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Genome-wide multi-parametric analysis of H2AX or γ H2AX distributions during ionizing radiation-induced DNA damage response

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Boston, MA, USA. 11-13 March 2013

Background

After induction of DNA double strand breaks (DSBs), the DNA damage response (DDR) is activated. One of the earliest events in DDR is the phosphorylation of serine 139 on the histone variant H2AX (γ H2AX) catalyzed by phosphatidylinositol 3-kinases-related kinases. Despite being extensively studied, H2AX distribution[1] across the genome and γ H2AX spreading around DSBs sites[2] in the context of different chromatin compaction states or transcription are yet to be fully elucidated.

Materials and methods

γ H2AX was induced in human hepatocellular carcinoma cells (HepG2) by exposure to 10 Gy X-rays (250 kV, 16 mA). Samples were incubated 0.5, 3 or 24 hours post irradiation to investigate early, intermediate and late stages of DDR, respectively. Chromatin immunoprecipitation was performed to select H2AX, H3 and γ H2AX-enriched chromatin fractions. Chromatin-associated DNA was then sequenced by Illumina ChIP-Seq platform.

HepG2 gene expression and histone modification (H3K36me3, H3K9me3) ChIP-Seq profiles were retrieved from Gene Expression Omnibus (accession numbers GSE30240 and GSE26386, respectively).

Results

First, we combined G/C usage, gene content, gene expression or histone modification profiles (H3K36me3, H3K9me3) to define genomic compartments characterized by different chromatin compaction states or transcriptional activity. Next, we investigated H3, H2AX and γ H2AX distributions in such defined compartments

before and after exposure to ionizing radiation (IR) to study DNA repair kinetics during DDR.

Our sequencing results indicate that H2AX distribution followed H3 occupancy and, thus, the nucleosome pattern. The highest H2AX and H3 enrichment was observed in transcriptionally active compartments (euchromatin) while the lowest was found in low G/C and gene-poor compartments (heterochromatin). Under physiological conditions, the body of highly and moderately transcribed genes was devoid of γ H2AX, despite presenting high H2AX levels. γ H2AX accumulation was observed in 5' or 3' flanking regions, instead. The same genes showed a prompt γ H2AX accumulation during the early stage of DDR which then decreased over time as DDR proceeded. Finally, during the late stage of DDR the residual γ H2AX signal was entirely retained in heterochromatic compartments. At this stage, euchromatic compartments were completely devoid of γ H2AX despite presenting high levels of non-phosphorylated H2AX.

Conclusions

We show that γ H2AX distribution ultimately depends on H2AX occupancy, the latter following H3 occupancy and, thus, nucleosome pattern. Both H2AX and H3 levels were higher in actively transcribed compartments. However, γ H2AX levels were remarkably low over the body of actively transcribed genes suggesting that transcription levels antagonize γ H2AX spreading. Moreover, repair processes did not take place uniformly across the genome; rather, DNA repair was affected by genomic location and transcriptional activity. We propose that higher H2AX density in euchromatic compartments results in high relative γ H2AX concentration soon after the activation of DDR, thus favoring the recruitment of the DNA repair machinery to those compartments. When the damage is

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repaired and γ H2AX is removed, its residual fraction is retained in the heterochromatic compartments which are then targeted and repaired at later times.

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Published: 18 March 2013

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doi:10.1186/1756-8935-6-S1-P58

Cite this article as: Natale *et al.*: Genome-wide multi-parametric analysis of H2AX or γ H2AX distributions during ionizing radiation-induced DNA damage response. *Epigenetics & Chromatin* 2013 **6**(Suppl 1):P58.

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