Production of Clustered DNA Damage by ^{125}I Decay

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To the Editor:

We previously found a large difference in DNA double strand break (dsb) induction when randomly incorporating ^{125}I or non-random association of ^{125}I with DNA at so-called 'estrogen response elements' (ERE) in a Chinese hamster ovary cell line transfected with human estrogen receptor (1). A relative biological effectiveness (RBE) value for DNA dsb induction (RBE_{dsb}) is calculated here for accumulated ^{125}I-iododeoxyuridine (^{125}IUdR) decays or ^{125}I-estrogen decays relative to γ radiation from a ^{137}Cs source. RBE_{dsb} values were 2.9 and 24 for ^{125}IUdR and ^{125}I-estrogen, respectively (1). RBE values for cell survival (RBE_s) were 3.1 and 3.9 for ^{125}IUdR and ^{125}I-estrogen, respectively (see Table 1).

Several lines of evidence support the notion of clustered DNA damage (or locally multiply damaged DNA or LMDs (2) produced by ^{125}I decay (1, 3). First, the production of clustered DNA damage or LMDs is indicated by a drop in the first fraction of elution profiles (1, 3), suggesting that ^{125}I decay produces complex lesions composed of small fragments of DNA which elute through the filter in the first fraction. Secondly, other workers have observed that RBE_{dsb} decreases relative to RBE_s with increasing LET of radiation used (4). The reduction in RBE_{dsb} suggests that dsb assays underestimate DNA dsb induction by not measuring all breaks in complex lesions such as LMDs or clustered lesions. For ^{125}IUdR, RBE_{dsb} is marginally less than RBEs. Repair of complex lesions may complicate this relationship. Thirdly, numerous physical models of Auger electron emission from ^{125}I decay predict energy deposition patterns consistent with production of clustered DNA damage.

Table 1

Comparison of RBE values

	^{125}IUdR	^{125}I-estrogen
RBE_s[1,3]	3.1	3.9
RBE_{dsb}[2,4,5]	2.9	24.0

^{125}I decays were converted to cGy using 0.742 cGy/^{125}I decay (11)

[1] RBE for cell survival = RBE_s = (dose of γ irradiation for D_o) ("dose" of ^{125}I required for D_o)

[2] RBE for DNA dsb induction = RBE_{dsb}

$$RBE_{dsb} = \frac{\text{(dose of } \gamma \text{ irradiation required to reduce the mean value to 50\%)}}{\text{('dose' of } ^{125}\text{I requried to reduce the mean value to 50\%)}}$$

[3] All survival data are own unpublihsed data. The D_o for γ irradiated CHOER cells equaled 232 cGy.

[4] The number of ^{125}I decays required to reduce the mean value to 50% for DNA dsb induction from accumulated^{125}IUdR and ^{125}IVME2 decays are from (1).

[5] The dose of γ radiation required to reduce the mean value to 50% for DNA dsb induction equaled 64 Gy (12).

The pattern of clustered DNA damage produced by ^{125}IUdR decay likely consists of short fragments of DNA on the order of 10–100s of base pairs. Martin & Haseltine (5) showed the highest frequencies of strand breaks in an oligonucleotide at about 10 base pairs upstream and downstream of the DNA-incorporated ^{125}IUdR and Panyutin & Neumann (6) found that approximately 1 DNA dsb is formed opposite to the site of ^{125}I incorporation and within 10 base pairs of the site of incorporation when they used a ^{125}I-labeled triplex forming oligonucleotide, which specifically binds to a particular nucleotide sequence in the major groove of double stranded DNA in vitro. Brenner & Ward (7) calculated that 2–6 ionizations are required within 1–4 nm volume of DNA for the formation of a DNA dsb. ^{125}I decay produces a cascade of 22–25 low-energy electrons per decay that could very well produce the requisite 2–6 ionizations within a 1–4 nm volume of DNA. Folding of the mammalian cell DNA into more compact chromatin structures, such as nucleosomes or solenoids, may place adjacent segments of DNA in close proximity to the incorporated radioactivity, resulting in strand breakage further than 10 bp from the site of ^{125}IUdR incorporation (about 100s of base pairs away since solenoids consist of about 1 kbp DNA).

In contrast to the data discussed above using ^{125}IUdR decay accumulation in cellular DNA, evidence suggests that the clustered DNA damage produced by accumulation of ^{125}I-estrogen decays in cellular DNA may comprise both the small fragments (10–100s bp) described above and large domain-sized fragments. In these studies, RBE_{dsb} from ^{125}I-estrogen decay accumulation studies are much higher than RBE_s (RBE_{dsb} was 24 and RBE_s was approximately 4) indicating that not only clustered DNA damage is produced but also a greater amount of additional DNA damage is induced by ^{125}I-estrogen than by ^{125}IUdR. The physical decay characteristics producing a high density of ionizations in DNA

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per ^{125}I decay does not change when using ^{125}IUdR or ^{125}I-estrogen but the target DNA does change. Other factors must be involved in the production of the'additional' DNA damage by ^{125}I-estrogen decay. This factor could be the nuclear location targeted by ^{125}1-estrogen. The nuclear target is genomic DNA at the nuclear matrix (8). The localization of ^{125}I-estrogen to the base of several looped domains of DNA could result in dense energy deposition at the base of looped domains of DNA that would produce DNA fragments approximately the size of looped domains of DNA (10–200 kbp) (9) in addition to the short fragments described above.

The significance of this so-called 'additional' clustered DNA damage (longer fragments equivalent to 100 kbp) produced by ^{125}I-estrogen may be in elucidating the non-homogeneous nature of the mammalian cell genome. The 'additional' clustered DNA damage produced by ^{125}I-estrogen decay saccumulation does not result in 'additional' cell killing. The RBE for cell killing by ^{125}I-estrogen compared to γ irradiation is optimally about the same as the RBE for cell killing by ^{125}IUdR compared to γ irradiation. Therefore, since there is no increase in effectiveness of ^{125}I-estrogn killing over ^{125}IUdR, we can speculate that the *extra* DNA damage that is observed with ^{125}I-estrogen is not likely lethal for the cell. Extension of this line of reasoning suggests that the type of clustered DNA damage produced by ^{125}IUdR (short fragments are produced) are the lethal lesions proudced by ^{125}I decay.

The notion of extra DNA damage produced by ^{125}I decay not contribuing to lethality is consistent with other studies labelling cells at speciifc sites in the nuclear genome, causing increased or decreased cell kill. These data and others confirm the idea that the entire nuclear genome is not uniformly sensitive to accumulated ^{125}I decay (10) although this is the first demonstration of *different DNA dsb damage* corresponding to similar levels of cell killing. Several models have been proposed to explain how ^{125}I decay can produce different degrees of cell killing. For example, some models include the postulation of, 1). Sensitive specific gene sequences, 2). Specific locations in chromatin, 3). Target versus non-target, 4). 2 targets versus multiple targets, and 5). Simple versus complex lesions. It is clear from the multitude of models proposed to explain differential cell killing by ^{125}I that further work needs to be done.

In conclusion, since the additional DNA damage produced by ^{125}I-estrogen decay accumulation does not correlate with increased cell killing, the additional DNA damage produced by ^{125}I-estrogen at the base of looped domains of DNA is probably not a primary target for radiaiton-induceed cell death. Therefore, these data show that additional DNA damage can be produced in the nuclear genome relative to chromatin but, the entire nuclear genome is not uniformly sensitive to DNA damage produced by ^{125}I decay.

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