OTKA 72762 grant final report

Summary of apoptotic changes

Morphological studies after genotoxic treatments suggest that the consequences of various chromatin injuries can be categorized based on the assessment of injury-specific chromatin changes. In a broader sense one can characterize external apoptotic changes caused by genotoxic agents and classify them according to their structural chromatin changes. Since the potential to distinguish among different chromatotoxic effects is of diagnostic significance, we have started to determine and systematize the effects of cadmium treatment (Banfalvi et al., 2005), gamma irradiation (Nagy et al., 2004) and UV irradiation (Ujvarosi et al., 2007). Genotoxic treatments may have multiple effects on different cell lines, therefore two different cell lines (Chinese hamster ovary cells and murine preB cells) were used for cadmium treatment and have seen the same large extensive disruptions and holes in the nuclear membrane and sticky incompletely folded chromosomes typical for cadmium treatment (Nagy et al., 2004; Banfalvi et al., 2007).

Preapoptotic changes upon γ-irradiation in CHO cells manifested as: (a) The cellular and nuclear sizes increased. (b) The DNA content was lower in each elutriated subpopulation of cells. (c) The progression of the cell cycle was arrested in the early S phase at 2.4 C value. (d) The chromatin condensation was blocked between the fibrillar chromatin and precondensed elongated chromosomal forms. (e) The number and size of apoptotic bodies were inversely correlated with the progression of the cell cycle, with many small apoptotic bodies in early S phase and less but larger apoptotic bodies in late S phase (Nagy et al., 2004). Similar observations were made in K562 cells after gamma irradiation (Banfalvi et al., 2007).

To correlate the presented data with those of others it was reported that UV can induce G_1 or G_2 cell cycle arrest in human keratinocytes and neuroblastoma cells (Gujuluva et al., 1994; Ceruti et al., 2005), while we have observed that chromatin condensation after UV irradiation was arrested in early S phase between 2.2 and 2.4 C-values. This discrepancy can be resolved by the fact that the C-value of G1 (2.0 C) is close to our observation (2.2-2.4 C). However, the small initial rate of DNA replication indicates that replication has started, thus the C-value is higher than 2.0 C. The G2 arrest means that chromosomes could not enter metaphase and condensed chromosomes were not

formed after UV irradiation. Indeed, metaphase chromosomes were not visible after irradiation, but this can be explained by the S phase block which is probably maintained throughout the S phase and in G2 phase. Results are in conformity with the view that UV irradiation generates first giant DNA fragments (Higuchi et al., 2003) which could be seen as a fibrillary cromatin cloud. The view of Higuchi et al. (2003) is shared that the absence of apoptotic cells is an indication of the formation of high molecular weight DNA which preceeds ladder-formation of internucleosomal DNA fragmentation associated with apoptosis through caspase activation.

Table 1.

The effect of gamma and UV irradiation on cells

Biochemical and morphological changes	Gamma irradiation	UVB irradiation
Replicative DNA synthesis	inhibited	inhibited
Repair DNA synthesis	elevated	elevated
Cellular size	increased	increased
Nuclear size	increased	increased
Apoptotic cells (shrinkage)	many	none
C-values during S phase	lowered	uniformly low
Arrest in S phase	2.4 C	2.2-2.4 C
Chromatin stage	fibrous	fibrillary
Apoptotic bodies	many	few
Metaphase chromosomes	visible	invisible

Morphological similarities and differences between UV and gamma irradiation

The effect of gamma and UV irradiation on mammalian cells are summarized in Table 1.

By summarizing the morphologic effects the similarities and differences between ultraviolet light and gamma irradiation are accentuated:

1. UV irradiation did not cause significant changes in cellular or nuclear size,

2. the DNA content expressed in C-value was lower in each synchronized cell population after UV irradiation,

3. the progression of cell cycle was arrested somewhat earlier in S phase (between 2.2 and 2.4 C), than after gamma irradiation (2.4 C),

4. UV irradiation blocked chromatin condensation at its fibrillary stage, nuclear structures were blurred and covered with fibrillary chromatin,

5. although, some apoptotic bodies were seen in mid S phase, they are not typical to UV rather to gamma irradiation,

6. the lack of metaphase chromosomes indicates that UV damage may release fibrillary chromatin at any stage during chromatin condensation and prevent the folding process.

Finally, data on the genotoxic effects of chemical (Trencsenyi et al., 2007), cadmium (Banfalvi et al., 2005), gamma (Nagy et al., 2004) and ultraviolet light irradiation support the notion that preapoptotic events can be categorized by fingerprinting the injury-specific chromatin changes.

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