The Role of RECQL4 Overexpression in Gene Regulation and Cancer Development: Implications of Effective Targeted Therapy

Mengyao Li

Abstract

The RECQL4 gene is located on human chromosome 8 and codes for a homonymous helicase. This gene plays a crucial role in DNA repair, replication, and recombination. Specifically, the coded enzyme participates in a wide variety of DNA repair pathways and preserves its structural integrity. However, such protective functions are often compromised by RECQL4 upregulation, which is potentially caused by mutation-induced gene amplification and deregulation. An overexpression of the RECQL4 gene leads to increases in cell proliferation and genomic instability and is highly correlated to cancer progression. Due to RECQL4 overexpression across multiple tumor types, the present study proposes that such a phenomenon has the ability to serve as a measure of cancer prognosis. To test this hypothesis, the level of RECQL4 expression across three types of cancer cell samples (HCC cells, breast and ovarian cancer cells) were measured via reverse transcription-quantitative polymerase chain reaction (RT-qPCR), and RECQL4 helicase abundance was assessed via western blotting. In addition, the RECQL4 genes within half of the cell samples were temporarily silenced, and the samples were analyzed via wound-healing assay to assess the effect of reduced RECQL4 activity on the cancer cells' self-repair mechanisms and invasiveness. The cell cycle of RECQL4-inhibited cells were then compared with original samples via flow cytometry. Lastly, the risk scores and IC50 values of sorafenib, doxorubicin, and cisplatin are then evaluated to determine the impact of RECQL4 inhibition on responsiveness of cancer cells to current treatments. Overall, the study revealed an unusually high level of RECQL4 expression in all three types of cancer cells, showed a decrease in the invasiveness of cells with an inhibited RECQL4 gene, and discovered an unexpected relationship between RECQL4 overexpression and drug resistance.

Keywords: RECQL4, Overexpression, Cancer Prognosis, RT-qPCR, Western Blotting, Wound-Healing Assay, Flow Cytometry, Drug Resistance

1. Introduction

1.1.1: Characteristics

As a member of the conserved RecQ family of DNA helicases, RECQL4 is found throughout the human body and is an inalienable part of DNA repair, replication, and recombination. Coded by a gene located on chromosome 8q24.3, RECQL4 consists of 1208 amino acids and does not have a well-defined shape. However, it does contain sequences with characteristics belonging to the RecQ-family, in other words, a highly conserved domain consisting of 380 amino acids and a Walker motif A responsible for phosphate binding {1}. Moreover, RECQL4 distinguishes itself from other RecQ helicases, as it contains neither a RecQ C-terminal (RQC) nor a Helicase and RNaseD C-terminal (HRDC), which is a typical feature of the RecQ family. Rather, RECQL4 harbors a unique C-terminal domain named RecQ4-Zn2+ (R4ZBD), as labeled olive on the RECQL4 model in Figure 1, as well as a Sld2-like N-terminal domain as labeled red in Figure 1, which recruits replication factors to initiate DNA replication {2}. It

should be emphasized that RECQL4, unlike other RecQ helicases that solely inhabit the nucleus, is localized to the cytoplasm and mitochondria {3}.

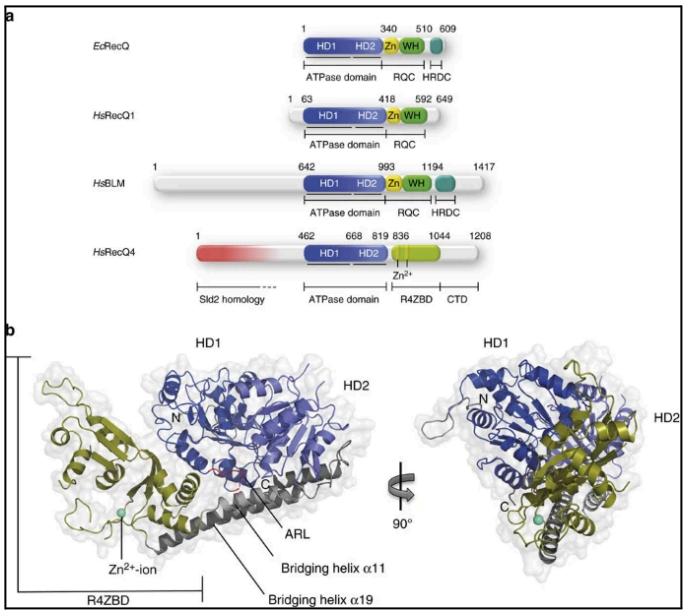


Figure 1: Comparison of the gene maps of four different RecQ helicases and the 3D structure of human RECQ4. The upper half of the model shows the comparison of domain architecture between E.coli RecQ helicase (EcRecQ), Homo sapiens (Hs) RecQ helicase 1 (HsRecQ1), BLM (HsBLM), and helicase 4 (HsRecQ4). The architecture of RECQ4 differs from other helicases due to the absence of RQC (color code: green) and HRDC (color code: turquoise). It incorporates the upstream Sld2-homology domain (color code: red) and the R4ZBD domain (color code: olive). The lower half of the model shows the 3D structure of human RECQ4 in two 90-degree rotations. Different regions are colored in correspondence to the above domain architecture models {2}.

1.1.2: Functions

RECQL4 participates in DNA replication and multiple central DNA repair pathways such as telomere maintenance and double-strand break repair (DSBR). Historically, the structural uniqueness of RECQL4, namely its absence of RQC and HRDC, fostered the belief that it did not possess helicase activity. This misconception was later disproved by in vitro helicase assays that detected helicase activity from RECQL4 {3}.

Past studies show that the Sld2-like N-terminus forms a protein complex containing core replication factors (e.g. MCM10, MCM2-7 helicase, CDC45, and GINS), and that the interactions between RECQL4 and these replication factors assemble active CDC45-MCM2-7-GINS replicative helicase to initiate DNA replication {4}. Telomeres are regions of repetitive DNA sequences located at chromosomal ends that prevent DNA degradation; their fragility can lead to genomic instability or apoptosis (programmed cell

death). RECQL4 deficiency has been associated with three autosomal recessive disorders: Rothmund-Thomson syndrome (RTS), Baller-Gerold syndrome, and RAPADILINO syndrome, all implicated in telomere maintenance {5}. It is confirmed that RECQL4, similar to the RecQ helicases WRN and BLM, is involved in this process. A study involving RTS patient cells has indicated that RECQL4 localizes at telomeres and stimulates WRN activity, and that there are more fragile telomeres in cells devoid of RECQL4. This supports the hypothesis that RECQL4 participates in telomere maintenance {5}.

DNA double-strand breaks are serious cell damages that, if repaired improperly, can lead to genomic instability and/or cell death. This may be repaired by two major pathways: homologous recombination (HR), and non-homologous end joining (NHEJ) {6}. RECQL4 is highly associated with NHEJ in human osteosarcoma cells {7}. It is also found to recruit CtIP, a protein that promotes DNA end resection in HR-dependent DSBR {7}.

1.2: HCC, Breast Cancer, and Ovarian Cancer

1.2.1: HCC and Sorafenib

HCC is currently the third leading cause of annual cancer-related deaths across the globe, and it accounts for 75% to 85% of diagnosed liver cancers {8}. It is primarily caused by cirrhosis induced by factors such as hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, alcohol consumption, aflatoxin exposure, diabetes, and tobacco usage {9}. When diagnosed at an early stage, surgical ablation and liver transplant can serve as curative treatments. However, if HCC progresses into an advanced stage, a mere life expectancy of months is expected.

For patients suffering from advanced stages of HCC when surgery and traditional chemotherapy are no longer effective, sorafenib is used to increase their survival rate. As an oral multikinase inhibitor with anti-angiogenic and antiproliferative effects, sorafenib suppresses the growth of HCC cells by blocking Raf-1, B-Raf, and kinase activity in the Ras/Raf/MEK/ERK signaling pathways {10}. However, sorafenib is costly and may not be prevalent amongst HCC patients.

1.2.2: Breast Cancer and Doxorubicin

Breast cancer is the most ubiquitous cancer among women, with 2.3 million new cases each year {11}. Females are more prone to developing breast cancer due to enhanced hormonal stimulation. Specifically, breast cells are especially sensitive to disruptions in the balance of circulating estrogens and androgens. Other risk factors include old age, family history of breast cancer, mutations in genes such as BRCA1 and BRCA2, and reproductive history {11}. Patients diagnosed with early-stage breast cancer usually undergo partial or total mastectomy (surgery to remove cancerous breast tissue), while those suffering from advanced-stage breast cancer often use chemotherapy, targeted therapy, and endocrine therapy to slow down tumor growth.

Doxorubicin is currently one of the most potent and efficient chemotherapy drugs to treat breast

cancer. It is commonly known as the “red devil” due to its characteristic red color and potential to cause severe side effects such as hair loss, nausea, and cardiotoxicity. Doxorubicin functions by embedding itself into the DNA of cancer cells, inhibiting tomoisomerase II, and disrupting mitochondria function {12}. It is also known to inhibit the proliferation of cancer cells by activating CREB3L1, a metastasis suppressor that initiates apoptosis in triple negative breast cancers {13}. However, it is observed that cancer cells could potentially develop resistance to doxorubicin by increasing the expression of P-glycoprotein that pumps doxorubicin out of the cell, and decreasing topoisomerase II expression while increasing the expression of its beta isoform, leading to failure in breast cancer treatment {14}.

1.2.3: Ovarian Cancer and Cisplatin

As the most elusive and lethal gynecological cancer, ovarian cancer has a typical 5-year survival rate of less than 30%. More than 70% of patients are diagnosed at an advanced stage, when tumor cells have metastasized to other pelvic organs such as the uterus, bladder, and even beyond the peritoneal cavity {15}. Ovarian cancer cells metastasize via two main routes: passive dissemination, or the separation of cancer cells from the primary tumor site into the peritoneal cavity by ascites (abnormal buildup of fluid in the abdominal cavity) and hematogenous metastasis, or cancer cells entering the blood circulation via intravasation {15}.

One of the most prominent chemotherapy drugs for treating ovarian cancer is cisplatin, a common form of anticancer drug that operates by damaging the genetic material, increasing the production of superoxides, and damaging mitochondrial DNA of cancer cells {16}. Current studies of cisplatin revolve around cisplatin resistance in ovarian cancer cells, such as one recent study that identifies the gene SH3RF2 as a driver of cisplatin resistance {17}.

1.3: RECQL4 Overexpression in Cancer Cells

Gene amplification refers to an increase in the number of copies of a gene in a genome and can be caused by environmental stressors or genetic instability. In the context of cancer biology, it serves as an effective machinery to promote tumorigenesis. Specifically, the amplification of certain oncogenes contributes to neoplasia. An example is MYC, an oncogene that is found to be amplified in many types of tumor cells. MYC gene amplification leads to overproduction of the MYC protein, which contributes to excessive cell proliferation in cancers such as osteosarcoma and small-cell lung carcinoma {18}. The same mechanism applies to the RECQL4 gene, which has shown in multiple studies to possess oncogenic potential upon being
overexpressed due to amplification. In cancer cells, RECQL4 plays a significant role in DNA replication and repair pathways, which may allow cancer cells to proliferate and perform telomere elongation {19}. Therefore, this study hypothesizes that RECQL4 overexpression can enhance cancer cells’ ability to migrate, thereby resulting in increased cell invasiveness and malignancy. In addition, RECQL4 overexpression is also shown to affect the cancer cell’s response to certain therapeutic agents.

2. Materials and Methods

2.1: Preparing Cell Samples

Frozen Hep3B, MCF7, and SKOV3 cell lines were obtained from the ATCC (Manassas, VA, USA) according to strict guidelines, such as informed consent for human-derived samples and biocontainment practices. All cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) and Minimum Essential medium, supplemented with 10% fetal bovine serum (FBS). The cells were incubated at 37℃ with media changes occurring every 48 hours {21}.

2.2: RT-qPCR

Total RNA extraction was performed on Hep3B, MCF7, and SKOV3 cells using TRIzol (Thermo Fisher Scientific, Waltham, MA, USA). Then, cDNA synthesis was performed using PrimeScript RT reagent kit with gDNA Eraser (TaKaRa, Otsu, Japan) according to instructions{21}. Specifically, the three RNA samples were mixed with master mix, Oligo dT Primer, Random 6 mers, and gene-specific primer. According to the TaKaRa manual, the reaction mixture was then incubated at 37℃ for 15 minutes, and then 85℃ for 5 seconds.

Protocol from a previous study was used for qPCR analysis {21}. The SYBR Premix Ex Taq (TaKaRa, Otsu, Japan) was used, and the levels of RECQL4 from half of the cell samples were normalized to β-actin expression using the 2−ΔΔCq method. The sequences of forward and reverse primers used are demonstrated in Figure 2.

RECQL4 (158bp, forward)	5′-TCAACATGAAGCAGAAACACTAC-3′
RECQL4 (158bp, reverse)	5′-CTGCTCGTTCAGGAAACAAGACT-3′
β-actin (142 bp, forward)	5′-GACAGGATGCAGAAGGAGATTACT-3′
β-actin (142 bp, reverse)	5′-TGATCCACATCTGCTGGAAGGT-3′

Figure 2: A table providing a more organized explanation of the types of forward and reverse primers used for RECQL4 and β-actin during qPCR. Primers are essential for qPCR since they hybridize with sample DNA, provide binding sites for DNA polymerase, and specify the regions to be amplified.

2.3: Western Blotting

Western blotting was performed according to a protocol used by a recent study {20}. Cancer cells were lysed using RIPA buffer for protein release. Protein samples were then separated on 7.5% SDS-PAGE gels and transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% non-fat milk in Tris-buffered saline and incubated overnight at 4℃ with rabbit anti-RECQL4 antibody and mouse anti-Beta Tubulin antibody. Protein bands were analyzed via the BioRad ChemiDoc ™ MP Imaging System.

2.4: Wound-healing Assay

Each cancer cell sample was divided into three groups: one group has functioning RECQL4 gene, and the other two are treated with either Si1 or Si3 that inhibits the gene. In accordance with the previously-mentioned protocol {20}, the cancer cells were then cultured in six-well plates until 95% confluence. A 10 μL pipette tip was then used to create scratch wounds in each well. Images captured at 0, 24, 48 hours were used to analyze cell migration patterns. It is noted that the scratch wound can not only be inflicted by mechanical methods (pipette tips and other sharp tools), but also through electrical (using electric current to induce apoptosis), thermal (damaging cells under heat stress), optical (using photosensitizers to generate localized O2), and chemical means.

2.5: Flow Cytometry

For a more comprehensive evaluation of the relationship between RECQL4 expression and cancer development, the cancer cell samples (with and without RECQL4 inhibition) were stained with antibodies, suspended in 70% ethanol, and passed through the FACS Calibur ™ flow cytometer. The data was then analyzed through the software FlowJo 10.

2.6: Analysis of Drug Sensitivity

According to the previously mentioned protocol {20}, the correlation between RECQL4 expression and IC50 values (half-maximal inhibitory concentration) for sorafenib, cisplatin, and doxorubicin was evaluated. A lower IC50 value signifies the drug to be a more potent inhibitor, since it shows that less of the substance is required to achieve the same level of cell inhibition (50%). The level of drug responsiveness in normal cancer cells and cancer cells with RECQL4 inhibition was then compared. It is presumed that the IC50 values are calculated in GraphPad Prism.

3. Predicted Results

3.1: RT-qPCR and Western Blotting

It is anticipated that the level of expression of RECQL4 mRNA in Hep3B is significantly greater than β-actin. In order to test this assumption, we evaluated several previous studies and found that it is indeed supported by a previous study, in which the mRNA levels of RECQL4 in HCC cancer cells (1.10 ± 0.07) is much higher than that of cells normalized to β-actin (0.33 ± 0.04, $P < 0.001$) {21}. The previously-depicted RT-qPCR result is visualized in Figure 3. A similar outcome is likely to apply to MCF7 and SKOV3. As evidenced by a clinical study involving breast cancer tumors, more than 50% of the cells exhibited high RECQL4 mRNA levels, which was found to be positively correlated to aggressive clinicopathological features {19}. Lastly, a separate study has observed RECQL4 amplification in 20~30% of ovarian cancer and is associated with a significantly poor prognosis and survivorship {22}. As a result of overexpressed RECQL4 mRNA, a corresponding increase in RECQL4 helicase expression across all three cancerous cell types is anticipated, which could be evaluated and confirmed by western blot analysis.

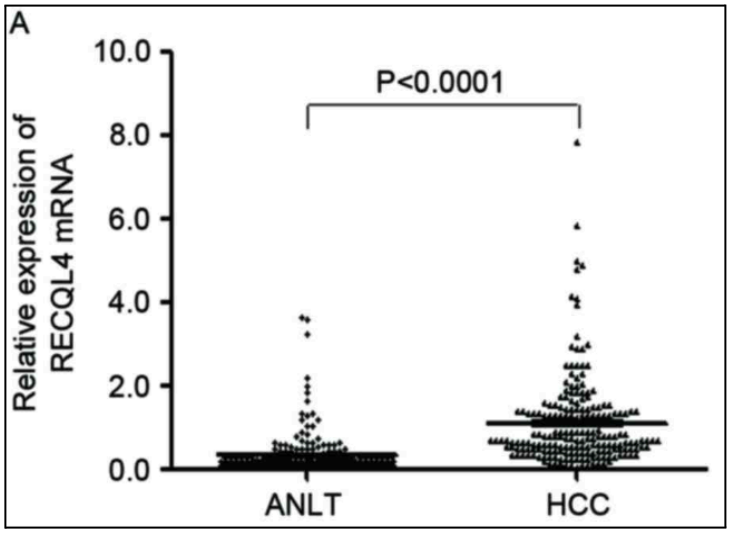
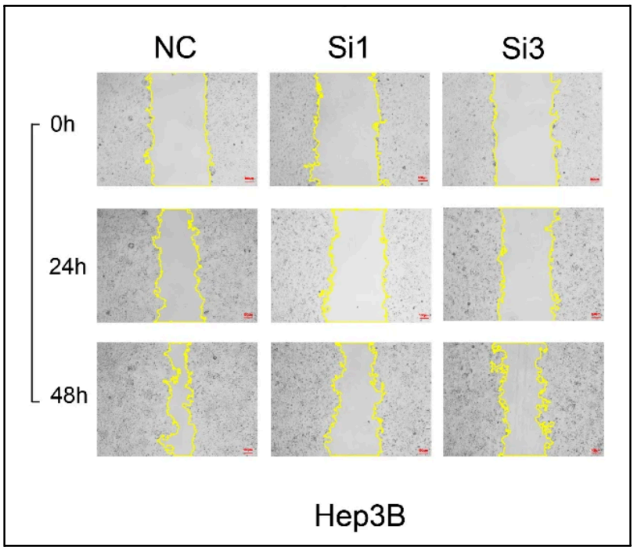


Figure 3: RT-qPCR result shows that the level of RECQL4 expression is significantly higher in HCC cells than normalized cells (ANLT). This graphic representation is based on 205 paired HCC cancer tissue and ANLT, and the mRNA expression levels were scored (P < 0.0001). According to the graph, RECQL4 mRNA is expressed more in HCC cells than ANLT. Study indicates that when RECQL4 is combined with AFP, a traditional biomarker for HCC detection, the diagnosis rate reached 85.37%. This shows that measuring the level of RECQL4 expression is an accurate method of cancer detection {21}.

3.2: Wound-Healing Assay

In order to determine how RECQL4 inhibition impacts the invasiveness of cancer cells, we conducted wound-healing assay on all three cancer cell samples as shown in Figure 4, in which cancer cells had either functional or artificially inhibited RECQL4 genes. The results show a significant increase in cell migration to the artificially created scratch wound in the plates containing cancer cells with functional RECQL4 genes. On the other hand, cells with inhibited RECQL4 genes may demonstrate significantly less migration to the wound. According to the recent study {20}, wound healing assays indicated that RECQL4 inhibition had impaired the migration capacity of Hep3B cell lines and reduced cell proliferation ($P < 0.05$). If RECQL4 overexpression is observed in MCF7 and SKOV3 cell lines, it may be speculated that cells with RECQL4 inhibited by siRNA will experience a more drastic reduction in migration to the scratch wound than cells without RECQL4 inhibition.

Figure 4: Images of wound-healing assay taken at the 0, 24, and 48hr time marks show that inhibiting RECQL4 gene decreases Hep3B's ability to migrate. The leftmost column shows the cell immigration progression of Hep3B in the negative control group at each time mark. At the 48hr mark, most of the "wound" has been inhabited by cancer cells. The middle and right columns show RECQL4 inhibited cells treated with siRNA. Through visual comparison, it is evident that the migration progression is significantly slower for cells depleted with RECQL4 functions. This further indicates that RECQL4 expression is correlated with increased invasiveness of certain cancer cells {20}.



3.3: Flow Cytometry

The complete cell cycle and apoptosis of normal cancer cells and cells with RECQL4 inhibition were evaluated by flow cytometry. In past studies, SKOV3 cancer cells with inhibited RECQL4 gene exhibited increased G1 arrest and decreased progression to S-phase, while the opposite was observed for cells with a functional RECQL4 gene {23}. The analysis also indicated that a larger proportion of apoptotic cells were observed upon RECQL4 inhibition. If the wound-healing assay for Hep3B and MCR7 demonstrated clear migration reduction in correspondence with RECQL4 inhibition, it is implied that they would exhibit similar outcomes with SKOV3 in flow cytometry.

3.4: Drug-Sensitivity

In order to determine the relationship between RECQL4 overexpression and the drug resistance level of cancer cells, we conducted quantitative analysis by evaluating the risk scores and IC50 values for sorafenib, cisplatin, and doxorubicin for HCC. The results show significantly greater cell responsiveness to these drugs when RECQL4 is upregulated {20}. This phenomenon is contradictory to the previously-held belief that RECQL4 overexpression will result in drug resistance. According to another study{23}, the IC50 value of cisplatin in ovarian cancer declined after RECQL4 gene was inhibited. We will further analyze and discuss this contradiction in the next section.

4. Discussion

4.1: Interpretation of Predicted Results

As explained in the previous section, the evaluation of Hep3B, MCF7, and SKOV3 cells via RT-qPCR and western blotting is expected to demonstrate an abnormally heightened level of RECQL4 gene and helicase expression in cancer cells. This aberrant characteristic suggests

that RECQL4 could potentially function as a prognostic tool in detecting specific types of cancer such as HCC, breast cancer, and ovarian cancer. In fact, high expression of RECQL4 mRNA was found to be “modestly correlated” with poor post-progression survival rate of breast cancer patients {24}, further corroborating the predicted outcome of the study.

Moreover, all three types of cells that underwent wound-healing assay are expected to show a decrease in the rate of migration progression for the samples that have RECQL4 silenced by siRNA. This result testifies the important role RECQL4 partakes in cancer cell proliferation and invasion previously mentioned in the Introduction section. A study involving the cGAS-STING pathway in HCC patients found that RECQL4 overexpression is significantly associated with increased cancer cell malignancy via epithelial-mesenchymal transition, which involves cancer cells acquiring mesenchymal traits with increased motility and invasiveness {25}. This supports the data collected from the wound-healing assay.

After the wound-healing assay, flow cytometry demonstrates the influence of RECQL4 on the cell cycle, as cells with silenced RECQL4 experienced decreased progression to S phase and increased possibility of apoptosis. In addition, a recent study revealed that RECQL4 inhibition helps maintain E-cadherin expression (adhesion of cancer cells), thereby preventing cell progression and metastasis {26}. This finding raises the potential of drugs targeting and inhibiting RECQL4 expression as a form of cancer therapy.

Interestingly, upon analyzing the risk scores and IC50 values, we deduced that RECQL4 expression increased sensitivity of HCC to sorafenib, cisplatin, and doxorubicin. However, a separate study shows that RECQL4 depletion increased sensitivity of ovarian cancer cells to cisplatin and olaparib {23}. As a result of the discrepancy, it may be deduced that the effect of RECQL4 overexpression on drug sensitivity varies between cancer types, implying a dual role in drug resistance and resulting in contradictory outcomes.

4.2: Overview of the Proposed Study

This proposed study aims to evaluate the role of RECQL4 overexpression in cancer progression and proliferation. It is also intended to provide insight into how RECQL4 upregulation correlates to current therapeutic drugs for certain cancers. The study involves three different cancer cell lines: Hep3B (HCC), MCF7 (breast cancer), and SKOV3 (ovarian cancer). RT-qPCR and western blotting were implemented to analyze the level of RECQL4 gene and protein expression in the cell samples. A wound-healing assay was then conducted to visualize the effect of RECQL4 inhibition on the migration progress of cancer cells. Then, the cell cycle of both regular and RECQL4 inhibited samples were analyzed and compared via flow cytometry. Lastly, the IC50 values of sorafenib, cisplatin, and doxorubicin were quantitatively analyzed to determine sensitivity of cancer cells towards therapeutic drugs under different levels of RECQL4 expression. The results indicate that RECQL4 overexpression contributes to cell proliferation and increased malignancy in HCC, breast cancer, and ovarian cancer and recognizes its potential of serving as a prognostic tool for measuring cancer progression. Moreover, different types of cancer cells exhibited contrasting behaviors to therapeutic drugs under RECQL4 overexpression, which could be further investigated.

4.3: Limitations and Future Implications

Studies involving RECQL4 and cancer are relatively new—most have been published within the past decade. Since RECQL4 partakes in a myriad of roles in gene regulation and repair, its exact mechanism in carcinogenesis is not yet fully understood. Studies toward the same hypothesis under different experimental settings have produced different outcomes, notably the involvement of RECQL4 overexpression in drug resistance of cancer cells shown by two separate studies {21},{23}. Furthermore, no research has yet investigated the role of RECQL4 overexpression in the G2/M phase compared to the G1/S phase. Thus, the vagueness of the function of RECQL4 in certain cellular pathways still needs to be clarified by future studies. Based on current studies, the future implication of RECQL4 research may center around understanding the role of RECQL4 in various cellular processes and advancing cancer therapy in accordance with its mechanisms.

5. References

1. Kellermayer, R. The versatile RECQL4. *Genetics in Medicine*, *8*(4), 213–216. https://doi.org/10.1097/01.gim.0000214457.58378.1a (2006).
2. Kaiser, S., Sauer, F., & Kisker, C. The structural and functional characterization of human RecQ4 reveals insights into its helicase mechanism. *Nature Communications*, *8*, 15907. https://doi.org/10.1038/ncomms15907 (2017).
3. Luong, T. T., & Bernstein, K. A. Role and Regulation of the RECQL4 Family during Genomic Integrity Maintenance. *Genes*, *12*(12), 1919. https://doi.org/10.3390/genes12121919 (2021).
4. Xu, X., Chang, C.-W., Li, M., Omabe, K., Le, N., Chen, Y.-H., Liang, F., & Liu, Y. DNA replication initiation factor RECQ4 possesses a role in antagonizing DNA replication initiation. *Nature Communications*, *14*(1), 1233. https://doi.org/10.1038/s41467-023-36968-1 (2023).
5. Ghosh, A. K., Rossi, M. L., Singh, D. K., Dunn, C., Ramamoorthy, M., Croteau, D. L., Liu, Y., & Bohr, V. A. RECQL4, the Protein Mutated in Rothmund-Thomson Syndrome, Functions in Telomere Maintenance*. *Journal of Biological Chemistry*, *287*(1), 196–209. https://doi.org/10.1074/jbc.m111.295063 (2012).
6. Aparicio, T., Baer, R., & Gautier, J. DNA double-strand break repair pathway choice and cancer. *DNA Repair*, *19*, 169–175. https://doi.org/10.1016/j.dnarep.2014.03.014 (2014).
7. Lu, H., Shamanna, R. A., Keijzers, G., Anand, R., Rasmussen, L. J., Cejka, P., Croteau, D. L., & Bohr, V. A. RECQL4 Promotes DNA End Resection in Repair of DNA Double-Strand Breaks. *Cell Reports*, *16*(1), 161–173. https://doi.org/10.1016/j.celrep.2016.05.079 (2016).
8. Qiu, S., Cai, J., Yang, Z., He, X., Xing, Z., Zu, J., Xie, E., Henry, L., Chong, C. R., John, E. M., Cheung, R., Ji, F., & Nguyen, M. H. Trends in Hepatocellular Carcinoma Mortality Rates in the US and Projections Through 2040. *JAMA Network Open*, *7*(11), e2445525–e2445525. https://doi.org/10.1001/jamanetworkopen.2024.45525 (2024).
9. Tunissiolli, N. M., Castanhole-Nunes, M. M. U., Biselli-Chicote, P. M., Pavarino, É. C., da Silva, R. F., da Silva, R. de C. M. A., & Goloni-Bertollo, E. M. Hepatocellular Carcinoma: a Comprehensive Review of Biomarkers, Clinical Aspects, and Therapy. *Asian Pacific Journal of Cancer Prevention : APJCP*, *18*(4), 863–872. https://doi.org/10.22034/APJCP.2017.18.4.863 (2017).

10. Tang, W., Chen, Z., Zhang, W., Cheng, Y., Zhang, B., Wu, F., Wang, Q., Wang, S., Rong, D., Reiter, F. P., De Toni, E. N., & Wang, X. The mechanisms of sorafenib resistance in hepatocellular carcinoma: theoretical basis and therapeutic aspects. *Signal Transduction and Targeted Therapy*, *5*(1), 1–15. https://doi.org/10.1038/s41392-020-0187-x (2020).
11. Łukasiewicz, S., Czeczelewski, M., Forma, A., Baj, J., Sitarz, R., & Stanislawek, A. Breast cancer—epidemiology, Risk factors, classification, Prognostic markers, and Current Treatment Strategies—an Updated Review. *Cancers*, *13*(17), 4287. https://pmc.ncbi.nlm.nih.gov/articles/PMC8428369/ (2021).
12. Kciuk, M., Gielecińska, A., Mujwar, S., Kołat, D., Kałuzińska, Ż., Celik, I., & Kontek, R. Doxorubicin—An agent with multiple mechanisms of anticancer activity. *Cells*, *12*(4), 659–659. https://doi.org/10.3390/cells12040659 (2023).
13. Mellor, P., Kendall, S., Smith, S., Saxena, A., & Anderson, D. H. Reduced CREB3L1 expression in triple negative and luminal a breast cancer cells contributes to enhanced cell migration, anchorage-independent growth and metastasis. *PLoS ONE*, *17*(7), e0271090–e0271090. https://doi.org/10.1371/journal.pone.0271090 (2022).
14. Chen, N.-N., Ma, X.-D., Miao, Z., Zhang, X.-M., Han, B.-Y., Ahmed Ali Almaamari, Huang, J.-M., Chen, X.-Y., Liu, Y.-J., & Su, S.-W. Doxorubicin resistance in breast cancer is mediated via the activation of FABP5/PPARγ and CaMKII signaling pathway. *Frontiers in Pharmacology*, *14*. https://doi.org/10.3389/fphar.2023.1150861 (2023).
15. Yeung, T.-L., Leung, C. S., Yip, K.-P., Au Yeung, C. L., Wong, S. T. C., & Mok, S. C. Cellular and molecular processes in ovarian cancer metastasis. A Review in the Theme: Cell and Molecular Processes in Cancer Metastasis. *American Journal of Physiology-Cell Physiology*, *309*(7), C444–C456. https://doi.org/10.1152/ajpcell.00188.2015 (2015).
16. Zoń, A., & Bednarek, I. Cisplatin in Ovarian Cancer Treatment—Known Limitations in Therapy Force New Solutions. *International Journal of Molecular Sciences*, *24*(8), 7585. https://doi.org/10.3390/ijms24087585 (2023).
17. Gong, T.-T., Liu, F.-H., Xiao, Q., Li, Y.-Z., Wei, Y.-F., Xu, H.-L., Cao, F., Sun, M.-L., Jiang, F.-L., Tao, T., Ma, Q.-P., Qin, X., Song, Y., Gao, S., Wu, L., Zhao, Y.-H., Huang, D.-H., & Wu, Q.-J. SH3RF2 contributes to cisplatin resistance in ovarian cancer cells by promoting RBPMS degradation. *Communications Biology*, *7*(1). https://doi.org/10.1038/s42003-023-05721-1 (2024).
18. Matsui, A., Ihara, T., Suda, H., Mikami, H., & Semba, K. Gene amplification: mechanisms and involvement in cancer. *BioMolecular Concepts*, *4*(6), 567–582. https://doi.org/10.1515/bmc-2013-0026 (2013).
19. Arora, A., Agarwal, D., Abdel-Fatah, T. M., Lu, H., Croteau, D. L., Moseley, P., Aleskandarany, M. A., Green, A. R., Ball, G., Rakha, E. A., Chan, S. Y., Ellis, I. O., Wang, L. L., Zhao, Y., Balajee, A. S., Bohr, V. A., & Madhusudan, S. RECQL4 helicase has oncogenic potential in sporadic breast cancers. *The Journal of Pathology*, *238*(4), 495–501. https://doi.org/10.1002/path.4681 (2016).
20. Li, Y., Yin, L., Liu, B., Liu, Y., He, D., Liu, X., & Liu, R. Targeting RECQL4 in hepatocellular carcinoma: from prognosis to therapeutic potential. *BMC Medical Genomics*, *18*(1). https://doi.org/10.1186/s12920-025-02107-6 (2025).
21. Li, J., Jin, J., Liao, M., Dang, W., Chen, X., Wu, Y., & Liao, W. Upregulation of RECQL4 expression predicts poor prognosis in hepatocellular carcinoma. *Oncology Letters*. https://doi.org/10.3892/ol.2018.7860 (2018).

22. Maity, J., Horibata, S., Zurcher, G., & Lee, J.-M. Targeting of RecQ Helicases as a Novel Therapeutic Strategy for Ovarian Cancer. *Cancers*, *14*(5), 1219. https://doi.org/10.3390/cancers14051219 (2022).
23. Guo, L., Li, Y., Zhao, C., Peng, J., Song, K., Chen, L., Zhang, P., Ma, H., Yuan, C., Yan, S., Fang, Y., & Kong, B. RECQL4, Negatively Regulated by miR-10a-5p, Facilitates Cell Proliferation and Invasion via MAFB in Ovarian Cancer. *Frontiers in Oncology*, *10*. https://doi.org/10.3389/fonc.2020.524128 (2020).
24. Zhu, X., Chen, H., Yang, Y., Xu, C., Zhou, J., Zhou, J., & Chen, Y. Distinct prognosis of mRNA expression of the five RecQ DNA-helicase family members – RECQL, BLM, WRN, RECQL4, and RECQL5 – in patients with breast cancer. *Cancer Management and Research*, *Volume 10*, 6649–6668. https://doi.org/10.2147/cmar.s185769 (2018).
25. Hong, W., Zhang, Y., Li, Z., Zeng, Z., & Du, S. RECQL4 Remodels the Tumor Immune Microenvironment via the cGAS-STING Pathway in Hepatocellular Carcinoma. *International Journal of Radiation Oncology*Biology*Physics*, *114*(3), e509–e510. https://doi.org/10.1016/j.ijrobp.2022.07.2081 (2018).
26. Huo, W., Huang, Y., Tian, B., Chen, X., Lu, J., Huang, X., Wu, M., Yu, J., Chen, D., & Wang, R. Unraveling the mechanisms of RECQL4-mediated cervical cancer progression through the PI3K/AKT pathway. *Translational Oncology*, *50*, 102146–102146. https://doi.org/10.1016/j.tranon.2024.102146 (2024).