DNA REPAIR GENES: FUNCTIONAL ANALYSIS AND EVOLUTION

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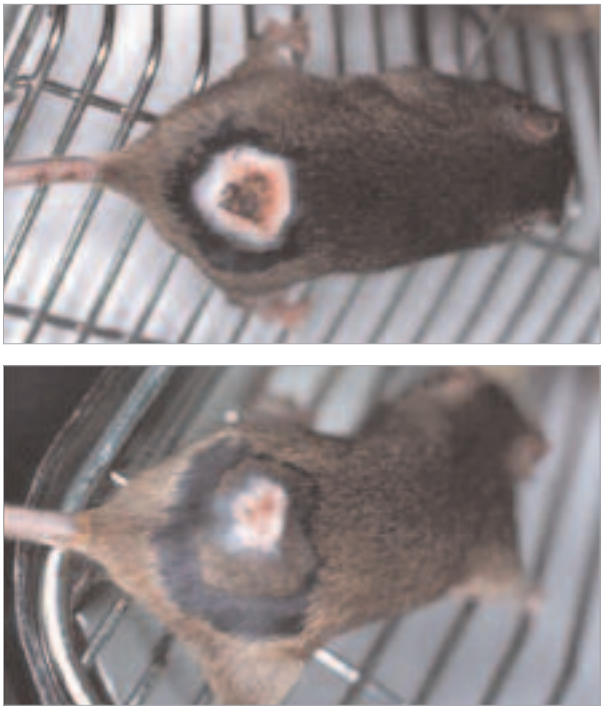

The mouse above, knockout mice (XPAKO) submitted to skin gene therapy with recombinant adenoviruses. Note that the animal that has been infected with the AdXPA virus (below) recovered faster from UV-B induced irradiation

Most of our research activities are based on the understanding of DNA repair and mutagenesis mechanisms in mammalian cells, as well as other organisms. With the recent enormous amount of data generated by genomic approaches, our project is including now an evolutionary point of view. In this Thematic Proposal we present 4 different subprojects, and 2 of them are based on studies of DNA repair in mammalian cells, giving priorities to primary cells from human beings. Much of this work will employ the well-succeeded recombinant adenovirus vectors, which were developed in this laboratory. These, carrying DNA repair and DNA repair-related genes, will be used to complement cells with human syndromes that present DNA repair deficiencies (such as xeroderma pigmentosum). The vectors can revert completely cells deficiencies and we are now initiating studies with the perspectives to analyze directly their ability to complement knockout mire for XP genes. Moreover, these vectors will also be used in order to diagnose and identify the genes involved in XP patients as well as in the studies of the dynamics of DNA repair proteins in the cells. Another endpoint to be investigated in mammalian cells is apoptosis induced by DNA damage, as we are trying to decipher the signals that lead the reli to trigger its program of cell death. Adenovirus vectors bearing the photolyase genes are being constructed and they will be tested on their ability to prevent apoptosis in human cells deficient for DNA repair. The involvement of RNA transcription, DNA replication, PARP and cell cycle in signaling for apoptosis in DNA damaged cells are also being investigated. In a different subproject, we are trying to understand the how plant cells defend their genomes from the presence of DNA damage. Two genes that were previously cloned (*AtXPB*1 e *Thi*1) have being targeted in this work, and our plan is to identify the roles of the proteins encoded by these genes, in the repair of DNA damage in nuclei (*AtXPB*) and organels (*Thi*1). We also plan to understand how these genes are expressed in plants. Finally, based on the knowledge of the complete genome sequence of *Caulobacter crescentus*, we intend to identify and investigate the genes involved in the DNA repair of this alpha proteobacteria. Basically, we propose to construct and analyze the phenotype of bacterial mutants on DNA repair related genes. The possibility to synchronize these bacteria may be useful to ascertain the effect of cell cycle on the repair functions in these cells. The differential gene expression along the cells cycle can also be investigated. To our knowledge, this is the first proposal for a genomic approach to investigate DNA repair in bacteria different from the classic *Escherichia coli* model. Subproject 1: Recombinant genetic vectors to study DNA repair in mammalian cells. Subproject 2: Signaling to apoptosis in mammalian cells with damaged genome. Subproject 3: DNA repair genes in plants and functional analysis of the genes *AtXPB*1 and *Thi*1. Subproject 4: Identification and function of genes related to DNA repair in *Caulobacter crescentus*.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

This Project basically aims at understanding how cells respond to and protect their genomes from environmental aggressions. The impact of these protection systems is dramatically illustrated with the human genetic syndrome xeroderma pigmentosum (XP). XP patients present high frequency of skin cancers in regions exposed to sunlight, early aging and developmental problems, which are mainly due to DNA repair defects in their cells. In this Project, we succeeded in developing recombinant adenovirus vectors which correct the genetic defects of the XP cells. This has been achieved both *in vitro* and *in vivo* by using XP-knockout mice, which simulate the XP syndrome. These adenoviruses were shown to prevent the appearance of skin tumors in mice irradiated with UVB light, opening potential gene therapy perspectives for these patients. More recently, work with vectors carrying genes of photolyases (specific DNA repair enzymes) has enabled us to identify the types of lesions that promote apoptosis by UV, thus answering a longstanding question. Interestingly, XP cells respond to a different lesion when compared to normal human cells, which indicates that the XP problem may be not only related to a quantitative number of unrepaired lesions, but also to different type of lesions.

In a different approach, we have been investigating the nature of the signaling process that lead to cell death after the induction of DNA lesions. The results clearly indicate the role of DNA replication as an important sensor mechanism that makes the cells respond to genetic damages. Also, in collaboration with Dr. Bernd Kaina (Mainz, Germany), we have identified DNA repair pathways involved in the protection of cells' genomes to DNA lesions induced by chemotherapeutic agents, with potential implications in tumor treatment.

Besides, DNA repair genes are highly conserved throughout evolution, and this led us to make significant and pioneer contributions in the identification of DNA repair genes in plants, homologs to those deficient in XP patients. In bacteria, we also were pioneer in the use of the bacterial model *Caulobacter crescentus* in studies of DNA repair mechanisms. These studies resulted in the identification of functions for a previously uncharacterized bacterial operon, which codes for a complex of proteins responsible for a translesion DNA polymerase role in the cell. Finally, studies with the genomes of the bacteria from the group of xanthomonadales led to the identification of several genomic islands, potentially originated from horizontal gene transfer. The beauty of the work is that the methodology employed allowed to determine that some of these islands were transferred a long time ago, and carry genes involved in the primary metabolism, adding essential genes to the list of genes that may be exchanged between bacteria during evolution.

MAIN PUBLICATIONS

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