

# RARG S427L attenuates the DNA repair response to doxorubicin in induced pluripotent stem cell-derived cardiomyocytes

Haojun Huang,<sup>1</sup> Effimia Christidi,<sup>1</sup> Sanam Shafaattalab,<sup>2</sup> Margot K. Davis,<sup>3</sup> Glen F. Tibbits,<sup>2,4</sup> and Liam R. Brunham<sup>1,5,6,\*</sup>

<sup>1</sup>Centre for Heart Lung Innovation, Department of Medicine, University of British Columbia, Vancouver, BC V6Z 1Y6, Canada

<sup>2</sup>Molecular Cardiac Physiology Group, Departments of Biomedical Physiology and Kinesiology and Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC V5A 1S6, Canada

<sup>3</sup>Division of Cardiology, Department of Medicine, University of British Columbia, Vancouver, BC V5Z 1M9, Canada

<sup>4</sup>Cellular and Regenerative Medicine Centre, British Columbia Children's Hospital Research Institute, Vancouver, BC V5Z 4H4, Canada

<sup>5</sup>Department of Medicine, University of British Columbia, Vancouver, BC V6T 1Z3, Canada

<sup>6</sup>Department of Medical Genetics, University of British Columbia, Vancouver, BC V6H 3N1, Canada

\*Correspondence: [liam.brunham@ubc.ca](mailto:liam.brunham@ubc.ca)

<https://doi.org/10.1016/j.stemcr.2022.03.002>

## SUMMARY

Doxorubicin is a commonly used chemotherapeutic drug, but its use is limited by doxorubicin-induced cardiotoxicity (DIC), which can lead to irreversible heart failure and death. A missense variant rs2229774 (p.S427L) in the retinoic acid receptor gamma (*RARG*) gene is associated with increased susceptibility to DIC, but the precise mechanism underlying this association is incompletely understood. We performed molecular dynamic simulations to determine the effect of this variant on *RARG* structure and then validated these predictions using CRISPR-Cas9-genome-edited, induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). We found that this variant leads to reduced activation of its target genes in response to doxorubicin, including gene pathways involved in DNA repair and consequently an inability to mediate DNA repair after exposure to doxorubicin. Our findings establish a role of *RARG* p.S427L in attenuating DNA repair in DIC and provide insight into the pathogenesis of this cardiotoxic effect.

## INTRODUCTION

Doxorubicin is a well-established and highly effective anthracycline chemotherapy drug. However, the use of doxorubicin is limited by dose-dependent and cumulative cardiotoxicity, leading to left ventricular dysfunction, arrhythmias, and heart failure (Singal et al., 1997; Volkova and Russell, 2011). The mechanisms of doxorubicin-induced cardiotoxicity (DIC) are incompletely understood, and we lack the ability to predict the risk of the cardiotoxic effect in individual patients.

Over 20 genetic association studies have identified genetic variants that are associated with the risk of DIC (Linschoten et al., 2018). Among these, a missense variant rs2229774 (p.S427L) in the retinoic acid receptor gamma (*RARG*) gene was identified in a genome-wide association study as being associated with increased risk of DIC (odds ratio [OR] = 4.7;  $p = 5.9 \times 10^{-8}$ ; Aminkeng et al., 2015; Schneider et al., 2017). Induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) from individuals with DIC who harbor *RARG*-S427L have increased susceptibility to doxorubicin-induced double-strand DNA breaks, reactive oxygen species production, and cell death compared with isogenic control cells (Christidi et al., 2020). These findings suggest a direct and causal role of *RARG*-S427L in the pathogenesis of DIC. However, the specific molecular mechanisms by which *RARG*-S427L leads to DIC remain to be elucidated.

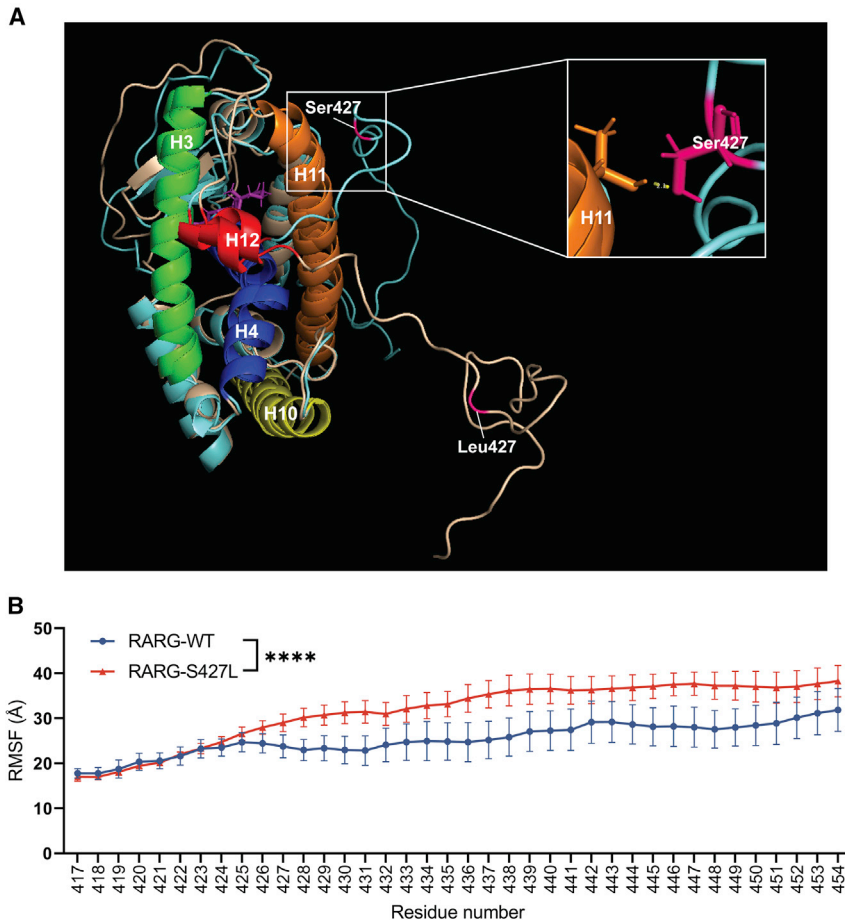
Here, we examined the functional consequences of *RARG*-S427L on retinoic-acid (RA)-responsive element (RARE) target gene expression in genome-edited iPSC-CMs. Our findings reveal a role for *RARG*-S427L in transcriptional response to doxorubicin in cardiomyocytes, leading to impaired activation of signaling pathways that are essential for protection against DIC.

## RESULTS

### Substitution of serine to leucine in residue 427 decreases F domain stability of *RARG*

*RARG* is a ligand-dependent transcription factor that plays critical roles in various biological processes, including heart development, skeletal growth, and matrix homeostasis (di Masi et al., 2015; Wiesinger et al., 2021). RA receptors (RARs) form heterodimers with retinoid X receptors (RXRs) and bind to specific RARE on the promoter of their target genes (Rochette-Egly and Germain, 2009). RARs can either repress or activate target gene transcription depending on the ligation with RA (Farboud et al., 2003; Farboud and Privalsky, 2004). Unliganded RARs repress gene transcription, while liganded RARs can activate target genes under conformational changes of helix 12 that release co-repressor and promote co-activator binding (Cordeiro et al., 2019). Since *RARG*-S427L is located in the F-domain,





**Figure 1. MD simulation of RARG with ligand**

(A) Molecular-dynamics-predicted RARG structure with ATRA (purple), highlighting interaction between residue Thr399 (on helix 11) and Ser427 (on F domain) of RARG-WT. Protein structure of RARG-WT is shown in blue and RARG-S427L in yellow. Key components are helix 3 (green), helix 4 (deep blue), helix 10 (light yellow), helix 11 (orange), helix 12 (red), and the variant site 427 (pink).

(B) C $\alpha$  RMSF of F domain (residues 417–454) of RARG-ATRA complex. MD experiments were run as independent triplicates. Analyses are shown as mean  $\pm$  SEM; n = 3; \*\*\*\*p < 0.0001; t test.

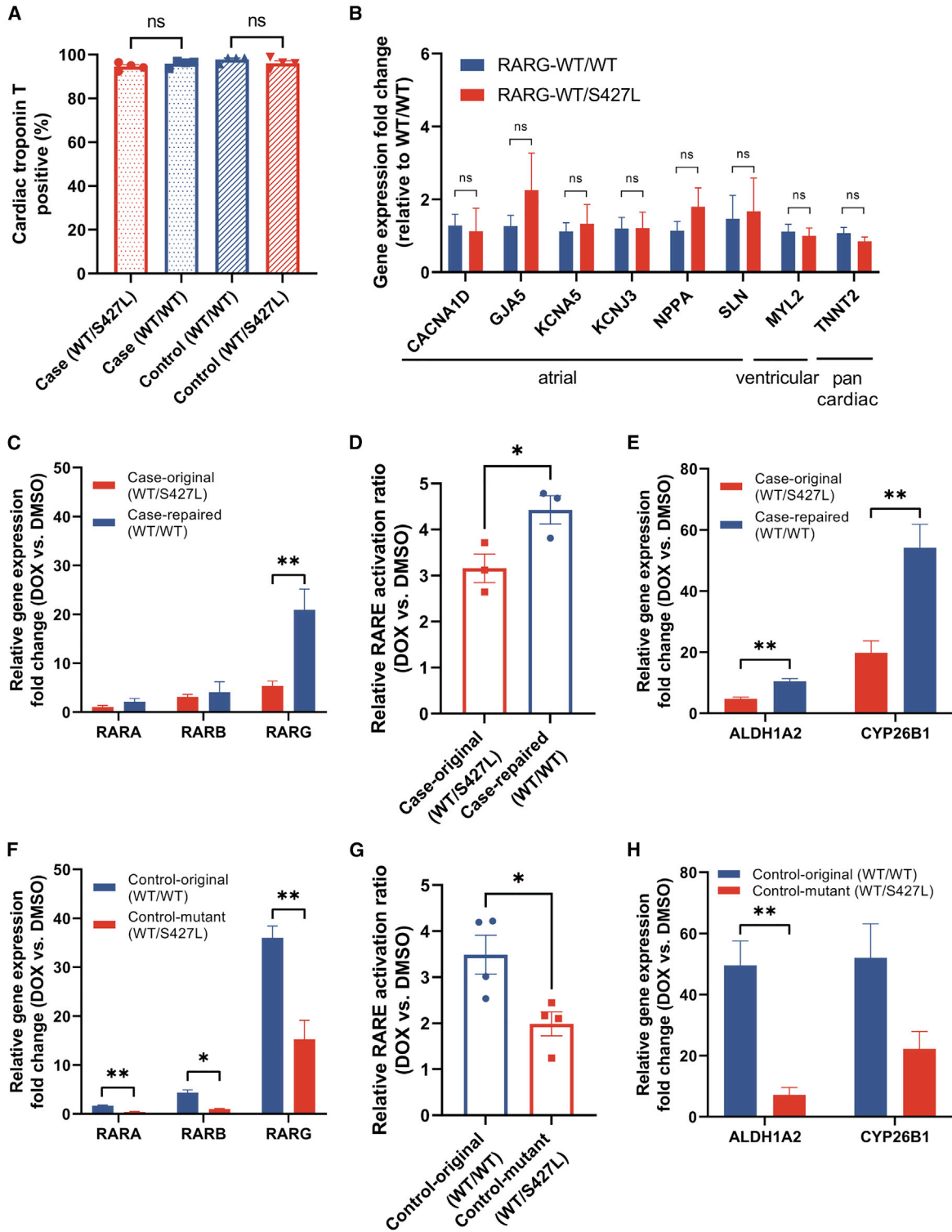
we hypothesized that it may impact the ability of the adjacent helix 12 to mediate transcriptional activation or repression of doxorubicin-responsive pathways. To test this, we used molecular dynamic (MD) simulations to predict the consequences of RARG-S427L on RARG protein structure and function. The crystal structure of wild-type (WT) RARG was obtained from PDB: 2LBD (Renaud et al., 1995) and modified at residue 427 from serine to leucine. MD simulations were performed on both RARG-WT and RARG-S427L models that docked with all-*trans* retinoic acid (ATRA) as the ligand.

The predicted RARG protein structures were clustered from each model system (Figures S1A–S1D). Among these predicted structures, the representative structures with the highest cluster coverage in each RARG-WT and RARG-S427L system were selected and aligned for comparison. As shown in Figure 1A, the WT serine 427 in the F domain of RARG forms a hydrogen bond with threonine 399 in helix 11, maintaining the F domain in close proximity to the protein surface. However, in the presence of leucine 427, the F domain was more disordered and did

not form hydrogen bonds with other protein regions, including helix 12. Comparing the F domain root-mean-square fluctuation (RMSF) between two model systems (Figures S1E and S1F), RARG-WT showed a significantly (p < 0.0001) lower average RMSF value than RARG-S427L starting at residue 422, which suggests that p.S427L impairs the stability of the F domain of RARG (Figure 1B). These observations indicate that RARG-S427L is predicted to decrease the stability of the F domain, which may impair the activation function of RARG.

### Doxorubicin treatment activates RA signaling in iPSC-CMs

To further investigate the effect of RARG-S427L on the ability of RARG to activate target genes in response to doxorubicin, we generated iPSC-CMs from patients treated with doxorubicin who did (case) or did not (control) develop DIC and performed CRISPR-Cas9-mediated genome editing to correct or introduce the RARG-S427L variant (Christidi et al., 2020). All genotypes were validated by Sanger sequencing (Figure S2A). The iPSC-CMs displayed high



**Figure 2. Assessment of retinoic acid signaling in cellular stress response to doxorubicin**

(A) Flow cytometry for cardiac troponin T (cTnT), indicating the percentage of cardiomyocytes present in different differentiation batches (n = 4). ns, not significant.

(legend continued on next page)



levels of cardiac troponin T (cTnT) positivity (>95% of cell cTnT positive), with no significant differences between genotypes, indicating that the *RARG* variant did not impact differentiation efficiency (Figures 2A and S2B). We further characterized the iPSC-CMs by immunostaining and observed high levels of cTnT and sarcomeric alpha-actinin expression (Figure S2C). Because *RARG* has been implicated in cardiac development (Wiesinger et al., 2021), we examined the expression of atrial and ventricular markers in the iPSC-CMs by qPCR. We observed no difference in the expression of these transcripts between genotypes, suggesting that the *RARG* variant did not influence cardiac cell sub-type specification (Figure 2B).

To test which RAR sub-types play the dominant role in doxorubicin response, we measured gene expression of *RARA*, *RARB*, and *RARG* by qPCR in both DIC-case and control lines (Figures 2C and 2F). In response to 1  $\mu$ M doxorubicin treatment in the DIC-case cell line, gene expression of *RARA* and *RARB* displayed minor differences between genotypes, whereas expression of *RARG* increased 21.0-fold in *RARG*-WT/WT versus 5.4-fold in *RARG*-WT/S427L iPSC-CMs ( $p = 0.009$ ; Figure 2C). The same trend was seen in the DIC-control cell line, with 32.0-fold increase of *RARG* gene expression in *RARG*-WT/WT versus 15.3-fold increase in *RARG*-WT/S427L iPSC-CMs ( $p = 0.004$ ; Figure 2F).

To further study the activity of RA signaling, we measured RARE activation by luciferase assay. RARE binding activity was increased in response to 1  $\mu$ M doxorubicin compared with no treatment control in iPSC-CMs (Figure 2D). Genetic correction of *RARG* from S427L to WT in a DIC case led to higher activation of RARE (3.2-fold versus 4.4-fold activation in *RARG*-WT/S427L versus *RARG*-WT/WT;  $p = 0.04$ ). Similarly, introducing the S427L variant in a DIC control cell line resulted in less RARE activation (3.5-fold versus 2.0-fold activation in *RARG*-WT/WT versus *RARG*-WT/S427L;  $p = 0.02$ ; Figure 2G).

We next measured the gene expression of two key molecular targets in RA signaling, aldehyde dehydrogenase one family member 2 (*ALDH1A2*) and cytochrome P450 26B1 (*CYP26B1*), in response to 1  $\mu$ M doxorubicin treatment (Figures 2E and 2H). The expression of both genes was increased upon doxorubicin treatment. *ALDH1A2* expression was increased to a significantly greater extent in *RARG*-WT/WT than *RARG*-WT/S427L in DIC-case (10.5-fold versus 4.7-fold;  $p = 0.005$ ) and DIC-control (49.6-fold versus 7.2-fold;  $p = 0.007$ ) iPSC-CMs. *CYP26B1* expression was induced to a significantly greater extent in iPSC-CMs

from DIC cases with the *RARG*-WT/WT as compared with *RARG*-WT/S427L (54.2-fold versus 19.8-fold;  $p = 0.007$ ). The difference was not statistically significant in DIC-control cells (52.0-fold versus 22.3-fold in *RARG*-WT/WT versus *RARG*-WT/S427L;  $p = 0.08$ ). This finding indicates that increased RAR transcriptional activation activity in response to doxorubicin treatment is mainly modulated by increased expression of *RARG* and that the presence of *RARG*-S427L impairs the up-regulation of *RARG* and other key RA signaling genes in response to doxorubicin.

### **RARG-S427L leads to reduced target gene activation in response to doxorubicin**

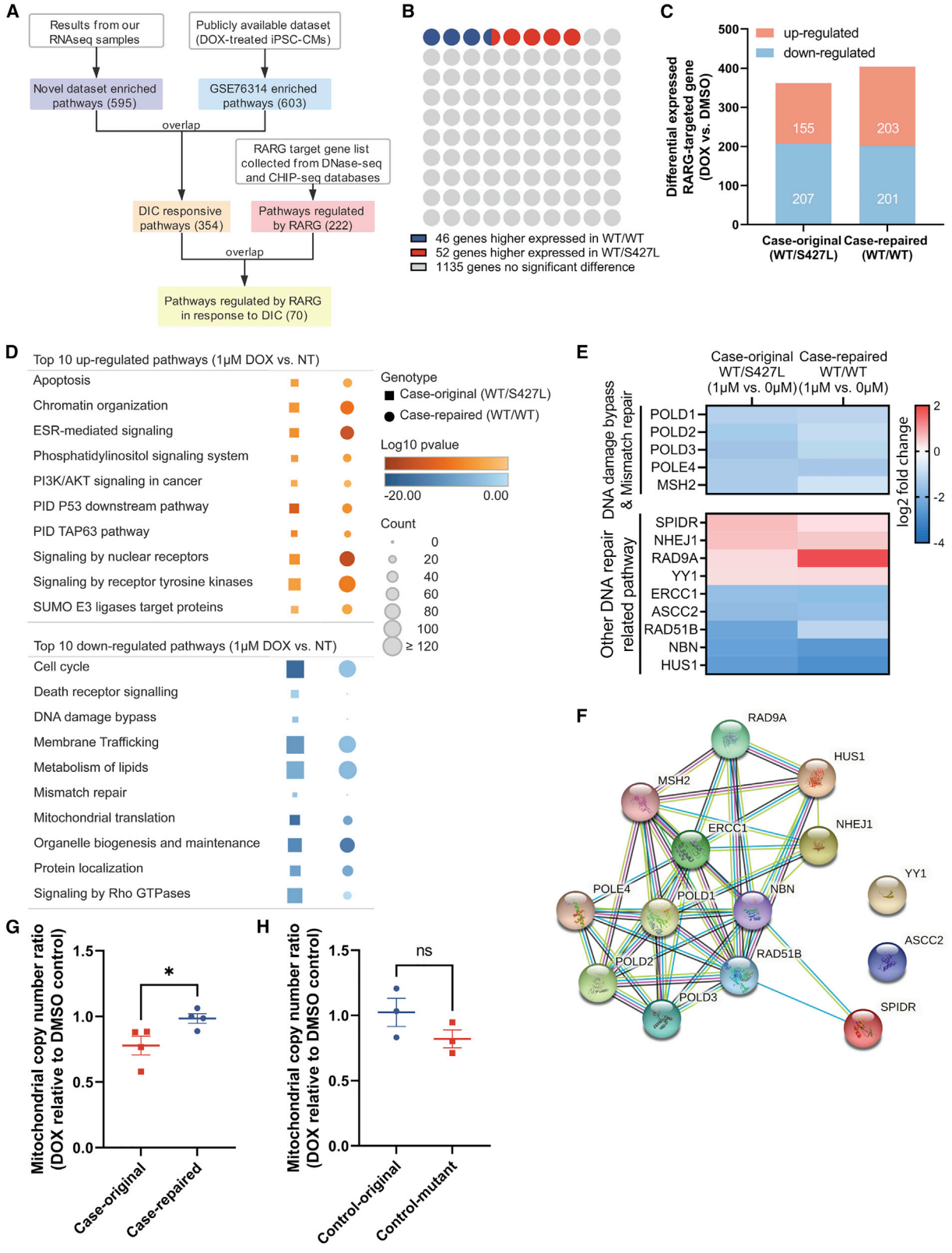
To more comprehensively assess the genes and pathways that are activated by RARG in response to doxorubicin, we performed RNA sequencing (RNA-seq) in DIC-case isogenic iPSC-CMs with and without *RARG*-S427L (Figure S3A). We co-analyzed a publicly available RNA-seq dataset (GEO: GSE76314) that was also generated from doxorubicin-treated, patient-specific iPSC-CMs for comparison (Burridge et al., 2016; Figure 3A). At baseline (vehicle control), less than 10% of RARG target genes were differentially expressed between *RARG*-WT/S427L and *RARG*-WT/WT (Figure 3B). When treated with 1  $\mu$ M doxorubicin, 29.4% (362/1,233) of RARG target genes in *RARG*-WT/S427L and 32.8% (404/1,233) in *RARG*-WT/WT were significantly differentially regulated, with 42 more RARG target genes up-regulated in the WT cell line (Figures 3C, S3B, and S3C). We observed similar patterns of up-regulation by doxorubicin of selected genes in the control isogenic lines, as measured by qPCR (Figures S4A–S4E).

We performed over-represented pathway analysis of doxorubicin-induced differentially expressed genes and RARG-targeted genes (collected from GTRD and hTFtarget databases; Kolmykov et al., 2021; Zhang et al., 2020). Pathways represented in both our dataset and GEO: GSE76314 were considered as common pathways that respond to doxorubicin in cardiomyocytes. Overlapping pathways between DIC responsive and RARG targeted were then investigated in greater detail (Figure 3D). Within these pathways, signaling of nuclear receptor, estrogens-mediated signaling, and signaling by receptor tyrosine kinases were significantly more activated in *RARG*-WT/WT group, while genes in the pathways of DNA repair, mitochondria functions, and Rho guanosine triphosphatases (GTPases) signaling were more down-regulated in *RARG*-WT/S427L group. We further investigated the genes presented in

(B) Gene expression of atrial, ventricular, and pan cardiac markers in two genotypes ( $n = 8$ ).

(C–H) Gene expression of RA receptors *RARA*, *RARB*, and *RARG* measured by qPCR in (C) DIC-case and (F) DIC-control cell lines ( $n = 3$ ). Luciferase assay results of (D) DIC-case ( $n = 3$ ) and (G) DIC-control ( $n = 4$ ) cell lines in response to doxorubicin are shown. Gene expression of RA signaling genes *ALDH1A2* and *CYP26B1* measured by qPCR in (E) DIC-case and (H) DIC-control cell lines ( $n = 3$ ). All experiments are biological, independent replicates and are shown as mean  $\pm$  SEM, \* $p < 0.05$ ; \*\* $p < 0.01$ ; t test.





(legend on next page)



DNA damage bypass and mismatch repair pathways that have predicted RARE-binding sites in their promoter regions as well as other RARG-targeted genes with over five RARE-binding sites in DNA-repair-related pathways (Figure 3E). The association network of these RARG-targeted, DNA-repair-related proteins is shown (Figure 3F). Since mitochondria-related pathway was more down-regulated in *RARG-WT/S427L* group, we also studied mitochondrial function between genotypes. Mitochondrial copy number was significantly increased in case *RARG-WT/WT* group (Figures 3G and 3H), but mitochondrial membrane potential between genotypes displayed no significant difference (Figures S4F and S4G). These findings indicate that S427L disrupts RARG target signaling pathway activation, potentially decreasing DNA repair activity following doxorubicin exposure.

### The impact of RARG-S427L on doxorubicin-induced DNA damage and repair

As our RNA-seq data implicated DNA repair pathways as being affected by impaired target signaling due to RARG-S427L, we next set out to validate the functional significance of this observation by performing single-cell electrophoresis (comet assay) to investigate DNA breaks after doxorubicin treatment and recovery. DNA breaks were categorized as mild, moderate, or severe based on the tail DNA percentage (Figures 4A and 4B). After 24 h of 1  $\mu$ M doxorubicin treatment in DIC-case iPSC-CMs, over 90% of the cells in *RARG-WT/S427L* and *RARG-WT/WT* showed a moderate or severe degree of DNA damage. With 6 and 24 h of recovery post-treatment, cell DNA damage levels were decreased in both genotypes. However, after 24 h of recovery, DNA damage levels remain significantly higher in *RARG-WT/S427L* compared with *RARG-WT/WT*: moderate (2.7-fold versus 0.86-fold;  $p = 0.009$ ) and severe (10.4-fold versus 1.2-fold;  $p = 0.03$ ). Similar results were seen in DIC-control isogenic lines with significantly higher severe level of DNA damage in *RARG-WT/S427L* in all time points (Figure 4C).

Finally, we assessed the expression of DNA repair gene *RAD9A* (RAD9 checkpoint clamp component A) with re-

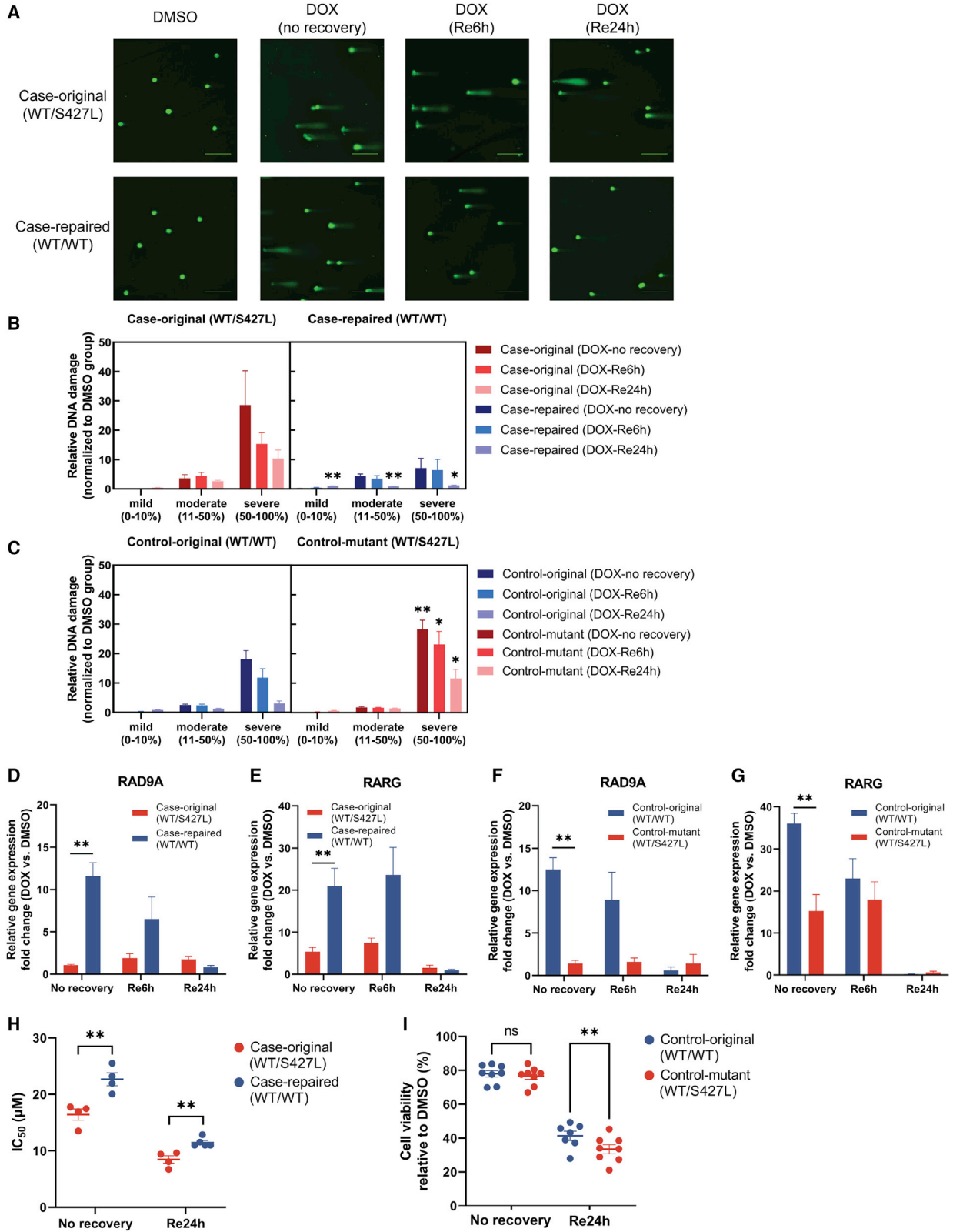
covery post-treatment. *RAD9A* was identified in both GTRD and hTFtarget databases as having a high abundance of RARE-binding sites in its promoter region (Kolmykov et al., 2021; Zhang et al., 2020). Using qPCR, *RAD9A* showed 11.6-fold versus 1.1-fold ( $p = 0.003$ ) increase in *RARG-WT/WT* versus *RARG-WT/S427L* after 24 h of 1  $\mu$ M doxorubicin treatment in DIC case (Figure 4D). After 24 h of recovery, gene expression of *RAD9A* had decreased in *RARG-WT/WT* but maintained a similar expression level as 6-h recovery in *RARG-WT/S427L*. The expression pattern of *RAD9A* was similar to that of *RARG*, in that both transcripts remained elevated in *RARG-WT/S427L* compared with *RARG-WT/WT* after 24 h of recovery (Figure 4E). Similar expression trends in *RAD9A* and *RARG* were also seen in DIC-control isogenic lines (Figures 4F and 4G). In addition, cell viability analysis revealed that cardiomyocytes continued dying after removal of doxorubicin and half-maximal inhibitory concentration ( $IC_{50}$ ) dropped from 13–25  $\mu$ M (no recovery) to 6–12  $\mu$ M (24 h of recovery). Cell viability was higher in *RARG-WT/WT* after recovery for 24 h than *RARG-WT/S427L* in both DIC-case and control isogenic lines (Figures 4H and 4I). These results suggest that RARG-S427L leads to greater cell death as a result of an impaired ability to mediate in DNA repair.

## DISCUSSION

*RARG-S427L* is associated with an increased risk of DIC (Aminkeg et al., 2015). In our previous study, we identified that the presence of the *RARG* p.S427L variant is necessary and sufficient to increase susceptibility to doxorubicin-induced cardiotoxicity and that the presence of this variant was associated with an increase in double-stranded DNA breaks, reactive oxygen species production, and cell death in iPSC-derived cardiomyocytes (Christidi et al., 2020). Here, we used patient-specific iPSC-CMs to investigate the structural and functional effects of this genetic variant. The specific advances of the current study are (1) the identification of the predicted structural consequences of *RARG-S427L* using molecular dynamic simulations; (2) providing direct evidence that *RARG-S427L* decreases the

### Figure 3. Differentially expressed genes and pathways measured by RNA-seq in doxorubicin-treated iPSC-CMs

- Summary of enriched pathways and selection process. ChIP, chromatin immunoprecipitation; DOX, doxorubicin.
- Differentially expressed gene ratio comparing non-treated case-original and case-repaired samples.
- Summary of up- and down-regulated RARG target genes expression under doxorubicin treatment.
- Top 10 up and down-regulated pathways that targeted by RARG and responded to doxorubicin. ESR, estrogen receptor; NT, no treatment; PI3K, phosphatidylinositol 3-kinase.
- Heatmap of RARG-targeted gene expression in DNA-repair-related pathways.
- Protein association network analysis of 11 highlighted RARG-targeted, DNA-repair-related genes.
- (G and H) Mitochondrial copy number ratio in iPSC-CM quantified by qPCR after doxorubicin treatment (normalized to DMSO control). All experiments are biological, independent replicates ( $n = 3-4$ ) and are shown as mean  $\pm$  SEM, \* $p < 0.05$ ; ns, not significant; t test.



(legend on next page)



binding affinity to target gene promoters; (3) performing global transcriptional analysis of RARG-S427L in response to doxorubicin treatment, which identified an inability to up-regulate genes involved in DNA repair in response to doxorubicin; and (4) providing evidence that RARG-S427L results in increased DNA damage in response to doxorubicin, which may underlie the increased susceptibility to doxorubicin-induced cell death. Collectively, these observations expand our understanding of the molecular mechanisms by which RARG protects from DIC by orchestrating a DNA repair response to doxorubicin and provides detailed insights into the pathogenesis of this adverse drug reaction.

RARs are transcription factors that can both activate or repress target genes in the presence or absence of ligands. However, RARG mainly plays an activation role of its target genes due to more stabilized contacts between helices 3 and 12 compared with isotype RARA (Farboud et al., 2003). Although deletion of the F domain of RARG was found to enhance co-activators binding *in vitro* (Farboud and Privalsky, 2004), we found the RAR activity was enhanced in RARG-WT/WT more than RARG-WT/S427L in response to doxorubicin treatment, which aligns with our prediction from MD results that the activation function of the protein is impaired in RARG-S427L.

We identified DNA damage and repair pathways as being among the most down-regulated pathways in RARG-WT/S427L cardiomyocytes, but these were not down-regulated in RARG-WT/WT cardiomyocytes (Figure 3D). Although not shown in the top regulated pathways, a subgroup of genes in the DNA repair pathway (R-hsa-73894) were significantly up-regulated in RARG-WT/WT (log p = -3.6), but not in RARG-WT/S427L. Among these genes, *RAD9A* is one of the top RARG-regulated genes that is activated in RARG-WT/WT and found to promote DNA damage repair through stimulating DNA polymerase beta binding at sites of DNA damage (Toueille et al., 2004). In addition to direct regulation effect of RARG on *RAD9A*, other key DNA repair genes, such as *MSH2*, *ERCC1*, and *RAD51B*, may also be affected via protein association networking with *RAD9A* (Figure 3F), indicating potential indirect effects of RARG on the DNA repair pathway through activating *RAD9A* expression. Combining these RNA-seq data with the results of lower RA singling activa-

tion and impaired ability to mediate DNA repair in RARG-WT/S427L, these results suggest that RARG-S427L attenuates the DNA repair response to doxorubicin in iPSC-CMs.

It is worth noting that, in addition to the DNA repair pathway we have highlighted, it is possible that RARG regulates other pathways that are also involved in DIC. We observed that mitochondrial number was reduced by doxorubicin to a greater extent in RARG-WT/S427L than RARG-WT/WT cardiomyocytes (Figures 3G and 3H), implicating this as another potential pathway by which RARG acts. We also specifically investigated the expression of genes involved in the apoptosis and p53 pathways and found that the degree of doxorubicin regulation was similar between genotypes (Figure 3D), which suggests that these are unlikely to be the major pathways by which the RARG variant acts. Similarly, although reactive oxygen species generation is an important component of DIC, we did not observe major changes in the regulation of genes in this process between genotypes. A recent study confirmed the important role of RARG-S427L in DIC (Magdy et al., 2021) and also reported an effect of RARG on DIC through suppression of topoisomerase 2 $\beta$  (*TOP2B*) expression and activation of the extracellular regulated kinase (ERK) pathway, highlighting that RARG may influence DIC through multiple pathways and mechanisms.

Our study has limitations that are worth noting. Firstly, iPSC-CMs are heterogeneous populations of cells with a mixture of atrial-, ventricular-, and nodal-like cardiac cells and express different transcriptional patterns across the differentiation stages (Karbassi et al., 2020). The heterogeneous cell population could introduce variability in the bulk RNA-seq and cellular assay we used. Also, our results were generated using two iPSC-CM lines, one from a DIC case and one from a DIC control, which both underwent genome editing to generate isogenic cell lines that differ only at the RARG-S427L variant. This approach has the advantage of allowing us to isolate the effect of the S427L variant by comparing each cell line with its genome-edited pair; however, it is also possible that RARG could have different effects in the context of different genetic backgrounds that may emerge when studying CMs from a larger number of iPSC lines.

In conclusion, we identify a mechanism by which RARG-S427L increases susceptibility to doxorubicin through reduced activation of the DNA repair pathway. These

#### Figure 4. Impact of RARG-S427L on doxorubicin-induced DNA damage and repair

(A) Comet assay results visualized under fluorescence microscope (scale bars represent 200  $\mu$ m).

(B and C) Relative DNA damage fold change in RARG-WT/WT and RARG-WT/S427L assay (n = 3; 100–150 cells were quantified per sample in each independent experiment).

(D–G) Gene expression of DNA repair gene (D and F) *RAD9A* and (E and G) *RARG* with 6 and 24 h of recovery after doxorubicin treatment in DIC-case cell lines measured by qPCR (n = 3).

(H and I) IC<sub>50</sub> and cell viability percentages after 24 h of doxorubicin treatment with no recovery time or with 24 h of recovery (n = 4–8). All experiments are biological, independent replicates and are shown as mean  $\pm$  SEM; \*p < 0.05; \*\*p < 0.01; t test.





results expand our understanding of the molecular mechanisms of DIC and suggest specific cellular pathways that could be targeted for the prevention or treatment of DIC related to DNA repair. In addition, by uncovering a cellular mechanism linking RARG-S427L to DIC risk, our results highlight the opportunity to identify patients at greatest risk for this devastating adverse drug reaction by pharmacogenetic profiling of this variant prior to doxorubicin treatment.

## EXPERIMENTAL PROCEDURES

A comprehensive description of the methodology is included in the [supplemental information](#).

### Cell culture and CRISPR-Cas9 genome editing

The iPSC lines used in this study were derived and described as part of a previous study (Christidi et al., 2020). In brief, we collected peripheral blood and performed re-programming to iPSCs from a patient who had experienced DIC (case) and a doxorubicin-treated individual who did not experience DIC (control). Then, we performed CRISPR-Cas9-mediated genome editing in the heterozygous case cell line (RARG-WT/S427L) to correct the genotype to homozygous WT. In the control cell line that was homozygous WT (RARG-WT/WT), we performed genome editing to introduce p.S427L in the heterozygous state (Figure S2A). All patient samples collection, process protocols, and experiments were approved by the University of British Columbia-Providence Health Care (UBC-PHC) Research Ethics Board.

### RNA sequencing

iPSC-CMs were replated in 12-well plates and treated with 1  $\mu$ M doxorubicin in the experiment group and vehicle (DMSO) in the control group for 24 h. Each condition has three replicates. Total RNA was extracted with illustra RNAspin Mini Isolation Kit (Cytiva) and used for library preparation following the standard protocol for the NEBnext Ultra ii Stranded mRNA (New England Biolabs) and sequencing that was performed on the NextSeq 500 (Illumina) with 30 million paired-end reads.

### Statistics

All statistical analyses were conducted with GraphPad Prism 8.3. In graphs, values are expressed as mean  $\pm$  SE of mean (SEM) and represent independent, biological repeats (at least three) unless otherwise stated. Statistical analyses were performed using Student's t test when comparing two groups.  $p < 0.05$  was considered statistically significant. Graphical abstract was created with [BioRender.com](#).

### Accession numbers

The accession number for the RNA sequencing datasets reported in this paper is GEO: GSE181517.

## SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.stemcr.2022.03.002>.

## AUTHOR CONTRIBUTIONS

Conceptualization, H.H., E.C., S.S., M.K.D., G.F.T., and L.R.B.; methodology, H.H., E.C., S.S., and M.K.D.; investigation, H.H., E.C., and L.R.B.; formal analysis, H.H.; writing – original draft, H.H. and L.R.B.; funding acquisition, L.R.B.; supervision, L.R.B.

## CONFLICTS OF INTEREST

The authors declare no competing interests.

## ACKNOWLEDGMENTS

This study was funded by the Canadian Institute of Health Research (grant PJT153118 to L.R.B.). L.R.B. is a Michael Smith Foundation for Health Research Scholar and a Canada Research Chair in Precision Cardiovascular Disease Prevention.

Received: August 25, 2021

Revised: March 2, 2022

Accepted: March 2, 2022

Published: March 31, 2022

## REFERENCES

- Aminkeng, F., Bhavsar, A.P., Visscher, H., Rassekh, S.R., Li, Y.L., Lee, J.W., Brunham, L.R., Caron, H.N., van Dalen, E.C., Kremer, L.C., et al. (2015). A coding variant in RARG confers susceptibility to anthracycline-induced cardiotoxicity in childhood cancer. *Nat. Genet.* *47*, 1079–1084. <https://doi.org/10.1038/ng.3374>.
- Burridge, P.W., Li, Y.F., Matsa, E., Wu, H.D., Ong, S.G., Sharma, A., Holmstrom, A., Chang, A.C., Coronado, M.J., Ebert, A.D., et al. (2016). Human induced pluripotent stem cell-derived cardiomyocytes recapitulate the predilection of breast cancer patients to doxorubicin-induced cardiotoxicity. *Nat. Med.* *22*, 547–556. <https://doi.org/10.1038/nm.4087>.
- Christidi, E., Huang, H.J., Shafaattalab, S., Maillet, A., Lin, E., Huang, K., Laksman, Z., Davis, M.K., Tibbits, G.F., and Brunham, L.R. (2020). Variation in RARG increases susceptibility to doxorubicin-induced cardiotoxicity in patient specific induced pluripotent stem cell-derived cardiomyocytes. *Sci. Rep.* *10*, 10363. <https://doi.org/10.1038/s41598-020-65979-x>.
- Cordeiro, T.N., Sibille, N., Germain, P., Barthe, P., Boulahtouf, A., Allemand, F., Bailly, R., Vivat, V., Ebel, C., Barducci, A., et al. (2019). Interplay of protein disorder in retinoic acid receptor heterodimer and its corepressor regulates gene expression. *Structure* *27*, 1270–1285 e1276. <https://doi.org/10.1016/j.str.2019.05.001>.
- di Masi, A., Leboffe, L., De Marinis, E., Pagano, F., Cicconi, L., Rochette-Egly, C., Lo-Coco, F., Ascenzi, P., and Nervi, C. (2015). Retinoic acid receptors: from molecular mechanisms to cancer therapy. *Mol. Aspects Med.* *41*, 1–115. <https://doi.org/10.1016/j.mam.2014.12.003>.
- Farboud, B., Hauksdottir, H., Wu, Y., and Privalsky, M.L. (2003). Isotype-restricted corepressor recruitment: a constitutively closed helix 12 conformation in retinoic acid receptors beta and gamma interferes with corepressor recruitment and prevents transcriptional repression. *Mol. Cell Biol.* *23*, 2844–2858. <https://doi.org/10.1128/Mcb.23.8.2844-2858.2003>.



- Farboud, B., and Privalsky, M.L. (2004). Retinoic acid receptor-alpha is stabilized in a repressive state by its C-terminal, isotype-specific F domain. *Mol. Endocrinol.* *18*, 2839–2853. <https://doi.org/10.1210/me.2004-0236>.
- Karbassi, E., Fenix, A., Marchiano, S., Muraoka, N., Nakamura, K., Yang, X., and Murry, C.E. (2020). Cardiomyocyte maturation: advances in knowledge and implications for regenerative medicine. *Nat. Rev. Cardiol.* *17*, 341–359. <https://doi.org/10.1038/s41569-019-0331-x>.
- Kolmykov, S., Yevshin, I., Kulyashov, M., Sharipov, R., Kondrakhin, Y., Makeev, V.J., Kulakovskiy, I.V., Kel, A., and Kolpakov, F. (2021). GTRD: an integrated view of transcription regulation. *Nucleic Acids Res.* *49*, D104–D111. <https://doi.org/10.1093/nar/gkaa1057>.
- Linschoten, M., Teske, A.J., Cramer, M.J., van der Wall, E., and Aselbergs, F.W. (2018). Chemotherapy-related cardiac dysfunction A systematic review of genetic variants modulating individual risk. *Circ. Genom. Precis. Med.* *11*, e001753. <https://doi.org/10.1161/CIRCGEN.117.001753>.
- Magdy, T., Jiang, Z., Jouni, M., Fonoudi, H., Lyra-Leite, D., Jung, G., Romero-Tejeda, M., Kuo, H.H., Fetterman, K.A., Gharib, M., et al. (2021). RARG variant predictive of doxorubicin-induced cardiotoxicity identifies a cardioprotective therapy. *Cell Stem Cell* *28*, 2076–2089.e7. <https://doi.org/10.1016/j.stem.2021.08.006>.
- Renaud, J.P., Rochel, N., Ruff, M., Vivat, V., Chambon, P., Grone-meyer, H., and Moras, D. (1995). Crystal structure of the RAR-gamma ligand-binding domain bound to all-trans retinoic acid. *Nature* *378*, 681–689. <https://doi.org/10.1038/378681a0>.
- Rochette-Egly, C., and Germain, P. (2009). Dynamic and combinatorial control of gene expression by nuclear retinoic acid receptors (RARs). *Nucl. Recept Signal.* *7*, e005. <https://doi.org/10.1621/nrs.07005>.
- Schneider, B.P., Shen, F., Gardner, L., Radovich, M., Li, L., Miller, K.D., Jiang, G., Lai, D., O'Neill, A., Sparano, J.A., et al. (2017). Genome-wide association study for anthracycline-induced congestive heart failure. *Clin. Cancer Res.* *23*, 43–51. <https://doi.org/10.1158/1078-0432.CCR-16-0908>.
- Singal, P.K., Iliskovic, N., Li, T., and Kumar, D. (1997). Adriamycin cardiomyopathy: pathophysiology and prevention. *Faseb J.* *11*, 931–936. <https://doi.org/10.1096/fasebj.11.12.9337145>.
- Touelle, M., El-Andaloussi, N., Frouin, I., Freire, R., Funk, D., Shevelev, I., Friedrich-Heineken, E., Villani, G., Hottiger, M.O., and Hubscher, U. (2004). The human Rad9/Rad1/Hus1 damage sensor clamp interacts with DNA polymerase beta and increases its DNA substrate utilisation efficiency: implications for DNA repair. *Nucleic Acids Res.* *32*, 3316–3324. <https://doi.org/10.1093/nar/gkh652>.
- Volkova, M., and Russell, R., 3rd. (2011). Anthracycline cardiotoxicity: prevalence, pathogenesis and treatment. *Curr. Cardiol. Rev.* *7*, 214–220. <https://doi.org/10.2174/157340311799960645>.
- Wiesinger, A., Boink, G.J.J., Christoffels, V.M., and Devalla, H.D. (2021). Retinoic acid signaling in heart development: application in the differentiation of cardiovascular lineages from human pluripotent stem cells. *Stem Cell Rep.* *16*, 2589–2606. <https://doi.org/10.1016/j.stemcr.2021.09.010>.
- Zhang, Q., Liu, W., Zhang, H.M., Xie, G.Y., Miao, Y.R., Xia, M.X., and Guo, A.Y. (2020). hTFtarget: a comprehensive database for regulations of human transcription factors and their targets. *Genom. Proteom. Bioinf.* *18*, 120–128. <https://doi.org/10.1016/j.gpb.2019.09.006>.