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## Dielectric Relaxation Spectroscopy and Dynamic Light Scattering Studies of Exosomes Released from Ionizing Radiation Exposed MG-63 Osteosarcoma Spheroids

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Exosomes are small membrane vesicles secreted by a variety of cell types into the extracellular environment. They have also been isolated from numerous body fluids such as plasma, serum and urine. Their presence in biological fluids suggests their involvement in diverse physiological and/or pathological events. In particular, it has been indicated that exosomes could be carriers for intercellular communications. In fact, they may shuttle lipids, proteins and mRNA or microRNA from exosome-producing cells to target cells. The cell-cell communication mediated by human cancer-derived exosomes seems to be involved in malignant progression and they are expected to play a role in the response of tumor cells to ionizing radiation (IR).

Multicellular tumor spheroids are an *in vitro* model which mimic numerous aspects of *in vivo* human solid tumors. In fact, the cell-cell interactions and microenvironmental conditions make spheroids comparable to micrometastases and avascular tumors. Thus, they can be extremely useful in studying the effects of IR on cancer cells and exosomes secreted *in vitro* from tumor spheroids may resemble in a more realistic manner the exosomes secreted by *in vivo* tumors.

The aim of the present study was to examine the effect of IR on the size and two electrical passive parameters of cell membrane (conductivity and permittivity) of exosomes secreted *in vitro* from MG-63 osteosarcoma spheroids. MG-63 spheroids were irradiated with 5 Gy and exosomes were isolated from spheroids at different times from irradiation. Dynamic light scattering technique and dielectric relaxation spectroscopy in the radiofrequency range were used to determine the size and the conductivity and permittivity of exosomes, respectively.

The results obtained show that IR influence the size of exosomes. In fact, exosomes isolated from non-irradiated spheroids are larger in diameter than exosomes isolated from irradiated spheroids at all times examined. Dielectric relaxation measurements reveal that IR induce changes in both conductivity and permittivity of exosomes. Interestingly, exosomes show the same dielectric characteristics of irradiated MG-63 spheroids from which they are released.

These data indicate that exosomes show a specific signature of the treatment at which tumor cells have been exposed. Exosomes could therefore represent an important tool to monitor the effects of anticancer treatments.

This work is dedicated to the memory of our colleague Maria Teresa Santini.

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