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Correspondence

Phosphorylation of H3 serine 10 by IKKα governs cyclical production of ROS in estrogen-induced transcription and ensures DNA wholeness

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Dear Editor,

A growing body of evidence indicates that several modifications on adjacent nucleosomal histone residues (marks) work in a combinatorial fashion to control access to DNA of multiple proteins involved in transcription, replication and repair.[1] In fact, the 'methyl/phos switch' hypothesis states that phosphorylation of serine 10 in histone H3 (H3S10) during activation of gene expression controls the methylation level of the preceding lysine (H3K9) by inhibiting recruitment of histone methyltransferase (HMT) factors to that site.[2] However, it has also been found that addition of the dimethyl group to the same lysine in synthetic H3 oligopeptides reduces approximately to one-third ability of the following serine to be phosphorylated *in vitro*.[2]

We have analyzed by chromatin immunoprecipitation (ChIP) the K9/S10 cross-talk in the estrogen receptor-expressing (ERα$^+$) human breast cancer MCF-7 cells depleted or not of the lysine-specific demethylase 1 (LSD1) that removes the methyl group from mono- and dimethylated lysine 9 in histone H3.[3] Permanence of H3K9me2 near the paradigmatic E2-responsive *pS2* promoter in LSD1-interfered cells challenged with 17β-estradiol (E2) prevented addition of the phosphoryl group to the following site, revealing to represent a pre-requisite for that modification, thus upgrading the current version of the 'methyl/phos' switch theory (Supplementary Figure 1A). Therefore, we have investigated the molecular mission of H3S10 phosphorylation in the transcriptional control by estrogens.

Upon parallel ChIPs in cells where the ERα-associated IKKα (that is the kinase responsible for phosphorylation of H3S10)[4] had been knocked down with siRNAs, we have observed that absence of H3S10ph accelerated of ~30 min targeting of the HMT Suv39H1 to *pS2* promoter. Moreover, in the same cells, H3K9me2 was constantly high, in spite of the concomitant presence of LSD1 (Supplementary Figure 1B). We imagined that this pattern could depend on the opposite action of LSD1 and Suv39H1, both present on chromatin, and consequently, as ERα-triggered demethylation of H3K9me2 by LSD1 produces reactive oxygen species (ROS) with oxidation of guanines (8-oxo-Gs) in flanking DNA,[5] we have assessed the accumulation of oxidized bases around *pS2* promoter. Silencing of IKKα in E2-challenged cells resulted into an increased presence of either 8-oxo-Gs as well as of the base-excision repair enzyme 8-oxo-guanine-DNA glycosylase 1 (OGG1, that cooperates in the removal of modified Gs upon formation of nicks on DNA)[6] and into a hampered transcription of *pS2* gene (Supplementary Figure 1B). On the basis of these observations, we have supposed that persistent and high levels of oxidized bases could damage DNA and that rupture of DNA integrity could trigger programmed cell death.

In fact, evaluation of poly(ADP-ribose) polymerase (PARP) cleavage as marker of apoptosis in cells challenged with E2 for 24 h in the presence or absence of the specific kinase inhibitor BAY11-7082 (BAY)[7] added for the first 6 h (a time sufficient to eventually accumulate ROS during cyclical activation of transcription by estrogens)[8] revealed that PARP fragmentation was clearly augmented by concomitant addition of BAY and E2 (Supplementary Figure 1C). This effect was mediated by the estrogen receptor, as it was not observed in the ERα$^-$ breast cancer MDA-MB-231 cell line, and by produced ROS, as revealed by treatment with their scavenger *N*-acetyl cysteine that reverted the increase of apoptotic cells treated with BAY and E2, a finding that was confirmed also by FACS analysis (Supplementary Figure 1C). Thus, inhibition of IKKα switched the effect of estrogens on MCF-7 cells from anti- to proapoptotic.

The role of phosphorylation of H3S10 in the transcriptional process governed by estrogens pictures a novel use of E2 that, in combination with the inhibition of IKKα activity, could be profitably tested in therapeutic trials for treatment of human hormone-responsive breast cancers.

Conflict of Interest

The authors declare no conflict of interest.

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