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R-loop Induced Epigenetic Remodeling in Human Diseases: A Systematic Review of Molecular Mechanisms and Pathological Consequences

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ADMINISTRATIVE INFORMATION

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Conflicts of interest - None declared.

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Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 13 June 2025 and was last updated on 13 June 2025.

INTRODUCTION

Review question / Objective This review aims to synthesize evidence on R-loop-mediated epigenetic remodeling in human diseases using PICOS: Population: Disease models (cancer/neurodegeneration) with R-loop alterations; Intervention: R-loop manipulation-induced epigenetic changes (methylation/histone marks); Comparison: Normal R-loop level controls; Outcomes: Primary: Epigenetic markers (ChIP-seq/WGBS verified)
Secondary: DNA damage foci, apoptosis rates, clinical progression; Study types: Experimental studies with molecular validation.

Condition being studied This systematic review focuses on human diseases characterized by dysregulated R-loop formation, which drives epigenetic remodeling leading to pathological consequences. R-loops are DNA-RNA hybrids that normally regulate gene expression but, when abnormally accumulated, trigger epigenetic alterations such as DNA methylation changes (e.g., hyper/hypomethylation at gene promoters) and histone modifications (e.g., H3K9me3 or H3K27ac shifts). These changes remodel chromatin structure, resulting in genomic instability, transcriptional dysregulation, and disease progression. Key conditions include cancer (e.g., breast or colorectal cancer with oncogene activation) and neurodegenerative disorders (e.g., amyotrophic lateral sclerosis or Alzheimer's disease linked to neuronal damage). The goal is to elucidate how R-loop-induced epigenetic disruptions contribute to these diseases, aiding in the identification of novel biomarkers and therapeutic targets. The description emphasizes the molecular-biological basis without overlapping with other review sections.

METHODS

Participant or population Participants include: In vitro models: Human cell lines (e.g., cancer lines: MCF-7, HeLa; neuronal lines: SH-SY5Y) with verified R-loop alterations (via RNase H1 modulation or DRIP-seq)

In vivo models: Genetically engineered animals (e.g., neurodegenerative mouse models expressing human TDP-43 mutants) Clinical specimens: Human tissue samples from patients with R-loop-associated diseases (e.g., breast cancer biopsies stratified by BRCA1 status)
All models must demonstrate R-loop dysregulation through molecular validation.

Intervention We define interventions as experimental manipulations directly altering R-loop homeostasis:
1) Molecular tools: RNase H1 modulation (transient/stable expression), SETX-targeting sgRNAs;
2) Compounds: DRB (10-100μM, 24h), HDACi (TSA 500nM);
3) Epigenetic disruptors: DNMTi (5-Aza 1μM, 72h).
All require orthogonal validation: DRIP-qPCR for R-loops, ChIP for histone marks, and bisulfite-seq for methylation changes. Doses/durations must align with original studies. Excluded: Non-quantified interventions or indirect modulators.

Comparator
Defined comparators:
(1) Genetic controls: Non-targeting CRISPR/siRNA groups with identical transfection protocols;
(2) Pharmacological controls: Vehicle-matched exposures (≤0.1% DMSO, same duration);
(3) Biological standards: RNase H1++ models (≥50% R-loop reduction verified).
Validation criteria: γH2AX foci counts comparable (±15%), methylation-sensitive PCR confirming baseline epigenetic status. Excluded: Non-validated controls or technical replicates without orthogonal assays.

Study designs to be included Included study designs: • Experimental studies: *In vitro* (cell line manipulations with CRISPR/siRNA) and *in vivo* (transgenic animal models) • Molecular profiling: Omics studies (ChIP-seq, ATAC-seq, DRIP-seq) with wet-lab validation (e.g., WB/qPCR) • Clinical mechanism: Patient-derived xenografts (PDX) and biospecimen analyses with R-loop detection Excluded: Literature reviews, case reports, pure computational models without experimental validation.

Eligibility criteria Additional inclusion criteria:
1. Technical validation:
Must report detection methods for R-loops (e.g., DRIP-seq/S9.6 antibody IF)
Epigenetic markers quantification (ChIP-seq peaks/WGBS coverage ≥10x)
2. Data availability:
Raw omics data in public repositories (GEO/SRA)
Full experimental protocols in supplements
3. Model relevance:
Disease models must have genetic evidence of R-loop involvement (e.g., BRCA1-/- cancers)

Additional exclusion criteria:
1. Studies using non-validated antibodies for R-loop detection
2. Samples with prolonged freeze-thaw cycles (>3 cycles)
3. High-throughput screens without orthogonal validation.

Information sources
Primary databases:
PubMed/MEDLINE
EMBASE
Web of Science Core Collection
Cochrane Central Register
Epigenetics databases: GEO, ArrayExpress.

Main outcome(s)
Primary molecular outcomes:
1. R-loop-induced epigenetic alterations:
• Quantitative changes: DNA methylation rates (β-value Δ ≥10% via WGBS), histone modification enrichment (ChIP-seq peaks ≥1.5-fold)
• Time metrics: Alterations at critical time-points (e.g., H3K9me3 accumulation at 48h post-RNase H1 knockdown)
2. Functional consequences:
• DNA damage markers: γH2AX foci counts (mean ±SEM), COMET assay tail moments
• Transcriptional dysregulation: RNA-seq verified expression changes (log2FC ≥1.0, FDR<0.05)

Secondary pathological outcomes:
• Disease progression metrics:
- Cancer: Invasion rates (Transwell assays, Δ% vs. control), chemoresistance IC50 shifts
- Neurodegeneration: Neuron loss counts (per mm^3 at 6-month endpoint)
• Effect size synthesis: Pooled SMD with 95%CI for molecular-pathology correlations

Timeframes:
In vitro: ≤72h post-intervention
In vivo: ≤6 months disease modeling
Clinical specimens: At initial diagnosis

Quality assessment / Risk of bias analysis We will assess risk of bias using modified SYRCLE tool with domain-specific criteria for epigenetic studies (antibody validation, ChIP-seq ENCODE standards), grading studies as low/moderate/high risk based on ≥4/2-3/≤1 criteria met, excluding high-risk studies from synthesis.

Strategy of data synthesis

Data synthesis strategy:
1. Molecular-level integration:
• Epigenetic remodeling: Pooled β-value differences (WGBS) and ChIP-seq peak enrichment (FRiP score synthesis)
• Causal inference: Mediation analysis via structural equation modeling (R-loops → epigenetics → phenotypes)

2. Pathological consequences:
• Meta-analysis: Random-effects model for DNA damage markers (γH2AX SMD with 95% CI)
• Subgroup stratification: Cancer vs. neurodegeneration using mixed-effects models

3. Mechanistic visualization:
• Interactive pathway mapping: Cytoscape network of R-loop-epigenetic targets (node degree ≥5)
• Spatial multi-omics integration: SPARK tool for 3D chromatin architecture changes.

Subgroup analysis

Planned subgroup analyses:
1. Disease mechanisms:
• Cancer vs. neurodegenerative models (e.g., BRCA1-deficient tumors vs. TDP-43 ALS models)
• Germline mutation carriers vs. somatic alterations (e.g., germline SETX vs. somatic AQR mutations)

2. Epigenetic layers:
• DNA methylation changes (WGBS data) vs. histone modifications (ChIP-seq H3K9me3/H3K27ac)
• Promoter-proximal vs. intergenic R-loop alterations

3. Technical stratification:
• CRISPR-based R-loop manipulation vs. chemical modulators (e.g., DRB/CX-5461)
• DRIP-seq validation depth: High-depth (>20M reads) vs. low-depth datasets

Analytical approach:
• Mixed-effects meta-regression for subgroup comparisons ($p<0.1$ for interaction)
• I^2 statistic for between-subgroup heterogeneity
• Forest plots with subgroup-specific SMDs (compatible with RevMan).

Sensitivity analysis

Sensitivity analyses:
Exclusion of studies with ENCODE RSC <0.8
Trim-and-fill method for publication bias.

Country(ies) involved Department of Emergency Medicine, Beijing Chaoyang Hospital, Capital Medical University, Beijing, China.

Keywords R-loop; epigenetic regulation; human diseases; Molecular mechanisms.

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