Journal of Nuclear Medicine, published on February 26, 2015 as doi:10.2967/jnumed.115.153841

γH2AX foci in peripheral blood lymphocytes to quantify radiation-induced DNA damage after ^{177}Lu-DOTA-octreotate peptide receptor therapy.

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In the paper by Denoyer et al. in the current issue of the Journal of Nuclear Medicine [1], the possibilities are explored to quantify radiation-induced DNA damage after internal treatment with ^{177}Lu-DOTA-octreotate peptide receptor therapy (LuTatePRRT) for metastatic or inoperable neuroendocrine tumors (NET). Radiation-induced DNA damage was quantified by analyzing the kinetics of γH2AX foci in peripheral blood lymphocytes (PBLs). 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, i.e. investigating the high dose end of the DNA damage and second, the secondary malignancy resulting from misrepaired damage leading to radiation induced mutations.

Radiation causes its main damage in the nucleus of cells. Clusters of ionizations in the DNA result in the damage that is typical for ionizing radiation. Most important in this perspective are base damage, DNA single strand breaks and DNA double strand breaks (dsb) [2]. If DNA double strand breaks are left unrepaired this does not lead to instantaneous cell death but leads to death after a limited number of cell divisions also known as clonogenic cell 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 extend [4]. The DNA damage response (DDR) consists of several pathways. These pathways lead to programmed cell cycle delays, DNA repair, cell death or combinations [2].

DNA repair can take place at several levels; base excision repair, single strand break repair and -most importantly- double strand break repair. Unrepaired DNA dsb's 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 histones (γH2AX) starts, which can be detected using immunostaining as specific nuclear γH2AX foci. After a rapid increase with maximum values usually observed after approximately one hour, DNA repair results in a decrease of these γH2AX foci with maximum repair at 24-48. Unrepaired foci at that time point are considered as residual damage and may be most important for tumor cell kill. These processes are dose-dependent, making assessment of γH2AX foci a useful tool for quantification of radiation induced DNA damage. Thus the number of γH2AX foci is considered as biomarker of exposure, remaining foci are a measure for repair capacity and are considered as a biomarker of susceptibility [5,6,7]. However, direct quantification of exposure has limited value because of the fast kinetics of decline and the wide variation of foci numbers between individuals, which is also clearly observed in the study by Denoyer et al [1,5]. Koch et al. showed that residual γH2AX 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 γH2AX foci. These baseline levels in expression of γH2AX foci show large variations and therefore need to be taken into account [5]. This constitutive expression of γH2AX has been investigated in a cohort of breast cancer patients, and found to be prognostic in triple negative patients [9]. It was highest in triple negative, BRCA1 and/or p53 mutated breast cancer cell lines [9]. Our own recent results 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 γH2AX positive tumors. However, constitutive expression of γH2AX might also be caused by aberrant repair signaling [12,13]. Abnormalities in DNA repair capability may indeed be detected by assessment of

γH2AX foci, which was for instance reported by Löbrich et al in patients that underwent a CT scan. A radiosensitive patient was shown to have relative high number of γH2AX foci after this procedure [14]. At very low doses, below 10mGy, assessment of γH2AX foci becomes imprecise which is related to difficulties to the kinetics of the decrease in γH2AX foci [15]. Finally, proteins that are involved in DNA repair are also involved in DNA replication. Therefore, misinterpretation of γH2AX quantification can be the result of how cell populations are distributed through the cell cycle. By assessment of baseline expression of γH2AX foci, i.e. just prior to application of the radiopharmaceutial drug, this can be limited. Denoyer et al. found that PBLs exhibit pre-treatment levels of γH2AX foci of between 0.06 and 0.75 foci/cell. Whether this variation is a sign for differences in baseline radiosensitivity, proliferation, telomere length, etc. remains to be established, but could be important for the correct interpretation of these results.

In conclusion, it is clear that quantification of γH2AX foci has a high potential for the application of quantifying genomic instability, and for quantifying radiation-induced toxicity both at a low dose for assessment of mutational effects of low dose exposure to ionizing radiation, as well as for quantifying DNA damage by high-dose ionizing radiation for therapeutic reasons. The study by Denoyer et al is another step forward with respect to the value of γH2AX foci for nuclear medicine purposes. γH2AX foci can be used to assess myelotoxicity and potentially induction of mutations leading to secondary malignancies even if the complex kinetics are taken into account that are involved in applying radiopharmaceuticals.

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