

### **In Vivo Study of Chromosomal Instability in Lymphocytes of Radiotherapy Patients**

V. Vinnikov<sup>a</sup>, N. Maznyk<sup>a</sup> and D. Lloyd<sup>b</sup>

<sup>a</sup>*Institute for Medical Radiology of the AMSU, Pushkinskaya St., 82, 61024 Kharkiv, Ukraine;* <sup>b</sup>*Radiation Protection Division, HPA-UK, Fermi Avenue, OX11 0RQ Didcot, Chilton, United Kingdom*

*lrcg.imr@mail.ru*

The problem of delayed genomic instability occurring in human cells in vivo has recently come to prominence as an important mechanism in radiation carcinogenesis. Considering cancer patients treated with partial body irradiation as a relevant group at risk of developing a second cancer, research has been carried out to quantify chromosomal instability (ChI) occurring in their lymphocytes. Ten patients with uterine cancer were selected at the IMR clinic according to local ethics procedures with minimum variations of age (55-65 y), tumor grade (T2a-cNxM0) and treatment method (only external Co-60 therapy given in 20 fractions, 2 Gy/ fraction, no chemotherapy). Control blood was taken 1-2 days before treatment and then post-treatment; 1-2 days after the final fraction and 1 year later (no chemotherapy during the follow-up period). The lymphocytes were cultured in the presence of 5-Bromodeoxyuridine for 50 and 100 h. Metaphase preparations were stained by fluorescence-plus-Giemsa (FPG) and the harlequin-fluorescence in situ hybridization method (h-FISH, highlighting chromosomes 2, 3 and 5 and all centromeres). Metaphases were analyzed for the presence of chromatid aberrations and chromosome-type rearrangements (on h-FISH also including translocations and insertions), with simultaneous determination of the 1st, 2nd, 3rd or later mitosis status of each cell. Both assays showed that the radiotherapy caused a significant increase of radiation-specific chromosome-type aberrations in patients' first mitosis lymphocytes together with ChI. The latter was concluded from the statistically excessive presence of chromatid aberrations in 6-8 % of those cells, which already contained previously induced chromosome-type rearrangements. That can be a result of an elevated DNA breakage in the G2 phase of the cell cycle, associated with either hyper-production/impaired scavenging of free radicals or hyper-expression of "hotspots" (fragile sites) in the chromatin, or both, caused by the presence of chromosome type rearrangement. The h-FISH assay showed that this form of ChI is transmissible through early cell cycles in vitro and can be seen in cells of 3rd and later mitoses, carrying stable translocations inherited from the irradiated predecessors. Probably, that mechanism was responsible for the in vivo persistence of the ChI, which was observed 1 year after radiotherapy and affected again 6-8 % of lymphocytes with stable aberrations. The possible relevance of this cytogenetic process to occurrence of second therapy-related cancers in progeny of cells irradiated within the treatment field will be discussed.