In-silico Characterization of the TP53 R175H (c.524G>A) Missense Mutation in the p53 Tumor Suppressor Gene

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Abstract

The TP53 gene encodes the tumor suppressor protein p53, a central regulator of apoptosis, senescence, and DNA repair. Mutations in TP53 are the most common genetic alterations in human cancers. Among them, the R175H (c.524G>A) missense mutation is a hotspot variant that disrupts the zinc-binding site of the p53 DNA-binding domain. In this study, we performed an in-silico simulation of the R175H mutation on TP53 isoform A using Biopython. Genomic, transcript, and protein-level consequences were mapped: Chr17:7675088 (GRCh38) at the genomic level, c.524G>A at the transcript level, and the codon change CGC→CAC (Arg→His) at the protein level. The computational workflow verified the mutation's location and consequence, illustrating the utility of reproducible pipelines for variant annotation. This framework can be extended to other hotspot TP53 mutations and integrated into larger cancer genomics studies.

Author Declarations

Author Approval: All authors have read and approved the manuscript.

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Declarations: This study is computational in nature and did not involve human participants, animal subjects, or patient data.

Data Availability Statement: All sequence data used in this study are publicly available through NCBI (NC_000017.11, GRCh38 assembly). The computational workflow (Python code and Jupyter notebook) is available in the Supplementary File S1.

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Introduction

The TP53 gene, located on chromosome 17p13.1, is widely regarded as the “guardian of the genome.” It encodes the tumor suppressor protein p53, a transcription factor that orchestrates cellular stress responses including apoptosis, DNA repair, and senescence. Mutations in TP53 are among the most common genetic alterations in human cancers, with more than 50% of tumors exhibiting at least one deleterious TP53 variant. One of the best-characterized mutations is R175H (c.524G>A), a hotspot missense mutation affecting the DNA-binding domain of p53. This substitution alters the arginine at codon 175, a key residue within the zinc-binding site, replacing it with histidine. The mutation disrupts structural stability, abrogates transcriptional activity, and endows the mutant protein with oncogenic properties such as promoting invasion and chemoresistance. In this study, we implemented an in-silico framework to replicate the R175H mutation computationally on the wild-type TP53 isoform A sequence. By mapping the mutation at the genomic, transcript, and protein levels, we demonstrate a reproducible workflow for variant characterization using publicly available sequence data and Biopython.

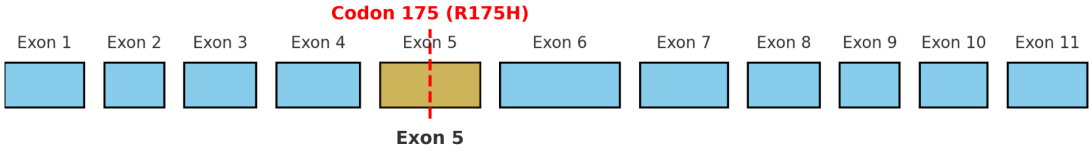
Methods

1. Data Retrieval: The genomic sequence of TP53 (GRCh38, NC_000017.11) was retrieved from NCBI GenBank.
2. Isoform Selection: Isoform A (canonical p53 protein) was chosen as the reference coding sequence (CDS).
3. Mutation Mapping: Using Biopython, the genomic position corresponding to codon 175 was identified. The wild-type nucleotide (guanine, G) was substituted with adenine (A) at position c.524 to simulate the c.524G>A transition.
4. Verification: Code outputs confirmed the raw sequence coordinate of the SNP and its correctness within the CDS context.

Results

- The genomic coordinate of the R175H mutation was confirmed at Chr17:7675088 (GRCh38).
- At the transcript level, the mutation was annotated as c.524G>A within exon 5.
- At the protein level, the codon change CGC → CAC resulted in the substitution of Arginine (R) → Histidine (H) at position 175.
- The mutation lies within the zinc-binding region of the DNA-binding domain, a site critical for stabilizing p53 structure.

- The in-silico mutated sequence for Isoform a, with annotations, is provided as a GenBank flatfile (see Supplementary File S2).



[Figure 1: Schematic of TP53 exon structure highlighting exon 5 and codon 175]

Position / Feature	Wild-type	Mutant (R175H)
Nucleotide at cDNA (c.524)	G	A
Codon (triplet)	**CGC** (codes for Arginine)	**CAC** (codes for Histidine)
Amino Acid	**Arginine (R)**	**Histidine (H)**
HGVS Protein Notation	p.Arg175	p.His175

[Figure 2: Wild-type vs. mutant codon and amino acid substitution table]

Discussion

The TP53 R175H mutation exemplifies the functional duality of p53 mutants: not only do they lose tumor suppressor activity, but they also acquire novel oncogenic functions. Our in-silico pipeline demonstrates that such mutations can be computationally replicated with high precision using Biopython, enabling reproducibility across studies. The framework outlined here is extendable to other hotspot mutations (e.g., R248Q, R273H) and could be integrated into variant annotation pipelines for cancer genomics. Furthermore, this method lays the groundwork for downstream structural modeling (e.g., molecular dynamics simulations) and functional prediction (e.g., protein-DNA binding assays in silico).

Conclusion

This study presents a reproducible computational workflow to map and characterize the TP53 R175H (c.524G>A) mutation across genomic, transcript, and protein levels. Such approaches are essential for bridging experimental cancer genomics with computational pipelines, paving the way for rapid hypothesis generation and precision oncology applications.

References/ Supporting Data

- Supplementary File S1: Biopython based Notebook to conduct in-silico R175H mutation on wild-type TP53 (https://www.kaggle.com/code/ramanbutta/tp53-mutation-analysis)
- Supplementary File S2: GenBank flatfile for TP53 R175H mutant isoform a (https://raw.githubusercontent.com/galaxyeagle/Bioinformatics-Project_Files/refs/heads/main/R175H%20Mutant%20TP53%20-%20GenBank%20Submission.txt)
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