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Keywords: Histone modification; Heterochromatin; Oncogene-induced senescence; plasmonic nanoparticles; plasmonic coupling effect

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Gold and Silver Plasmonic Nanoprobes Trace the Positions of Histone Codes

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Keywords : Histone modification; Heterochromatin; Oncogene-induced senescence; plasmonic nanoparticles; plasmonic coupling effect; single-cell imaging

Abbreviations : ChIP-seq, Chromatin-Immunoprecipitation-sequencing; H3K9me3, tri-methylation of the 9th lysine residue of histone 3; H3K27me3, tri-methylation of the 27th lysine residue of histone 3; HP1, heterochromatin protein 1; OIS, oncogene-induced senescence; SAHF, senescence-associated heterochromatin foci

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Abstract

We visualized the distribution of heterochromatin in a single nucleus using plasmonic nanoparticle-conjugated H3K9me3 and H3K27me3 antibodies. Due to distance-dependent plasmonic coupling effects between nanoprobes, their scattering spectra shift to longer wavelengths as the distance between heterochromatin histone markers reduced during oncogene-induced senescence (OIS). These observations were supported by simulating scattering profiles based on considerations of particle numbers, interparticle distances, and the spatial arrangements of plasmonic nanoprobes. Using this plasmon-based colourimetric imaging, we estimated changes in distances between H3K9me3 and H3K27me3 during the formation of senescence-associated heterochromatin foci in OIS cells. We anticipate that the devised analytical technique combined with high-spatial imaging and spectral simulation will eventually lead to a new means of diagnosing and monitoring disease progression and cellular senescence.

Histones are the most abundant, highly charged polycationic globular proteins located in cell nuclei. DNA strands wrap histone octamers (two sets of H2A, H2B, H3, and H4) to form a structural unit called a nucleosome (Figure 1a upper). Tens of modifications in histone isoforms have been found and new modifications continue to be identified. The possible combinations of modifications in individual histone molecules, that is, in histone code, exceed trillions. Considering that each histone octamer is wrapped by a 147 bp long nucleotide with a unique DNA sequence, in combination histone and DNA sequences provide enormous information capacity. The histone code determines which gene should be expressed, while DNA sequences of a gene contain information on the functional structures of proteins and RNAs. ChIP-seq analyses using an antibody, such as an antibody specific for H3K9me3 (trimethylated 9th lysine of histone), provide an address written in DNA A, C, G, T bases where H3K9me3 locate on genome. It might be considered that if H3K9me3 and H3K27me3 share the same DNA address, they are located near each other, perhaps in the next nucleosome or the same molecule. However, the DNA-sequence address of a modified histone does not give information about its spatial distribution in a nucleus.

Regardless of limited resolution, microscopic imaging of heterochromatin-specific histone modifications such as H3K9me3 and H3K27me3 has shown the presence of distinct spatial distributions that can differentiate senescent cellular phenotypes. Hutchinson-Gilford Progeria Syndrome is a rare, fetal genetic disease that induces premature senescence, and the syndrome is characterized by a defect in Lamin A protein (a scaffold protein of the nuclear envelope). Furthermore, instability of the nuclear envelope exhibits results in dramatic heterochromatin rearrangement. Oncogene-induced senescence (OIS) also dramatically disturbs heterochromatin structure and the nuclear envelope. Oncogenes such as Ras, Raf, and Myc paradoxically induce cellular senescence when there are no additional mutations of tumor suppressor genes. We found that plasmonic nanoprobe-based immunostaining of OIS cells using an antibody specific for H3K9me3 revealed distinct clustered patterns of senescence-associated heterochromatin foci (SAHF) (Figure 1a lower). Furthermore, our observations that (i) Lamin A protein interacts with Heterochromatin-Protein 1 (HP1); (ii) HP1 specifically binds H3K9me3, and (iii) HP1 proteins oligomerize may explain how H3K9me3 locates along the nuclear envelope in normal growing cells. On the other hand, in senescent cells, H3K9me3 displayed a punctate intranuclear pattern. During OIS, H3K27me3 spatially rearranged to produce SAHF. These observed spatial arrangements of histone modifications in the nucleus appear to represent unique features of epigenetic status that might be useful for determining

cellular status, whereas conventional histone imaging methods using organic fluorescent dyes are limited by photo-bleaching, poor resolution, and the obligatory use of secondary antibodies.

We first employed gold and silver plasmonic nanoparticles as probes to detect the spatial distributions of histone modifications. When two or more plasmonic nanoparticles are placed in close proximity, they exhibit plasmonic coupling, which induces spectral shifts in absorbance and scattering (Figure 1b). These spectral shifts are sensitively dependent on interparticle distance, arrangement, and type of the nanoparticles (Figure 1c). We utilized plasmonic nanoparticles conjugated with primary antibodies for H3K9me3 and H3K27me3 to achieve high-spatial and colourimetric imaging of histone markers in a single nucleus. The distance-dependent plasmonic coupling effect between the nanoprobes allowed distances between histone modifications to be estimated and interpreted. The proposed method based on spectral analyses of plasmonic probes provides a novel means of visualizing spatial changes of modified histones and other biomarkers at the single cell level and of predicting disease progression or the status of senescence.

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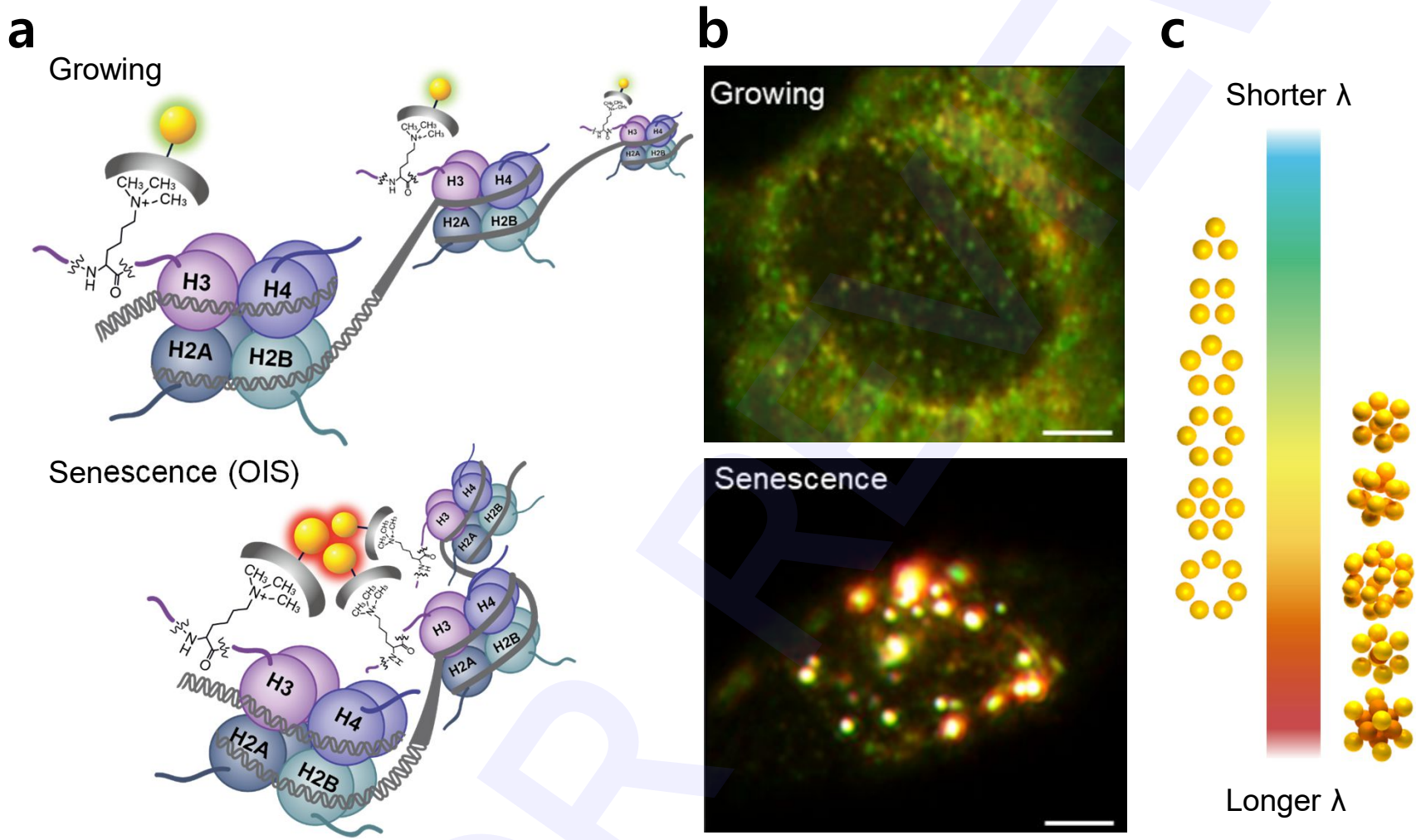
Figure Legends

Figure 1 Spatial distribution of histone modifications detected using plasmonic nanoparticles conjugated to histone antibodies. (a) Schematic diagram of nanoprobe-conjugated H3K9me3 antibody and of the specific arrangements of histone octamers in young growing cells (upper) and oncogene-induced senescent (OIS) cells (lower). (b) Representative dark-field scattering images of plasmonic nanoprobes targeting heterochromatin histone markers in a young growing cell (upper) and a senescent (lower) cell. Scale bar, 10 μm. (c) Relationship between the spectral peak (λ_{max}) of simulated scattering and the spatial arrangements of plasmonic nanoprobes.


a
Growing
H3
H4
H2A
H2B
Senescence (OIS)
b
Growing
Senescence
c
Shorter λ
Longer λ