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ORIGINAL ARTICLE

WRN-2310 Serves as a Predictive Short Peptide for Microsatellite Instability Colorectal Cancer Prognosis and Clinical Treatment

Weiping Fan[a,d], Menglin Li[a,d], Xinna Du[c], Yonghua Bian[a], Hu Zhang[b*]

[A]Institute of Department of Immunology and Microbiology, Jiangsu Medical College, Yan Cheng, China

[B]Department of Physiology and Biochemistry, Jiangsu Medical College, Yan Cheng, China

[C]Department of Pathology, Jiangsu Medical College, Yan Cheng, China

[D]Weiping Fan And Menglin Li Contributed Equally to This Work. Author Order Was Determined by the Type of Their Contributions

Corresponding Author: Hu Zhang

Abstract:
Microsatellite instability (MSI) colorectal cancer (CRC) is a malignant tumor with a poor prognosis. Significant advancements have been achieved in the field of peptide drug development in recent years, propelled by innovative manufacturing processes, modification and analytical techniques. Thus, further research is needed to identify predictive biomarkers and promising short peptide targets. In this study, multiple bioinformatics methods were used to mine TCGA and other tumor databases to analyze the differential expression of Werner syndrome protein (WRN) genes and their prognosis. To further explore the effects of WRN-2310 on the proliferation and migration of MSI colorectal cancer cells HCT116/RKO, and predict its functional mechanism. The results revealed that the level of WRN protein in colorectal cancer tissues was higher than that in normal tissues, which was beneficial to the prognosis of patients and was a risk factor affecting the prognosis of patients. Meanwhile, further study of antagonist peptide WRN-2310 inhibited both proliferation and migration of MSI colorectal cancer cells RKO (p<0.01). This study demonstrated that WRN is a potential prognostic and diagnostic marker and that WRN-2310 could be a new short peptide target for the clinical treatment of MSI colorectal cancer.

Keywords: WRN, MSI, CRC, prognosis, short peptide

Introduction

Globally cancer incidence and associated mortality are rapidly increasing, with colorectal cancer (CRC), which includes colon and rectum adenocarcinoma (COADREAD), being one of the most common gastrointestinal tumors, ranking almost third and second in terms of morbidity and mortality, respectively [1]. With the development of targeted therapies and immunotherapies [2], the 5-year survival rate of colorectal cancer patients remains worrisome. Due to the heterogeneity of colorectal cancer, patients with the same clinical presentation may have significantly different outcomes [3]. Therefore, there is an urgent need to develop new diagnostic markers and therapeutic targets to improve the clinical outcomes of CRC patients.

Microsatellite instability (MSI) is a distinctive feature of certain genetic disorders that affect the accuracy of DNA replication. This condition arises from a dysfunction in the DNA mismatch repair (MMR) system, which is crucial for maintaining the fidelity of DNA during replication and for repairing errors that occur after replication [4]. In the case of colorectal cancer (CRC), MSI contributes to the development of approximately 10% to 20% of all cases, indicating that a significant proportion of these cancers are linked

to defects in the mechanisms that ensure the precision of DNA replication and subsequent repair processes [5]. MSI is involved in the pathogenesis of many types of tumors, both hereditary and sporadic, including CRC [6], bladder cancer [7], gastric cancer [8, 9], and ovarian cancer [10, 11]. Among them, 45% to 60% of cancers are unresponsive to immune checkpoint antagonism [12, 13]. Therefore, new therapeutic approaches are needed for MSI tumors.

The WRN gene is responsible for the production of Werner Syndrome Protein, which is a component of the RecQ family of DNA helicases. This gene is vital for preserving the integrity of the genome, as it is involved in various critical processes such as DNA repair, replication, transcription, and the upkeep of telomeres [14, 15]. When there are mutations or defects in the WRN gene, it can lead to Werner Syndrome [16, 17], a condition inherited in an autosomal recessive pattern. This syndrome is marked by premature aging and an increased susceptibility to specific types of cancer.

Chan EM et al. [18] showed that WRN is a synthetic lethal target in MSI cancer. WRN deficiency selectively causes double-stranded DNA breaks in MSI cancer cells, which promotes apoptosis and cell cycle arrest. Defects in DNA damage repair genes may be synthetically lethal in relation to the inactivation of the WRN gene to kill cancer cells [19], suggesting that WRN is a potential therapeutic target for MSI colorectal cancer. In recent years, immune checkpoint blockade (ICB) therapies have made significant achievements in clinical outcomes for colorectal cancer patients. However, not all patients benefit from ICB. Certain peptides that have been modified have shown to possess excellent stability. In a notable instance, Carvajal et al. [20, 21] developed stable α-helical peptides that function as inhibitors of the MDM2 and MDMX proteins. These peptides are significant in the therapeutic approach for cancers that rely on the p53 pathway.

In recent years, peptides have emerged as a novel category of therapeutic agents, distinguished by their unique biochemical properties and promising therapeutic applications. In this study, a bioinformatics approach was used to analyze the correlation between WRN and the occurrence, development and prognosis of CRC. To better understand the effect of WRN on the prognosis of MSI colorectal cancer, we analyzed the effects of WRN antagonist peptides on the proliferation and migration of MSI colorectal cancer cells as a way to screen for new targets and develop novel antitumor drugs.

Material and Methods

Data retrieval and Manipulation

The University of ALabama at Birmingham CANcer data analysis Portal [22, 23] (UALCAN) is a comprehensive, user-friendly, and interactive web resource for analyzing cancer OMICS data. It is built on PERL-CGI with high quality graphics using javascript and CSS. The website (https://ualcan.path.uab.edu/) was used to download RNA express ion profile and clinical data of CRC in the Cancer Genome Atlas dataset (TCGA). WRN expression data were converted by Log2 for intergroup comparison.

Expression and Prognostic Analysis of WRN

Data analysis was performed using UALCAN. Set the conditions: ①Analysis ②Scan my genes ③WRN; other settings are the database defaults. Click on the cancer abbreviations shown in red to analyze the gene differential expression and survival curves.

Analysis of the Clinical Significance of WRN

LinkedOmics: Select the cancer type "TCGA_COADREAD", retrieve the dataset "HiSeq RNA", enter the gene "WRN", select "Clinical" for the dataset, select "Non-parametric T test" for the statistical method, and analyze the results.

Docking with WRN molecules, short peptides with binding energy scores better than -10 kcal/mol

In this study, we used ZDOCK 3.0.2 to predict the binding of 620 short peptides of 16-30 amino acid sequences to WRN proteins. Before the docking, we obtained WRN protein structure files and short peptides with 16-30 amino acid sequences (620 in total) from AlphaFold [24, 25] database (https://alphafold.ebi.ac.uk/) and CPPsite 2.0 [26] database (http://crdd.osdd.net/raghava/cppsite/), respectively. Subsequently, the proteins obtained from the above downloads were optimized using the Protein Preparation Wizard of the meastro13.0 software, including the removal of non-ligand

molecules and water molecules, the addition of hydrogen atoms and the optimization of the structure using the OPLS2005 force field to remove intermolecular collisions.

For docking, amino acids in the range of 1370-1409 aa [27] at the C-terminus of WRN were defined as the active site, and the docking study was carried out with the default configuration of ZDOCK 3.0.2. After completion of docking energy minimization was performed using AMBER18 at ff14SB force field. Finally, the short peptide and protein complex conformations after energy minimization were evaluated for binding energy using the prodigy tool. The binding model based on the best binding energy was visualized and analyzed using PyMOL 2.5.5.

Subsequently, we commissioned Jiangsu Jitai Biological Company to synthesize the membrane penetrating peptides TAT: RKKRRQRRR, 2093: RKKRRQRRR YTFGLKTSFNVQ and 2310: RKKRRQRRRYARAAARQARAKALARQLGV AA.

WRN-2093/2310 short peptide and HCT116/RKO cell proliferation assay

Cell suspensions (100μL/well) were inoculated in 96-well plates, and the plates were pre-cultured in an incubator (37°C, 5% CO_2). After the cells were treated, 10μL Cell Counting Kit 8 (CCK8) solution was added to each well (be careful not to generate air bubbles in the wells, as they will affect the optical density (OD) reading), and the plates were incubated in an incubator for 1-4 hours, and the absorbance at 450 nm was measured by an enzyme meter. If the OD value is not measured for the time being, 10μL of 0.1M HCl or 1%w/v sodium dodecyl sulfate (SDS) solution can be added to each well, and the plate should be covered and stored at room temperature to protect the plate from light, and the absorbance will not change when measured within 24 hours. (three biological replicates; α=0.05; ns, $p\geq0.05$; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$).

WRN-2310 and RKO cell wound healing assay

Colorectal cancer RKO cells with a cell density of 5-10 $\times10^5$ cells /mL were spread on 24-well plates (500μL per well), add RPMI-1640 culture medium containing 10% fetal bovine serum, and incubate for 16~24h to form a monolayer of cells. The single-layer cells were marked with the word "one" with the tip of a 10μL pipette gun and cleaned with PBS for 3 times. After incubation for 24h, the cells were replaced with the RPMI-1640 culture medium containing 10% fetal bovine serum, which was incubated for 24h, observed and photographed. (two biological replicates; α=0.05; ns, $p\geq0.05$; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$).

Statistical Analyses

Overall survival was measured by Log-rank, and the effect of WRN expression difference on survival of colorectal cancer patients was analyzed. The difference of WRN expression in normal colorectal and colorectal cancer tissues and the influence of clinical indexes on WRN expression were detected by nonparametric T test. Moreover, cell proliferation and cell wound healing were analyzed using Graphpad prism 7 software. Significance was determined with a Oneway ANOVA followed by Bonferroni's Multiple Comparison test comparing to control or blank. (ns, $p\geq0.05$; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$).

Results

Differential Expression of WRN

To study the potential clinical significance of WRN, we used the TCGA dataset pan-cancer to analyze the differential expression of WRN in 24 tumors and normal tissues, which was significantly up-regulated in COAD, and not significant in READ (Figure 1A). In COAD, analysis of the TCGA dataset alone showed that the expression level of WRN was significantly up-regulated ($p<0.001$), which was consistent with the results of the pan-cancer data analysis (Figure 1B). Identically, none of the WRN expression levels were statistically significant in READ (Figure 1C).

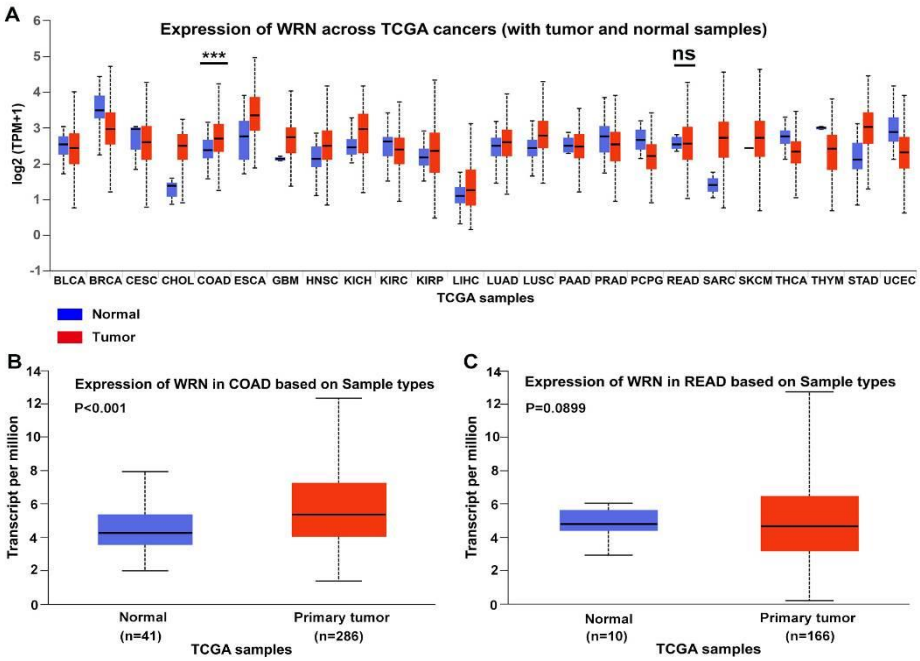

Figure 1. Differential expression of WRN in colorectal cancer. (A) Comparison of WRN expression between tumor and normal samples in 24 types of cancer; (B) Expression of WRN in colon adenocarcinoma tissues and normal tissues; (C) Expression of WRN in rectum adenocarcinoma tissues and normal tissues. ns, $p \geq 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Prognosis significance of WRN

To further investigate the relationship between WRN expression levels and prognosis, we performed a survival correlation analysis for colorectal cancer, including overall survival (OS) and disease-free survival (DFS). The results of survival curve analysis all showed that low expression of WRN gene was detrimental to the prognosis of patients and was a risk factor for poorer prognosis of colorectal and colon cancer (Figure 2A, C). Additionally, the difference between WRN expression and disease- free survival of colorectal cancer and prognosis of Rectum adenocarcinoma was not statistically significant (Figure 2B, D).

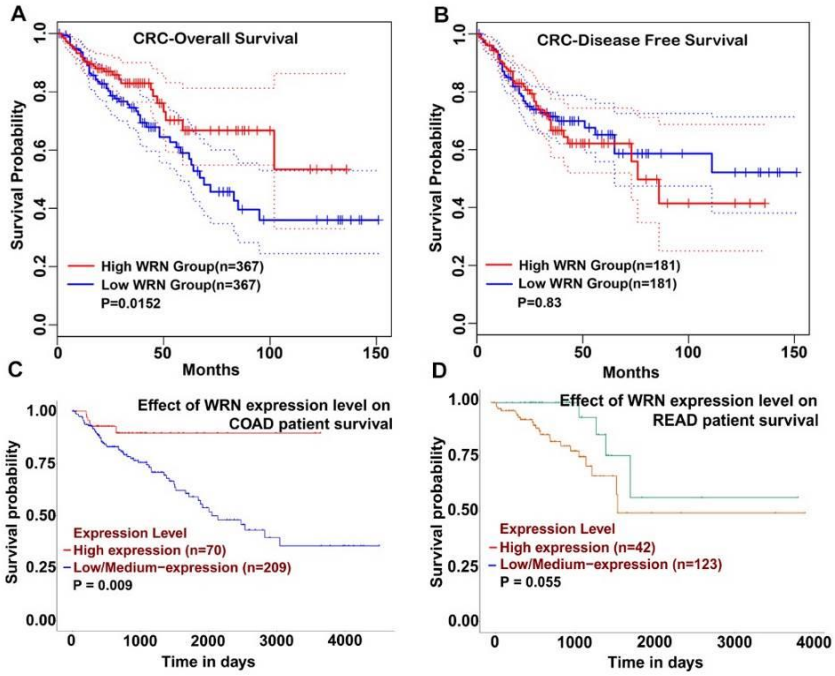

Figure 2. WRN gene expression and patient prognosis. Kaplan-Meier analyzed the relationship between

WRN expression and (A) OS and (B) DFS in CRC patients; (C-D) Kaplan-Meier analyzed the effect of WRN expression level on survival of COAD/READ patients. *, p<0.05; **, p<0.01.

Correlation between WRN expression and clinical indicators of CRC

To explore the clinical significance of WRN expression in colorectal cancer and to analyze the correlation between WRN expression and patients' clinical indexes. In CRC, WRN expression was affected by lymph node number (p=0.0124), pathologic stage (p=0.0206) and pathology N stage (p=0.0456) (Figure3 A-C). In COAD, WRN expression based on lymph node metastatic status (Normal vs N0/Normal vs N1/Normal vs N2, p<0.001; N0 vs N1, p<0.05) was different for different N stages (Figure 3D); different individual cancer stages (Normal vs Stage1/Normal vs Stage2/Normal vs Stage3, p<0.001; Stage1 vs Stage2, p<0.05; Stage1 vs Stage4, p<0.01), the expression of WRN was different (Figure 3E); the expression of WRN was also affected by the sex of patients (Normal vs Male/Normal vs Female, p<0.001) (Figure 3F). In READ, there was a difference in WRN expression according to nodal metastasis status (N0 vs N2, p<0.05) (Figure 3G); there was no statistically significant effect of individual cancer stage and patient gender on WRN expression (Figure 3H-I).

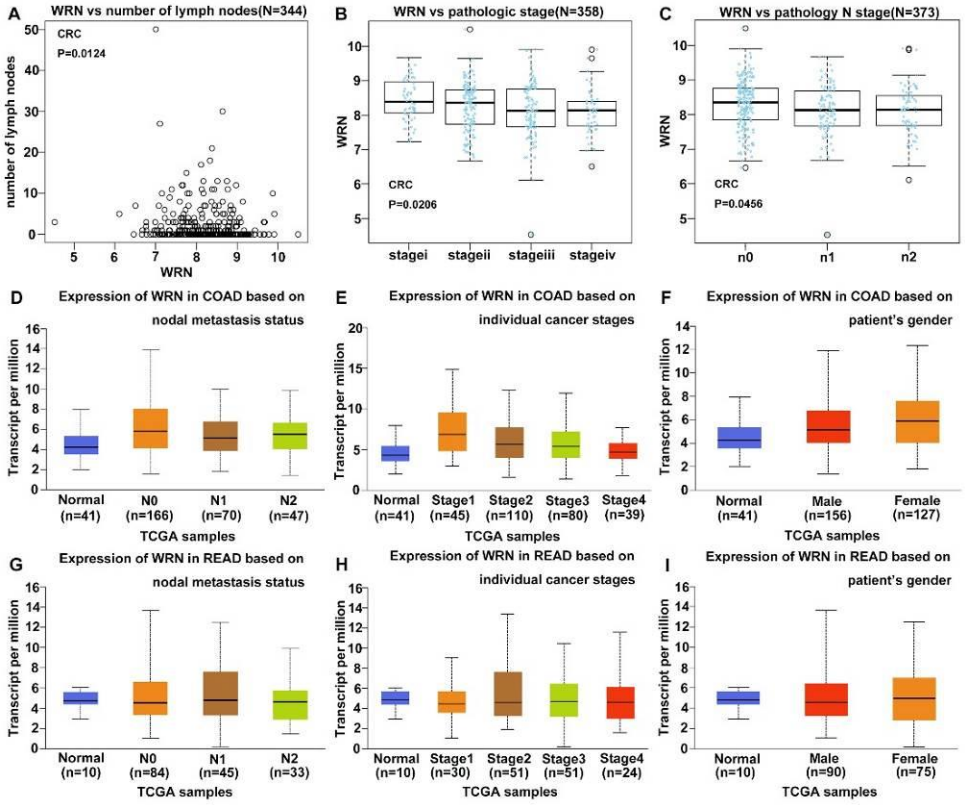

Figure 3. Correlation between WRN expression and clinical indicators of CRC. (A-C) Expression of WRN in lymph node number, pathology and N stage of colorectal cancer; (D-F) Expression of WRN in COAD based on nodal metastasis status(Normal vs N0/Normal vs N1/Normal vs N2, p<0.001; N0 vs N1, p<0.05)、individual cancer stages(Normal vs Stage1/ Normal vs Stage2/Normal vs Stage3, p<0.001; Stage1 vs Stage2, p<0.05; Stage1 vs Stage4, p<0.01) and patient's gender(Normal vs Male/ Normal vs Female, p<0.001); (G-I) Expression of WRN in READ based on nodal metastasis status(N0 vs N2, p<0.05)、individual cancer stages(p>0.05) and patient's gender(p>0.05).

Screening of WRN short peptides with binding energy scores better than -10 kcal/mol

Short peptides have been gaining popularity in various fields such as biology, chemistry, and

medicine because of their unique properties. Their wide range of structures, coupled with their ability to adopt various shapes, allows for precise control over how they engage with specific receptor sites. This results in peptides exhibiting a high degree of selectivity, as they interact in a very specific manner with their intended targets [28]. In this study, we used CPPsite 2.0 to predict the binding scores of 620 short peptides with sequences of 16-30 amino acids and WRN proteins as shown in Supplementary Table 1. Then, ZDOCK 3.0.2 software was used to screen out a total of 11 WRN short peptides with binding energies greater than -10 kcal/mol. The screened high scoring short peptides bound to each other with amino acids in the range of 1370-1409 aa at the C-terminal end of the WRN, and their names, sequences, and binding energies with the WRN are shown in Table 1.

Table 1. Total of 11 WRN short peptide binding energy scores better than -10 kcal/mol.

Peptide	Sequence	Binding energy (kcal/mol)
1301	LLIIARRRIRKQAHAHSK	-10.2
1753	AAVACRICMNFSTRQARRNHRRRHRR	-10.3
1806	GRKKRRQRRRPPQTYADFIASGRTGRRNAI	-10.3
2003	IYLATALAKWALKQGFGGRRRRRRR	-10.2
2093	YTFGLKTSFNVQYTFGLKTSFNVQ	-10.9
2152	GLFKALLKLLKSLWKLLKAGGC	-10.1
2310	YARAAARQARAKALARQLGVAA	-10.9
2829	YSSYSAPVSSSLSVRRSYSSSSGS	-10.6
2854	KKLALHALHLLALLWLHLAHLALKK	-10.9
2943	HRLRHALAHLLHKLKHLLHALAHRLRH	-10.1

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High binding energy WRN short peptide that can hydrogen bond to sites ARG-1403

Peptides are able to finely regulate the binding mode to specific receptor sites due to their diverse structures and multiple morphologies. To further explore how high binding energy short peptide bind to WRN, we analyzed the microstructure of high binding energy short peptides. The high-scoring short peptides we screened bound to amino acids in the range of 1370-1409 aa [27] at the C-terminal of WRN. Among them, 2093, 2310, 2829 and 2854 can hydrogen bond with site ARG-1403, which implies that 2093, 2310, 2829 and 2854 are important short peptides screened (Figure 4A-D). It is worth noting that 2093 has 9 binding sites (Figure 4A), 2310 and 2829 have 4 binding sites (Figure 4B, D), while 2854 has only 2 binding sites (Figure 4C), so 2854 is excluded based on the fact that the more binding sites there are, the stronger the binding. Interestingly, both 2310 and 2829 have four binding sites and their sequences are shown in Table 2.

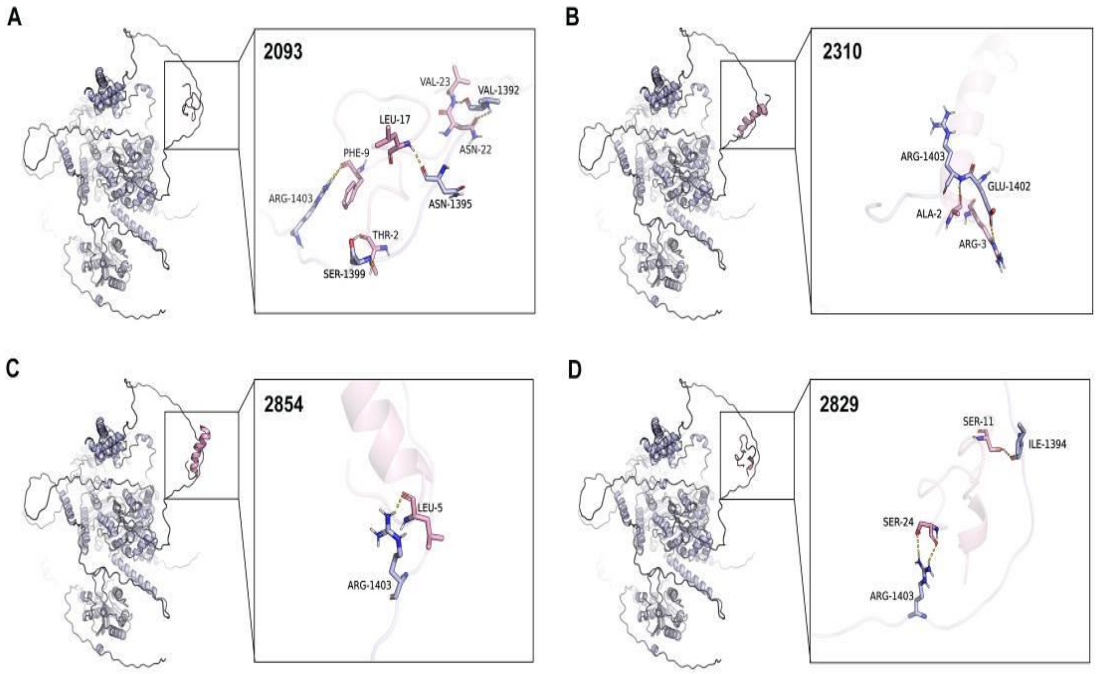

Figure 4. High binding energy WRN short peptide that can hydrogen bond to site ARG-1403. (A-D) Among them, 2093, 2310, 2829, 2854 can hydrogen bond with site ARG-1403, implying that 2093, 2310, 2829, 2854 are important short peptides screened.

Table 2. Further screening of the short peptides 2310 and 2829.

Peptide	Sequence	Number of repeated sequences	Total amino acids	Binding energy (kcal/mol)
2310	YAR***AAA***RQARAKALARQLGV***AA***	2	22aa	-10.9
2829	Y***SS***YSAPV***SSS***LSVRRSY***SSSS***GS	3	24aa	-10.6

It can be found that 2829 has one more repeat sequence than 2310 and also has 2 more amino acids, and the binding stability is poor with many repeat sequences, so 2829 is excluded.

Based on the above analysis, it can be found that 2829 has one more repeat sequence than 2310 and also has 2 more amino acids, and the binding stability is poor with many repeat sequences, so 2829 is excluded. According to the above analysis, 2093 and 2310 were finally selected as short peptides for subsequent experiments. This suggests that the high binding energy short peptide recognizes and binds precisely to the WRN molecule, just as one key can only open one lock.

Correlation analysis of WRN-2093/2310 short peptide with proliferation of HCT116/RKO cells

In order to verify whether the screened WRN-2093/2310 has an effect on MSI colorectal cancer cells HCT116/RKO, we performed a CCK8 cell proliferation assay. The experimental results showed that at a concentration of 20μM, WRN-2310 had a significant effect on the proliferation of RKO cells, WRN-2093 had no effect on RKO cells, and neither WRN-2093/2310 had any effect on HCT116 cells (Figure 5A-B); different concentrations of WRN-2310 significantly affected the proliferation of RKO cells, especially at WRN-2310 40μM (p<0.01) and 80μM (p<0.001), while none of them affected HCT116 cells (Figure 5C-D). This suggests that WRN-2310 plays a role by affecting the proliferation of MSI colorectal cancer cells, thereby regulating the occurrence and development of MSI colorectal cancer.

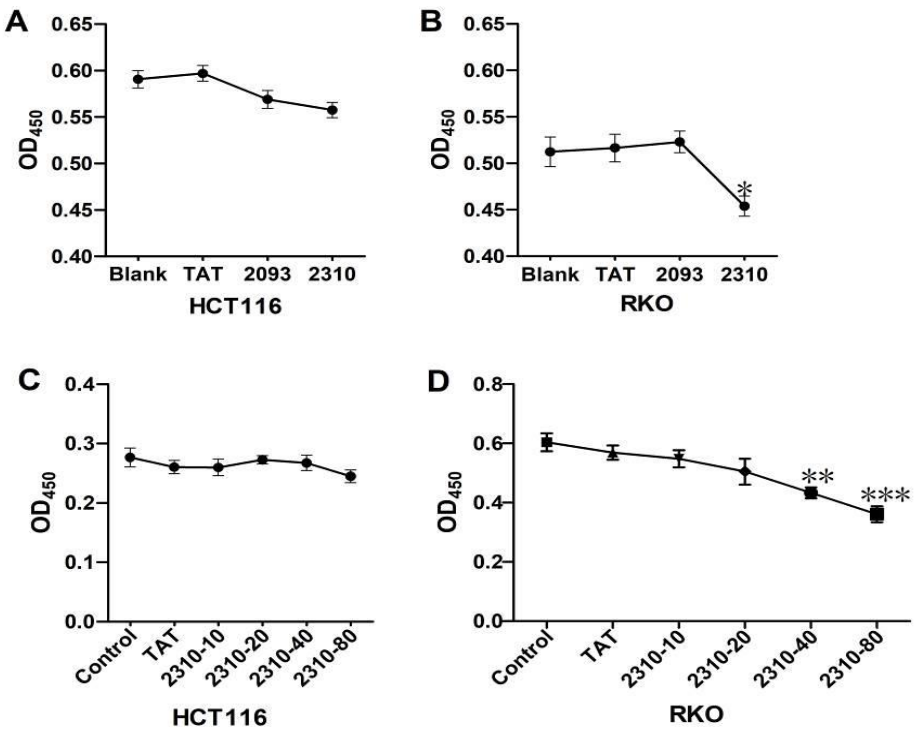

Figure 5. WRN-2093/2310 short peptide and HCT116/RKO cell proliferation assay. (A-B) Effects of WRN-2093/2310 short peptides on proliferation of HCT116/RKO cells, respectively; (C-D) Effects of different concentrations of WRN-2310 on proliferation of HCT116/RKO cells respectively. **, $p<0.01$; ***, $p<0.001$.

Correlation analysis of WRN-2310 with cell Wound healing of RKO cells

Whether the effect of WRN-2310 on MSI colorectal cancer cells is other than inhibition of proliferation was tested by applying cell scratch assay. Cell scratch assay showed that wound closure was slowed down in the WRN-2310 40μM and WRN-2310 80μM groups compared to the control group (Figure 6A), i.e., reduced migration ability; the migration rate of RKO cells varied with the concentration of WRN-2310, especially at WRN-2310 40μM ($p<0.01$) and 80μM ($p<0.001$) (Figure 6B), the migration rate was lower, i.e., inhibited RKO cell migration. It is hypothesized that WRN-2310 plays a role in RKO cell migration to affect MSI colorectal cancer development and may be a new therapeutic target for MSI colorectal cancer.

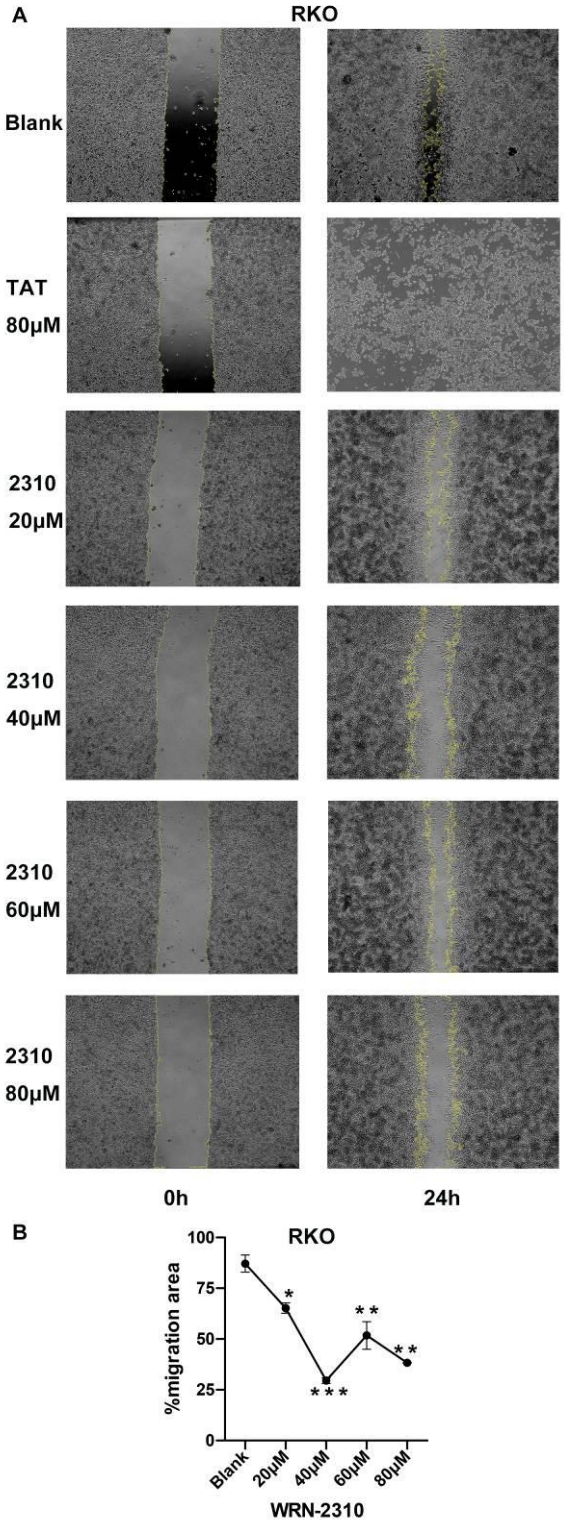

Figure 6. WRN-2310 short peptide and RKO cell Wound healing assay. (A) Wound healing assay under microscope (40×); (B) The graphical data represented the percentage of migration area determined by wound healing assay.

Discussion

CRC is the third most common cancer in the world, with a higher incidence in recent years posing a serious impact on patients' quality of life [29]. Different types of CRC patients present varied prognosis, which means they responded differently to the same treatment plan [30, 31]. MSI, which stands for microsatellite instability, is a significant mechanism that contributes to the

development of colorectal cancer, being present in around 15% of non-hereditary cases [32]. To date, the role of MSI is currently used to select patients with colorectal cancer for immunotherapy, but its efficacy varies and only some cancer patients may benefit from it [33, 34]. Recently, therapeutic peptides have risen to prominence as a unique class of pharmaceutical agents consisting of an ordered series of amino acids, usually with molecular weights of 500-5000Da [35]. Therefore, it is urgent to actively screen new pharmaceutical targets and develop novel antitumor drugs in the clinic.

Werner syndrome protein (WRN), also known as WRN RecQ Like Helicase, is a protein-coding gene that is mainly localized in the nucleus and cytoskeleton of cells. Our study found that WRN gene is highly expressed in colorectal cancer, and the trend of this expression level was analyzed in TCGA pan-cancer and TCGA normal and tumor tissues, respectively, suggesting that WRN is involved in colorectal carcinogenesis and progression. The RecQ DNA helicase WRN has been reported to be a potential therapeutic target for cancers characterized by microsatellite instability, which is a type of genetic instability resulting from defects in the mismatch repair system [18, 36, 37]. When WRN is removed from cells with MSI, it triggers extensive DNA double-strand breaks, which can cause the cells to stop dividing or apoptosis[4]. This further validates our study that high WRN expression is an independent risk factor for CRC. Survival curve analysis showed that low WRN expression was closely associated with poorer OS in colorectal cancer and colon cancer, which is contrary to our study, and it is possible that part of the colorectal cancer cells are dependent on the WRN gene for survival [38], and in particular, MSI colorectal cancer cells are more dependent on the WRN gene for survival [37].

In this paper, we found that in colorectal cancer, the expression of WRN was affected by the number of lymph nodes, pathological stage and pathological N stage, reflecting the heterogeneity of colorectal cancer. Tumor heterogeneity refers to the fact that different tumor cells can exhibit different morphological and phenotypic characteristics, including cell morphology, gene expression, proliferation and metastasis potential. Increasing evidence suggests that tumor heterogeneity and the intra- and extra-tumor environments are both independent of and interact with each other, and Wang, et al. [39] reported that the relationship between intra- and extra-CRC factors and CRC heterogeneity. In COAD, there were differences in WRN expression in different N and S stages, suggesting a role in the different progression of colon cancer.

Currently, peptides are showing significant promise as versatile agents in the field of cancer treatment. A variety of peptides, both naturally occurring and artificially created, are designed to bind to their native receptors for both diagnostic and therapeutic purposes. Synthetic peptides, in particular, can be readily modified to enhance their stability within the body, their ability to cross cell membranes, and their specificity for targets, often drawing on the properties of well-established natural peptide families [40, 41]. In this paper, we performed molecular docking using ZDOCK 3.0.2, and the screened highly scored short peptides bound to amino acids in the range of 1370-1409 aa [27] at the c-terminus of the WRN, of which, 2093 and 2310 were able to form a hydrogen bond with the ARG-1403 site, suggesting that 2093 and 2310 are important short peptides for the screen. The results of cell proliferation assay showed that WRN-2310 had a significant effect on the proliferation of RKO cells compared with WRN-2093; different concentrations of WRN-2310 significantly affected the proliferation of RKO cells, suggesting that WRN-2310 plays a role in regulating the development of MSI colorectal cancer by affecting the proliferation of MSI colorectal cancer cells. At the same time, RKO cell migration also depends on WRN-2310 concentration, especially at WRN-2310 40μM (p<0.01) and 80μM (p<0.001). We hypothesized that WRN-2310 is involved in RKO cell migration affecting MSI colorectal cancer development and may be a new therapeutic target for MSI colorectal cancer. This is consistent with the fact that WRN helicase is a promising synthetic lethal target for MSI colorectal cancer [18].

Notably, when doing the wound healing assay of WRN-2310 on RKO cells, we found that the membrane-penetrating peptide TAT 80μM was not effective on RKO cells, and the reason might be related to its delivery mechanism and

application conditions. TAT is a short-chain polypeptide rich in basic amino acids with a strong ability to penetrate cell membranes, and has been called a "biological missile". However, despite the ability of TAT to bring nucleic acid molecules and drug-protein molecules into the cell to act, its application is not unconditional. For example, although TAT has been found to deliver molecules, including nucleic acids, proteins, and small-molecule drugs, through non-covalent linkages and other forms, no study has yet compared differences in the ability of TAT to deliver proteins [42]. This suggests that the poor effect of TAT when used as a control may be related to the choice of delivery method, and it is further hypothesized that the amount of the membrane-penetrating peptide TAT that enters the RKO cells cannot be determined, which, in turn, affects the wound-healing ability of the RKO cells. Moreover, TAT is rich in basic amino acids and the amino acid sequence is usually positively charged. This characteristic enables TAT to carry a variety of bioactive substances of different sizes and properties into cells, including peptides, proteins, plasmid DNA, siRNA, etc [43, 44]. However, this broad applicability does not mean that optimal results are achieved under all experimental conditions, especially when used in conjunction with specific drugs or therapeutic approaches, which may require more refined optimization of conditions and experimental design.

Another phenomenon was that WRN-2310 inhibited RKO in MSI colorectal cancer cells at 40μM and 80μM better than WRN-2310 at 20μM and 60μM, which may be related to the sequence and concentration dependence of 2310 protein domain peptide [45, 46]. Firstly, the 2310 sequence is YARAAARQARAKALARQLGVAA, in which there are two adjacent repeating A, with a total of 22 amino acids. If 2310 is further improved by increasing or decreasing the number of amino acids, 2310 presents a variety of structures and morphologies, it will be able to finely regulate the way of binding with WRN molecules, which may affect the effect of WRN-2310 on MSI colorectal cancer cell RKO effects. Secondly, different concentrations of WRN-2310 may affect the rate and amount of entry into RKO cells, and thus affect the effect on RKO cells.

Based on the above inference, we make a bold speculation that protein molecules such as WRN, a cancer-related multifunctional DNA helicase with nuclear localization signals, synthesized in the cytoplasm and localized in the nucleus, which plays an important role in DNA repair, replication, transcription, and telomere maintenance, can be designed into antagonistic peptides to inhibit cancer development, promote clinical prognosis, and become a new target for cancer treatment.

Conclusion

In summary, this study comprehensively analyzed the role of high WRN expression in prognosis, clinical staging in colorectal cancer. Meanwhile, the effect of WRN-2310 antagonistic peptide on MSI colorectal cancer cells (HCT116/RKO) was designed and verified, indicating that WRN may become a new target for anti-MSI colorectal cancer therapy and presents a novel approach to combating cancer.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting information

Table S1. CPPsite 2.0 predicts the binding fraction of 620 short peptides of 16-30 amino acid sequences to WRN proteins.

Peptide	Binding_energy(kcal/mol)
1002	**-7**
1003	**-7.2**
1004	**-6.4**
1038	**-6.6**
1045	**-8.9**
1046	**-7**
1047	**-8.5**
1048	**-6.1**
1049	**-9.1**
1050	**-5.3**
1051	**-6.6**
1052	**-5.2**
1053	**-6.2**
1054	**-7.7**

1055	**-6**
1056	**-4.7**
1057	**-9.1**
1058	**-4.8**
1059	**-7.8**
1061	**-5.5**
1062	**-5**
1063	**-4.8**
1064	**-5.6**
1065	**-6.5**
1066	**-4.8**
1067	**-4.4**
1068	**-5.2**
1069	**-4.9**
1070	**-5.2**
1071	**-4.2**
1072	**-6.7**
1073	**-5.6**
1074	**-5**
1075	**-5.1**
1078	**-6.2**
1079	**-5.9**
1080	**-9.6**
1081	**-6.5**
1082	**-5.4**
1083	**-6.5**
1084	**-6.7**
1085	**-8.5**
1086	**-6.7**
1088	**-4.9**
1089	**-7.2**
1091	**-7.7**
1093	**-8.3**
1094	**-6.9**
1115	**-9.2**
1116	**-7.4**
1117	**-6.6**
1118	**-6.3**
1119	**-6.7**
1120	**-7.8**
1121	**-7.7**
1122	**-7.4**
1123	**-6**
1124	**-8.7**
1125	**-7.8**
1126	**-6.9**
1127	**-7.3**
1128	**-7.6**
1129	**-7**
1130	**-6.8**

1131	**-9.2**
1132	**-8.3**
1135	**-8.6**
1139	**-7.3**
1140	**-8.4**
1141	**-7.1**
1206	**-6.8**
1207	**-7**
1209	**-5.9**
1210	**-5.6**
1211	**-7.5**
1212	**-6.5**
1214	**-7.1**
1215	**-8.4**
1238	**-9.5**
1239	**-7.8**
1240	**-8.1**
1241	**-8.6**
1242	**-9.4**
1243	**-7.1**
1244	**-8.4**
1246	**-6.9**
1247	**-5.8**
1249	**-8.1**
1251	**-6.2**
1252	**-7.9**
1253	**-9.6**
1254	**-7.8**
1255	**-6.8**
1256	**-5.8**
1258	**-7.3**
1259	**-7.7**
1260	**-8.4**
1261	**-8.4**
1296	**-6.5**
1297	**-6.7**
1298	**-6.1**
1299	**-6.1**
1300	**-5.6**
1301	**-10.2**
1302	**-5**
1303	**-4.9**
1304	**-7.2**
1305	**-5.8**
1306	**-8**
1307	**-6.2**
1308	**-6.8**
1309	**-5.8**
1310	**-5.1**
1311	**-5.4**

1312	**-6.6**
1313	**-6**
1314	**-6.9**
1315	**-6.5**
1316	**-6.8**
1321	**-6.1**
1322	**-6.3**
1323	**-5.2**
1324	**-6.2**
1325	**-5.8**
1330	**-7.5**
1331	**-6.3**
1332	**-6**
1333	**-7.6**
1334	**-8.1**
1335	**-6.9**
1336	**-8.3**
1337	**-6.3**
1338	**-7**
1339	**-5.6**
1341	**-6.8**
1342	**-7.5**
1343	**-5.9**
1344	**-6.5**
1345	**-6.3**
1346	**-7.5**
1347	**-6.7**
1350	**-7.6**
1351	**-7.3**
1352	**-6.9**
1353	**-7.9**
1358	**-8.3**
1365	**-5.9**
1369	**-5.8**
1370	**-6.9**
1371	**-6.5**
1378	**-6.5**
1380	**-5.4**
1381	**-6.6**
1382	**-8.7**
1389	**-5.2**
1390	**-4.7**
1391	**-4.7**
1396	**-9**
1398	**-7.2**
1399	**-7.3**
1400	**-6.7**
1401	**-5.4**
1402	**-6.3**
1404	**-7.6**

1405	**-7.3**
1406	**-6.9**
1407	**-6.6**
1408	**-7.7**
1409	**-6.4**
1411	**-7.2**
1412	**-7.5**
1416	**-7.6**
1417	**-7.4**
1418	**-9**
1420	**-7.6**
1421	**-8.9**
1423	**-5.8**
1424	**-4.5**
1429	**-4**
1430	**-5.8**
1431	**-4.8**
1433	**-4.4**
1434	**-5.3**
1435	**-5.4**
1436	**-5.5**
1447	**-6.7**
1449	**-5.2**
1458	**-7.1**
1459	**-7.1**
1460	**-6.6**
1470	**-5.3**
1471	**-6**
1472	**-8.3**
1475	**-5.4**
1476	**-6.6**
1477	**-7.6**
1478	**-7.7**
1480	**-6.9**
1481	**-7.8**
1484	**-6.3**
1486	**-6.8**
1487	**-5.6**
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1492	**-8.6**
1493	**-7.7**
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1502	**-4.4**
1506	**-7.1**
1507	**-4.9**
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1514	**-5.1**
1517	**-6.1**
1519	**-7.7**

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1524	**-6.2**
1531	**-5.1**
1532	**-5.4**
1533	**-8.3**
1538	**-3.1**
1540	**-7.9**
1541	**-4.4**
1542	**-4.7**
1543	**-5.6**
1544	**-6.5**
1545	**-7.3**
1546	**-7.1**
1547	**-8.5**
1548	**-6.6**
1549	**-7.7**
1550	**-6.4**
1551	**-8.7**
1556	**-8.3**
1557	**-8.5**
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1559	**-8.5**
1560	**-7.7**
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1563	**-7.1**
1564	**-8.5**
1565	**-8.6**
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1713	**-6.4**
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1753	**-10.3**
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1763	**-8.9**
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1766	**-7**
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1794	**-9.3**
1800	**-7.2**
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1806	**-10.3**
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1833	**-9**
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1836	**-7.3**
1837	**-7.6**
1838	**-6.5**
2001	**-7.4**
2002	**-6.6**

2003	**-10.2**
2004	**-7.3**
2005	**-8.1**
2006	**-5.5**
2007	**-6.1**
2011	**-6.1**
2013	**-4.5**
2014	**-7.6**
2015	**-6**
2034	**-6**
2035	**-6.7**
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2041	**-8.9**
2042	**-8.5**
2064	**-8.3**
2071	**-9.2**
2084	**-5.6**
2085	**-6.7**
2086	**-5.3**
2087	**-5.7**
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2095	**-8.1**
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2123	**-6.2**
2124	**-6.3**
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2135	**-8**
2136	**-8.4**
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2152	**-10.1**
2153	**-4.4**
2154	**-6.7**
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2157	**-8.1**
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2163	**-7.1**
2164	**-6.5**
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2166	**-8.6**
2172	**-5.5**
2176	**-6.6**
2177	**-5.2**

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2196	**-6.3**
2197	**-5.3**
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2220	**-8.3**
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2290	**-6.3**
2297	**-5.8**
2300	**-6.7**
2301	**-8.1**
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2313	**-7.6**
2322	**-6.5**
2325	**-6.5**
2326	**-5.8**
2327	**-5.7**
2331	**-8.4**
2333	**-6.5**
2335	**-6.1**
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