PREFACE

This book presents and discusses topical research in the study of DNA, genetics and cell biology. Topics discussed include DNA damage related to exposure to oil spills; mutation detection in TP53 gene; DNA and protein sequence analysis; the role of effectors on hypoxia due to nitric oxide production in human alveolar epithelial cells and plasma cell dyscrasias.

Chapter I - One of the major challenges involved mainly in searching of point mutations for clinical relevance is the technology used; in particular for cancer research will be focused on the gains or loss-of-suppression function, v.gr. in cancer genes as *RET*, *TP53*, *RAS*, etc. *TP53* has been used as an excellent model for point mutation detection, because its more than 20,000 different mutations, being this gene the most frequently found in many human cancers.

Furthermore, there are uncommon somatic and germline mutations that might be related to specific cancers or predispositions. In particular case, the precise nature of the *TP53* mutation presents both challenges and opportunities for alternate treatment strategies in specific cancers. These highlight the clinical need to accuratelly identify often unknown inherited aberrations or infrequently represented mutations in mixed populations of DNA molecules.

Chapter II - *TP53* is the most commonly mutated gene in human cancers, and the p53 protein is a potent inhibitor of cell growth, arrestingcell cycle progression at several points and inducing apoptosisof cells undergoing uncontrolled growth. The loss of p53 function by mutation is too common in cancer. However,most natural p53 mutations occur at a late stage in tumor development,and many clinically detectable cancers have reduced p53 expression but no p53 mutations.

Since, approximately 90% of the *TP53* gene mutations are localized between domains encoding exons 5 to 8. Much research suggests that *TP53* mutations have prognostic importance and sometimes are a significant factor in clinical Oncology. The presence of specific p53 mutational hotspots in different types of cancer implicates environmental carcinogens and endogenous processes in the etiology of human cancer. Oxidative stress and the generation of reactive species may cause mutations in cancer-related genes, and affect key regulator proteins of DNA repair, cell cycle, and apoptosis.

This review gives a brief perspective of some of the landmark discoveries in mutation research. The molecular and biochemical characteristics of *TP53* and p53 are then covered,

followed by an overview of how it can be studied in the laboratory. Finally, the implications of mutational hotspotsof *TP53* gene at level of DNA damage are discussed.

Chapter III - Oil spills are one of the most frequent ecological disasters giving rise to the pollution of enormous coastal areas and affecting also the local flora and fauna. As a direct consequence of them, a large number of individuals is always involved, as they take part in the different tasks derived from the need of cleaning the spilled oil and recovering the natural environments. From a toxicological point of view, oil is a complex mixture of compounds that can penetrate into the body burden through dermal, respiratory or digestive routes. Despite the huge number of spills occurred all around the world, the international literature dealing with the harmful effects of this exposure is very scarce and restricted to the acute and psychological effects. This seems paradoxical attending to the large number of carcinogenic agents composing this mixture.

So, after the *Prestige* oil spill (November 2002, NW of Spain) and taking into account the seriousness of the catastrophe, the large number of individuals involved, the damaging character of oil components and especially the lack in the scientific literature of reports considering the consequences of this exposure from a genotoxic or carcinogenic point of view, the author developed an extensive biomonitoring study including effect and susceptibility biomarkers. Three groups of exposed individuals whose exposure during the recovery of *Prestige* oil polluted areas differed quantitatively (short and acute or more prolonged in time) and qualitatively (as a consequence of the different methods used for this purpose) were included. Environmental concentrations of volatile organic compounds were evaluated by means of passive dosimeters in each exposure group. Two types of effect biomarkers were applied: the comet assay, characterized by its high sensitivity in population studies and for reflecting also DNA repair phenomena, and two well established cytogenetic assays, micronucleus test and sister chromatid exchanges. Moreover, due to the fact that individual differences in terms of susceptibility to xenobiotics have been extensively reported and mainly attributed to some polymorphisms in genes encoding for biotransformation enzymes and DNA repair proteins with functional consequences, a complete set of the most relevant were also included in this study.

Chapter IV - In an age of molecular genetics, viable but not culturable organisms, and highly sophisticated DNA sequence analysis technologies, is there a chance for a new breakthrough? It is quite extraordinary that 99% of microorganisms not only can't be cultured but are totally unknown to the scientific community. Many bacterial gene functions and identifications in the environment have been uncovered by recent advances in metagenomics or culture-independent genomic analysis in addition to the DNA microarray technology. Our knowledge of detecting and classifying bacterial isolates has been also improved by cutting-edge molecular tools such as biosensors and molecular subtyping as well as phage recombinant probes. Microbial communities, many to be discovered, are slowly revealed by these sharp scientific discoveries.

Chapter V - The effect of hypoxia on cell viability, proliferation and possible induced apoptosis in human alveolar epithelial cells and human hepatocytes is not clearly understood.In cultured human alveolar epithelial cells, IL-1β, TNF-α and INF-γ stimulated the nitric oxide production and resulted with inflammation manifested by hypoxia and apoptosis.In cultured hepatocytes,enhanced expression of superoxide dismutase and glutathione reductase enzymes indicated hypoxia associated with the enhanced level of *cAMP* dependent phosphodiesterase, NADPH dependentCytochrome C Oxidase enzyme activities

due to energy insufficiency in hepatocyte cultured medium.Both hypoxia and energy insufficiency reduced hepatocyte viability.The author propose that extent of inflammation leading to hypoxia initially and programmed cell death later both can enhance the MRI visible edema fluid content in lungs due to oxygen and energy insufficiency to surviving inflammatory alveolar epithelial cells.The mechanism of A549 cell damage due to nitric oxide (NO) production included: Cytokines regulated the expression of nitric oxide synthase (NOS) through NF-κB activation; Cofactor tetrahydro biopterin-catalyzed synthesis of iducible nitric oxide synthase (iNOS) enzyme.The NO production and increased level of NOS expression lead to Na^+ ion transport and cell proliferation with differentiation or apoptosis.Hypoxic human alveolar epithelial cells showed the possibility of NO production associated with the decrease of their viability and rapid increase of apoptosis. In conclusion, increased level of NO production can be a cause of hypoxia induced apoptosis and possibly MRI visible indicator of inflamed alveolar cell viability. High resolution MRI can track these sites of inflammation in lungs.

Chapter VI - Cancer is a disease resulting from the breakdown of several checkpoints and tumor-suppressing mechanisms. In cancer research, the development of new technologies, which have produced genomic tools indispensable for understanding how gene products are regulated in normal and diseased conditions on a global genome scale; one of these technologies is the DNA arrays. Although the most common use of DNA arrays is gene expression profiling and mutation detection, scientists have successfully used them for multiple applications, including genotyping, re-sequencing, DNA copy number analysis and DNA-protein interactions mainly.

This section then will be dedicated to the use of a public sequence database that can be accessed, and the design of DNA oligonucleotide probes for oligoarrays derived from sequences of special interest. Single probe and stacking hybridization are explored as possible microarray designs. Then the use of thermodynamic models and *in silico* hybridization are explored in order to access the sensitivity and specificity of the oligoarray and how its design can be improved. However, many commercial and public applications do not consider that the hybridization between target DNA's and microarray probes is a chemical reaction which is influenced by several thermodynamic parameters. One of the most important of such parameters is the thermal stability of the nucleic acid duplexes, which are formed as a result of the hybridization. According to the reaction conditions, these duplexes can be perfectly or imperfectly paired, which is of critical importance when a diagnostic kit is developed in order to assess its sensitivity and specificity.

In silico hybridization can be used to avoid undesired hybridization events (such as multiple target-probe interactions and stable mismatched hybrids). Also, the strategy can facilitate (through use of different length probes) selection of a probe set that has a narrow duplex stability allowing maximal specificity under a single hybridization condition.

Chapter VII - Plasma cell dyscrasias (PCDs) include plasmacytomas and various forms of plasma cell myeloma (multiple myeloma-MM). Diagnosis of plasmacytoma requires demonstration of a monoclonal/aberrant plasma cell (PC) population in a tissue biopsy; whereas, diagnosis of a PCD involving the bone marrow (BM) (i.e., various forms of MM) requires quantitation of the PCs in the BM in addition to the demonstration of monoclonality/aberrancy. This chapter will describe the definitions of the various forms of PCD (including the International Staging System-ISS), the morphological variants and immunophenotypic features of neoplastic PCs, the diagnostic laboratory techniques useful in

establishing a diagnosis (including immunophenotypic techniques), and the ancillary studies that may aid in diagnosis, prognostication, and therapeutic decision-making (i.e., immunophenotypic features, as well as cytogenetic and molecular findings).

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