

REVIEW ARTICLE



RHNO1: at the crossroads of DNA replication stress, DNA repair, and cancer

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The DNA replication stress (DRS) response is a crucial homeostatic mechanism for maintaining genome integrity in the face of intrinsic and extrinsic barriers to DNA replication. Importantly, DRS is often significantly increased in tumor cells, making tumors dependent on the cellular DRS response for growth and survival. Rad9-Hus1-Rad1 Interacting Nuclear Orphan 1 (RHNO1), a protein involved in the DRS response, has recently emerged as a potential therapeutic target in cancer. RHNO1 interacts with the 9-1-1 checkpoint clamp and TopBP1 to activate the ATR/Chk1 signaling pathway, the crucial mediator of the DRS response. Moreover, RHNO1 was also recently identified as a key facilitator of theta-mediated end joining (TMEJ), a DNA repair mechanism implicated in cancer progression and chemoresistance. In this literature review, we provide an overview of our current understanding of RHNO1, including its structure, function in the DRS response, and role in DNA repair, and discuss its potential as a cancer therapeutic target. Therapeutic targeting of RHNO1 holds promise for tumors with elevated DRS as well as tumors with DNA repair deficiencies, including homologous recombination DNA repair deficient (HRD) tumors. Further investigation into RHNO1 function in cancer, and development of approaches to target RHNO1, are expected to yield novel strategies for cancer treatment.

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INTRODUCTION

The DNA replication stress (DRS) response is a highly orchestrated cellular process that ensures the accurate and efficient replication of DNA. Both intrinsic and extrinsic sources of DRS, including DNA secondary structures and cancer chemotherapy, respectively, present barriers to faithful DNA replication [1, 2]. The DRS response helps maintain genomic integrity and is crucial for preventing genetic mutations that promote cancer development [3]. Paradoxically, cancer cells exploit the DRS response in the face of DNA damage, allowing their continued growth and survival [4, 5]. The reliance of cancer cells on the DRS response makes proteins integral to the DRS response attractive cancer therapeutic targets. These proteins include Ataxia Telangiectasia and Rad3 related protein (ATR), Checkpoint kinase 1 (Chk1), Wee1, and Protein kinase, membrane-associated tyrosine/threonine 1 (PKMYT1) [5–8]. Among the proteins implicated in the DRS response, Rad9-Hus1-Rad1 Interacting Nuclear Orphan 1 (RHNO1, previously called C12orf32 and RHINO) is novel and understudied [9, 10]. Early seminal work on RHNO1 function indicated that it promotes ATR-Chk1 signaling following DNA damage [10, 11]. Furthermore, RHNO1 has been shown to promote oncogenic phenotypes and chemoresistance [10, 12–14]. Intriguingly, recent data suggest that RHNO1 also promotes DNA repair, by facilitating theta mediated end joining (TMEJ) (also known as alternative end joining (A-EJ) and microhomology mediated end joining (MMEJ)) [13]. Herein, we review the biochemical functions of RHNO1 as well as its emerging potential as a cancer therapeutic target.

RHNO1 STRUCTURE

In humans, *RHNO1* contains three exons, and is translated as two different isoforms (accession number NM_001252499.3 and NM_001252500.3) [15]. The first isoform, considered the canonical form of RHNO1, consists of 238 amino acids. The second isoform, generated by alternative splicing, is 224 amino acids long, missing the amino acids from positions 43 to 56. It is unclear whether the absence of these amino acids affects the function of the protein. Several regions of RHNO1 are highly conserved in mammals (Fig. 1), including the DNA binding domain (DBD) known as the APSES motif (Asm1p, Phd1p, Sok2p, Efg1p and StuAp). The APSES motif in RHNO1 helps to mediate RHNO1's role in the DRS response [10, 16]. Within this motif, seven conserved amino acids (aa 55–61) were shown to be required for RHNO1 binding to Rad1, a component of the ring-shaped heterotrimeric DNA binding protein complex 9-1-1 (Rad9-Rad1-Hus1) [10]. These seven amino acids bind Rad1 in an extended, random coil conformation with binding driven primarily by hydrophobic interactions mediated by the side chains of RHNO1 residues Try 54, Val 57, Pro 59, and Phe 61. An additional 11 highly conserved amino acids (aa 88–99) of RHNO1 were recently shown to mediate binding to Rad9 at the same site bound by Rad17, a protein serves as a clamp loader that facilitates the loading of the 9-1-1 complex onto DNA at sites of damage (Fig. 1) [17, 18]. Similar to that observed for the RHNO1/Rad1 interaction, the RHNO1 88–99 peptide adopts an extended random coil conformation with RHNO1/Rad9 complex formation being driven by hydrophobic interactions formed by the side chains of

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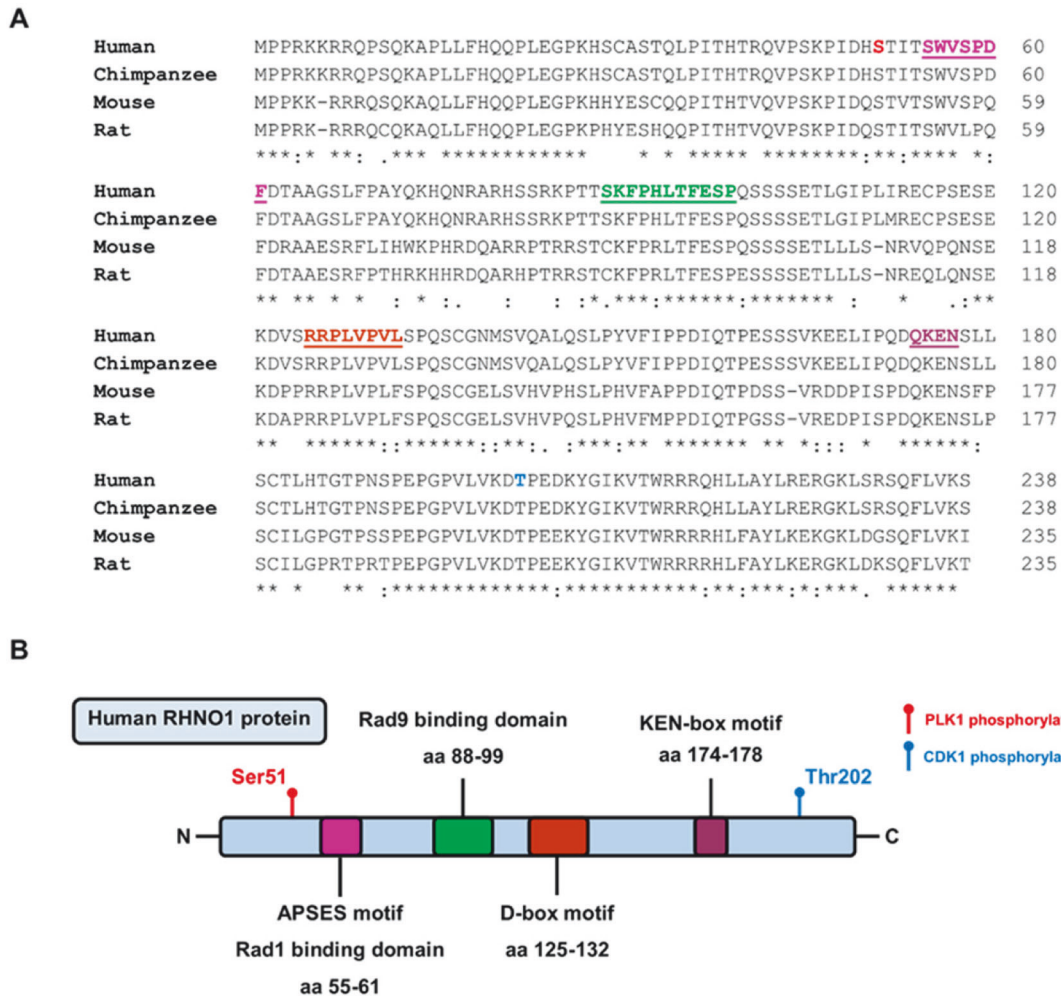


Fig. 1 Sequence alignment of RHNO1 in vertebrate species, and human RHNO1 structure and functional domains. A Sequence alignment of the RHNO1 in common vertebrate species. The APSES motif, Rad9 binding domain, D-box and KEN-box motifs, and the POLQ binding residue Ser51 and TopBP1 binding residue Thr202, are indicated by colored characters matching the same domains or phosphorylation sites diagrammed in panel B. The alignment highlights conserved regions using the following symbols: * = identical amino acid across all sequences, := conserved residues with strongly similar properties, = conserved residues with weakly similar properties, blank space = non-conserved. **B** Schematic of human RHNO1 protein with identified functional domains and regulatory components.

RHNO1 residues Phe 91, Leu 94, and Phe 96. Based on mutagenesis experiments, the formation of the quaternary RHNO1/9-1-1 complex appears more likely to rely on RHNO1 interaction with Rad1 as opposed to Rad9 [10, 17, 19]. The multivalent interaction between RHNO1 and Rad9/Rad1 may fine-tune overall complex stability or may prevent the 9-1-1 complex unloading from DNA that is mediated by Rad17 recoupling [17]. Additionally, RHNO1 may directly compete with the C-terminal domain of RAD9 for binding to a hydrophobic pocket on RAD9, which thus serves to facilitate the DNA binding activity of 9-1-1 [17].

Separate from its interaction with 9-1-1, RHNO1 binds to DNA topoisomerase II binding protein (TopBP1), a key component of the ATR/Chk1 signaling pathway [20]. RHNO1’s interaction with TopBP1 occurs via a conserved phosphorylation site, Thr 202, located in the C-terminal region of RHNO1 [21] (Fig. 1). Phosphorylated Thr 202 RHNO1 binds the BRCT1 (BRCA1 C Terminus) domain in TopBP1 with high affinity, thereby activating the ATR/Chk1 signaling cascade [11, 21]. Although the specific kinase responsible for phosphorylating RHNO1 at Thr 202 is unknown, it is likely to be a CDK or another proline-directed protein kinase, given the similarity in the sequence motif to that involved in Rad4/TopBP1 interaction via BRCT1 and BRCT2 domains [22]. Interestingly, the recruitment of TopBP1 to DNA

damage sites can be facilitated by Rad9 independent of RHNO1 [23]. Nevertheless, RHNO1’s function in ATR/Chk1 activation at stalled replication forks appears to be related to TopBP1 recruitment mediated by 9-1-1 [10, 11, 23].

Disorder prediction models suggest that RHNO1 lacks defined three-dimensional structure over most of the 238 residues, with a 84.9% consensus disorder over the length of the protein [24, 25] (Fig. 2). Four of these models predict disorder throughout the entire protein, but most identify at least two regions, residues 138–160 and the C-terminal 40 residues, that have defined structure (Fig. 2). Even in the models predicting disorder in the 138–160 region, this portion of RHNO1 is predicted to interact with another protein and is defined as a Linear Interacting Peptide (Lip) region. Indeed, RHNO1 is predicted to possess as many as 10 Lip regions, including seven conserved amino acids within the APSES motif required for RHNO1 binding to Rad1, mentioned above.

RHNO1 SHOWS CELL CYCLE SPECIFIC EXPRESSION AND IS FREQUENTLY OVEREXPRESSED IN CANCER

The expression of RHNO1 has been shown to vary across different phases of the cell cycle [9, 13]. An initial study conducted in breast

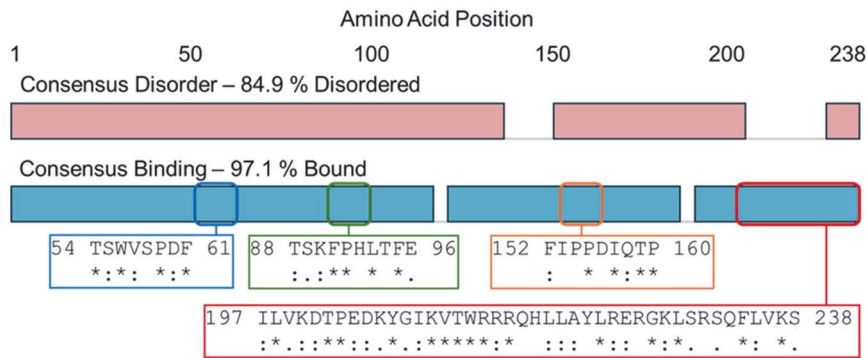


Fig. 2 Consensus predictions for human RHNO1 disorder (pink) and binding (blue) regions. The most highly conserved regions at the amino acid level are indicated in the colored boxes. The conservation given reflects the 85 most closely related RHNO1 orthologs. Numbers indicate human RHNO1 amino acid positions. Key: * = identical amino acid, := conserved substitution, = a semi-conserved substitution, blank space = non-conserved.

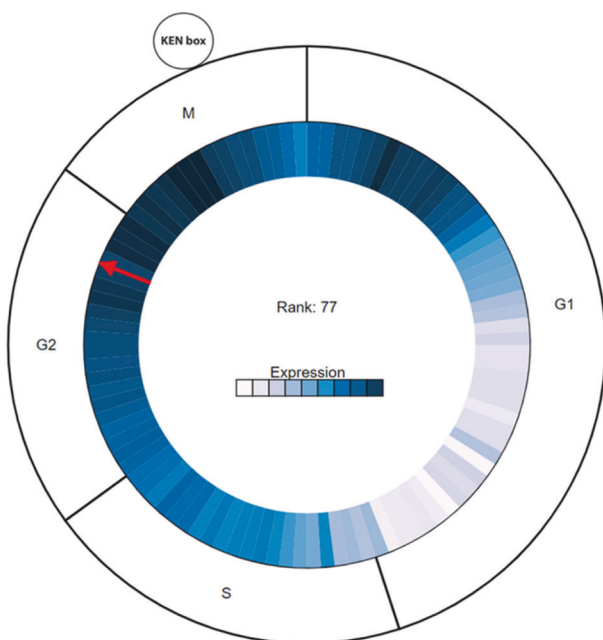


Fig. 3 Cyclebase data of RHNO1 mRNA expression in HeLa cells throughout cell cycle phases. RHNO1 mRNA expression is enriched in late S, G2, M, and early G1 phases of HeLa cells. The red arrow indicates the estimated time of the highest expression.

cancer cell lines observed that RHNO1 exhibited high expression and nuclear localization during interphase, but gradually decreased in expression and became diffusely expressed as cells entered M phase [9]. Conversely, a recent investigation using the DLD-1 colon cancer cell line with an N-terminal Flag-tag knocked into the endogenous RHNO1 locus reported that RHNO1 was predominantly expressed in M phase and tightly regulated by the Anaphase-Promoting Complex or Cyclosome (APC/C), which is regulated by CDK1 phosphorylation during late M phase [13, 26]. APC/C recognizes specific degron motifs, including D-box and KEN-box sequences, that are present in RHNO1 (Fig. 1) [13]. Notably, the degron motifs found in RHNO1 are conserved in vertebrates (Fig. 1). Based on these conflicting data, we re-examined the expression of RHNO1 throughout cell cycle phases of HeLa cells using Cyclebase [27]. Cyclebase data indicate that RHNO1 is highly expressed in mitosis but also is expressed throughout S and G2 phases, consistent with its role in ATR/Chk1 signaling (Fig. 3). Further investigation is needed to determine if there are cell context specific differences in RHNO1 expression, e.g., cell cycle deregulation in the context of oncogenic insults. Notably,

RHNO1 expression during the cell cycle has yet to be examined in non-cancerous cells.

RHNO1 is overexpressed in several types of cancer, at both the mRNA and protein levels. In the first published study on RHNO1 (then called C12orf32), Kim et al. reported that RHNO1 expression was increased in breast cancer cell lines and tissues at both mRNA and protein levels as compared to normal tissues [9]. More recently, similar findings were reported in hepatocellular carcinoma (HCC) and elevated RHNO1 expression was associated with reduced survival of HCC patients [14]. In our recent studies, we found that RHNO1 is overexpressed in high grade serous ovarian cancer (HGSO) cell lines as well as in both primary and recurrent HGSO tumors [12]. Moreover, RHNO1 was overexpressed in pancreatic tissues as compared to normal control tissues, and this included both primary, metastatic, and recurrent tumor tissues [12]. Thus, the emerging data suggest that RHNO1 overexpression is a frequent characteristic of human cancer.

Human RHNO1 is located on chromosome 12p13.33, a genomic region that frequently shows amplifications and copy number increases in cancer, including in HGSO and triple negative breast cancer (TNBC) [12, 28, 29]. We previously investigated this genomic locus in HGSO based on interest in the oncogenic transcription factor FOXM1, which is located in this amplicon [28–30]. Remarkably, from these studies, we discovered that FOXM1 is arranged in a bidirectional, or head-to-head, gene arrangement with RHNO1 [12]. Further work revealed FOXM1 and RHNO1 are co-regulated by a bidirectional promoter (named F/R-BDP) [12]. In both HGSO and TNBC, chromosome 12p13.33 copy number gain directly correlated with overexpression of FOXM1 and RHNO1 [12]. We also observed that the F/R-BDP is evolutionarily conserved in vertebrates and that FOXM1 and RHNO1 directly correlated in every biological setting examined, including at the single cell level. This led us to hypothesize that FOXM1 and RHNO1 may have evolved to work cooperatively, which is a common characteristic of bidirectional gene pairs (Fig. 4A) [12, 31]. Supporting this idea, FOXM1 increases DRS [32], while RHNO1 promotes the DRS response by promoting ATR/Chk1 signaling [12]. Additionally, emerging data linking RHNO1 to TMEJ [13] (discussed below), suggests that the functional interplay of RHNO1 and FOXM1 may go beyond DRS resolution and may also involve the preservation of mitotic integrity, which is consistent with the fact that FOXM1 both promotes mitotic progression and is highly elevated in cancer cells [33].

RHNO1 PROMOTES ATR/CHK1 SIGNALING IN RESPONSE TO DRS

The cellular DRS response promotes a diverse set of cell physiological responses including cell cycle exit, replication fork stabilization, DNA

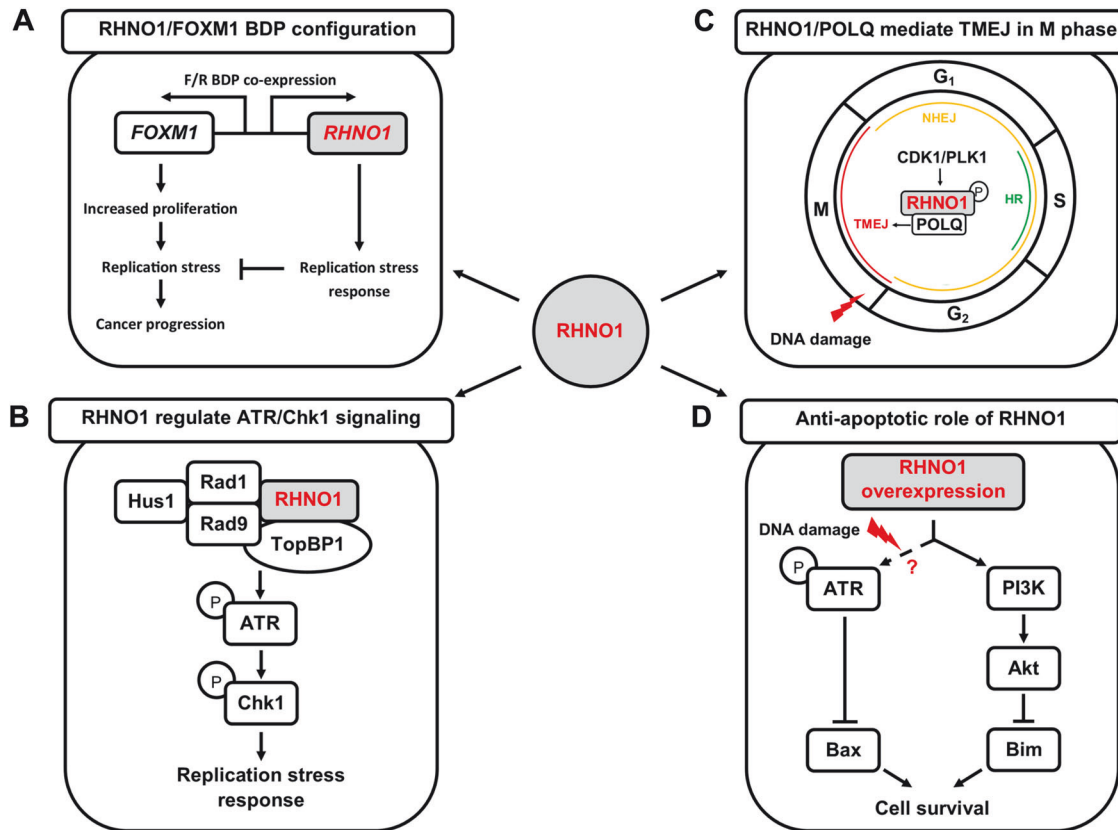


Fig. 4 Functional interplay of RHNO1 with oncogenic pathways in cancer. RHNO1 may contribute to oncogenesis by virtue of at least four functions: **A** Co-expression with FOXM1 as a result of its bidirectional gene (BDG) gene configuration, which helps to mitigate DRS caused by FOXM1, **B** Binding to 9-1-1 and TopBP1, which promotes ATR/Chk1 dependent DRS signaling, **C** Direct interaction with POLQ, which promotes TMEJ in response to mitotic DNA damage, and **D** Augmenting PI3K/Akt signaling, which leads to reduced pro-apoptotic protein expression and increases cell survival.

repair, and fork restart [1, 2]. The nexus of the DRS is the protein kinase ATR [34]. ATR is recruited to replication protein A (RPA)-coated single stranded DNA (ssDNA) generated at DNA break points or stalled replication forks [34, 35]. There are two canonical ATR activators, Ewing tumor-associated antigen 1 (ETAA1), which recognizes RPA-coated ssDNA, and TopBP1, which is recruited to RPA-coated ssDNA by ATR-interacting protein (ATRIP) and the RHNO1/9-1-1 complex [19, 34, 36]. ATR activation leads to Chk1 phosphorylation, which in turn acts as the amplifier for the ATR/Chk1 signaling pathway [34]. Phosphorylated Chk1 mediates a multifaceted response to maintain DNA integrity at stalled replication forks, including cell cycle arrest during S phase and G2/M, promotion of DNA damage repair by homologous recombination (HR), and replication fork stabilization [10, 12, 37, 38].

Seminal work by Cotta-Ramusino et al. revealed that RHNO1 plays in regulating ATR/Chk1 signaling (Fig. 4B) [10]. In this study, *RHNO1* was identified using a siRNA screen in U2OS cells as a transcript required for normal cell cycle arrest following DNA damage by ionizing radiation, and RHNO1 was shown to localize to sites of DNA damage. RHNO1 knockdown in cancer cells resulted in the partial loss of the G2/M checkpoint, and this failure allowed DNA damage incurred during interphase to progress into mitosis [10]. Under homeostatic conditions, when DNA damage occurs during S phase, ATR promotes phosphorylation of Chk1 and initiates fork preservation and DNA repair. However, following knockdown or knockout of RHNO1, there is a reduction in Chk1 phosphorylation, partially compromising the integrity of the DNA damage or S phase checkpoint [10–12]. Furthermore, reduced ATR/Chk1 signaling elicited by RHNO1

depletion reduced HR, compromising the DNA damage response (DDR) [10, 12].

RHNO1 forms a stable complex with 9-1-1 and TopBP1 on DNA even in the absence of DNA damage lesions; however, these interactions are increased upon UV irradiation or hydroxyurea treatment, i.e., conditions that promote DRS [11, 12]. As noted above, *RHNO1* is commonly overexpressed in multiple type of cancers, thus it is plausible that RHNO1 overexpression in cancer functions to hyperactivate ATR/Chk1 signaling, promoting cell survival in the face of the sustained DNA damage and genomic instability that is characteristic of cancer cells and tumors [5, 12]. Targeting RHNO1 may thus hold promise as a novel cancer therapeutic strategy.

EMERGING FUNCTIONS OF RHNO1

Recent studies suggest that RHNO1 has important biological functions beyond promoting ATR activation [9, 13, 14]. Most notably, Brambati et al., reported that RHNO1 is required for TMEJ, a DNA repair mechanism dependent on DNA polymerase theta (Pol θ , POLQ) (Fig. 4C) [13, 39]. In this study, a CRISPR-Cas9 screen was conducted in cells that were deficient in HR and the classical non-homologous end joining (NHEJ) pathway, to identify factors required for TMEJ [13]. Interestingly, this led to the identification of both 9-1-1 component genes and *RHNO1* as important TMEJ players, which agrees with an earlier Repair-seq study showing that loss of 9-1-1 components phenocopies POLQ loss [13, 40]. In particular, *RHNO1* deletion was found to significantly impair TMEJ activity, resulting in synthetic lethality in BRCA1/2 mutated cancer

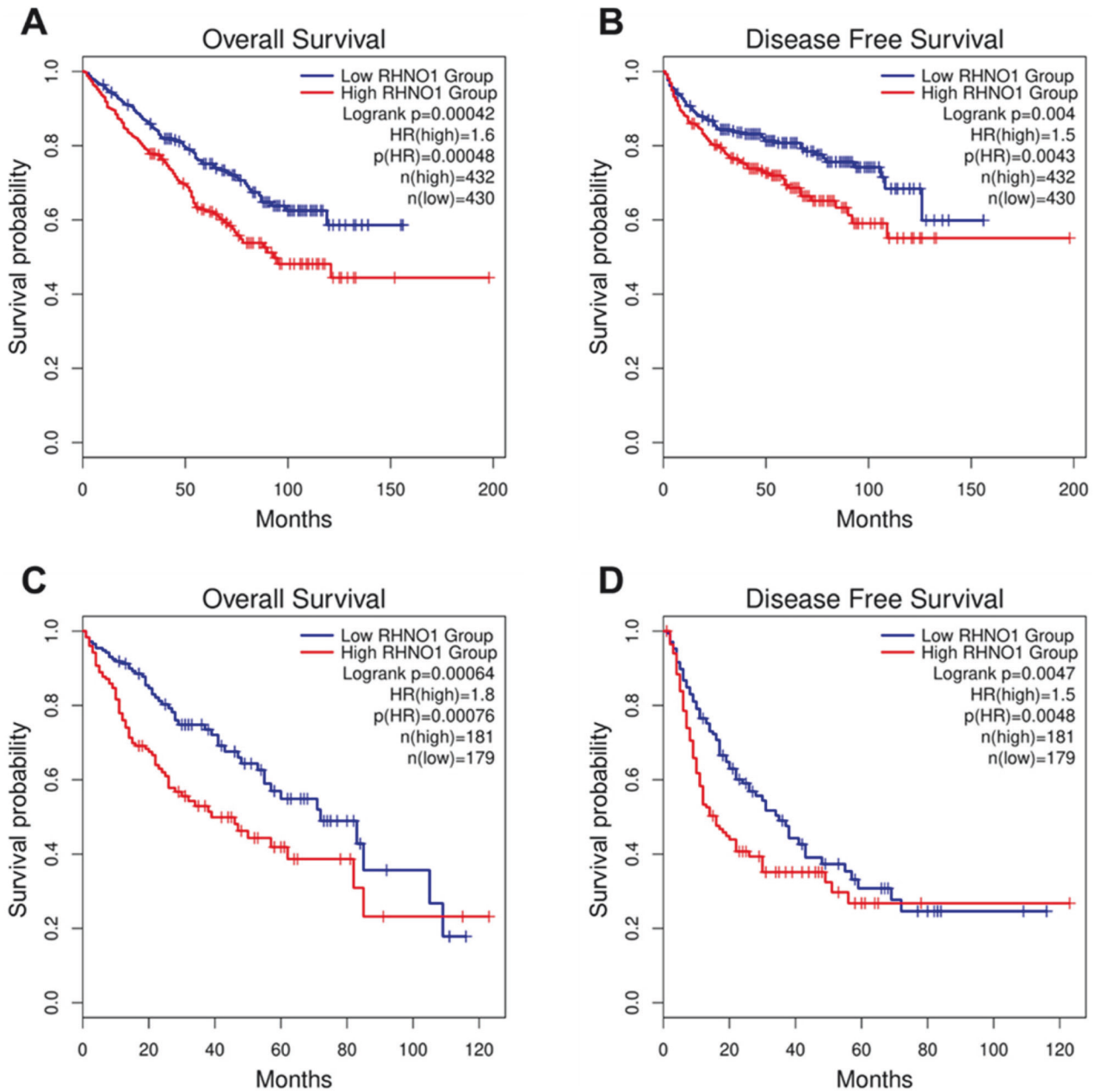


Fig. 5 *RHNO1* expression is associated with reduced survival in renal cancer and hepatocellular carcinoma (HCC). **A** Overall survival and **B** disease-free survival of renal cancer patients based on dichotomized *RHNO1* expression. **C** Overall survival and **D** disease-free survival of HCC patients based on dichotomized *RHNO1* expression. Data from TCGA were generated using GEPIA2.

cells, which are HRD [13]. TMEJ is considered a back-up DNA damage repair pathway when both HR and NHEJ are inactive [41–43]. Because TMEJ is an error-prone pathway resulting in genomic insertions and deletions, its hyperactivity may increase genomic instability and contribute to cancer progression and chemotherapy resistance [41].

Using a DLD-1 human colorectal cancer cell line model dependent on TMEJ for survival, Brambati et al., identified RHNO1/9-1-1 proteins as crucial TMEJ mediators while, in contrast, ATRIP and ETAA1 were dispensable, suggesting that TMEJ activation by RHNO1/9-1-1 is unrelated to ATR/Chk1 activation [13]. Subsequent studies revealed that RHNO1, but not 9-1-1, directly interacted with purified and cellular POLQ protein [13]. Additionally, the authors reported that, in DLD-1 cells, RHNO1 shows highest expression during M phase, where it interacts with POLQ [13]. Phosphorylation of RHNO1 at Ser51 by

Polo-like Kinase 1 (PLK1) appears to promote the interaction of RHNO1 with POLQ, and this phosphorylation event is primed by CDK1-dependent RHNO1 phosphorylation [13]. RHNO1 depleted cells exhibited DNA damage during M phase, which was manifested by increased micronuclei-containing cells following ionizing radiation treatment. The addition of a POLQ inhibitor did not increase the frequency of micronuclei in RHNO1 depleted M phase cells, suggesting that POLQ and RHNO1 act in the same TMEJ pathway for mitotic DNA repair [13]. In contrast, RHNO1 depletion and POLQ inhibitor treatments did not result in an increased frequency of micronuclei in irradiated interphase cells, implying that these proteins specifically function to protect against mitotic DNA damage [13].

In another recent study, RHNO1 was reported to function in the Phosphoinositide 3-Kinase Alpha/AKT Serine/Threonine Kinase 1 (PI3K/Akt) signaling pathway, promoting hepatocellular carcinoma

(HCC) progression (Fig. 4D) [14]. *RHNO1* was identified as commonly overexpressed in HCC cells and tissues, and depletion of *RHNO1* from HCC cell lines led to apoptosis, which coincided with increased proapoptotic protein expression and activation of the intrinsic mitochondrial apoptosis pathway [14]. Gene set enrichment analysis (GSEA) revealed that upregulation of the PI3K/Akt/mTOR pathway correlates with *RHNO1* expression in HCC tumors, along with additional pathways such as G2/M checkpoint and mitotic spindle genes [14]. The authors specifically focused on PI3K/Akt and found that *RHNO1* depletion caused reduced phosphorylated PI3K and phosphorylated Akt in HCC cell lines [14]. Importantly, intratumoral injection of *RHNO1* siRNA into subcutaneous HCC xenografts inhibited tumor growth without affecting mouse body weight. This observation constitutes the first demonstration that *RHNO1* promotes tumorigenesis in vivo [14]. Consistent with the results obtained from studies with cell lines, HCC tumor cells isolated from mice after treatment with *RHNO1* siRNA demonstrated lower phosphorylated Akt, while levels of the proapoptotic protein Bim increased [14]. Although this study highlighted a possible oncogenic role for *RHNO1* via PI3K/Akt activation, it is important to note that it was previously shown that ATR plays a direct antiapoptotic role in the mitochondrial apoptosis pathway [44]. Thus, further investigation is needed to determine whether *RHNO1* directly participates in the PI3K/Akt signaling or whether this function is a downstream consequence of its impact on ATR/Chk1 signaling.

RHNO1 IS A POTENTIAL CANCER THERAPEUTIC TARGET

As described above, data from several different investigations indicate that *RHNO1* has functions consistent with a potential role as an oncoprotein (Fig. 4). In particular, *RHNO1* contributes to both the DRS response and the DDR in cancer cells. Moreover, *RHNO1* has been shown in multiple settings to promote cancer cell survival, both in vitro and in vivo [9, 12, 14]. In light of these observations, we investigated the potential of *RHNO1* expression as a cancer prognostic marker using The Cancer Genome Atlas (TCGA) data and Gene Expression Profiling Interactive Analysis (GEPIA) [45]. We observed that *RHNO1* expression is associated with an unfavorable prognosis in both renal cancer and HCC, further suggesting its potential as a cancer therapeutic target (Fig. 5).

Interestingly, in BRCA1/2-deficient cancer cells, *RHNO1*-mediated ATR/Chk1 signaling was shown to play a crucial role in promoting cell survival. Genetic high-throughput screening in BRCA2-deficient ovarian cancer cells identified *RHNO1*, as well as other ATR pathway components, as synthetic lethal interactors [46]. Moreover, hyperactivation of the ATR/Chk1 pathway has been identified as common mechanism of PARP inhibitor resistance in HRD tumors [5]. We reported that *RHNO1* depletion in HGSOC cell lines reduces ATR/Chk1 signaling and increases DNA damage in S and G2/M phase cells, consistent with its role in the DRS response [12]. Moreover, *RHNO1* knockdown sensitized HGSOC cell lines to both olaparib and carboplatin [12]. Notably, HGSOC cells selected for PARPi resistance that exhibit ATR pathway activation, (UWB1.289 cell clones SyR12 and SyR13) were re-sensitized to olaparib by co-suppression of *RHNO1* and *FOXM1* [5, 12].

In addition to the acute interest in impairing the ATR/Chk1-dependent DRS response as a cancer treatment, emerging data supports targeting POLQ, especially in HRD tumors, as a potentially promising therapeutic strategy [41]. As *RHNO1* appears to be integral for POLQ recruitment to DNA damage lesions in mitotic cells, inhibiting *RHNO1* function may serve as an alternative approach to inhibit TMEJ for therapeutic benefit [13, 41]. This strategy may be highly significant in breast and ovarian cancers, as TMEJ is required for cell survival in HRD tumors, providing a potential opportunity to achieve a synthetic lethal interaction [47].

Another potential effect of *RHNO1* targeting relates to cancer immunotherapy. Inhibiting DDR responses often results in the accumulation of micronuclei and cytosolic DNA which activate the

innate immune response [48–50]. Consistently, small molecule inhibitors of DRS/DDR proteins including ATR, Chk1, and POLQ have the capacity to activate cytosolic nucleic acid sensors such as mitochondrial antiviral-signaling protein (MAVS) and cyclic GMP-AMP synthase/stimulator of interferon genes (cGAS/STING) [49–51]. Activation of these innate immune components can increase cancer cell immunogenicity by upregulating HLA class I and II and by promoting the activation of antigen presenting cells in the tumor microenvironment. Moreover, such agents can act to sensitize cancer cells to immune checkpoint inhibitors [48]. It is plausible that targeting *RHNO1*, which augments both ATR/Chk1 signaling and POLQ-mediated TMEJ, may be an attractive means to promote anti-tumor immune recognition through this pathway. Moreover, HGSOC, which harbors *RHNO1* overexpression and high levels of DRS [12], is well known to be insensitive to immune checkpoint inhibitors [52]. Targeting *RHNO1* might augment responses to these agents in HGSOC and other tumors with elevated dependence on *RHNO1*, including HCC and breast cancer [9, 14, 53].

CONCLUSION

While several aspects of *RHNO1* function remain elusive, the current evidence argues for a significant role in the ATR/Chk1 dependent DRS response, in promoting DNA repair, and in increasing cancer cell survival. The accumulating structural insights into *RHNO1* and its interactions with partners in ATR/Chk1 pathway may enable the design of the first small molecule inhibitor of *RHNO1*. To date, ATR/Chk1 inhibitors have exhibited limited success in clinical trials, in part due to toxicity [54]. Impairing *RHNO1* function might be a promising alternative approach to impair the DRS response with reduced toxicity, as *RHNO1* augments ATR/Chk1 signaling but is not essential for it. Further, as *RHNO1* is implicated in POLQ-mediated TMEJ, targeting *RHNO1* might offer a two-pronged approach to induce DNA damage in cancer cells by both preventing an effective DRS response and DDR. Future studies should be focused on determining the physiological functions of *RHNO1* in somatic tissues and during tumorigenesis. In particular, in vivo models such as *RHNO1* conditional knockout (CKO) mice may provide crucial insight into the function of *RHNO1* and its potential as a cancer therapeutic target. As alluded to earlier, it is also worthwhile to explore if and in what context *RHNO1* inhibition may impact innate immune signaling and if this might augment responses to immune checkpoint inhibitors. In summary, continued investigation of *RHNO1* functions and development of therapeutic strategies to inhibit those functions are fertile topics for future research.

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NJ and ARK drafted, edited, and prepared the final version of the manuscript. SFB, DRR, and GG edited the manuscript. NJ, ARK, and DRR prepared figures. All authors approved the final version of the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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