Clustered DNA Damage Spectrum in Primary Human Hematopoietic Stem Cells from Smokers is Similar to the Radiation-Induced Damage Spectrum

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Clustered DNA Damages Induced by Low Radiation Doses

Irradiation of cells with low doses of X- or γ-rays induces clustered damages in mammalian cells with a linear dose response, indicating that a cluster is produced by one radiation 'hit,' i.e., an ionizing photon or particle and its accompanying cloud of radicals.

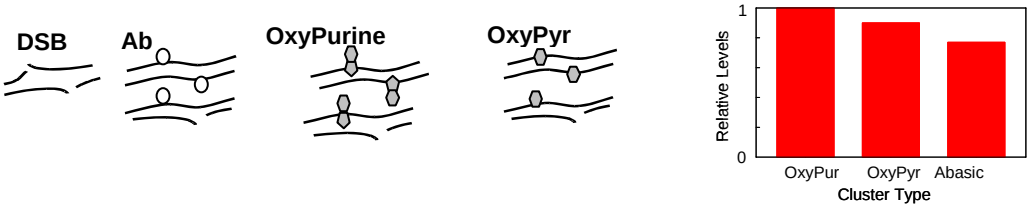

Figure 1. (Left) Diagram of DSB and Abasic clusters, OxyPurine clusters and OxyPyrimidine clusters. Sutherland et al., Proc. Natl. Acad. Sci. USA, 97, 103-108. (2000); Right, DNA damage spectrum (relative cluster levels) for clusters produced by low LET radiation.

X-rays induce substantial levels of all cluster classes, and a DNA Clustered Damage Spectrum (relative cluster levels) of 1 Oxidized Purine clusters: 0.9 Oxidized Pyrimidine Cluster: 0.77 Abasic Cluster.

Endogenous Clusters in Human Cells

Endogenous cellular processes induce oxidative stress and radicals, and thus might induce damage clusters. However, cellular DNA (isolated in argon to prevent induction of artifactual clusters) from most established hematopoietic cell lines contain low levels of endogenous clusters of any kind.

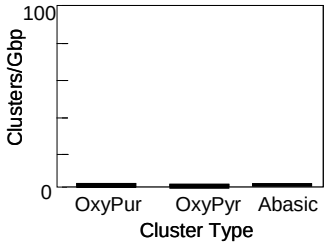

Figure 2. Clustered damage levels for four unirradiated human hematopoietic cell lines. Bennett et al., *Free Radic Biol Med* 37, 488-99 (2004).

Two lines—TK6 and WI-L2-NS—reported to be repair deficient, contained both OxyPurine and OxyPyrimidine clusters, but no abasic clusters. Thus even these repair deficient cells did not accumulate abasic clusters.

Endogenous Clusters in Primary Human Hematopoietic Stem and Progenitor Cells

To determine the level of endogenous damages in human hematopoietic tissue, we tested bone marrow stem and progenitor cells (non-cultured samples) from normal healthy human

donors. Cells from three donors contained very low levels of clusters. In contrast, DNA from stem cells of smokers (otherwise healthy donors of similar ages and both genders) contained high levels of OxyPurine clusters (~ 50/Gbp), OxyPyrimidine clusters (~ 22/Gbp), and of Abasic clusters (~ 42/Gbp). These data suggested two possibilities: that the level of Ape1, the human repair enzyme with activity towards abasic sites was decreased in smokers, or that the level of stressors in smokers was so high that the constitutive levels of Ape1 were unable to remove the accumulating clusters. Initial Ape1 activity assays of extracts of smokers and non-smokers suggest that the former is the case.

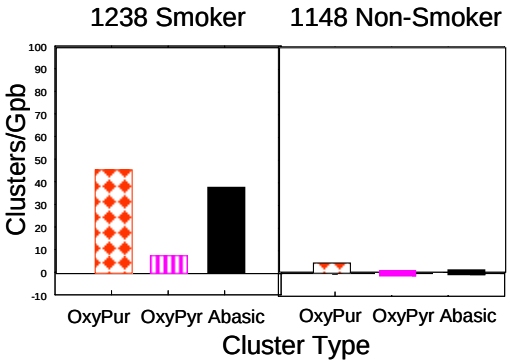

Figure 3. Endogenous cluster levels in primary human hematopoetic stem and progenitor cells from the bone marrows of two healthy donors, one a smoker (Donor 1238) and one a non-smoker (Donor 1148). Similar results were obtained from three other smoker donors and two additional non-smoker donors. Small filled symbols, individual measurements; large open symbols, averages; Error Bars, SD.

Since some preparations of Nfo protein (used to detect and quantify Abasic clusters) can also cleave at oxidized pyrimidines, we characterized the activity of the Nfo protein (purified to homogeneity in our laboratory) using synthetic oligonucleotides with a single lesion. The Nfo protein used in these determinations was purified according to a conventional procedure (Ljungquist, 1977). It has been shown that this preparation of Nfo had extremely low activity towards oxidized pyrimidines (Ishchenko et al., 2006). Thus the abasic clusters we measure by using this Nfo are indeed true abasic clusters.

Conclusions

Two characteristics are strikingly different in the hematopoietic stem and progenitor cells of the smokers vs. the non-smokers. First, the absolute yields of all types of clustered damages were significantly higher in the stem cells from smokers than from non-smokers. In addition, the spectrum of DNA damages in the smokers included high relative levels of abasic clusters, similar to that found in directly irradiated cells.

Acknowledgments

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References

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Ljungquist, S. (1977) A new endonuclease from Escherichia coli acting at apurinic sites in DNA. *J. Biol. Chem.*, **252**, 2808-2814.