News

Genetic and Epigenetic Determinants of Premalignant and Malignant Phenotype

THE SIXTH biannual Sardinian International Meeting was held on 15–18 October 1991 in Alghero, Italy. The primary emphasis was the relevance of genetic and epigenetic modifications in carcinogenesis. The meeting began with a special lecture by Peter Bannasch (Heidelberg) on the relevance of animal models for the elucidation of early stages of neoplastic development. From studies in several animal models of carcinogenesis, particularly from hepatocarcinogenesis induced in rodents by chemicals, the concept of preneoplasia, indicative of an increased risk for neoplastic development, has been inferred. Although phenotypic cellular changes found in preneoplastic lesions are heterogeneous, most reflect early metabolic aberrations. Similar preneoplastic lesions have been identified in laboratory animals and in humans. Thus, elucidation of biochemical and molecular aspects of preneoplasia are relevant to the mechanism of neoplastic conversion as well as to early diagnosis and cancer prevention.

Molecular basis of aberrant differentiation and progression in human cancer

The first plenary session was opened by Carlo M. Croce (Philadelphia) who discussed the role of chromosome translocations in the pathogenesis of human hematopoietic malignancies. In most B and T cell leukaemias and lymphomas the translocations involve juxtaposition of cellular protooncogenes with the loci for human immunoglobulins or T cell receptors, respectively. In pre-B cell leukemias with the t(1:9) chromosome translocation and in myeloid leukaemias chromosome translocations lead to gene fusion and to expression of aberrant chimeric protein products. The molecular mechanism of chromosome translocation in non-B and non-T hematopoietic malignancy is not known. The analysis of chromosome rearrangements in human hematopoietic malignancies has permitted the isolation and characterisation of a number of genes involved in the pathogenesis of human leukaemias and lymphomas. The discovery of a new oncogene (PTC) in human thyroid papillary carcinomas was reported by Giancarlo Vecchio (Naples). PTC is activated in 20–25% of human thyroid papillary carcinomas. It was isolated by DNA transfection of NIH/3T3 cells. PTC results from the fusion of the tyrosine kinase domain of *ret* protooncogene with an unknown 5′ sequence, designated as H-4. This rearrangement occurs via a chromosomal inversion and is present in the original tumour DNA and lymphonodal metastases, but not in normal thyroid DNA from the same patient, in other types of thyroid cancer or in any other human cancer. Both *ret* and H-4 were mapped to chromosome 10 (*ret* to band q11.2, H-4 to band q2.1). Amplification, overexpression and rearrangement of protooncogene c-*met* and expression of structurally altered proteins occur in various tumors and in preneoplastic lesions in colonic carcinogenesis. C-*met* encodes four major RNAs of 9.7, 5.2 and 3.4 kb. The c-*met* protein ($p190^{c\text{-}met}$) is a heterodimer of two disulphide linked α (50 kd) and β (145 kd) chains. According to Paolo Comoglio (Turin), it is a truncated form of receptor whose β-chain lacks the cytoplasmic kinase domain. Kinase activity of $p190^{c\text{-}met}$ is regulated by tyrosine kinase autophosphorylation. Hepatocyte growth factor (HGF) binds c-*met* receptor and stimulates its intrinsic tyrosine kinase activity, indicating that c-*met* plays a role in the control of cell proliferation. As an introduction to experimentally induced cervical carcinomas utilising human cervical epithelial cells. J. DiPaolo (Bethesda) pointed out that a specific subset of human papilloma virus (HPV), detectable in over 90% of cervical cancers, can immortalise human epithelial cells. Moreover, binding of HPV encoded E7 oncoprotein to the retinoblastoma protein (Rb), causes loss of Rb protein function, and binding of E6 protein to p53 results in a complexing with p53 *in vitro*. This type of interaction, however, does not represent the only mechanism of cell transformation by these viral proteins. DiPaolo reported that high-titre of E6 and E7 oncoproteins, encoded by recombinant retroviruses, stimulated cell proliferation and delayed differentiation. A correlation between the magnitude of these changes and levels of E7 expression suggested a causal relationship. Thus, perturbation of cell differentiation by HPV oncoproteins represents an early change predisposing to malignant progression. Cell immortalised by recombinant HPV 16 became tumorigenic after transfection with a fragment of herpes simplex virus 2. This observation supports epidemiological evidence that papillomaviruses are not the only causal agents in carcinogenesis.

Carmen Sapienza (La Jolla) closed the section by reviewing genome imprinting, a process that results in the gamete-of-origin-dependent modification of phenotype. One line of evidence agrees with the hypothesis that in many paediatric tumours a suppressor gene is inactivated by genome imprinting. The second allele is lost, by non-disjunction or mitotic recombination, and tumour occurs. If this hypothesis is correct, the inactivation of tumour suppressor allele depends on the activity of genes controlling the imprinting process. Thus, in families in which genome imprinting is not involved, the disease (i.e. retinoblastoma) will cosegregate with markers linked to a tumour suppressor gene. No such cosegregation occurs when genome imprinting is involved.

DNA methylation and gene expression in carcinogenesis

This session commenced with the dynamic DNA changes during embryonic development, illustrated by Aharon Razin (Jerusalem). Methylation patterns of gene sequences in mammals are cell and tissue specific; genes are generally hypomethylated at their 5′ ends in all tissues, including sperm, and unmethylated in tissues that express the specific gene. Evidence was presented indicating that gene-specific methylation patterns depend on interplay between *de novo* methylation and demethylation at some embryonic early stages. Polymerase chain reaction (PCR) assay of methylation status of housekeeping and tissue-

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specific genes, expressed in embryo or only in adult tissues, revealed that many sites undergo demethylation at the morula stage and a gradual *de novo* methylation in the gastrula stage. Some sites become methylated very late in embryonic development or after the birth. Walter Doerfler (Köln) observed that the inactivation by methylation of late E1A and E2A promoters of adenovirus type 12 and 2 DNA, respectively, can be partially reversed by the E1A-encoded 289 aminoacid transactivator of Ad2 or by strong immediate early enhancer of human cytomegalovirus, without the concomitant loss of 5-methylcytosine. *De novo* methylation of integrated adenovirus genomes is initiated in the middle of the integrated viral genome and spreads across the entire genome reaching the junction to cellular DNA. Spreading of *de novo* methylation is affected by the presence of protein bound to DNA in the late EA2 promoter of Ad2 DNA. The hypothesis was proposed that *de novo* methylation represents a cellular defence mechanism against the expression of foreign DNA serendipitously integrated into mammalian host DNA.

The role of DNA methylation in carcinogenesis was also discussed. Hypomethylation of specific sequences in gene promoter is a condition necessary, though not sufficient, for gene expression. Interplay between demethylation and *de novo* methylation may regulate the expression of genes critical for carcinogenesis. Hypermethylation of regulatory sequences of suppressor genes and hypomethylation of regulatory sequences of oncogenes could occur during carcinogenesis.

One way to circumvent the difficulty of studying the effect of DNA methylation in the initiation step, is the evaluation of the methylation pattern of oncogenic viruses or *in vitro* transforming genes. Jiudith K. Christman (Detroit) inserted bacterial CAT gene into hepatitis B virus (HBV) enhancerII/promoter to test the hypothesis that a long latency period of HBV-associated hepatocellular carcinoma (HHC) is linked to a methylation-mediated silencing of virus-regulated sequences. Evidence was provided indicating that methylation of enhancer/promoter regions may suppress HBV gene expression by blocking formation of complexes with positive regulatory proteins and enhancing binding of negative regulatory proteins.

As discussed by J. Locker (Pittsburgh), most tumours show multiple abnormalities in DNA methylation, such as global hypomethylation, increased methylation, and/or a marked increase in DNA methyltransferase activity. A common feature of premalignant colonic polyps and of precancerous liver lesions is a proportional loss of methylation at all sites in a gene. However, there is no compelling evidence to suggest that reduced methylation results in heritable epigenetic changes that are essential features of carcinogenesis. The role of DNA hypomethylation in liver carcinogenesis has been evaluated in rats fed methyl-deficient diets (MDD). Elsie Wainfan (New York) stressed a possible role of DNA hypomethylation in cancer promotion by MDD. Prolonged intake of MDD is carcinogenic for the liver of male F344 rats and B6C3 mice. Choline and methionine supplementation prevents or diminishes the effects of some chemical carcinogens. 1 week of feeding a MDD led to hypomethylation of rat liver DNA and tRNA, and expression and hypomethylation of p53, c-*myc*, c-*fos*, and c-Ha-*ras*. These changes persisted or were amplified by 4 weeks of MDD feeding which, however, resulted in decreased levels of mRNAs for epidermal growth factor (EGF) and its receptor. After return to a complete diet, the rates at which the various changes in methylation and gene expression were reversed were not the same for all of the genes or all of the processes studied. J. Locker has a divergent explanation. MDD induces p53 mutation and c-*myc* gene amplification in liver precancerous cells, indicating that methyl deficiency may be considered in a genetic and an epigenetic context. DNA adducts are not detected in the liver of animals treated with MDD alone, thus excluded direct chemical modification of DNA by MDD. A MDD induces cell damage, cell death and compensatory cell proliferation, and increases the probability of inducing mutation. Methyl deficiency would also impair strand discrimination during DNA repair. Some evidence in favour of a role of DNA methylation in liver cancer promotion and progression was discussed by Francesco Feo (Sassari). A fall in S-adenosylmethionine (SAM) liver content and SAM/S-adenosylhomocysteine (SAH) ratio occurs in liver of carcinogen-treated rats fed a diet containing adequate lipotrope amounts, during the development of early preneoplastic lesions and in liver modules. This change is associated with a fall in global DNA methylation and hypomethylation and overexpression of c-*myc*. c-Ha-*ras* and c-Ki-*ras*. Treatment with exogenous SAM causes a dose-related recovery of SAM level, SAM/SAH ratio, DNA methylation and inhibits DNA synthesis and c-*myc*, c-Ha-*ras*, and c-Ki-*ras* expression. A fall in the incidence of preneoplastic and neoplastic lesions occurs, even several months after SAM treatment. The observation that SAM effect is partially overcome by 5-azacytidine, an inhibitor of DNA methyltransferases, suggests a role of DNA methylation. Lionel A. Poirier (Jefferson) showed that rats chronically fed a MDD, with/without an initiating dose of diethylnitrosamine (DENA), developed cells resembling hepatocytes in small clusters around the islet of Langerhans. 5-Azacytidine induced transdifferentiation of *in vitro* cultured pancreatic acinar cells to hepatocyte-like cells characterised by increased proliferation of endoplasmic reticulum, peroxisomes and Golgi apparatus, increased growth in soft agar, global DNA hypomethylation and hypomethylation and overexpression of c-Ha-*ras*. Similar changes were found by transfecting a SV-40 activated c-Ha-*ras* gene into primary cultures of pancreatic cells. Thus, c-Ha-*ras* activation may be both an effect and a cause of DNA hypomethylation. It is thus apparent that altered methylation can affect the differentiation changes in preneoplastic and neoplastic tissues.

Oncogenes, anti-oncogenes and cancer development

Alterations of cell proliferation may involve abnormalities in oncogene and anti-oncogene function, growth factors, growth factor receptors and signal transduction. Some of these mechanisms were discussed. An overview of the RB gene in tumorigenesis was given by Philip W. Hinds (Cambridge). Deletion of the Rb gene is responsible for retinoblastoma development and the progression of various tumours types. Rb gene encodes a 105 kd nuclear protein; phosphorylation occurs in late G1 and S and is reversed in M. pRb is bound by at least three tumour virus oncoproteins, two of which preferentially associate with hypophosphorylated pRB *in vitro*. Both phosphorylation and oncoprotein binding inactivate the putative function of pRb as a suppressor of cellular proliferation. In many human tumours the oncoprotein-binding domain is altered by mutation. Phosphorylated or mutant forms of pRb bind weakly to the nucleus. Only nuclear-bound hypophosphorylated pRb is functional, but this interaction may not be sufficient for pRb function. Tumour suppressor gene activity has been well established for Rb and p53 genes, but only inferred for other genes. Eric J. Stanbridge (Irvine) has provided functional evidence for the existence of multiple suppressor genes, derived from cDNA transfection and monochromosome transfer studies. A novel tumour suppressor

gene for neuroblastoma mapped to chromosome 17, and for Wilms' tumour to 11p15. Interestingly, although multiple tumour suppressor gene defects were found in colorectal carcinoma cells, the correction of a single tumour suppressor gene defect was sufficient to induce tumour suppression. Michael A. Tainsky (Houston) investigated skin fibroblasts from Li-Fraumeni familial cancer syndrome (LFS) for genetic changes responsible for full tomorigenic transformation. LFS patients have inherited certain point mutations in one of their p53 genes, and immortal LFS fibroblasts have lost the remaining wild type p53. This loss correlated with immortalisation, but was not sufficient for cells become susceptible to transformation by activated Ha-*ras* or N-*ras*. Perhaps, the loss of *ras* tumour suppressor is necessary for tumorigenic transformation. Thomas J. Gill III (Pittsburgh) has developed a model for tumour suppressor genes in the rat involving genes linked to the major histocompatibility complex in the growth and reproduction complex which influences growth and susceptibility to chemical carcinogens. The susceptible animals have a 70-kb deletion in the major histocompatability complex-linked region, suggesting the loss of a suppressor gene. The deleted region contains a TL-like gene, designated as RT(5.8), whose sequence is similar to those of the TL genes in several mouse strains. Similar types of genes seem to exist in humans. A different study of tumour susceptibility was presented by Tommaso Dragani (Milan) who studied the incidence of Ha-*ras* gene mutations at codon 61, in spontaneous or DENA- or urethan-induced liver tumours of genetically susceptible and genetically resistant mouse strains. Liver tumours induced by DENA, urethan or spontaneously occurring have a different pattern of Ha-*ras* mutation at codon 61. These mutations were an infrequent molecular alteration in the pathogenesis of liver tumours in genetically resistant strains.

Dominant cancer genes may have multiple roles in carcinogenesis. Ubaldo Armato (Verona) exposed rat fetuses to dimethylnitrosamine (DMNA) *in utero*. A persistent rise in expression of c-*myc* and of the gene encoding the nuclear poly(ADP-ribose) polymerase (pADPRP) occurred in primary cultured of postnatal hepatocytes. Treatment of DMNA-initiated cells or normal neonatal hepatocytes with a single dose of phenobarbital (PB), after 4 days *in vitro* had no effect on c-*myc* expression, but induced within 0.5–2 h expression of c-*fos* and c-*jun* genes, and a further increase in pADPRP gene expression at the 4th h, when histone H3 mRNA was expressed. Thus, c-*myc* and pADPRP gene hyperexpression seem to mark DMBA initiation of hepatocytes. Expression of c-*fos*, c-*jun*, H3 and pADPRP genes could be related to the specific mitogenic response elicted by PB. The role of growth hormone (GH) regulation of c-*myc* and c-*ets*-2 genes during sex differentiated carcinogenesis was emphasised by Inger Porsch-Hällström (Huddinge). C-*myc*, but not c-*ets*-2, is overexpressed in early hepatocyte nodules (11 weeks after initiation) of male but not female rats, subjected to the Solt/Farber protocol. C-*myc* behaved similarly, 8 months after initiation. At this time, c-*ets*-2 was overexpressed in both male and female rats. 3 months later, the expression of both genes was increased in nodules and carcinomas of male and female rats. C-*myc* overexpression was down-regulated by GH in early, but not in late nodules of male rats. Thus, hormone regulation of c-*myc* may have a role in relatively early stages of rat liver carcinogenesis, while c-*ets*-2 could be implicated in progression. As reported by Rosa Pascale (Sassari), in the case of ornithine decarboxylase gene, accumulation of 1.8, 2.1 and 2.6 kb mRNA, in late liver nodules and HCC, is coupled with gene rearrangement, which could be implicated in the progression of liver nodules to cancer.

Skin carcinogenesis is the consequence of an activating mutation in the c-Ha-*ras* gene. As reported by Stuart H. Yuspa (Bethesda), *in vitro* introduction of the v-Ha-*ras* oncogene into normal keratinocytes during a defective retrovirus induces the phenotypic characteristics of benign neoplasia, and production of tumours after grafting recipient cells *in vivo*. In v-Ha-*ras* kerotinocytes, terminal differentiation in response to 1 mmol/l Ca^{2+} is inhibited, and the expression of differentiation-specific proteins, induced in 0.12 mmol/l Ca^{2+} medium, is blocked. Expression of TGFα, basal phosphatidyl inositol (PI) turnover, and diacylglycerol content increased in v-Ha-*ras* keratinocytes. These cells responded aberrantly to phorbol esters, but only minor changes were found in protein kinase C (PKC) profile when mRNA from normal and v-Ha-*ras* cells were examined. Many phenotypic changes produced by v-Ha-*ras* were reproduced *in vitro* by stimulating selectively PI turnover. Thus the earliest changes in squamous neoplasia induced by *ras* gene activation could be alterations in PKC mediated pathways, which influence the cellular response to growth factors and signals for terminal differentiation.

Because *ras* activation is a frequent feature of human pancreatic duct carcinoma, Dante G. Scarpelli (Chicago) studied *ras* activation in *N*-nitrosamine-induced pancreatic ductal carcinogenesis, in the hamster. Transfection of genomic DNA into NIH 3T3 cells resulted in tumorigenic cells. Southern blot analysis revealed a specific band for hamster Ki-*ras* G to A transversion in the second position identical to that observed in human pancreatic ductal carcinoma. Analysis of precancerous lesions indicated that Ki-*ras* activation is an early event in ductal carcinogenesis. A second hit, probably involving a suppressor gene, may be required to express the malignant phenotype. Relatively few metastatic carcinomas exhibited activated Ki-*ras*. They may reflect the loss of the mutated allele as a consequence of tumour progression. Molecular events linking Ki-*ras* mutations were described by J. L. Bos (Utrecht). These mutations abolish the intrinsic GTPase activity of the encoded protein resulting in malfunction of p21 *ras*. Bos demonstrated, by using rat fibroblasts, the involvement of p21 *ras* in the insulin signal transduction pathway. This was confirmed in NIH/3T3 cells overexpressing the insulin receptor. Insulin stimulation causes, in these cells, the conversion of *ras*GDP into *ras*GTP. Interestingly, dominant inhibitory mutants of p21 *ras* inhibit insulin-induced activation of c-*fos* promoter. Thus, p21 *ras* appears involved in insulin-induced signal transduction. H. Yamasaki (Lyon) correlated the role of *ras* gene mutations and alterations of intracellular communications (IC) in multistage carcinogenesis. With a BALB/c 3T3 cell transformation model, an A to T transversion at the 61st codon of Ki-*ras* gene in transformed foci produced by dimethylbenzantracene (DMBA) was identified. DMBA-induced A^{182} to T transversions were also present in Ki-*ras* and Ha-*ras* genes, but only Ki-*ras* mutation plays a crucial role in BALB/c 3T3 cells transformation. 12-*O*-Tetradecanoyl-phorbol-13-acetate greatly increased morphologic transformation of initiated cells, probably by blocking IC between initiated and surrounding cells. Quantitative measurements of gap-junctional IC (GJIC) in liver freshly removed from rats or humans, showed that loss of homologous or heterologous GJIC is a relatively early event in tumour promotion/progression.

Interaction between retinoids and cell differentiation has been the object of DeLuca's talk (Bethesda). Tracheal epithelium of the Syrian golden hamster, which under normal conditions is pseudostratified, becomes stratified upon exposure to chemical carcinogens or vitamin A deficiency, as well as in organ and cell cultures. Normal tracheal epithelia showed the keratin pair

K5/K14 exclusively in a discontinuous layer of basal cells. The first identifiable stage in the process eventually leading to squamous metaplasia, a preneoplasia lesion, induced by vitamin A-deficiency, is the so-called minimal morphological change, characterised by a continuous layer of K5/K14-positive basal cells. These cells do not stain with antibodies to K6 and K13. However, in the presence of a severe vitamin A deficiency normal epithelium is separated from the basement membrane by several layers of squamous cells forming the squamous metaplastic focus. The entire thickness of the focus stains with anti-K5/K14 antibodies, while K6 and K13 staining only occurred at the suprabasal level. This indicates that either basal cells acquire the ability to express K6 and K13 upon leaving the basement membrane or that a separate population of cells contributes to squamous lesion at different epithelial heights.

Cell proliferation and cell death

The relationships between gene expression and cell cycle were investigated by Christiane Guguen-Guillouzo (Rennes). In primary culture of hepatocytes exposed to EGF, c-*myc* is highly expressed before entrance into S-phase (48 h), while c-Ki-*ras* mRNA was detected only at the transition G1/S. Cdc2 protein was detected only after 48 h and increased thereafter reaching a peak at 84 h. No protein was detected during G1, and quantitation of the tyrosine 15 phosphorylated form within cdc2 protein showed an accumulation during S phase that gradually disappeared, indicating progression toward G2 and M. Thus, cdc2 protein may play a role in the G2/M, but not in G1/S, transitions. Different proliferative stimuli may act through different signal transduction pathways. Whether these differences reflect differences in the ability of various stimuli to support carcinogen-induced initiation is not known. Amedeo Columbano (Cagliari) analysed the response of "immediate early genes" following (1) compensatory growth induced by partial hepatectomy (PH) or CCl_4 and (2) mitogen-induced hyperplasia following treatment with cyproterone acetate (CPA), nafenopin (NAF), lead nitrate and ethylene dibromide (EDB). Different growth stimuli elicited different responses: transient and sequential expression of c-*jun*, c-*fos*, and c-*myc* occurred after PH or CCl_4 treatment, while only c-*myc* mRNA increased during liver hyperplasia induced by lead nitrate, and EDB. None of the above genes was overexpressed in liver hyperplasia induced by CPA. Among regulatory mechanisms of DNA synthesis, cholesterol (CH) synthesis plays a crucial role. Lipid acylation and farnesylation are essential for signal transduction and biological activity of GTP binding proteins such as G-proteins and *ras* oncoproteins (p21). K. N. Rao (Pittsburgh) has proposed that balanced co-operation between negative growth controls, such as circulating lipoproteins, and positive growth controls, such as hormones and growth factors, regulates cell proliferation. During carcinogenesis low density lipoproteins (LDL), hormone and growth factor receptors are down regulated. Oncoproteins bypass the G-protein-mediated signal transduction and stimulate continuously cell proliferation. In the absence of an LDL receptor pathway, *de novo* CH synthesis and farnesylation of p21 is not inhibited. Modulation of *de novo* CH synthesis may thus provide a new background for cancer therapy.

Cell death by apoptosis may modulate development of neoplasia. Rolf Schulte-Hermann (Vienna) observed that transforming growth factor (TGF)β1 and especially its precursor pro-TGFβ1 are present in apoptotic cells of liver, preneoplastic foci and HCC. Pro-TGFβ1 accumulated 3 h before chromatin condensation. Exogenous TGFβ1 induces apoptosis in cultured hepatocytes. TGFβ1 could be involved in the induction of apoptosis in certain epithelial normal and tumoral cells. W. Bursch (Vienna) pointed out the difficulty in recognising apoptotic cells by single morphological or biochemical features. Indeed, a mammary carcinoma cell line (MCF-7) showed nuclear condensation after treatment with the anti-oestrogen tamoxifen, but DNA of MCF-7 cells was not degraded into nucleosomes by glucocorticoids. Thus, nuclear changes associated with chromatic condensation may differ in diverse cells suggesting a non-apoptotic mechanism, or a different mechanism of genome inactivation in tamoxifen-treated MCF-7 cells. Francesco Baccino (Turin) studied apoptosis in monolayers of L cells exposed to synchronising agents, such as excess of thymidine and aphidicolin, an inhibitor of polymerase α, and etoposide, a chemotherapeutic compound which interferes with the religation step in DNA topoisomerase II action. The comparison between the effects of these compounds showed that except for the morphologic characteristics, no unifying concept emerges to describe apoptosis comprehensively. The results suggest a possible link of apoptosis and cell proliferation.

Thus, epigenetic mechanisms play an important role in the development of preneoplastic and neoplastic lesions. D. S. R. Sarma (Toronto) revised possible roles of genetic and epigenetic mechanisms in multistage carcinogenesis. Compensatory cell proliferation occurs during liver carcinogenesis initiation. One mechanism for liver cell proliferation is formation of hypomethylated stretches in certain genes. In fact, replication of carcinogen-modified DNA results in hypomethylated stretches and hypomethylating agents potentiate initiation of liver carcinogenesis. SAM, which methylates liver DNA, inhibits or even reverses liver carcinogensis, and hepatic nodules have hypomethylated sites in genes related to cell cycle and resistance to xenobiotics. Promotion selectively amplify initiated cells. Alternatively, a promoter may target surrounding non-initiated cells and selectively inhibit them, thus permitting initiated cells to respond to growth stimuli. In this case initiated cells may be resistant to the mitoinhibitory effect of the promoter and are spared of any further genetic or epigenetic change induced by the promoter. Available data favours a model based on mitoinhibition of surrounding cells as a mechanism by which orotic acid promotes liver carcinogenesis. However, the role of selection, as an epigenetic mechanism for carcinogenesis promotion is rather complex, as shown by Ezio Laconi's (Cagliari) observation that hepatic preneoplastic nodules are resistant to the necrogenic effect of galactosamine, but galactosamine is unable to promote liver carcinogenesis in DENA-initiated rats.

In closing remarks, J. DiPaolo (Bethesda) noted that the meeting represented a world-wide shift in the study of carcinogenesis. The multistage carcinogenesis process involves altered regulation of gene function leading to aberrant cell differentiation. Induction of terminal differentiation or the reversal of a blocked pathway which leads to the undifferentiated state should be a topic for future meeting. Such an approach presents a remedy for cancer which can be called anticarcinogenesis or antimutagenesis because inhibitors of proteins encoded by dominant oncogenes will either replace or bypass damaged tumour suppressor genes and oncogenes.

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