Overall, the innovative approach of combining SEC data with cysteine-directed ABPP has opened up new possibilities for identifying chemical probes that can perturb protein complexes in human cells. Not only have they successfully targeted historically undruggable protein classes, but the potential for scaling up this approach to profile larger electrophilic compound libraries (e.g., DOS-constructed libraries) is enormous and has broad implications in chemical biology and drug discovery.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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Prometheus unshackled: Liver regeneration makes you young

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In this issue of *Molecular Cell*, Yang and colleagues[1] discover age-dependent increases in broad regions of the repressive histone modification H3K27me3. They also demonstrate partial reversion to younger H3K27me3 patterns and gene expression upon resection of older livers.

Chromatin modifications are essential for maintaining cellular identity. Accordingly, as organisms and tissues lose cellular identity with age, there is accompanying global chromatin modification dysregulation across species and cell types.[2] However, the specific chromatin modifications that change, the magnitude and directionality of these changes, and the precise genomic loci affected have proven to be highly context- and cell-type-specific. While a long-standing theory postulates that aging is characterized by a loss of repressive chromatin modifications that results in spurious transcriptional upregulation in aged cells,[3] the universality of this theory has been challenged by conflicting findings in different tissues and organisms. In *Caenorhabditis elegans*, there is an age-dependent decrease in the repressive histone methylation mark H3K27me3.[4,5] Similarly, heterochromatin decreases with age in cells from older people[6] or in cells from patients with the premature aging disease Hutchinson-Gilford progeria syndrome. On the other hand, global levels of H3K27me3 increase with age in muscle stem cells of old mice[7] and in the skeletal muscle of African Killifish.[8] The effects of manipulating the enzymes that deposit these histone modifications for lifespan and aging phenotypes are similarly complex. Reducing expression of the H3K27me3 demethylase UTX-1 increases lifespan in *C. elegans*,[4,9] while decreasing expression of MES-2, the putative H3K27me3 trimethylase, also extends the lifespan of sterile *C. elegans*.[5]

In order to gain greater clarity on chromatin changes in aging, Yang

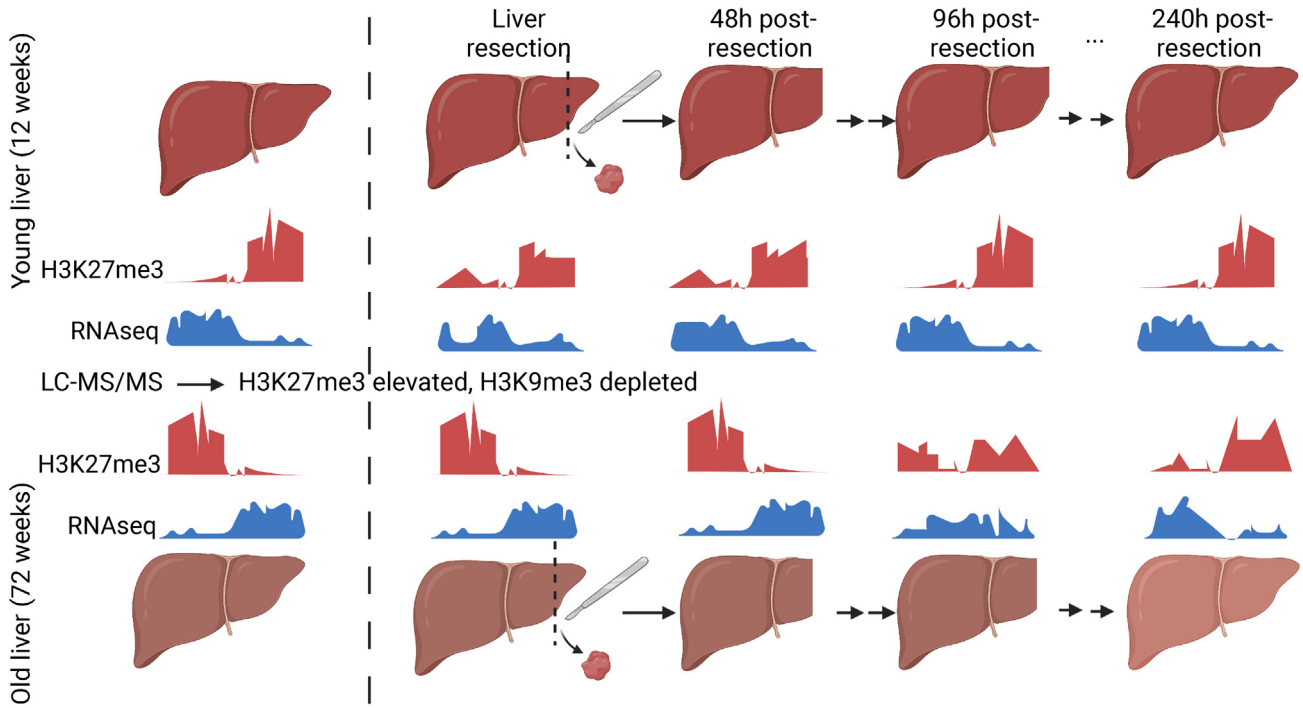

Figure 1. H3K27me3 and gene expression revert to more youthful state in mouse livers upon regrowth in response to resection
LC-MS/MS of young liver (12 weeks) and old liver (72 weeks) revealed a global increase in H3K27me3 and decrease in H3K9me3.[1] The elevated H3K27me3 was further confirmed by ChIP-seq and RNA-seq (left panel). Resection of mouse livers revealed that while young mouse livers regenerated more rapidly, old mouse livers upon regeneration reverted to a more youthful expression of genes and H3K27me3 pattern[1] (right panels). This figure was created using Biorender.com.

et al. performed an unbiased assessment of histone modifications by quantitative mass spectrometry in young and aged mouse livers. The authors found elevated repressive histone post-translational modifications, most notably H3K27me3, in the aged mouse liver. A more detailed age gradient found that H3K27me3 increased directly after the optimal reproductive age (~11 months in mice) and remained elevated for the remainder of the lifespan in mice livers. Importantly, the authors corroborate these mass spectrometry measures by orthogonal methods, including western blot and immunofluorescence. By performing H3K27me3 chromatin immunoprecipitation followed by sequencing (ChIP-seq) using spike in normalizations, the authors discovered several striking changes with aging, including a loss of locus-specific H3K27me3 at the promoters of individual genes and an increase in megabase scale regions of H3K27me3 across gene-poor regions (termed "age-domains"). H3K27me3 age-domains could also be identified in aged kidney, heart, and muscle tissues. The authors complement these genomic assays by a set of biochemical fractionations and single molecule atomic force microscopy, which together demonstrate that samples from older animals have larger and more compact chromatin arrays. The consequences of these changes in chromatin structure on gene transcription was several fold, including de-repression of specific genes encoding neuronal and cardiac lineage regulators in liver and a global suppression of gene transcription.

Among the more intriguing aspects of this study, the authors took advantage of the regenerative nature of the liver to ask whether regeneration of aged livers reverses aspects of age-dependent chromatin changes. Notably, while older mice livers were slower to regenerate than younger mice livers, the authors discovered that the chromatin and transcriptional landscapes of older livers post-resection were more similar to younger livers than pre-resection (Figure 1). This result highlights the potential of regeneration to reverse aging states in specific tissues. Indeed, it would be interesting to determine whether these regenerative H3K27me3 domains are conserved in highly regenerative species, such as zebrafish, axolotl, or starfish.

Many groups now have identified detailed maps of chromatin changes that occur with aging.[2] The study by Yang et al. significantly adds to this body of work and raises interesting future questions about the regulation and conservation of chromatin changes during aging. Are conserved "age-domains" less likely to show changes in species with similar genomes but significantly longer lifespans and slower aging clocks than mice, such as the naked mole rat or bats? How do these "age-domains" behave in more divergent species such as the jellyfish (*Turritopsis dohrnii*) or the fresh-water polyp (*Hydra vulgaris*), which have been purported not to age, or in populations of supercentenarian humans? Comparative species analyses could point to truly conserved mechanisms of regulation rather than passenger changes that accompany aging.

Due to the global dysregulation of chromatin modifications with age,

reversing age-induced chromatin modifications present attractive targets for combating age-dependent diseases. However, questions remain about whether inducing specific chromatin modifications at precise gene loci can be implemented without affecting other essential properties of cellular identity or stimulating cancer. Bridging the gap between genome-wide analyses of chromatin structure and gene expression and the role of individual genes that are critical for regulating aging is clearly a remaining challenge for the coming years. The regenerative liver provides a powerful system for performing directed manipulations of chromatin and examining the functional consequences. Utilizing nuclease null Cas9 fused with H3K27 methyltransferases[10] or demethylases at critical loci will help transform the field from correlative observations toward critical causal occurrences.

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