INVITED PERSPECTIVE

γ-H2AX Foci in Peripheral Blood Lymphocytes to Quantify Radiation-Induced DNA Damage After 177Lu-DOTA-Octreotate Peptide Receptor Radionuclide Therapy

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The paper by Denoyer et al. in the current issue of The Journal of Nuclear Medicine (1) explores the possibility of quantifying radiation-induced DNA damage after internal treatment with 177Lu-DOTA-octreotate (LuTate) peptide receptor radionuclide therapy for metastatic or inoperable neuroendocrine tumors. Radiation-induced DNA damage was quantified by analyzing the kinetics of phosphorylated histone variant H2AX (γ-H2AX) foci in peripheral blood lymphocytes. The results showed a significant correlation between peak-foci number and absorbed dose to tumor and bone marrow. The authors suggest that as a next step, studies are needed in two directions: first with respect to acute toxicity, that is, investigating the high dose end of the DNA damage; and then with respect to the secondary malignancy resulting when misrepaired damage leads to radiation-induced mutations.

Radiation produces its main damage in the nucleus of cells. Clusters of ionization in the DNA result in the damage typical of ionizing radiation. Most important from this perspective are base damage, DNA single-strand breaks, and DNA double-strand breaks (2). If DNA double-strand breaks are left unrepaired, it is not instantaneous cell death that occurs but death after a limited number of cell divisions, also known as clonogenic death of the so-called doomed cells (3). Radiation has always been around us. From an evolutionary point of view, cells had to cope with this damage, and a machinery of repair processes is available for this purpose. Jeggo et al. made it clear that “Double strand breaks represent the most biologically significant lesion induced by ionizing radiation,” and cells are able to repair this damage to some extent (4). The DNA damage response consists of several pathways. These pathways lead to programmed cell-cycle delays, DNA repair, cell death, or combinations of these (2). DNA repair can take place at several levels: base excision repair, single-strand break repair, and—most important—double-strand break repair. Unrepaired DNA double-strand breaks can lead to cell death, and misrepaired DNA damage can lead to chromosomal translocations, mutations, and subsequently carcinogenesis.

Within a few minutes after irradiation, phosphorylation of H2AX histone (γ-H2AX) starts, as can be detected using immunostaining as specific nuclear γ-H2AX foci. After a rapid increase, with maximum values usually observed after approximately 1 h, DNA repair results in a decrease in these γ-H2AX foci, with maximum repair at 24–48. Unrepaired foci at that time point are considered residual damage and may be most important for tumor cell kill. These processes are dose-dependent, making assessment of γ-H2AX foci a useful tool for quantification of radiation-induced DNA damage. Thus, the number of γ-H2AX foci is considered a biomarker of exposure, and the remaining foci are a measure of repair capacity and are considered a biomarker of susceptibility (5–7). However, direct quantification of exposure has limited value because of the fast kinetics of decline and the wide variation in foci numbers between individuals, as is also clearly observed in the study by Denoyer et al. (1,5). Koch et al. showed that residual γ-H2AX foci are more likely to predict local tumor control after radiotherapy than initial damage or the kinetics of repair (8).

Even without irradiation, (tumor) cells can exhibit γ-H2AX foci. These baseline levels in expression of γ-H2AX foci show large variations and therefore need to be considered (5). This constitutive expression of γ-H2AX has been investigated in a cohort of breast cancer patients and was found to be prognostic in triple-negative patients (9). Expression was highest in triple-negative, BRCA1- or p53-mutated breast cancer cell lines (9). Recent results from my own group indicate that there is a correlation with telomere length (10). Shorter telomeres have recently been described in BRCA-related tumors (11). These shorter telomeres—signs of telomere dysfunction—could also explain the poorer prognosis of triple-negative breast cancer patients with γ-H2AX–positive tumors. However, constitutive expression of γ-H2AX might also be caused by aberrant repair signaling (12,13). Abnormalities in DNA repair capability may indeed be detected by assessment of γ-H2AX foci, as reported, for instance, by Löbrich et al. in patients who underwent CT. A radiosensitive patient was shown to have a relatively high number of γ-H2AX foci after this procedure (14). At very low doses, below 370 MBq (10 mGy), assessment of γ-H2AX foci becomes imprecise because of difficulties in the kinetics of the decrease.

See page 505
in γ-H2AX foci (15). Finally, proteins that are involved in DNA repair are also involved in DNA replication. Therefore, misinterpretation of γ-H2AX quantification can be the result of how cell populations are distributed through the cell cycle. By assessment of baseline expression of γ-H2AX foci, that is, just before application of the radiopharmaceutical drug, misinterpretation can be limited. Denoyer et al. found that peripheral blood lymphocytes exhibit pretreatment levels of γ-H2AX foci of between 0.06 and 0.75 foci per cell. Whether this variation is a sign of differences in baseline radiosensitivity, proliferation, telomere length, or other factors remains to be established but could be important for the correct interpretation of these results.

In conclusion, it is clear that quantification of γ-H2AX foci has high potential for quantifying genomic instability, as well as for quantifying radiation-induced toxicity both at a low dose for assessment of the mutational effects of low-dose exposure to ionizing radiation and for quantifying DNA damage by high-dose ionizing radiation for therapeutic reasons. The study by Denoyer et al. is another step forward in demonstrating the value of γ-H2AX foci for nuclear medicine purposes.

DISCLOSURE

No potential conflict of interest relevant to this article was reported.

REFERENCES

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