yeast mutants in Rad3 are unable to express damage-induced genes, and they do not delay cell cycle progression in response to DNA damage. Two regions of homology define the genes involved in these pathways.
Cad3/ATM family of proteins: (a) the Rad3 homology region of unknown biochemical function; and (b) a COOH-terminal kinase domain related to that of the PI3K. Truncation and frameshift mutations of the ATM protein in AT patients eliminate the PI3K function at the COOH-terminal region of the protein. The PI3K domain of ATM can phosphorylate the nuclear c-Abl tyrosine kinase at a specific serine site, and this phosphorylation activates the c-Abl tyrosine kinase. In ATM-deficient human or mouse cells, IR does not activate c-Abl. A physiological substrate of c-Abl is the COOH-terminal repeated domain of RNA polymerase II. Exposure to IR causes an increase in the phosphotyrosine content of RNA polymerase II, and this increase is dependent on ATM as well as c-Abl. The role of c-Abl in damage-induced gene expression, in the regulation of S-phase response to DNA damage, and in the damage-induced inhibition of terminal differentiation was discussed. pRB, a negative regulator of proliferation, binds to a number of cellular transcription factors to repress their function. The nuclear c-Abl tyrosine kinase is a target for inhibition by pRB. Thus, c-Abl is negatively regulated by pRB and positively regulated by ATM. DNA damage-induced G₁ checkpoint response is dependent on pRB dephosphorylation, which can be induced by the CDK inhibitor p21cip1. With S-phase and G₂-phase cells, DNA damage also triggers checkpoint responses. Whereas IR causes a transient inhibition of DNA synthesis, treatment with cisplatin leads to the prolonged inhibition of S-phase progression. Cisplatin causes pRB dephosphorylation in S-phase cells without inhibiting the activity of CDK2 or Cdc2. Microinjection of the constitutively active pRB into S-phase cells leads to a block in DNA synthesis that cannot be overcome by cyclin E or cyclin A. Thus, dephosphorylated RB can inhibit both S-phase entry and S-phase progression. It was also pointed out that c-Abl can be activated by methyl methane sulfonate only in S phase and that nuclear c-Abl plays a role in methyl methane sulfonate-induced myodifferentiation. Nuclear localization of c-Abl is affected by cell adhesion, and cell adhesion is required for G₁-S-phase transition. Thus, it appears that cell adhesion-dependent mechanisms influence DNA damage responses. With the elucidation of DNA lesion-specific signaling pathways, one may eventually be able to apply DNA-damaging agents to tumor cells in a more rational manner, matching defects with drugs to increase the efficacy of cancer therapy.

C. Prives (Columbia University, New York, NY) focused her discussion on signaling to p53 by covalent and noncovalent modifiers. Wt p53 serves as a multifunctional recipient of signals from a wide spectrum of agents of cellular stress. Once activated by such signals, it can induce either cell cycle arrest or apoptosis. There are several ways in which p53 can be regulated. DNA-damaging agents signal to p53 through as yet unidentified posttranscriptional mechanisms. Phosphorylation of human p53 at serine 15 occurs after DNA damage, resulting in reduced interaction of p53 with its negative regulator, the oncoprotein MDM2. Using purified p53-DNA, it was demonstrated that phosphorylation of p53 at serine 15 and serine 37 impairs the ability of MDM2 to inhibit p53-dependent transactivation. These effects are most likely due to a conformational change induced upon phosphorylation of p53. The induction of p53 can be modified by DNA-PK in response to DNA damage. Phosphorylation of p53 at Ser-15 after irradiation is not carried out by either DNA-PK or ATM. The cyclin H/CDK7/p36 (MAT-1) CAK complex can phosphorylate p53 at its NH₂ terminus at serine 33 that appears to be constitutively rather than inducibly phosphorylated in vitro. Latent p53, which binds inefficiently to DNA, is as effective in activating transcription from a p53-responsive template in HeLa nuclear extracts as active p53 that binds DNA efficiently. HeLa nuclear extracts can stimulate DNA binding by latent p53; a p53-stimulating protein purified from them was determined to be the product of the Ref-1 redox/repair gene. Oxidized forms of full-length and COOH-terminally truncated p53 (p53Δ30), which are inactive for DNA binding, are both stimulated by Ref-1 protein. In the presence of reducing agent, Ref-1 is a potent stimulator of full-length p53, but not of p53Δ30. A peptide spanning the NH₂-terminal 40 residues of Ref-1 is capable of stimulating p53, although to a much lesser extent than the full-length Ref-1 protein. Full-length Ref-1 binds to p53 in vitro, and residues mapping between amino acids 127 and 190 are required for the interaction. Thus, Ref-1 protein was found to stimulate p53 by both redox-dependent and - independent means and to play a key role in p53 regulation. Ref-1 can stimulate p53 transactivation function in vitro. The ability of p53 to transactivate target promoters is markedly reduced when endogenous Ref-1 is down-regulated. In conclusion, a novel noncovalent protein modifier of p53 was identified that is likely to play an important role in the regulation of p53 in cells. HMG-1 was found to be another regulator of p53 in HeLa nuclear extracts. HMG-1 can stimulate both wt and p53Δ30, indicating that stimulatory activity does not involve the relief of repression by the p53 COOH terminus. HMG-1 stimulates the formation of higher-order protein-protein complexes on DNA. The possibility that HMG-1, a DNA-bending protein, can facilitate DNA binding to p53 by providing it with prebent DNA was discussed. The expression of p53 can sensitize cells to apoptosis by treatment with agents that damage DNA. p53-null H1299 cell lines were generated that express wt or mutant p53 under a tetracycline-regulated promoter. The induction of wt p53 sensitizes cells to apoptosis upon exposure to diverse chemotherapeutic agents. Many agents also cooperated effectively with a transcriptionally defective p53, p53(Gln22Ser23), but an exception was seen with 5-FU. Exposure to 5-FU sensitized cells to apoptosis mediated by wt p53 but not by p53(Gln22Ser23), suggesting that apoptosis can occur through at least two mechanisms, one dependent on and one independent of the transactivation/repression activities of p53. A p53 protein lacking the final 30 amino acids efficiently cooperated with the DNA-damaging agents, including 5-FU. Although transcriptionally competent, a p53 mutant lacking the pro-domain between residues 63 and 90 failed to cooperate with any compounds tested. The results suggested that at least two genetically separable functions of p53 are capable of inducing cell death. Strategies for future study may examine simultaneous treatment of tumors with chemotherapy and p53 gene transfer.

Animal Models

R. DePinho (Dana Farber Cancer Center, Boston, MA) discussed cancer development in the telomerase RNA knockout mouse. First, he outlined some recent work with the 9p21 locus that encompasses both INK4b (p15INK4b) and INK4a; the latter encodes both the CDK inhibitor p16INK4a and p19ARF, which binds to MDM2 and thus spares p53 from MDM2-mediated inactivation. An emerging concept in cancer biology speculates that pRB and p53 pathways are inactive in most forms of cancer. Consequently, a homozygous deletion of 9p21 would simultaneously disrupt both the pRB and p53 pathways (through p16 and p19ARF, respectively). Next, in collaboration with Carol Greider, he discussed the consequences of rendering mice telomerase-deficient by deleting the gene encoding the telomerase RNA component (mTR) were examined. Mice homozygous null for mTR lacked detectable telomerase activity, yet they were viable and fertile; mTR−/− cells retain the ability to express telomerase activity after reintroduction of the wt mTR gene by transient transfection. In collaboration with Peter Lansdorp, telomere lengths were examined by fluorescence in situ hybridization in serially passaged mTR−/− cells and in cells/tissues derived from successive generations of mTR−/− mice. The estimated rate of loss determined from these studies was ~100 bp/cell division, close to the rate of loss/cell division in human cells. The loss of telomere signal and the appearance of chromosomal...
rearrangements provided direct experimental support for the concept that the absence of telomerase activity leads to telomere shortening and chromosome instability in mammalian cells. The possibility that telomerase-deficiency and/or telomere erosion impact the ability of cells to immortalize was assessed. Surprisingly, the mTR−/− MEF cultures yielded immortal cell lines. Whereas these studies established that telomerase does not serve an essential role in cellular immortalization, it remained possible that telomerase deficiency and/or telomere function impact the rate of spontaneous immortalization. A high rate of immortalization is noted in INK4a (p16)−/− fibroblasts. Colony formation of fifth generation mTR−/− (G5) MEFs that were also INK4a−/− was compared to INK4a−/− MEFs. In the INK4a−/−, mTR−/− (G5) MEF cultures, a 10- to 100-fold reduction in the number of colonies after low density seeding was noted. Thus, maintenance of telomere function, although not essential for immortalization, plays an important role in facilitating this cellular transition.

In collaboration with Maria Blasco and Carol Greider, it was demonstrated that despite telomerase deficiency and accompanying genomic instability, MEFs derived from mice up to the sixth generation (G6) were still able to be transformed by viral oncogenes (T-Ag or E1a) plus RAS; these T-Ag/RAS or E1a/RAS mTR−/− cell lines were able to generate tumors in nude mice. It will also be essential to determine the impact of telomerase-deficiency on the rate of cellular transformation by cellular proto-oncogenes and on the incidence and clinical behavior of tumors arising in various mouse cancer models. To begin to examine the importance of the telomerase pathway in tumorigenesis in vivo, initial efforts were focused on the INK4a-deficient mouse model that develops fibrosarcomas and B-cell lymphomas early in life. Mice doubly null for INK4a and mTR from one to five generations (G1 through G5, respectively) were produced. The telomerase deficiency did not affect the INK4a-null phenotype through the first three mTR−/− generations. However, in the fourth and fifth generations, tumor incidence had decreased significantly. An increase in survival was also evident. The decline in tumor formation and increased survival in the later generations are likely due to the lack of an efficient telomere maintenance mechanism and attendant genetic instability. These findings support the concept that telomerase inhibition may be of use in an antioncologic regimen. It is possible that impaired host functions are the basis for the decline in tumor formation in the late-generation mice. The latter is likely not the case, based upon the diminished transformation efficiency of INK4a−/−, mTR−/− (G5) MEFs compared with INK4a−/−, mTR−/+ MEFs in cell culture studies. In fact, a 3- to 10-fold reduction in Myc/RAS foci for the double-null MEFs were observed; the decreased foci numbers and tumorigenic potential could be rescued by adding back mTR to the Myc/RAS cotransfections. Thus, it appears that the efficiency with which cells acquire an immortal or tumorigenic phenotype is compromised in the setting of severe telomere erosion.

P. Demant (The Netherlands Cancer Institute, Amsterdam, the Netherlands) discussed a genetic dissection of cancer susceptibility in the mouse. The genetic basis of cancer is evident in numerous familial cancer syndromes with Mendelian inheritance of cancer predisposition. The genes responsible for these syndromes have very high penetrance; these genes are selected against, and hence these syndromes are responsible for a very small proportion of cancer cases. Nevertheless, a large proportion of apparently sporadic cancer is likely to occur in a different type of genetically predisposed individuals. The responsible susceptibility genes have a lower penetrance than those causing the familial cancer. The genetic analysis of cancer with a low-penetrance genetic component is virtually impossible in humans, given the current techniques, but can be studied in mice and rats, analyzing tumor susceptibility as a qualitative trait. Subsequently, one can search for homologous loci in humans. The availability of congenic strains, recombinant inbred strains, and RCSs of mice and rats allowed a more detailed study of the phenomenon of tumor susceptibility than observations of family syndromes or population studies in man. Genetic differences in tumor susceptibility have been detected in all species studied. The evidence suggests that there might be considerable polymorphism among the tumor susceptibility genes. Whether this represents a large number of tumor susceptibility genes with a small number of alleles or a limited number of tumor susceptibility genes with a large number of alleles at each locus remains to be investigated. Susceptibility to tumors in different organs usually exhibits a different strain distribution pattern, indicating that different subsets of genes affect the tumor susceptibility in each organ. For example, BALB/c mice are susceptible to mammary tumors but resistant to tumors of the small intestine, but the reverse is true for C57BL/6 mice, whereas both of these strains are resistant to colon tumors, to which STS/A mice are susceptible. To what extent these different sets of genes share certain common loci remains to be seen. Tumor susceptibility genes operate within the target organ, but there is evidence that at least some tumor susceptibility genes have a systemic effect. Individual tumor susceptibility genes affect some but not all aspects of tumor phenotype. Thus, histological type or propensity for malignant progression may be under the control of a different subset of genes than tumor multiplicity or incidence. The bulk of the data obtained until now indicates that the major susceptibility genes are not identical to the major genes known from somatic analysis of cancer cells such as oncogenes, tumor suppressor genes, or mismatch repair genes. As the tumor susceptibility genes are revealed by virtue of the biological effects of their germ-line polymorphism, they should be identifiable by positional cloning. To efficiently map the multiple quantitative trait loci controlling tumor susceptibility, a novel analytic system, the RCSs, was developed. The advantage of the RCS system is that a complex phenomenon controlled by multiple genes can be efficiently analyzed by separating nonlinked genes that control a trait into different strains having largely the same genetic background. A series of RCSs is produced by crossing two standard inbred strains, one of which serves as a background strain, and the other serves as a donor strain. Two generations of backcrossing to the background strain, followed by brother-sister mating, produce a series of new homozygous strains (the RCSs), each of which carries a random fraction of only about 12.5% of the genome from the donor strain and 87.5% of the genome from the common background strain. The use of the RCS system has led to the mapping of cancer susceptibility genes (21 loci) and resistance to infectious diseases [Mycobacterium tuberculosis, L. major (5 loci)] as well as control of lymphocyte function (7 loci), apoptosis (3 loci), and lipid metabolism (1 locus). The positional cloning is most advanced with the Scc1 (susceptibility to colon cancer 1) locus, which has been mapped to an interval of approximately 300 kb. The superior power of the RCS system revealed the phenomenon of mutual interactions of tumor susceptibility genes. For alleles at some tumor susceptibility loci, the characteristic of being susceptible or resistant is not their intrinsic property but depends on the interaction with additional susceptibility loci. Currently, the major loci controlling susceptibility to colon cancer, lung cancer, mammary tumors, and radiation-induced leukemia are being mapped.
determinants of tumor development and progression. Induction of angiogenesis begins before solid tumors or invasive cancers appear. The incidence of apoptosis rises concomitant with hyperproliferation in premalignant stages and falls successively, indicating that acquired resistance to apoptosis is crucial for carcinogenesis. A genome-wide screen was performed to identify additional loci important for transformation; LOH was found on chromosomes 16 and 9. It was found that Loh16 is lost at the angiogenic islet stage at the same ~32% frequency as end-stage tumors. The loss on chromosome 9 only occurs (~22%) in the end-stage tumors. Evidence presented implicated Loh9 as a potential inducer of apoptosis and Loh16 as an angiogenesis suppressor; intriguingly, a new p53 homologue (p63) maps near Loh16. A combination of fine structure mapping, candidate genes, ESTs, and expression profiling on microchip arrays is being used to identify candidate tumor suppressor genes contained within these loci.

T. Van Dyke (University of North Carolina Medical School, Chapel Hill, NC) discussed the p53 tumor suppression mechanisms with emphasis on the apoptosis induced in a CP carcinoma model upon p53 activation consequent to DNA damage or other triggering cellular events. A transgenic mouse model was developed in which epithelial brain tumors are initiated by inactivation of pRB family proteins with an oncprotein derived from SV40 T antigen (T_{121}). T_{121} induces the normally nondividing CP cells to proliferate, and p53-dependent gene expression and apoptosis can occur. p53 inactivation results in an 85% reduction in apoptosis, accelerated tumor growth, and a 7-fold reduction in lifespan. This in vivo system is used to study the molecules that act both upstream and downstream of p53. Expression of bax, p21, and MDM2 is induced in the T_{121} CP cells concomitant with p53-dependent apoptosis. When both bax alleles were inactivated, the tumor growth rate increased significantly, resulting in a 3-fold reduction in survival time. Accelerated tumor development in the absence of Bax correlated with a 50% drop in the apoptotic index. Hence, approximately half of the T_{121}-induced p53-dependent apoptosis is mediated through Bax. Thus, Bax can function as a tumor suppressor as a component of a p53-dependent pathway. Comparing brain tumor progression in T_{121} mice heterozygous for bax or p53, it was shown that p53 inactivation contributes tumor suppressor functions in addition to apoptosis. The role of the E2F-1 transcription factor in the brain tumor model was studied. E2F-1 deficiency, similar to a p53 deficiency, causes an 80% reduction in apoptosis. Transcriptional activation of p53 target genes is also impaired without E2F-1, suggesting that E2F-1 acts upstream of p53. For instance, p21 increases induced by p53 are E2F-1 dependent. E2F-1 is not a tumor suppressor because tumor growth is not accelerated in its absence, as it is in the absence of p53. Without E2F-1, the tumor cell cycle is impaired. In conclusion, E2F-1 is required in the induction of p53-dependent apoptosis and in tumor cell proliferation. These observations provide an explanation for the apparent paradox that E2F-1 can act as an oncogene and a tumor suppressor and indicate that E2F-1 may provide a specific target for cancer drug development.

Penetrance versus Expressivity

A. Balmain (Onyx Pharmaceuticals, Richmond, CA) discussed the genetic basis of tumor predisposition and progression in mice. Indeed, the development of cancer in mammals requires the accumulation within a single somatic cell of mutations in a series of critical growth-controlling genes. Genes that have been shown to suffer mutations in both mouse and human tumors include members of the ras family of small GTPases, p53, p16, and APC. Thus, mouse tumor development often represents a valid model of human cancer genetics. Using transgenic and knockout animals, it was found that different target genes exert their functions at different stages of tumorigenesis. Ras is mutated early in the process of chemical carcinogenesis, at the stage of initiation, and the introduction of ras genes into the germ-line using keratin promoters can recapitulate most of the steps of skin tumor development. Expression of ras in the more differentiated cell populations gives rise to promoter-dependent tumors that do not progress to malignancy, whereas targeting of the same mutant ras allele to the putative stem cell population in the hair follicles generates benign tumors that arise spontaneously and show frequent malignant progression. Cyclin D1 is expressed at the stage of promotion. p53 is mutated in chemically induced tumors at the time of the benign-malignant transition; different subpopulations of cells respond by induction of G1 arrest or apoptosis in a p53-dependent manner after exposure to DNA-damaging agents. The p16/p19ARF/p15 locus is homozygously deleted at the latest stages of carcinogenesis in the skin, suggesting that in this system, p16 is more likely to be involved in metastasis than simply involved in the loss of G1 growth control. Many genes can confer a strong predisposition to tumor development when inherited in mutant form through the human or mouse germ-line. Such mutations are normally highly penetrant and give rise to familial cancer syndromes such as Li-Fraumeni syndrome (p53), familial melanoma (p16), or colon cancer (APC). Low-penetration genes may contribute to the development of sporadic cancers. Mapping and cloning of these low-penetration genes is likely to be simpler in mouse models than in the corresponding human disease. Tumor predisposition in different strains of mice is controlled by multiple loci, which probably control fundamental processes such as tumor growth rate, apoptosis, ability to stimulate angiogenesis, or invasive properties. Genetic background also controls the incidence of tumors. For instance, a ras transgene on a FVB mouse background gives rise to a large number of skin tumors, whereas the same ras transgene generates markedly fewer tumors on a C57Bl6 mouse background. The strongly tumor-resistant Mus spretus species was used to map several genes that control different stages of skin tumorigenesis. Genes on chromosome 7 (Spr1 and Spr2) confer resistance to development primarily of benign tumors, whereas a locus on chromosome 5 (Spr3) can also inhibit the formation of malignant carcinomas. An extensive allelotypic analysis of the genetic alterations that take place during tumor development in interspecific hybrid mice was carried out. Some of the predisposition loci show allele-specific loss or imbalance in the tumors, indicating that they act as bona fide tumor suppressor genes. Spretus mice are also resistant to the development of several other chemically induced types of tumors, suggesting that they may be a good source of polymorphic cancer modifier genes.

The ret mutations and associated developmental and cancer syndromes were outlined by Bruce Ponder (Cambridge University, Cambridge, United Kingdom). Ret encodes a receptor tyrosine kinase that is expressed during development in a small number of tissues that include the neuroectodermal lineages that give rise to thyroid C cells, the sympathetic ganglia, and the gut autonomic nervous system, and the developing kidney and the pharyngeal endoderm (from which the parathyroids arise). Gain of function mutations of ret are associated with the inherited cancer syndrome multiple endocrine neoplasia type 2. Loss of function mutations of ret are associated with the inherited cancer syndrome multiple endocrine neoplasia type 2. Loss of function mutations are associated principally with Hirschsprung disease (absence of the gut autonomic nerve plexuses) and with abnormalities in kidney development. Some mutations can cause gain and loss of function in the same individual. Gain and loss of function phenotypes show variable expression between and within families. Different specific gain of function mutations of ret are associated with different phenotypic pictures in the multiple endocrine neoplasia type 2 syndrome. Although the mechanisms of ret function in normal development and in tumorigenesis remain unclear, it is known that Ret cysteine 634 mutation leads to covalent dimerization;
in familial disease, cysteine 634 is frequently mutated; in sporadic disease, it appears in only 23% of the cases. As a consequence of these mutations, ligand-independent activation of the Ret tyrosine kinase can occur. A framework to account for the different phenotypic effects of different mutations can be proposed, based on abnormalities in the timing of ret signaling during development, the intensity of signaling, and altered specificity of the signaling pathway.

The genetic, cellular, and tissue interactions affecting the intestinal epithelium and its neoplasms were discussed by W. F. Dove (University of Wisconsin, Madison, WI). The importance of the APC molecule in regulating normal and abnormal growth, the role of other factors, and the interactions affecting the neoplastic process was emphasized. The Min strain of the BL6 laboratory mouse and its derivatives were used to study factors that regulate the transition between normal and neoplastic growth. In general, the Min mouse can live only about 120 days before succumbing to intestinal tumors. Using appropriate crosses, it was found that B6 dominance increases allelic loss in APC and penetrance. When adenomas form in the Min mouse, both copies of the APC gene must be inactivated. One copy is mutated by the nonsense APC allele carried in heterozygous form in this strain, and the other copy can be silenced by any of several mechanisms. A somatic mutation at a second locus may constitute a two-hit genetic process as initially proposed by Knudson; for instance, loss of APC determined the formation of adenomas, and then loss of P53 determines progression to carcinomas. The kinetic order for the transition to adenoma may be still higher than two if polyclonal adenomas require stronger interactions than passive fusion. The incidence of desmoid fibroma is increased in p53-null mice, but results in APC-null mice show that APC is also a determinant; thus, there are overlapping lesions. The severity of the intestinal neoplastic phenotype of the Min mouse is dependent upon loci other than APC. Mom1 encodes an active resistance conferred by a secretory phospholipase. Mom1 acts locally within a crypt lineage, not systemically. When tumors arise in Mom1 heterozygotes, the active resistance allele is maintained in the tumor. Indeed, the secretory phospholipase is synthesized by postmitotic Paneth cells, not by the proliferative cells that presumably generate the tumor. Other loci can modify the severity of the Min phenotype. It is important to find ways to identify the full set of genes that interact with the intestinal cancer predisposition of the Min mouse strain. Indeed, when comparing human familial adenomatous polyposis (FAP) families with the Min mouse strain. Indeed, when comparing human familial adenomatous polyposis (FAP) families with the Min mouse strain. Indeed, when comparing human familial adenomatous polyposis (FAP) families with the Min mouse strain. Indeed, when comparing human familial adenomatous polyposis (FAP) families with the Min mouse strain. Indeed, when comparing human familial adenomatous polyposis (FAP) families with the Min mouse strain.

**Mutator Phenotype**

R. Kolodner (Ludwig Institute for Cancer Research, La Jolla, CA) discussed the phenomena of genomic instability mutator genes and cancer susceptibility. Mutator phenotypes and genome instability are characteristics of inherited and sporadic cancers. The lack of mismatch repair defects in human cells with the Min mouse strain, it was seen that multiple colon polyps, small G1 adenomas, desmoid tumors, and epidermoid cysts occur in both. The ENU-mutagenized Min mouse is an excellent model to study the genetics of human colon neoplasia. With appropriately phenotyped human families, one can investigate by a candidate approach which modifying factors influence the epidemiology of human colon cancer. Modifier activities discovered by mouse genetics provide candidates for chemopreventive and/or therapeutic modalities in the human.
The importance of environmental factors in human cancer has long been evident, resulting in estimates that up to 80% of all cancer in the United States is potentially preventable or avoidable. Additional indications come from the shifts in the cancer experience of migrant populations whose rates approximate those of host country, the geographic patterns of cancer within the United States, the changing incidence of certain cancers over time, the racial/socioeconomic differentials for certain cancers, and the epidemiological evidence linking carcinogenic risks to a variety of lifestyle and other environmental exposures. Recent progress in identifying and characterizing highly penetrant but relatively rare susceptibility genes in familial cancer has magnified our understanding of genetic mechanisms and their critical importance in cancer etiology. Potentially more significant to the public health burden are the common polymorphic susceptibility genes that confer low relative and absolute risks but high population attributable risks in the presence of relevant environmental exposures. The two classes of genes represent parts of a continuum, for even the highly penetrant genes responsible for hereditary cancer may involve environmental exposures for expression. This point is illustrated by the susceptibility to radiogenic tumors in hereditary RB and Li-Fraumeni syndrome and by the accelerated risk of hereditary melanoma with p16 mutations from sunlight exposure. Discoveries of polymorphic genes and their functions can also be parlayed into a better understanding of environmental carcinogenesis. For instance, a recent case-control study of oral cancer showed that the risk among heavy drinkers was greatest in subjects homozygous for the 1-1 genotype of alcohol dehydrogenase-3, an enzyme that rapidly metabolizes ethanol to acetaldehyde. Because acetaldehyde but not alcohol is carcinogenic in animal experiments, a plausible mechanism by which alcohol drinking may induce various types of cancer is suggested. Whereas family studies linkage analyses have been highly successful in identifying highly penetrant genes, population-based studies may offer greater promise in clarifying the risks associated with common polymorphic genes and their interactions with exposures. In planning these studies, it is important to carefully consider the candidate genes selected for testing and the particular study design (case control or cohort) to detect associations, along with the statistical power of the study, which depends on sample size and the amount of error involved in measuring exposure and genotype. By integrating careful exposure assessment and mechanistically plausible susceptibility genes into epidemiological studies, it should be possible to clarify the efforts of dietary and nutritional factors, environmental pollutants, and various other exposures involving complex mixtures of agents, and low levels of relative risk. The application of molecular technology tools to study populations with biospecimen collections (molecular epidemiology) should help expedite our understanding of gene-gene and gene-environment interactions in cancer etiology and thus hasten the development of preventive interventions.

The symposium ended with a discussion of future projections in genetic and molecular epidemiology by D. Goldgar (IARC, Lyon, France) and D. Easton (Strangeways Research Laboratories, Cambridge, United Kingdom). Both indicated the importance of highly accurate exposure and genotyping data in the study of gene-environment interactions as well as large sample sizes with sufficient statistical power to detect interactions. The need to develop and apply new technologies to permit genome-wide searches for discovery of susceptibility genes, to coordinate multicentered studies including large cohorts with biospecimen collections such as EPIC, and to evaluate the efficiency of alternative family-based design vis-a-vis population-based approaches was also emphasized.

In addition to the oral presentations summarized above, 16 poster presentations were submitted and discussion sessions were held.

Louise Strong (University of Texas M. D. Anderson Cancer Center, Houston, TX) discussed the cancer risk in Li-Fraumeni syndrome as a model for hereditary cancer susceptibility. In 80% of the cases, p53 missense germ-line mutations are seen; in Li-Fraumeni families, both males and females have an increased breast cancer risk. Breast cancer families with or without p53 mutations were studied.

Louise Strong described results of her study of Li-Fraumeni syndrome and p53 germ-line mutation in a series of systematically ascertained childhood sarcoma patients. Overall, p53 germ-line mutations were rare and, as in tumor-specific mutations, were most often (80%) missense and associated with greatly increased cancer risk. She showed that the cancer risk associated with a p53 germ-line mutation varied by age, gender, and generation, but surprisingly not by p53 mutation type as defined by missense or truncating. Of the highly cancer-prone kindreds identified by the kindred score method from segregation analysis, only about half show p53 germ-line mutations. P53 has been ruled out as the cancer susceptibility locus by mutation analysis (SSCP, direct sequencing), a yeast functional assay, and/or linkage analysis in some classic Li-Fraumeni Syndrome kindreds, suggesting evidence for additional broad tissue specificity cancer susceptibility loci.

Gene-environment interactions in the etiology of cancer were discussed by J. Fraumeni Jr. (National Cancer Institute). The importance of environmental factors in human cancer has long been evident, resulting in estimates that up to 80% of all cancer in the United States is potentially preventable or avoidable. Additional indications come from the shifts in the cancer experience of migrant populations whose rates approximate those of host country, the geographic patterns of cancer within the United States, the changing incidence of certain cancers over time, the racial/socioeconomic differentials for certain cancers, and the epidemiological evidence linking carcinogenic risks to a variety of lifestyle and other environmental exposures. Recent progress in identifying and characterizing highly penetrant but relatively rare susceptibility genes in familial cancer has magnified our understanding of genetic mechanisms and their critical importance in cancer etiology. Potentially more significant to the public health burden are the common polymorphic susceptibility genes that confer low relative and absolute risks but high population attributable risks in the presence of relevant environmental exposures. The two classes of genes represent parts of a continuum, for even the highly penetrant genes responsible for hereditary cancer may involve environmental exposures for expression. This point is illustrated by the susceptibility to radiogenic tumors in hereditary RB and Li-Fraumeni syndrome and by the accelerated risk of hereditary melanoma with p16 mutations from sunlight exposure. Discoveries of polymorphic genes and their functions can also be parlayed into a better understanding of environmental carcinogenesis. For instance, a recent case-control study of oral cancer showed that the risk among heavy drinkers was greatest in subjects homozygous for the 1-1 genotype of alcohol dehydrogenase-3, an enzyme that rapidly metabolizes ethanol to acetaldehyde. Because acetaldehyde but not alcohol is carcinogenic in animal experiments, a plausible mechanism by which alcohol drinking may induce various types of cancer is suggested. Whereas family studies linkage analyses have been highly successful in identifying highly penetrant genes, population-based studies may offer greater promise in clarifying the risks associated with common polymorphic genes and their interactions with exposures. In planning these studies, it is important to carefully consider the candidate genes selected for testing and the particular study design (case control or cohort) to detect associations, along with the statistical power of the study, which depends on sample size and the amount of error involved in measuring exposure and genotype. By integrating careful exposure assessment and mechanistically plausible susceptibility genes into epidemiological studies, it should be possible to clarify the efforts of dietary and nutritional factors, environmental pollutants, and various other exposures involving complex mixtures of agents, and low levels of relative risk. The application of molecular technology tools to study populations with biospecimen collections (molecular epidemiology) should help expedite our understanding of gene-gene and gene-environment interactions in cancer etiology and thus hasten the development of preventive interventions.

The symposium ended with a discussion of future projections in genetic and molecular epidemiology by D. Goldgar (IARC, Lyon, France) and D. Easton (Strangeways Research Laboratories, Cambridge, United Kingdom). Both indicated the importance of highly accurate exposure and genotyping data in the study of gene-environment interactions as well as large sample sizes with sufficient statistical power to detect interactions. The need to develop and apply new technologies to permit genome-wide searches for discovery of susceptibility genes, to coordinate multicentered studies including large cohorts with biospecimen collections such as EPIC, and to evaluate the efficiency of alternative family-based design vis-a-vis population-based approaches was also emphasized.

In addition to the oral presentations summarized above, 16 poster
presentations contributed significantly to stimulating interactions at the meeting.

L. Masramon, R. Arribas, G. Capella, and M. A. Peinado (Institut de Recerca Oncològica & Hospital de Sant Pau, Barcelona, Spain) provided data substantiating the fact that genomic instability characterizes clonal divergence in tumor cells and is responsible for the development of tumor cell heterogeneity. Genetic clonal divergence was evaluated in three established human cell lines: (a) SW480 (mutated p53; hypodiploid; RER−); (b) LoVo (wt p53; hyperdiploid; RER+); and (c) HCT116 (wt p53; diploid; RER+), which are representative of different pathways of tumor progression in colorectal cancer. Genomic divergence has been investigated by a DNA fingerprinting technique. The relative degree of heterogeneity was measured as the percentage of DNA bands differentially displayed in the clones and subclones with regard to the parental cell line. A higher degree of genetic divergence was found in the clones of the LoVo and SW480 cell lines in comparison with those of the HCT116 cell line. The LoVo cell line and its clones show no heterogeneity at the ploidy level but showed heterogeneity at the chromosome level as visualized by cytogenetic and comparative genomic hybridization analyses.

N. Neff, J. Geman (New York Blood Center, New York, NY), T. Ye (Memorial Sloan-Kettering Cancer Center), M. Pyrochva, M. Sanez (New York Blood Center), and S. Ciocci (Memorial Sloan-Kettering Cancer Center) presented data on the genomic instability evident in BS and the role of the BLM helicase in maintaining genomic stability. The hypermutability of BS cells is manifest at metaphase by increased chromosome breakage and chromatid exchange. In addition, bromodeoxyuridine-treated BS cells exhibit an excessive rate of SCEs. Recently, the BS gene BLM was isolated and shown to encode a 1417-amino acid protein homologous to the bacterial DNA helicase RecQ. The RecQ helicases include the human genes RECQL and WRN, the S. cerevisiae gene SGS1, and the S. pombe gene Rqh1. The function of the RecQ helicases in DNA replication and repair is unknown. The wt BLM cDNA was transfected into BS cells. The SV40-transformed BS fibroblast cell line GM08505B was used as a recipient for a full-length cDNA cloned in an expression vector (pOPRSVI-BLM). The SCE frequency of GM08505B cells is high. In pOPRSVI-BLM-transfected cells, the SCE frequency was corrected toward normal. This level of correction in GM08505B cells was similar to that observed when these cells were hybridized to normal cell lines or when a chromosome 15 carrying a normal BLM gene was introduced into them. Three mutations have been introduced in BLM by site-directed mutagenesis. The mutated proteins have no in vitro helicase activity. Transfection of the mutated BLM cDNAs into GM08505B cells fails to correct their high-SCE phenotype. Therefore, helicase activity is required for BLM function. Thus, it was demonstrated that helicase activity is necessary for correction of the high-SCE phenotype in BS cells.

M. Castellano (Present address: Istituto Nazionale Tumori, Milan, Italy) P. M. Pollack, B. G. Gabrielli, P. G. Parsons, and N. K. Hayward (Joint Experimental Oncology Program, Queensland Institute of Medical Research, Herston, Australia) presented a study of the status and the inactivation mechanisms of CDKN2A, a melanoma susceptibility gene that encodes the p16 protein and is homozygously deleted or mutated in a large proportion of tumor cell lines and, to a lesser extent, in primary tumors, including melanomas: 60 cell lines derived from sporadic melanomas were used. Twenty-six of the melanoma cell lines resulted to be homozygously deleted for CDKN2A, and an additional 15 cell lines carried missense, nonsense, or frameshift mutations. Eleven cell lines expressed a wt protein, but in five of them, microsatellite analysis revealed LOH at the markers immediately surrounding CDKN2A, and in one cell line, the R24C mutation of CDK4 was identified. The overall data indicated that 55 of 60 melanoma cell lines demonstrated some aberration of CDKN2A or CDK4, thus suggesting that this pathway is a primary genetic target in melanoma development.

A. Kindler-Röhborn, B. U. Kölsch (University of Essen Medical School, Essen, Germany), C. Fischer, and M. F. Rajewsky (University of Heidelberg, Heidelberg, Germany) presented evidence that two distinct loci on chromosome 10 may be involved in strain-specific susceptibility and progression of ENU-induced schwannomas in the rat. In fact, whereas BDIX rats develop schwannomas with an incidence of >98% after ENU exposure on postnatal day 1, BDIV rats are almost entirely resistant. The T:A→A:T transversion mutation at N^2012 of the neu/erbB-2 gene located on chromosome 10q32.1 is diagnostic for ENU-induced rat schwannomas. (BDIX × BDIV) F1 and F2 generation rats were treated with ENU. The development of schwannomas was strongly suppressed in the F1 generation. LOHs on chromosome 10 were found in 100% of F2 trigeminal schwannomas with a telomeric consensus region excluding neu/erb B-2. Targeted linkage analysis for markers on chromosome 10 mapped schwannoma susceptibility to a region located 10 cM centromeric of neu/erbB-2 that is also deleted in 94% of F1 schwannomas.

L. Lazaravec, L. Collavin, R. Uterra, D. Delia, and C. Schneider (LNCIB, AREA Science Park, Trieste, Italy and Instituto Nazionale Tumori, Milan, Italy) provided evidence for a novel p53-inducible gene encoding for a microtubule-localized protein with G2-phase specific expression. Recently, increasing evidences have accumulated suggesting that wt p53 is also involved in the control of the G2 phase. By a subtractive hybridization approach in Balb/c Val5 fibroblasts, six genes were isolated that are markedly induced by wt p53. One of them, named B99, encodes for a novel protein that was shown to localize to the microtubule network. Evidence was obtained that B99 is indeed a direct target for transcriptional regulation by p53 and that it is cell cycle regulated even in the absence of DNA damage or other p53 stimuli. When B99 was ectopically expressed in murine and human cells, it inhibited cell growth and caused an increase in the fraction of cells with 4 n DNA content.

T. Raveh, E. Feinstein, O. Cohen, B. Inbal, J. Kissil, H. Berissi, R. DePinho (present address: Dana Farber Cancer Institute, Harvard Medical School, Boston, MA), and A. Himchi (The Weizmann Institute of Science, Rehovot, Israel) studied DAP-kinase, which is a positive mediator of IFN-induced programmed cell death and also mediates Fas and tumor necrosis factor-induced apoptosis. The kinase is associated with the actin microfilament system. Overexpression of DAP-kinase in the absence of external signals induced apoptosis, for which the catalytic activity was essential. An expression library of randomly fragmented DAP-kinase cDNA was generated and applied to a functional selection in HeLa cells treated with IFN. Protein fragments and peptides were derived from different functional domains of the protein, which acted in a dominant negative manner to inhibit the proapoptotic activity of the endogenous DAP-kinase; the COOH-terminal death domain module was one of the critical elements. Overexpression of the entire death domain module enhanced the growth of Rat-1 cells in soft agar. Several lines of evidence link DAP-kinase to the negative control of carcinogenesis and link the loss of DAP-kinase function to decreased apoptosis in restrictive environments.

S. Giordano, A. Maffé, P. Michieli, C. Basilio, P. Longati, A. Bordelli, and P. M. Comoglio (University of Turino, School of Medicine, Institute for Cancer Research and Treatment, Candiolo, Italy) discussed the induction of biological responses by MET mutants identified in human PRC. Recently, germ-line and somatic mutations were identified in the tyrosine kinase domain of the MET gene in
affected members of hereditary PRC families and in a subset of sporadic papillary PRC. MET cDNAs containing the point mutations found in hereditary PRC and PRC were stably expressed in fibroblasts and epithelial cells. Mutants with up-regulated kinase activity transformed NIH 3T3 fibroblasts. However, increased enzymatic activity did not correlate with the acquisition of different biological properties. Correlations between the activation of specific pathways and the acquisition of different biological properties were presented.

M. Falchetti, C. D’Amico (University “La Sapienza,” Rome), A. Amorosi (University of Florence, Florence, Italy), C. Saieva (Careggi Hospital, Florence, Italy), G. Masala (ISI-CSPO, Florence, Italy), L. Frati (University “La Sapienza,” Rome, and IMNS, Pozzilli, Isernia), A. Cama (University “Gabriele D’Annunzio,” Chieti, Italy), D. Palli (Careggi Hospital), and R. Mariani-Costantini (University “Gabriele D’Annunzio”) studied 50 gastric cancer cases of MSI+ status to verifying the associations between MSI at dinucleotide repeats and mutations at coding mononucleotide runs within the TGF-βRII, IG-FIIR, and BAX genes that are involved in cell growth control and within the mismatch repair genes hMSH6 and hMSH3. In addition, a trinucleotide coding repeat within E2F4 was analyzed, as was a mononucleotide coding repeat within BRCA2. In 44 tumors, the status of BAT-26 and of a poly(A)8/(T)15 repeat within the 3’ UTR of the E-Cadherin gene were examined. Cases that were negative for instability at BAT-26 and at the E-Cadherin 3’ UTR repeat but positive for mutations at two or more dinucleotide microsatellites and/or at coding mononucleotide repeats were further analyzed using BAT-40. The TGF-βRII, IG-FIIR, BAX, hMSH6, hMSH3, and E2F4 repeats were altered in 11, 5, 4, 16, 5, and 5 cases, respectively. Mutations occurred only in MSI+ tumors and correlated with increasing MSI levels. No alterations of the BRCA2 repeat were found. Mutations in genes other than hMSH6 were strongly associated with hMSH6 mutations, suggesting a key role of this gene. A subset of tumors with MSI at two or more dinucleotide microsatellites and/or at coding mononucleotide repeats suggested the possibility of independent pathways controlling mononucleotide and dinucleotide stability. The overall results were consistent with the model of progressive mutator mutations that drives a cascade of genomic alterations at sequence repeats.

W. Zheng, M. Gross, D. Campbell, P. Mink, J. R. Cerhan, L. Kushi, T. Sellers, K. Anderson, W. Oetting, and A. R. Folsom (South Carolina Cancer Center, Columbia, SC) presented data on the polymorphisms of the NAT2, cytochrome p450 1A1 (CYP1A1), and glutathione S-transferase (GSTM1) genes in relation to the risk of breast cancer. A nested case-control study was conducted in the Iowa Women’s Health Study on a prospective cohort study of 41,837 women (ages, 55–69 years). Information on cigarette smoking and other breast cancer risk factors was obtained from the baseline survey conducted in 1986. Genomic DNA samples were assayed for polymorphisms of the NAT2, CYP1A1, and GSTM1 genes using PCR/RFLP techniques. None of the individual enzyme genotypes were found to be associated with the risk of breast cancer. There were suggestive elevated risks of breast cancer for women with combined risk genotypes of CYP1A1 and GSTM1 and CYP1A1, GSTM1, and NAT2. The findings suggested that the genetic polymorphisms investigated in this study may not be major susceptibility factors for breast cancer among postmenopausal Caucasian women.

M. G. V. DeBenedetti (Istituto Nazionale Tumori) outlined an analysis of the BRCA1 and BRCA2 genes in Italian breast and/or ovarian cancer patients and the characterization of 26 novel and 22 recurring germ-line mutations. Thus far, more than 300 distinct mutations and polymorphisms have been described in BRCA1, and more than 100 distinct mutations and polymorphisms have been described in BRCA2. Recently, p53 somatic mutations have been reported in breast carcinomas that are associated with BRCA1 germ-line mutations. A total of 199 probands were analyzed for exons 2, 5, 8, 11, 12, 16, and 20 of the BRCA1 gene, and 234 probands were screened for exons 10 and 11 of BRCA2 by the protein truncation test, SSCP, and sequence analysis. All cases had family history or tumor onset at an early age. Twenty-nine of 48 distinct mutations were deletions, 12 were insertions, and 7 were base substitutions that produced a stop codon. Analysis of BRCA1 or BRCA2 was extended to relatives of probands carrying a germ-line mutation. There were 71 symptomatic and 28 asymptomatic BRCA1 mutation carriers and 16 symptomatic and 6 asymptomatic BRCA2 mutation carriers. Six recurring BRCA1 mutations were detected in more than one family. Twelve and six males were carriers of a germ-line mutations in the BRCA1 and BRCA2 genes, respectively.

R.-A. Risques, E. Marcuello, S. Tortola, R. Arribas, G. Capella, and M. P. Peinado (Institut de Recerca Oncologica & Hospital de Sant Pau) described a study aimed at characterizing and quantitating genomic damage (other than that produced by the microsatellite mutator phenotype) in human colorectal tumor samples by DNA fingerprinting. Colorectal tumor samples (119) and the paired normal mucosa were analyzed. Correlations between the GDF and the molecular and clinicopathological parameters were investigated. Tumors with p53 mutations showed a higher GDF. Mutations in the p53 gene were an indicator of poor prognosis, whereas GDF was not; however, in cases with a p53 mutation, increased levels of GDF were associated with diminished survival.

C. Ruivenkamp, T. van Wezel, F. Stassen, and P. Demant (The Netherlands Cancer Institute) discussed gene interactions in multigenic susceptibility to colon cancer. The genetics of susceptibility to colon tumors in mice was studied using the C57Bl6j/D2 Rcs. Each of these strains contains a different random subset of approximately 12.5% of genes from susceptible strain STS/A and 87.5% of genes from the relatively resistant strain BALB/c. Ten loci controlling susceptibility to chemically induced colon tumors have been mapped on chromosome 2, chromosome 1, chromosome 17, and chromosome 18. Recently, five additional loci have been mapped to chromosomes 5, 3, 8, 10, and 11, respectively. Most and possibly all of these loci differed from the oncogenes, tumor suppressor genes, and mismatch repair genes known to be mutated in tumors. Unexpectedly, several of these loci exhibited the phenomenon of reciprocal interaction, in which an allele is not intrinsically susceptible or resistant, but its effect depends on the genotype at the interacting locus. Therefore, the products of both loci are likely to interact functionally, which could facilitate their identification.

I. Gonzalez, S. Tortola, E. Marcuello, V. Moreno, M. A. Peinado, and G. Capella (Institut de Recerca Oncologica & Hospital de Sant Pau, Barcelona, Spain) prospectively evaluated the prognostic significance of p53 and K-ras gene mutations in colorectal cancer. They analyzed 140 patients. Eight tumors with the microsatellite mutator phenotype were excluded. Mutations at the K-ras and p53 genes were detected and characterized by RFLP, SSCP, and sequencing as appropriate. p53 mutations were detected in 66 of 132 patients, and K-ras mutations were detected in 54 of 132 patients. In 26 cases, ras and p53 mutations coexisted, and in 38 cases, neither mutation was found. Survival was strongly correlated with the presence of p53 mutation, alone or in combination with K-ras mutations.

T. Dittmar, F. Entschladen, A. Paschka, B. Niggemann, and K. S. Zänker (Institute of Immunology, Witten, Denmark) studied the functional involvement of the tumor promotor PMA in the migration of different tumor cell lines. PMA induces PKC-dependent migratory activity in T lymphocytes. The locomotory behavior of breast adenocarcinoma cell lines MDA-NEO and MDA-HER2 and one melanoma...
cell line, MV3, was observed. The MDA-NEO and MDA-HER2 cell lines were generated by stable transfection with a control vector (MDA-NEO) and a c-erbB-2 expression plasmid (MDA-HER2). PMA treatment of MDA-NEO cells showed a tremendous increase of migratory activity. In contrast, the c-erbB-2-positive variant MDA-HER2 showed only a minor increase in locomotory activity that could be induced by EGF stimulation. The EGFR-positive and c-erbB-2 negative MV3 melanoma cells reacted to PMA with decreased migratory activity but enhanced development of pseudopodia; thus, polarity is a prerequisite for migration, but it does not necessarily include migratory activity. The in vivo activator of PKC is diacylglycerol, which is produced by the activity of phospholipase C-γ, and this enzyme, in turn, is activated by receptor tyrosine kinases of the EGFR family. PKC and EGFR are thereby tightly connected in signaling pathways, but the c-erbB-2-negative MDA-NEO cells increased locomotory activity after PMA treatment but not after EGF treatment, whereas the c-erbB-2-positive MDA-HER2 cells increased locomotory activity after EGF treatment but not after PMA treatment. Thus, the signaling pathways that lead to migration are independent from each other.

F. Contegno, S. Marchini, and M. Broggini (Mario Negri Institute, Milan, Italy) described the mechanism of action of a new, nonalkylating minor groove DNA binder derivative of dystamycin A (PNU 151807). Genes potentially involved in drug-induced cytotoxicity or resistance were evaluated by the MICRO ARRAY (GENOMESYSTEM) technology. mRNA has been purified through an oligodeoxythymidylic acid column from A2780 ovarian cancer cell lines after PNU 151807 treatment at doses equivalent to its IC50. Data obtained after phosphorimager analysis revealed that there were at least 15 cDNAs that were differentially expressed. Among these, the hPMS2 cDNA appeared to be induced after PNU 151807 treatment; two consensus sequences for the p53 protein are present in the hPMS2 promoter.

L. Scopsi (Fosdinovo, Italy) and F. Tacconi outlined problems related to information and counseling on hereditary tumors as accessed through the Internet. The experience gained with an Italian Web site devoted to information and counseling on hereditary and familial tumors was described. During the period from July to December 1997, there were more than 21,000 requests for documents from the site, which means an average of 120 requests daily. Of the inquiries, 75% concerned oncology, and 45% were specifically about syndromes with increased susceptibility to cancer. Genes for susceptibility to common diseases are being cloned with increasing frequency, thus anticipating the ability to determine an individual’s genetic predisposition to cancer, infections, or degenerative diseases. In the face of this situation, the illiteracy health professionals manifest in the field is a serious matter of concern.
Tenth Annual Pezcoller Symposium: The Genetics of Cancer Susceptibility

Enrico Mihich, Louise Strong and Richard Klausner


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