Electronic microscopy in MCF7 cells: Cd - 5-Fu effects

Yolande Asara[1], Paola Tolu[1], Vittorio Mazzarello[1], Juan A. Marchal[2], Andrea Montella[1], Roberto Madeddu[1,3]

[1] Biomedical Sciences Department, University of Sassary, Italy
[2] Anatomy and Embryology Department, University of Granada, Spain
[3] Istituto Nazionale Biostrutture e Biosistemi (INBB), Italy

Cadmium (Cd) is known as a highly toxic metal wich represents a major hazard to the environment The extremely long biological half life (30-35 years) makes it a cumulative toxin; therefore long-term past exposure could still result in direct toxic effects. The toxicity of this metal contributes to a large variety of health conditions, including major diseases such as heart disease, cancer and diabetes. The fluoropyrimidines, especially 5-Fluorouracil (5-Fu) are antimetabolite inhibitors of the novo purine and pyrimidines syntheses. 5-Fu play an important role in standard chemotherapy protocols for a range of solid tumors, including breast and colorectal cancers. Breast cancer represent the most common tumor of female in many industrialized countries. The MCF7 human breast cancer cell line had been used as an excellent experimental model to improve the efficacy of different therapies before use in patients. In order to elucidate the mechanism of both Cadmium and 5-Fu effects, we compared the morphological transformations with observation by TEM and SEM electronic microscopy.

MCF7 cells were grown at 37° C in an atmosphere containing 5% of Co_2 with RPMI 1640 Medium supplemented with 10% of fetal bovine serum. For Elettronic Microscopy, MCF7 cells were treated with Cd (5uM, 20uM, 40uM) and 5-Fu (1,5uM, 50uM) for different time of incubation (6h, 24h, 48h). The cells, to be observed by TEM, were fixed in 2% glutharaldehyde and postfixed in 1% OsO_4, washed and then embedded in 1.5% low-melting-point agarose. Samples were stained, after ultrathin sections, with 4% uranyl acetate and lead citrate. Culture cells to be observed by SEM were fixed in 2.5% glutaraldehyde, then washed and post fixed in 1% OsO_4. Samples were dehydrated in ethanol, incubated in hexamethyldisilazane and examined in low vacuum using a SEM FEI Quanta 200.

In the cells treated with Cd already to small concentrations and brief times of incubation it increases the number of the vesicles, probable peroxisoms that begins to polish up the cells from the cadmium. To tall concentrations of Cd (20uM -40uM) we note notable increase of the cytological disorder, the nucleuses are very evident and globular. The addition of 5-fu (1,5uM) to cells treated with Cd (5 uM- 20 uM) brings to a reduction of the number of the secretion's vesicles and the number of filopods, in fact to 48h the cells are introduced flat with a smoother cellular surface. Under extreme conditions (cd 40, 48h), the addition of the anti-tumoral substance doesn't meaningfully modify the cellular disorder observed with only Cd.

Key words
Cadmium, 5-Fluorouracil, Breast cancer, MCF7, Electronic Microscopy