



UNIVERSITÀ DEGLI STUDI DI SALERNO



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Techniques of proteomic analysis as tools for studies in biomedical field

Dottorando

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Abstract

It is known that prenatal exposure to pollutants and particularly heavy metals can have long term damaging consequences on infants, due to their accumulation in-body. Since the 1990s, ten million tonnes of waste have been illegally dumped in the area around Caserta and Naples. Thus, direct exposure to waste and heavy metals during the last two decades was very frequent in the so-called “Lands of fires”. The number of children suffering from cancer and of malformed fetuses in Italy's "Land of Fires", an area where toxic waste has been dumped by the mafia, is reported significantly higher than elsewhere in the country. In this thesis we examined the proteome of the umbilical cords from malformed fetuses obtained by therapeutic abortions, after mothers' being exposed to the pollution on “land of fire” during early pregnancy, and analyzed the differences between umbilical cords from malformed fetuses to healthy ones. The main goals were to understand the impact of the contamination by heavy metals on the fetus development, and to identify new putative biomarkers of exposure to metal contaminants.

All umbilical cords were obtained in Campania region (Naples and Caserta, mainly in the “land of fires”). The collection of the biological samples was carried out in collaboration with the Caserta Hospital “Sant’Anna e San Sebastiano” and with the Avellino Hospital “San Giuseppe Moscati”. A proteomic approach based on Filter-Aided Sample Preparation (FASP) method was set up and performed. This bio-analytical strategy combines the advantages of in-gel and in-solution digestion for mass spectrometry-based proteomics, greatly reduces the time required for sample preparation and enables more flexibility in sample processing. Protein identification and quantification were performed by matching mass spectrometry data in on-line protein database, using the MaxQuant 1.5.2.8 software. Statistical analyses were employed to identify proteins whose levels were sensibly different in the umbilical cords from malformed fetuses. Gene Ontology (GO) classification was used in order to obtain functional information of the differentially expressed proteins and to correlate them to the embryonic development. Finally, Matrix Metalloproteinases (MMPs) have been shown to play significant roles in a number of physiological processes, including embryogenesis and angiogenesis, but they also contribute to the development of pathological processes. Thus, gelatin zymography technique was performed to detect MMPs enzymatic activity in the umbilical cords. Our results support a significant role of MMPs in the fetus development.