VI Seminario Nazionale per Dottorandi in Farmacologia e Scienze Affini
Siena, Certosa di Pontignano, 23 - 26 Settembre 2002

MUTATIONS OF P53 TUMOR SUPPRESSOR GENE COULD INDUCE CHANGES OF PROTEIN EXPRESSION INVOLVED IN CHEMORESISTANCE

Capponcelli S., 1° anno di corso del Dottorato in Farmacologia e Tossicologia, XVII ciclo. Durata del Dottorato in anni: 4. Sede di servizio: Istituti Ortopedici Rizzoli, Laboratorio di Ricerca Oncologica.

Alterations of specific genic *loci* are often related to antineoplastic chemotherapy failure. The p53 tumor suppressor gene is mutated in over 50% of human cancers and cells carrying p53 mutations often present chemoresistance both in vitro and in vivo.
The wild-type p53 protein can exert a variety of antiproliferative effects, including induction of cell cycle arrest and apoptosis. These effects are usually shown in cells exposed to stress (like DNA damage) and could cause accumulation and biochemical activation of p53 protein.
p53 gene alterations can induce abrogation of cellular wild type p53 activity. Moreover, several p53 mutants can "gain" oncogenic functions such as tumorigenicity and resistance to therapy that could play a substantial role in patients prognosis.
Most of p53 mutations are localized in the DNA binding domain, expecially in some codons, called hot spots. We'll analyse specifically mutation on 175 codon, from Arginine to Histidine, one of the must frequent p53 alteration in human cancers and mutation on 220 codon, from Tyrosine to Serine, found in families bringing Li-Fraumeni syndrome and showed a very aggressive clinical phenotype.
Murine p53-knock out fibroblasts were trasfected with the p53 mutant p53- Ser220 or p53-His175. These mutated proteins were produced by site direct mutagenesis. Chemosesitivity assays (IC^{50} and clonogenicity tests) showed that fibroblasts carrying the mutations present an enhanced resistance to drugs, expecially to Doxorubicin. Chemoresistance could be connected to the activation, by mutant p53, of transcriptional pathways, synthesis of proteins and protein-to-protein interactions different from wild – type p53.
To investigate the molecular mechanisms underlying chemoresistance to Doxorubicin of these p53 mutants we used proteomic tecniques, both classic and functional.
Aim of the first year PhD research is the evaluation of protein expression in cells carrying a wild-type p53 and in fibroblasts with a p53 gene mutated at the 220 and 175 codons and to assess if the treatment with Doxorubicin is able to modify this expression.
I used coimmunoprecipitation with p53 specific monoclonal antibodies and SDS-acrylamide gel electrophoresis, in order to identify proteins that, binding to mutant p53, could play a role in chemoresistance.
Fibroblasts carrying the p53 220 mutation present extra-bands (compared to the wild type ones) 24 hours after treatment with Doxorubicin. These bands could represent proteins involved in chemoresistance.
Whereas, fibroblasts carrying the p53 175 mutation do not show some bands present in the p53 wild-type cell lines. This evidence could mean that this mutation inhibit the interaction with p53-wild-type interacting proteins. These proteins could be involved in the cellular cycle arrest and the decrease of their levels could explain the aggressiveness of this mutation.

We are in the process of identifying these proteins using classic proteomic techniques in collaboration with Dr. Angela Bachi from the Protein MicroSequencing Facility, D.I.B.I.T., Ospedale S.Raffaele, Milano.
