Nuclear patterns of DNA damage response and DNA replication, studied with voxel correlation.

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Inhibition of topoisomerase I and II leads to formation of double-strand DNA breaks (DSBs) in cells which are carrying out DNA replication in the S-phase of cell cycle. Phosphorylation of histone H2AX on serine 139 (γH2AX) is an early step in cellular response to DSBs, followed by recruitment of p53-binding protein (53BP1), which controls the pathway of DSB repair. Patterns of replicating DNA, nuclear distribution of γH2AX [1-2] and 53BP1 [3] can be imaged at high resolution with 3D fluorescence microscopy. It has been demonstrated (using analysis of centroid distances) that DNA replication foci correlate in space with the high-intensity γH2AX foci, but not those of low intensity [1-2]. Therefore, only the former may be regarded as markers of DSBs [1-3]. This approach is limited in case of corresponding 53BP1 pattern where only high-intensity foci can be reliably segmented. One may note that such these foci are detectable also in non-replicating cells (G-phase). Therefore, we quantify pattern of nuclear 53BP1 distribution using spatial correlation of voxels in a range of intensity classes. We demonstrate that blocking of topoisomerases induces redistribution of the protein, depending on the phase of cell cycle and the inhibitor. We apply this technique to characterize change of respective patterns of γH2AX and newly replicated DNA. Finally, we study effects of topoisomerase inhibition on relationship between distributions of the two proteins and newly replicated DNA with voxel cross-correlation. These data reveal distinct types of DNA damage inflicted by topoisomerase inhibitors in cell cycle dependent manner.

1. K. Berniak, P. Rybak, T. Bernas, M. Zarębski, E. Biela, H. Zhao, Z. Darzynkiewicz, J. W. Dobrucki. Relationship between DNA damage response, initiated by camptothecin or oxidative stress, and DNA replication, analyzed by quantitative 3D image analysis. (2013) Cytometry, 83 (10), 913 - 924
2. T. Bernas, K. Berniak, P. Rybak, M. Zarębski, H. Zhao, Z. Darzynkiewicz, J. W. Dobrucki. Analysis of spatial correlations between patterns of DNA Damage Response and DNA replication in nuclei of cells subjected to replication stress or oxidative damage. (2013) Cytometry, 83 (10), 925 - 932
3. P. Rybak, A. Hoang, L. Bujnowicz, T. Bernas, K. Berniak, M. Zarebski, Z. Darzynkiewicz, J. Dobrucki Low level phosphorylation of histone H2AX on serine 139 (γH2AX) is not associated with DNA double-strand breaks. (2016) Oncotarget 7(31):49574-49587