**RADIOTHERAPY OF ENDOTHELIAL CELL AND CAPILLARIES AS BIOLOGICAL MARKERS IN RADIOLOGICAL PROTECTION**

Manti Lorenzo¹, Scampoli Paola¹, Grossi Gianfranco¹, Pennarola Raffaele², Porzio Giuseppe², Russo Spena Francesco³,

**Matarazzo Giuseppe**³

1 Dipartimento di Scienze Fisiche, Università di Napoli Federico II and Istituto Nazionale di Fisica Nucleare (INFN) Sezione di Napoli, Italy
2 Centro di Servizio Radioprotezione e Fisica Sanitaria- Università di Napoli “Federico II”
3 Azienda Ospedaliera A.Cardarelli, Napoli

**I N T R O D U C T I O N**

Endothelial cell and capillaries are particularly sensitive to radiation. The importance of alterations in endothelial cell and capillaries caused by ionizing radiation has been evident since the first observations by researchers. Radiosensitivity of endothelial cell and biological markers of effects such as, particularly, premature cellular senescence assessed by β-galactosidase assay and the behaviour of the capillary loops in subjects radioexposed recorded with “in vivo capillaroscopy” are the topic of this research. Over the last 20 years there has been a considerable development of the procedures involving risk of exposure to ionising radiation in departments undertaking interventions under radiological monitoring.

**M a t e r i a l s and Methods**

The induction of SIPS (stress-induced premature senescence) and its relationship with telomere length, which drives physiological senescence, were investigated in vitro by exposing a model system (HUVEC or human vein endothelial cells) to the GSI carbon ion beam used for therapeutic purposes, at both the LET values incurred by normal (plateau region) and tumour (SOBP or Spread-Out Bragg Peak) cells. X-ray irradiation was used as reference.

The examination in vivo of capillaries was carried out in 16 workers (radiologists and medical staff) aged between 42 and 67 years and length of service between 9 and 22 years exposed to radiation during the procedures of interventional radiology, using a ‘Videocap’ (DS-Medica, Milan) video-capillaroscope with optical probes of 200x. The multiparametric capillaroscopic examination has been integrated with the classification of different profiles with reference to morphology and hemorheology.

**R e s u l t s**

As early as 2 weeks from irradiation, many more cells stain positive for β-galactosidase in the progeny of irradiated cells than in that from controls. At such early times, doses as low as 0.1-0.5 Gy from the plateau region of the Bragg curve were more effective than 2 Gy SOBP at inducing SIPS. In general, plateau-irradiated HUVECs showed more senescent cells than those from x ray irradiation. A part of senescing cells significantly higher than control was also observed following SOBP irradiation despite the incidence of lethal damage being associated with its higher LET. The protocol adopted in radio-exposed subjects during the procedures of interventional radiology, has shown an augmentation of abnormalities of the capillaroscopic examinations suggesting intraclinic effects of ionising radiation connectible with length of service.

Assays were performed at regular intervals in the descendants of irradiated and control cells. Onset of senescence was assessed by β-galactosidase assay and by measurements of telomere length by Interphase Quantitative (IQ-FISH). Expression of the senescence-specific enzyme β-galactosidase allowed discrimination of the senescent fraction from proliferating cells. Cells positive for enzyme activity looked brightly green-stained. For IQ-FISH, interphase cells were labelled by a pan-telomeric fluorescent probe (cy3-PNA, DAKO) and a centromere-directed one (FITC, Vysis) used as internal standard. Fluorescence signal distribution was acquired by means of an automated system scanning each cells over the z-axis. Relative telomere length was derived from the fluorescence intensity ratio telomere to centromere (T/C) between the two markers.

**C o n c l u s i o n**

Ionising radiation efficiently cause SIPS in the HUVEC model system, exhibiting a dependence upon LET; The senescent phenotype occurs at early times post irradiation and is induced by doses as low as 0.1-0.5 Gy of 12C ions from the plateau region of the Bragg curve; Telomere length attrition may be a driving molecular mechanism for SIPS after low LET-irradiation but is of no apparent relevance for 12C ion-induced senescence. In the medical staff radioexposed, the use of in vivo capillaroscopy seems an effective way of controlling the effects of chronic professional radiation doses accumulated over many years of service and it seems of great interest as a biological indicator of effects for radiological prevention.