INTRODUCTION: Osteoarthritis (OA) is a multifaceted pathological condition with an etiology that remains enigmatic. While OA involves numerous transcription-associated events, the precise mechanisms underlying these events are not fully understood. This challenges the identification of transcriptional regulatory targets essential for developing targeted OA therapies. Interestingly, triplex nucleic acid structures known as R-LOOPs, resulting from the hybridization of nascent RNA with its DNA counterpart, play roles in myriad biological processes. However, their involvement in OA remains unexplored. In this study, we utilized DRIP-Seq and RNA-seq to determine the genome-wide presence of R-LOOP and investigated its potential role in OA pathogenesis. Notably, based on DRIP-Seq findings, we probed the regulatory influence of the R-LOOP structure in the Keap1 promoter concerning ferroptosis in OA chondrocytes, offering promising therapeutic avenues for OA.

METHODS: The study received ethical approval from the Ethics Committee of the Second Affiliated Hospital of Guangzhou Medical University. We procured paired cartilage samples from the medial (OA) and lateral (control) condyles during total knee replacement procedures. Employing DRIP-seq and RNA-seq, we mapped the genomic distribution of R-LOOP and its dynamic alterations. Bioinformatics techniques pinpointed primary regulatory R-LOOP structures, subsequently confirmed through in vitro transcriptional regulation experiments. In vivo, we created RnaseH1 conditional knockout mice, RnaseH1 fl/fl-; Col2a1-2A-CreERT2 (cKO). Prior to establishing the OA model, the target gene's R-LOOP structure was removed. Comprehensive analyses using RT-qPCR, Western Blotting, HE staining, immunofluorescence, and immunohistochemistry enabled a profound understanding of the R-LOOP's physiological and pathological roles in OA, including the Keap1 promoter R-LOOP structure's impact on chondrocyte ferroptosis. We utilized the Student's t-test and the Mann-Whitney-Wilcoxon test to discern significant differences in continuous variables between groups. (*P < 0.05, **P < 0.01, ***P < 0.001; n.s., not significant).

RESULTS SECTION: Our findings revealed that R-LOOP structures are predominantly situated in promoter regions, specifically within CpG islands. Significant disparities were observed between OA and control groups (Fig 1 A). Whole-transcriptome sequencing emphasized the strong association between R-LOOP formation and transcriptional patterns in OA. Conjoint analyses indicated that promoter-associated R-LOOP formation, aligned with augmented transcriptional activity, occurred frequently (Fig 1 B). Remarkably, post-OA induction functional metrics in the Rnase H1-cKO mouse model were diminished compared to its Rnase H1-WT counterpart (Fig 2 A, B). This was accompanied by inadvertent R-LOOP accumulation, compromised DSBR repair in OA cartilage, leading to elevated apoptosis and ferroptosis events. The Keap1 gene promoter's R-LOOP structure showcased significant confidence and peak variances (Fig 1 C). In vitro assessments revealed that Erastin-treated chondrocytes exhibited elevated Keap1 expression (Fig 3 A, B). However, upon RnaseH1 overexpression, Keap1, IL-6, IL-1β, and TNF-α expressions were significantly reduced (Fig 3 B). Additionally, changes related to Ferroptosis were evident, including decreased mitochondrial alterations, cell death rates, and expression of Ferroptosis markers (Fig 3 C). CoIP assays demonstrated that Rnase H1 overexpression reduced the binding affinity between Keap1 and NRF2, inhibiting NRF2 degradation and subsequently activating the NRF/HO-1 pathway (Fig 3 D).

DISCUSSION: Our research unveils the intricate relationship between R-LOOP formation and transcriptional regulation in OA. This association is pivotal for the typical DSB repair mechanisms. The Keap1 promoter's R-LOOP structure augments Keap1 transcription, elevating its expression, intensifying the Keap1-Nrf2 binding, promoting Nrf2 degradation, inhibiting the Nrf2/HO-1 signaling cascade, and hastening ferroptosis in OA chondrocytes.

SIGNIFICANCE/CLINICAL RELEVANCE: This study is the first to chart the genomic and temporal landscape of R-loop structures in OA. We underscored the profound interplay between R-loop formations and transcriptional dynamics in OA. Additionally, our findings illuminate that abnormal R-loop accumulation in OA leads to genomic instability, compromising DSBR repair mechanisms. This research establishes a theoretical groundwork for R-LOOP targeting as a potential therapeutic strategy for OA.

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