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INDUCTION OF DNA BREAKAGE IN X-IRRADIATED NUCLEOIDS SELECTIVELY STRIPPED OF NUCLEAR PROTEINS IN TWO MOUSE LYMPHOMA CELL LINES DIFFERING IN RADIOSENSITIVITY

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DNA in the nucleus of eukaryotic cells is tightly associated with histones and other nuclear proteins and folded into a higher order chromatin structure that is anchored to the nuclear matrix. Chromatin proteins are important not only for the maintenance of chromatin structure but also may protect DNA against exogenous damage. Regions of chromatin of more open conformation, like transcriptionally active DNA, are more susceptible to ionizing radiation than inactive regions [1,2]. Proteins may also protect DNA against radiation-generated free radicals, or may serve as a source of reducing equivalents for chemical repair of DNA radicals.

To examine the role of nuclear proteins in protection of DNA against ionizing radiation and their contribution to the radiation sensitivity, an alkaline version of comet assay, which detects overall DNA damage i.e. DNA breaks and alkali-labile sites, was used to estimate DNA damage [3]. The cellular model consisted of two L5178Y (LY) mouse lymphoma cell lines, LY-S and LY-R, differing in sensitivity to ionizing radiation; D_0 values of survival curves (the dose required to reduce the surviving fraction by the factor e^{-1}) are 0.5 Gy and 1 Gy, respectively. Sequential removal of nuclear proteins by an extraction with different concentrations of NaCl resulted in an increase of DNA damage in LY-R nucleoids. Removal of histone HI and a part of non-histone proteins (NHP's) (0.8 M NaCl) caused 1.1 fold increase in the mean DNA content in the comet tails of the irradiated nucleoids, as compared to nucleoids treated with 0.14 M NaCl ($p<0.01$). Total removal of histones and NHP's (2.5 M NaCl) resulted in 1.25-fold increase in DNA damage of irradiated nucleoids ($p<0.01$, Fig.1). In contrast, in the radiation sensitive LY-S cell line, depletion of nuclear protein practically did not affect DNA damage (Fig.1).

In control (non-irradiated) cells the sequential selective removal of chromatin proteins from the nucleoids did not result in any significant changes in DNA mobility. However, we found significantly more DNA in the comet tail of control LY-R cells than in LY-S cells ($p<0.05$). This phenomenon may reflect a higher level of endogenously generated DNA damage in LY-R cells due to the higher steady-state transition metal ion content (for discussion see [4]) and has repeatedly been observed by us in the comet assay with LY cells. Another explanation of this phenomenon may be a different chromatin organisation in LY sublines, previously proposed in view of the different supercoiling properties of nucleoids from LY cells [5]. Nevertheless, the initial DNA damage induced in unextracted chromatin with 1.5 Gy of X radiation was similar in the two cell lines. A similar level of initial DNA breakage was also found previously in non-exposed cells [6].

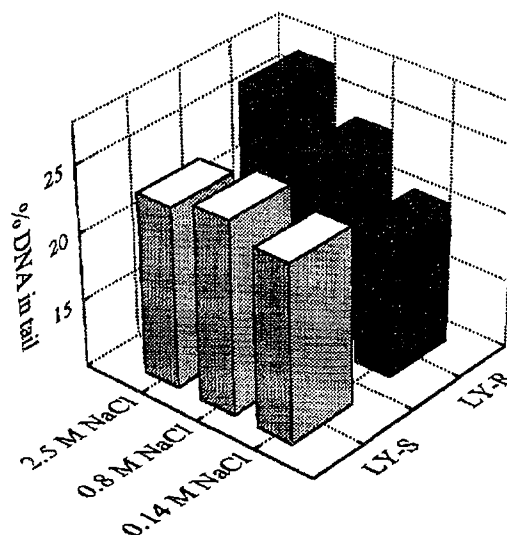

Fig.1. DNA damage in protein-stripped nucleoids of L5178Y cell lines expressed as a percentage of DNA in the comet "tail". Data represent mean values for 50 comets after subtraction of the mean value for 50 control comets.

Although there is no doubt that the main cause of LY-S cells' sensitivity to ionizing radiation is a defect in the repair of DSB's, our data support the concept that chromatin organisation may contribute to the cellular susceptibility to DNA damaging agents.

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