GENOMICS

Humanized mouse models provide new insights into human evolution

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As a species, we possess unique biological features that distinguish us from nonhuman primates (NHPs), including our closest relatives the chimpanzee and bonobo. Uniquely human traits are the result of genetic changes between humans and NHPs, but identifying these critical changes is challenging. Comparative genomics studies have identified DNA sequences known as Human Accelerated Regions (HARs) that are unique to humans and could contribute to the phenotypic differences between human and NHP. However, functional studies are needed to validate the role of these DNA regulatory regions in human evolution.

In a new study, Emily Dutrow and colleagues at Yale School of Medicine used knock-in mouse models to investigate the biological role of *HACNS1*, a HAR harboring a strong human-specific signature and also known as HAR2 and 2xHAR.3. Previous work has shown that *HACNS1* acts as a transcriptional enhancer and can drive increased expression of a LacZ reporter gene in the embryonic mouse limb compared to its NHP orthologs. Here, the investigators used homologous recombination to replace the orthologous mouse locus with 1.2 kb sequence of *HACNS1* or its chimpanzee counterpart in mice, and compare the impact of the mouse, chimpanzee and human sequences on limb development.

Using in-situ hybridization and transcriptomic analysis, the team showed that *HACNS1* induces unique spatial and quantitative changes in gene expression in the developing limb compared to the mouse and chimpanzee sequences, including changes in the expression of nearby gene *Gbx2*. Single-cell sequencing analysis notably revealed that *Gbx2* expression was increased in hindlimb bud of *HACNS1* embryos compared to the chimpanzee ortholog line and wild-type embryos. *Gbx2*, which encodes a transcription factor, was notably co-regulated with genes expressed in mesenchymal cells that would become chondrocytes. These results suggest that *HACNS1* maintains its human-specific enhancer activity in the mouse embryo and regulates chondrocyte differentiation.

"These findings support the long-standing hypothesis that discrete regulatory changes altering expression of pleiotropic developmental regulators in specific tissues contribute to the evolution of phenotypic differences—in the case of this study, molecular phenotypes—across species," write the investigators in their report. The team, who did not observe major morphological changes in the limb of *HACNS1* embryo, also explains that further analyses might reveal more subtle changes in the limb of humanized embryos and adult mice.

Altogether this study demonstrates the value of using genetically humanized mouse models to link genetic changes that arose during human evolution to the unique traits that make us humans.

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