Changes in Chromatin Conformation and PARP-1 Activity Induced by Cisplatin in Rat Liver

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I. G. Artsruni
K. S. Matinyan
L. H. Demirkhanyan
E. S. Gevorgyan

Faculty of Biology, Dept of Biophysics
Yerevan State Univ., Alex Manoogian 1
Yerevan, Armenia 0049
GANA@ysu.am

The formation of ladder configuration of the nucleosomal DNA fragmentation is a biochemical hallmark of apoptosis in different cell types. To date, the regulation of apoptotic DNA fragmentation has been well explained by the CAD/ICAD system operating in dying cells. Nevertheless, in some cases the internucleosomal DNA fragmentation in apoptosis is mediated by Ca^{2+}/Mg^{2+} endonuclease. It was suggested that suppression of Ca^{2+}/Mg^{2+} endonuclease activity contribute to formation of resistance of cancer cells to chemotherapeutic drugs by inhibition of non-random DNA degradation in nuclei.

Coming from the knowledge that the character and intensity of DNA degradation in chromatin are determined by its accessibility for cleaving endonucleases, we suppose that DNA internucleosomal fragmentation in apoptosis could be modulated by definite epigenetic changes in chromatin structure and architecture caused by chemotherapeutic drug cisplatin.

To assess the ability of cisplatin to alter chromatin structure in a manner that recapitulates inhibition of internucleosomal DNA fragmentation, we assessed whether the drug is capable to affect the accessibility of liver chromatin to endogenous Ca^{2+}/Mg^{2+} endonuclease activity. Taking into the account that poly-ADP-ribosylation plays a prominent role in determination of chromatin architecture and regulation of basic chromatin-associated functions we examined the effect of cisplatin administration on rat liver nuclei poly(ADP-ribose)polymerase-1 (PARP-1) activity.

As it was shown in our previous study, the addition of Ca^{2+} and Mg^{2+} ions into incubation media of naked rat liver nuclei caused rapid activation of Ca^{2+}/Mg^{2+} endonuclease and eventually internucleosomal cleavage of nuclear DNA. In present study we revealed that in 24 hour of cisplatin administration to out-breed white rats (intraperitonial, 10 mg/1kg weight) the internucleosomal DNA ladder generated by endogenous Ca^{2+}/Mg^{2+} endonuclease in liver nuclei loses its characteristic sharpness. We detect so called "smearing" of corresponding DNA bands, visualized by agarose gel electrophoresis. Importantly, changes in DNA ladder configuration were accompanied by suppression of PARP-1 activity in the liver nuclei of cisplatin treated animals.

These data suggest that cytotoxic effect of cisplatin can be mediated by DNA-cisplatin interactions that occur in linker regions of chromatin.

Chromatin Higher Order Structure and Regulation of its Compaction

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Daniela Rhodes*
Sara Sandin
Andrew Routh
Philip Robinson

MRC Laboratory of Molecular Biology
Hills Road, Cambridge, CB2 0QH, UK
*rhodes@mrc-lmb.cam.ac.uk

During the past decade it has become evident that histone and DNA modifications are key regulators of all nuclear processes whose substrate is DNA. While the effects of, for instance, histone post-translational modification on transcription are well-documented, there is no mechanistic understanding of how such modification regulate chromatin condensation directly, or indirectly. Such an understanding is dependent on knowledge of the three-dimensional structure of chromatin. Although the structure of the first level of DNA folding, the nucleosome core, is known at atomic resolution, the structure of the second level of folding, whereby a string of nucleosomes folds into a fiber with an approximate diameter of 30 nm – the '30-nm' chromatin fiber, remains undetermined. I will describe our studies on the higher orders structure of chromatin with two primary aims:

1.) Determination of the structure of the '30nm' chromatin fiber to provide an understanding of fiber topology and nucleosome-nucleosome interactions.