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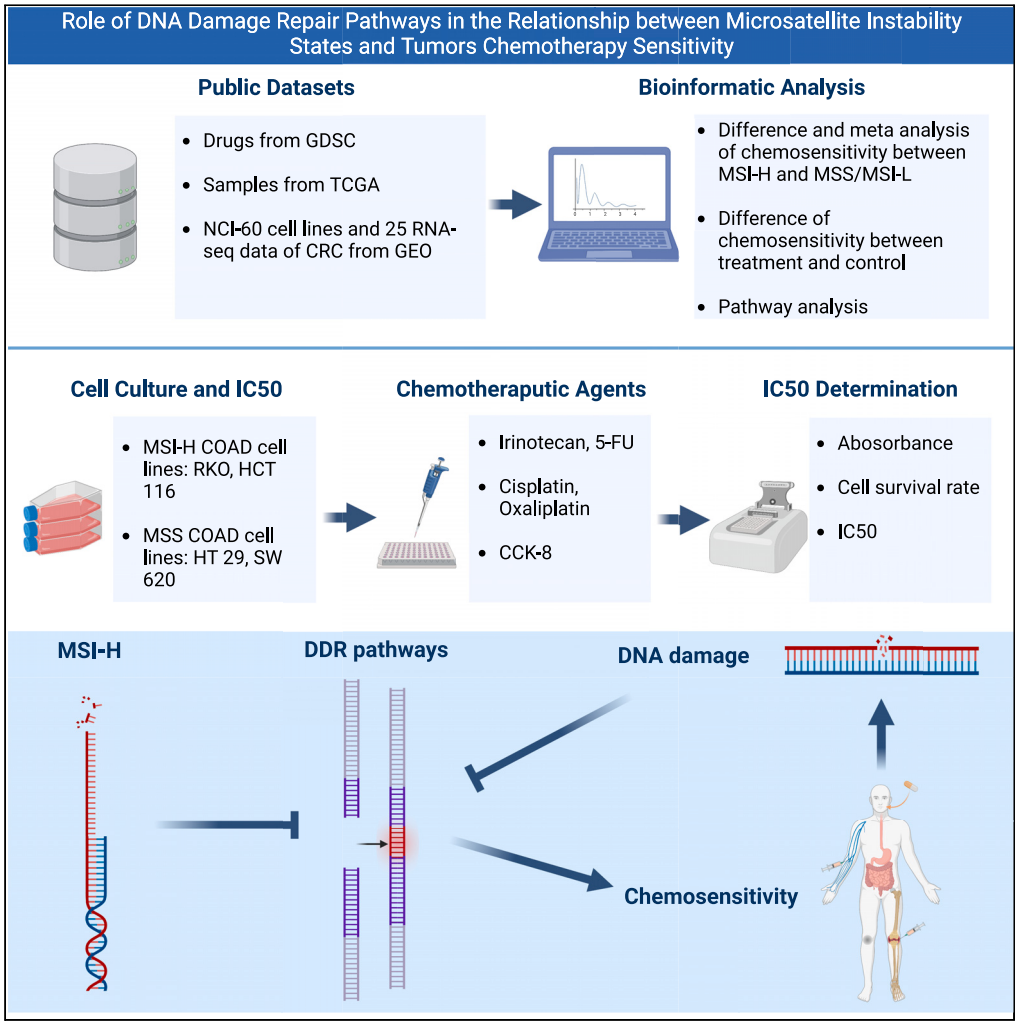


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## Highlights

MSI-H tumors were more sensitive to various chemotherapy drugs than MSS/MSI-L tumors

DDR pathways are related to the drug sensitivity of tumors mediated by MSI-H status

NHEJ is also a protective factor in terms of CRC patient prognosis

Drugs could inhibit DDR pathways such as NHEJ, further increasing drug sensitivity

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# Microsatellite instability states serve as predictive biomarkers for tumors chemotherapy sensitivity

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## SUMMARY

**There is an urgent need for markers to predict the efficacy of different chemotherapy drugs. Herein, we examined whether microsatellite instability (MSI) status can predict tumor multidrug sensitivity and explored the underlying mechanisms. We downloaded data from several public databases. Drug sensitivity was compared between the high microsatellite instability (MSI-H) and microsatellite-stable/low microsatellite instability (MSS/MSI-L) groups. In addition, we performed pathway enrichment analysis and cellular chemosensitivity assays to explore the mechanisms by which MSI status may affect drug sensitivity and assessed the differences between drug-treated and control cell lines. We found that multiple MSI-H tumors were more sensitive to a variety of chemotherapy drugs than MSS/MSI-L tumors, and especially for CRC, chemosensitivity is enhanced through the downregulation of DDR pathways such as NHEJ. Additional DNA damage caused by chemotherapeutic drugs results in further downregulation of DDR pathways and enhances drug sensitivity, forming a cycle of increasing drug sensitivity.**

## INTRODUCTION

Cancer became the second leading cause of death in the world in 2018, accounting for an estimated 9.6 million deaths, or one in six deaths.[1] Almost all proliferating cells have the potential to become malignant,[2] which can lead to the development of hundreds of tumor types, and even more subtypes may be identified as more detailed, novel, and even molecular-level classification methods are developed. Due to the random nature of tumorigenesis at the genomic level and the variability among different tumors, for the vast majority of tumors, complete prevention is almost impossible, and it is difficult to design specific and effective therapeutic agents for each tumor subtype. Even though new therapeutic approaches, such as targeted therapy[3] and immunotherapy[4] are emerging, they are ineffective for the vast majority of tumors, especially for tumors that tend to undergo systemic dissemination and tumors that are in an intermediate to advanced stage, which are thus unresectable; chemotherapy, which has a nonselective killing effect and strong cytotoxicity, remains the most important treatment to improve patient prognosis and even save patients' lives.[5]

However, drug resistance is a key challenge in cancer chemotherapy.[6] The causes of the resistance are diverse. (1) Cancer cells can reduce the damage caused by chemotherapy by controlling the process of drug entry and exit from the cell membrane to reduce the concentration of drug in the cell. For example, some cancers can encode ATP binding cassette transporter (ABC) proteins to regulate drug transport across the membrane, leading to increased drug excretion and drug resistance.[7] (2) When cancer cells receive DNA damage caused by chemotherapeutic agents, they can activate the DDR network to prolong damage repair by blocking the cell cycle,[8] repair DNA damage sites by activating the DDR pathway, and mediate apoptosis when damage cannot be repaired to prevent the transmission of the wrong genome to offspring cells.[9] (3) Chemotherapeutic agents can lead to the acute activation of certain prosurvival signals, such as the PI3K/PTEN/Akt pathway, in cancer cells, which can promote cancer cell survival and regulate growth, proliferation and other cellular processes.[10] (4) Cancer cells can also regulate their own genomic expression by regulating the transcription of certain noncoding RNAs to mediate drug resistance.[11,12] These aforementioned mechanisms vary among individuals. Cancer heterogeneity often affects the

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effectiveness of chemotherapy,[13] so it is important to achieve precision chemotherapy.[14] As new chemotherapeutic drugs and treatment methods become increasingly available,[15] there is greater demand for predictive markers that are universally applicable to multiple drugs.[16,17] Predicting the chemosensitivity of cancer patients before treatment can improve their prognoses.[18]

There are also many studies on the development of markers to predict chemotherapy response based on these mechanisms, and these markers include the homologous recombination deficiency (HRD) score,[19] heat shock protein 90α (HSP90α),[20] and Kirsten rat sarcoma virus (KRAS) gene.[21] However, the most widely used biomarker in clinical practice is MSI status.[22]

A microsatellite is a short tandem repeat DNA sequence of one to four base pairs distributed in the human genome. Due to their repetitive structure, microsatellites are particularly prone to replication errors. These errors are usually repaired by the DNA mismatch repair (MMR) pathway.[23] If any MMR protein is not expressed properly, the MMR pathway does not function properly and incorrect base pairings accumulate, resulting in MSI.[24] MSI is a strongly mutated-associated phenotype.[25] Features associated with MSI can lead to the formation of a variety of tumors, including CRC (15% with MSI), stomach adenocarcinoma (STAD, 15% with MSI), uterine corpus endometrial carcinoma (UCEC, 20–30% with MSI) and ovarian serous cystadenocarcinoma (OV, 12% with MSI).[26] MSI status has multiple applications: it can be used in multiomics studies[27] and for the prediction of prognosis,[28,29] immunotherapy efficacy prediction,[30] radiotherapy efficacy,[31] and chemotherapy efficacy.[32] However, most research on MSI and chemotherapy sensitivity has focused only on the sensitivity to a single drug. At present, there is a lack of systematic studies mechanistically analyzing the effect of MSI status on the sensitivity to multiple drugs, including drugs used for the treatment of multiple cancer types. Therefore, exploring the relationship between MSI status and pancancer drug sensitivity may provide new insight, which may improve treatment strategies for cancer patients.

In the present study, we used multiple approaches and multiple databases, such as The Cancer Genome Atlas (TCGA) and Genomics of Drug Sensitivity in Cancer[32](GDSC), and found that for multiple cancers, including CRC, STAD, and UCEC, MSI-H tumors had significantly higher drug sensitivity than MSS/MSI-L tumors. We also confirmed the value of MSI status in predicting the CRC response to chemotherapy by meta-analysis. Further study revealed that MSI status was closely associated with DDR pathways. In MSI-H CRC, we found that sensitivity to multiple chemotherapeutic agents, especially DNA damage-related chemotherapeutic drugs, was likely mediated by downregulation of the NHEJ pathway. We also elucidated a new mechanism of secondary tumor changes after chemotherapy. DDR pathway changes in tumor cell lines after chemotherapy depend largely on the chemotherapeutic drugs used. We note that there may be a cycle of increasing drug sensitivity in CRC; that is, downregulation of the NHEJ pathway in MSI-H CRC leads to increased chemosensitivity, and the DNA damage induced by chemotherapeutic drugs leads to further downregulation of the NHEJ pathway, thus creating a positive cycle of increasing chemosensitivity in CRC. In conclusion, our study provides guidance for the selection of chemotherapy drugs for cancer patients and provides a new theoretical basis for related research on secondary drug resistance, which could be used to potential cancer treatment strategies.

## RESULTS

### Relationship between MSI status and chemosensitivity across cancers

Tumor cell lines from the GDSC database were divided into the MSI-H group and the MSS/MSI-L group. The Mann-Whitney U test showed a significant correlation between MSI status and chemotherapeutic drug IC50 for CRC, STAD, OV, and UCEC. The IC50 values for cytarabine, mitoxantrone, topotecan, and gemcitabine in STAD; oxaliplatin and 5-FU in OV; and nelarabine, oxaliplatin, mitoxantrone, cisplatin, and vinblastine in UCEC were significantly lower in the MSI-H groups than in the MSS/MSI-L groups (Figure 1A, Table S3, $p < 0.05$). All MSI-H CRC cell lines had significantly lower IC50 values than MSS/MSI-L cell lines for all chemotherapy drugs except paclitaxel. This indicates that MSI-H CRC cell lines are more sensitive than MSS/MSI-L cell lines to a variety of chemotherapeutic drugs, including 5-FU, oxaliplatin, and irinotecan, which are included in standard CRC chemotherapy regimens (Figure 1B). Moreover, the IC50 values for chemotherapy drugs related to DNA damage, such as oxaliplatin, mitoxantrone, 5-FU, cisplatin, and gemcitabine, were more significantly different between the MSI-H and MSS/MSI-L groups than those for other classes of chemotherapy drugs ($p < 0.01$). We then predicted the response of patients in the TCGA

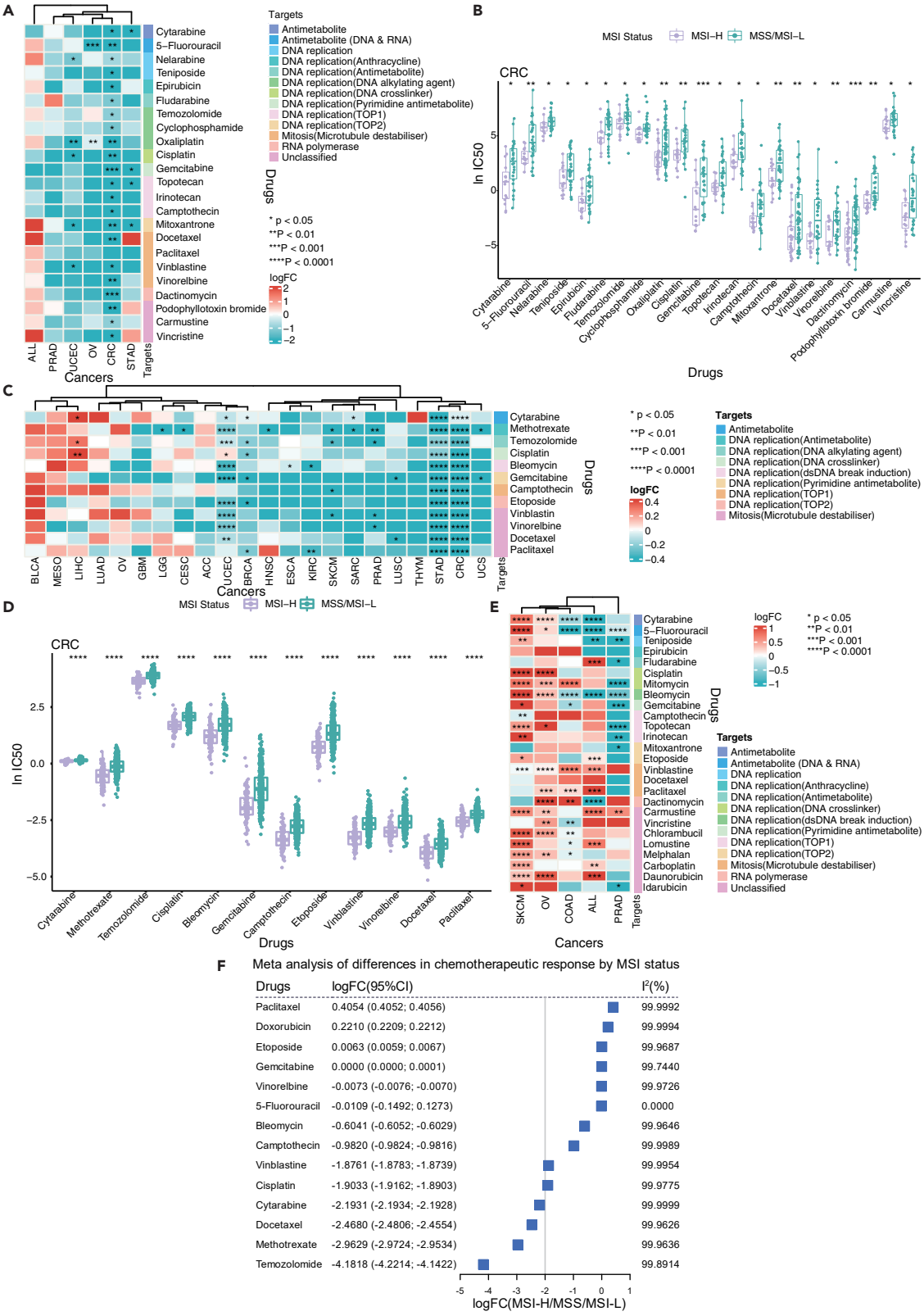
A
Cytarabine
5−Fluorouracil
Nelarabine
Teniposide
Epirubicin
Fludarabine
Temozolomide
Cyclophosphamide
Oxaliplatin
Cisplatin
Gemcitabine
Topotecan
Irinotecan
Camptothecin
Mitoxantrone
Docetaxel
Paclitaxel
Vinblastine
Vinorelbine
Dactinomycin
Podophyllotoxin bromide
Carmustine
Vincristine
ALL
PRAD
UCEC
OV
CRC
STAD
Targets
Cancers
Targets
Antimetabolite
Antimetabolite (DNA & RNA)
DNA replication
DNA replication(Anthracycline)
DNA replication(Antimetabolite)
DNA replication(DNA alkylating agent)
DNA replication(DNA crosslinker)
DNA replication(Pyrimidine antimetabolite)
DNA replication(TOP1)
DNA replication(TOP2)
Mitosis(Microtubule destabiliser)
RNA polymerase
Unclassified
Drugs
* p < 0.05
**P < 0.01
***P < 0.001
****P < 0.0001
logFC
2
1
0
−1
−2
B
MSI Status
MSI−H
MSS/MSI−L
CRC
ln IC50
5
0
−5
Drugs
C
BLCA
MESO
LIHC
LUAD
OV
GBM
LGG
CESC
ACC
UCEC
BRCA
HNSC
ESCA
KIRC
SKCM
SARC
PRAD
LUSC
THYM
STAD
CRC
UCS
Cancers
Cytarabine
Methotrexate
Temozolomide
Cisplatin
Bleomycin
Gemcitabine
Camptothecin
Etoposide
Vinblastine
Vinorelbine
Docetaxel
Paclitaxel
Drugs
Targets
* p < 0.05
**P < 0.01
***P < 0.001
****P < 0.0001
logFC
0.4
0.2
0
−0.2
−0.4
Targets
Antimetabolite
DNA replication(Antimetabolite)
DNA replication(DNA alkylating agent)
DNA replication(DNA crosslinker)
DNA replication(dsDNA break induction)
DNA replication(Pyrimidine antimetabolite)
DNA replication(TOP1)
DNA replication(TOP2)
Mitosis(Microtubule destabiliser)
MSI Status
MSI−H
MSS/MSI−L
D
CRC
ln IC50
2.5
0.0
−2.5
−5.0
Cytarabine
Methotrexate
Temozolomide
Cisplatin
Bleomycin
Gemcitabine
Camptothecin
Etoposide
Vinblastine
Vinorelbine
Docetaxel
Paclitaxel
Drugs
E
SKCM
OV
COAD
ALL
PRAD
Cancers
Cytarabine
5−Fluorouracil
Teniposide
Epirubicin
Fludarabine
Cisplatin
Mitomycin
Bleomycin
Gemcitabine
Camptothecin
Topotecan
Irinotecan
Mitoxantrone
Etoposide
Vinblastine
Docetaxel
Paclitaxel
Dactinomycin
Carmustine
Vincristine
Chlorambucil
Lomustine
Melphalan
Carboplatin
Daunorubicin
Idarubicin
Drugs
Targets
logFC
1
0.5
0
−0.5
−1
* p < 0.05
**P < 0.01
***P < 0.001
****P < 0.0001
Targets
Antimetabolite
Antimetabolite (DNA & RNA)
DNA replication
DNA replication(Anthracycline)
DNA replication(Antimetabolite)
DNA replication(DNA crosslinker)
DNA replication(dsDNA break induction)
DNA replication(Pyrimidine antimetabolite)
DNA replication(TOP1)
DNA replication(TOP2)
Mitosis(Microtubule destabiliser)
RNA polymerase
Unclassified
F
Meta analysis of differences in chemotherapeutic response by MSI status
Drugs
logFC(95%CI)
I²(%)
Paclitaxel 0.4054 (0.4052; 0.4056) 99.9992
Doxorubicin 0.2210 (0.2209; 0.2212) 99.9994
Etoposide 0.0063 (0.0059; 0.0067) 99.9687
Gemcitabine 0.0000 (0.0000; 0.0001) 99.7440
Vinorelbine -0.0073 (-0.0076; -0.0070) 99.9726
5-Fluorouracil -0.0109 (-0.1492; 0.1273) 0.0000
Bleomycin -0.6041 (-0.6052; -0.6029) 99.9646
Camptothecin -0.9820 (-0.9824; -0.9816) 99.9989
Vinblastine -1.8761 (-1.8783; -1.8739) 99.9954
Cisplatin -1.9033 (-1.9162; -1.8903) 99.9775
Cytarabine -2.1931 (-2.1934; -2.1928) 99.9999
Docetaxel -2.4680 (-2.4806; -2.4554) 99.9626
Methotrexate -2.9629 (-2.9724; -2.9534) 99.9636
Temozolomide -4.1818 (-4.2214; -4.1422) 99.8914
-5
-4
-3
-2
-1
0
1
logFC(MSI-H/MSS/MSI-L)

**Figure 1. Analysis of differences in chemotherapeutic response between tumors grouped by MSI status**

(A) Heatmap of the differences in IC50 values between the MSI-H and MSS/MSI-L groups of pancancer cell lines treated with 23 chemotherapeutic agents.

(B) Boxplots with individual data points from for the analysis of differences in IC50 values between the MSI-H and MSS/MSI-L groups of CRC cell lines treated with chemotherapeutic drugs; only drugs with significant differences are shown.

(C) Heatmap of the differences in the predicted response to 12 chemotherapeutic agents for the MSI-H and MSS/MSI-L groups of TCGA cancer types.

(D) Boxplots with individual data points from differential analysis of predicted drug responsiveness between the MSI-H and MSS/MSI-L groups of CRC cell lines. Only drugs with significant differences are shown.

(E) Heatmap of the differences in IC50 values between the MSI-H and MSS/MSI-L groups of NCI-60 cell lines treated with 28 anticancer drugs. Targets in the heatmap annotations were obtained from drug information in the GDSC database, and the horizontal and vertical coordinates of heatmaps are clustered according to the results of log2 (FC) and targets, respectively.

(F) Forest plot of meta-analysis of the difference in the predictive values of chemotherapeutic drug response between the MSI-H and MSS/MSI-L groups of CRC cell lines. (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; and ****$p < 0.0001$; log2(Fold changes); Wilcoxon Rank-Sum test).

database to chemotherapy based on their MSI status. There were significant differences in the predicted sensitivity to some chemotherapeutic drugs between the MSI-H and MSS/MSI-L groups for the following cancers: CRC, STAD, UCEC, uterine carcinosarcoma (UCS), prostate adenocarcinoma (PRAD), breast invasive carcinoma (BRCA), head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC), lung squamous cell carcinoma (LUSC), liver hepatocellular carcinoma (LIHC), esophageal carcinoma (ESCA), skin cutaneous melanoma (SKCM), and sarcoma (SARC) (Figure 1C, Table S4, $p < 0.05$). The predicted drug sensitivity results were in agreement with our IC50 results based on analysis of the GDSC CRC dataset; both analyses indicated that MSI-H tumors were more sensitive to the chemotherapeutic drugs analyzed (Figure 1D). For additional verification, we analyzed differences in IC50 values between MSI-H and MSS/MSI-L cell lines in the NCI-60 panel. The differences in IC50 for cytarabine, 5-FU, bleomycin, and gemcitabine in COAD samples grouped based on MSI status were in agreement with those in CRC, although the differences in IC50 for cytarabine, cisplatin, and gemcitabine in SKCM and those for 5-FU in OV showed the opposite trends (Figure 1E, Table S5, $p < 0.05$). We therefore focused on CRC for our further studies. To further demonstrate the practical significance of MSI as a biomarker for stratification based on chemotherapy sensitivity, we performed a meta-analysis of 25 CRC expression profiles from the GEO database for the prediction of chemotherapy sensitivity in cell lines with different MSI statuses. As with the previous GDSC database analysis, the results showed that cell lines in the MSI-H CRC group had significantly lower IC50 values (logFC < 0, 95% CI < 0, Figure 1F, Table S8) than cell lines in the MSS/MSI-L CRC group for almost all chemotherapeutic agents except paclitaxel, doxorubicin, and etoposide, which means that CRC cell lines with MSI-H status are more sensitive to a variety of chemotherapeutic agents, including 5-FU and oxaliplatin, which are part of the standard CRC chemotherapy regimen, and are also more sensitive to other DNA damage-related chemotherapeutic agents, such as temozolomide, methotrexate, camptothecin, and bleomycin. Taken together, these results demonstrate that MSI status is a predictive marker of chemotherapeutic drug sensitivity for multiple cancers, including CRC, STAD, LIHC, ESCA, OV, UCEC, UCS, PRAD, BRCA, DLBC, HNSC, KIRC, LUSC, LIHC, ESCA, SKCM, and SARC, and in most cases, MSI-H indicates greater chemosensitivity.

## Experimental analysis of chemotherapeutic drug IC50 in COAD cells based on MSI status

To further examine the sensitivity of COAD cells with different MSI statuses to a variety of chemotherapeutic drugs, we determined the IC50 values for five chemotherapeutic drugs in two MSI-H COAD cell lines (RKO and HCT116) and two MSS/MSI-L COAD cell lines (HT29 and SW620). For irinotecan and oxaliplatin, the IC50 values were lower in MSI-H COAD cells than in MSS/MSI-L COAD cells (Figures 2A and 2B, $p < 0.05$). For cisplatin and doxorubicin, RKO and HCT116 cells were more chemosensitive than HT29 cells but less chemosensitive than SW620 cells (Figures 2C and 2D, $p < 0.05$). There were no significant differences in 5-FU IC50 values between MSI-H and MSS/MSI-L COAD cell lines (Figure 2E).

## Relationship between MSI status and DDR pathway and its prognostic significance in pancancer patients

Due to the relationship between MSI status and DNA damage-related chemotherapeutic drug sensitivity and the correlation between DDR pathways and chemosensitivity,[33] we further explored the role of DDR pathways in the chemotherapy response of MSI-H and MSS/MSI-L tumors. The CRC cohort of TCGA was first analyzed by GSEA. The base excision repair (BER), Fanconi anemia (FA), nucleotide excision repair (NER), NHEJ, and single-strand break (SSB) pathways, which are DDR pathways, were significantly downregulated in MSI-H CRC and significantly upregulated in MSS/MSI-L CRC (Figure 3A, p value < 0.05, ES < 0). OV cell line data from NCI-60 were also divided into MSI-H and MSS/MSI-L groups for GSEA.

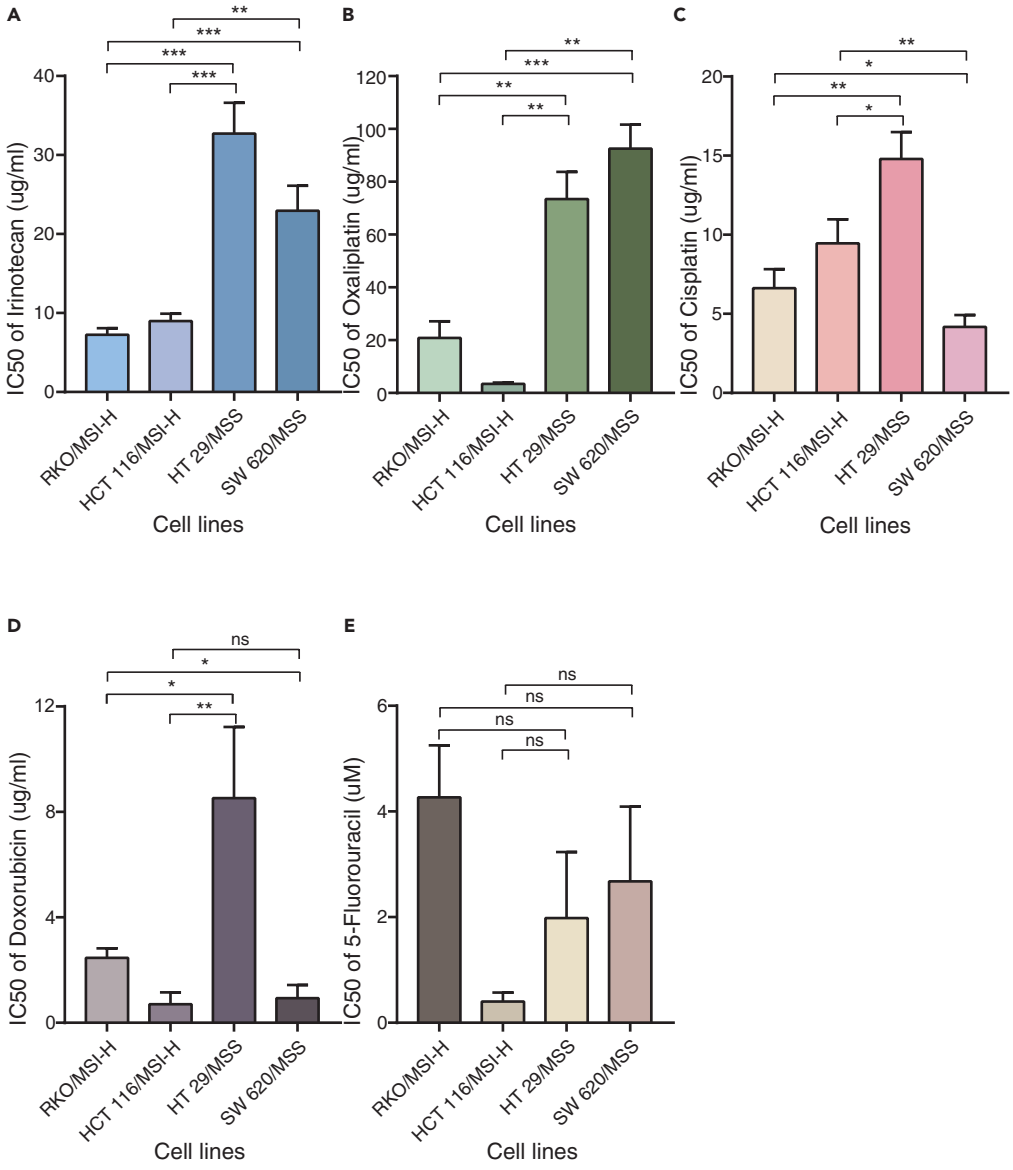


**Figure 2. Sensitivity of COAD cells with different MSI statuses to various chemotherapeutic drugs**

(A–E) show the results for irinotecan, oxaliplatin, cisplatin, doxorubicin, and 5-FU (ns > 0.05; *p < 0.05; **p < 0.01; ***p < 0.001; and ****p < 0.0001).

The BER and NHEJ pathways were significantly downregulated in the MSI-H group and significantly enriched in the MSS/MSI-L group (Figure S1A, p value < 0.05, ES < 0). We also divided pancancer samples into MSI-H and MSS/MSI-L groups with a MANTIS score cut-off value of 0.4 for GSEA. We observed significant but opposite correlations between MSI status and multiple DDR pathways for PRAD and UCEC, and in many tumors, especially CRC and OV, the NHEJ and BER pathways were significantly associated with MSI status. We next further explored the role of these DDR pathways in patient prognosis. In the univariate Cox regression analysis of ssGSEA scores obtained from TCGA pancancer cohort expression data, we found that the association between DDR pathways and patient prognosis was closely related to tumor type (Figures 3B and 3C). For example, in tumors such as UCEC, DDR pathway enrichment was often a risk factor, whereas in other tumors such as STAD, multiple DDR pathways were significantly protective factors. Generally, downregulation of the NHEJ pathway may be an important mechanism of chemosensitivity in MSI-H CRC, and upregulation of NHEJ may play a positive role in patient prognosis by inhibiting tumor development.

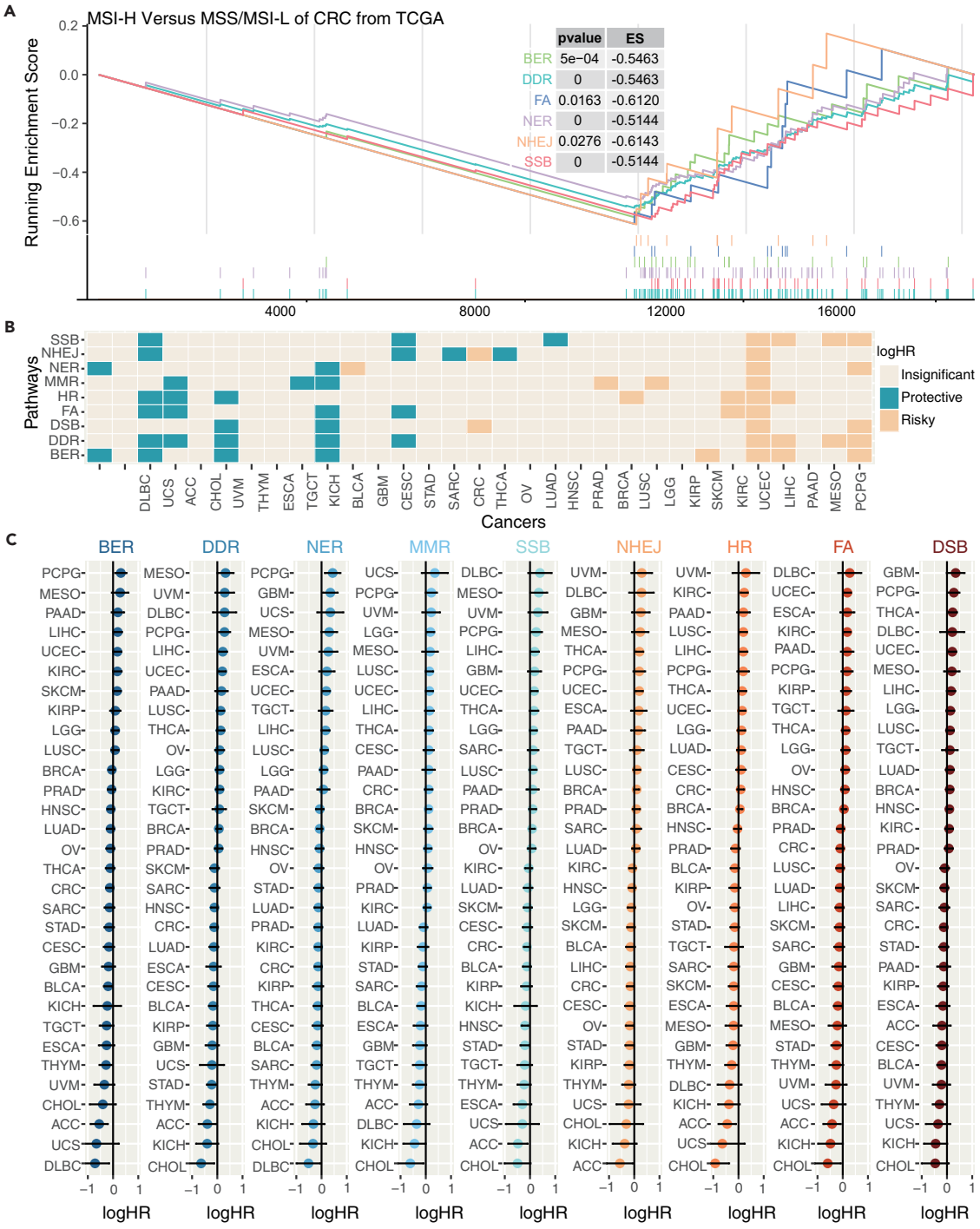


**Figure 3. Pathways differentially enriched in MSI-H and MSI-L and its impact on the prognosis of patients with tumors**

(A) GSEA map for MSI-H and MSS/MSI-L groups in the TCGA-CRC cohort; only gene sets related to DDR pathways and with significant differences between the MSI-H and MSS/MSI-L groups are displayed.

(B) Annotated plot of the univariate Cox regression analysis of DDR pathways as a covariate with patient survival data from TCGA pancancer datasets, with hazard ratio (HR) and p value-related information (protective: logHR < 0; risk: logHR > 0; nonsignificant: p value > 0.05).

(C) Forest plots drawn from the results of univariate Cox regression analysis of DDR pathways as a covariate with s patient survival data from TCGA pancancer datasets.

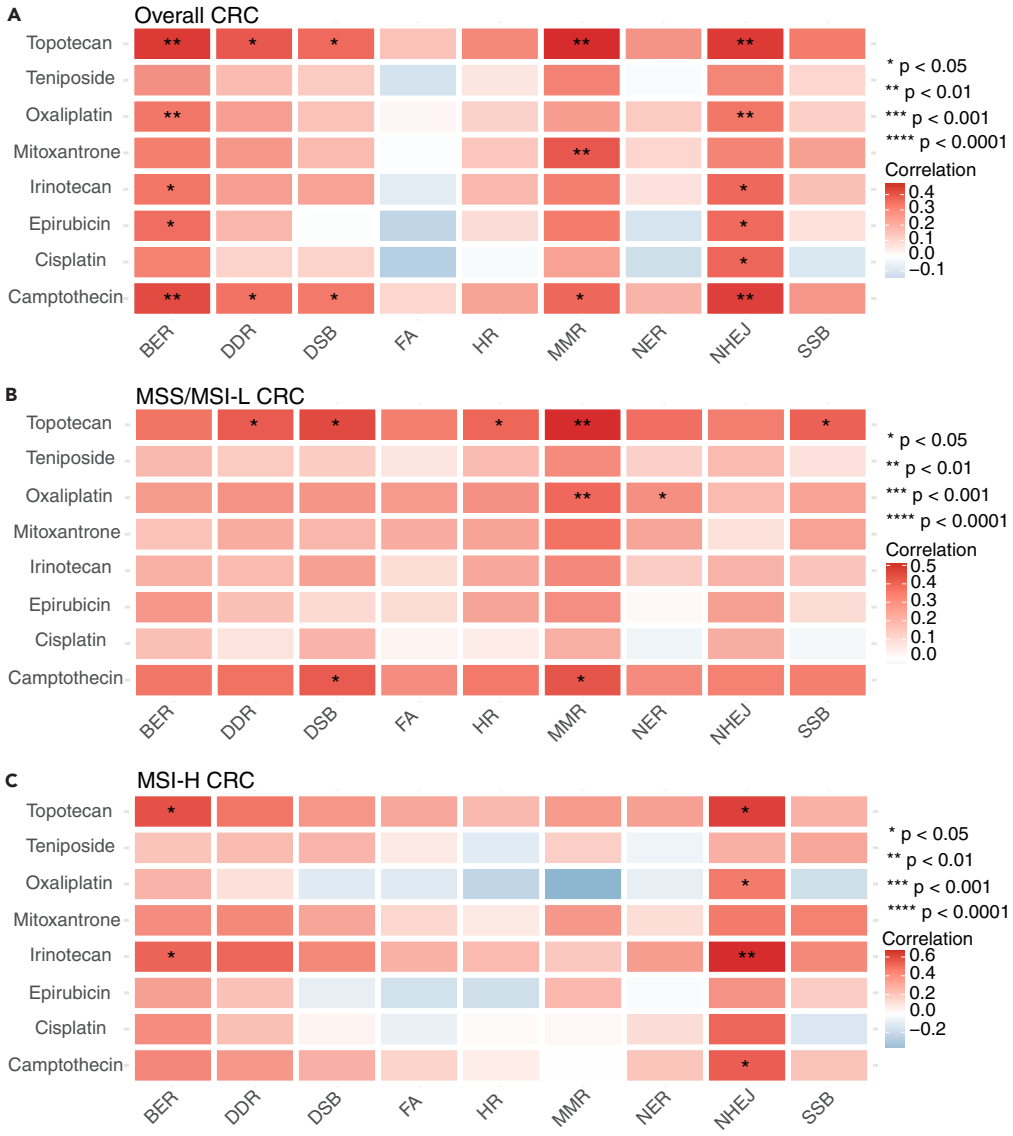


**Figure 4. Correlation between DDR pathway ssGSEA scores and ln(IC50) of DNA damage response-related chemotherapeutic agents**

(A–C) Heatmap of the correlation between DDR pathway ssGSEA scores and ln(IC50) of DNA damage response-related chemotherapeutic agents in the CRC cohort of TCGA, clustered by R values on the horizontal and vertical axes. A-C depict the results of the overall group, MSS/MSI-L group, and MSI-H group. (*p < 0.05; **p < 0.01; ***p < 0.001; and ****p < 0.0001).

## The role of MSI status in CRC in the relationship between DDR and chemosensitivity

We analyzed the correlation between DDR pathway ssGSEA scores and the ln(IC50) values of drugs related to the DDR in the CRC cohort of GDSC (overall group). DDR pathway ssGSEA score was significantly positively correlated with ln(IC50) for chemotherapeutic drugs (Figure 4A, Table S6, R > 0, p value < 0.05). The NHEJ pathway especially showed a strong and significant correlation with the ln(IC50) values for all DNA damage-responsive chemotherapeutic agents except teniposide and mitoxantrone (R > 0.3, p value < 0.05). This indicates that upregulation of DDR pathways is associated with chemotherapy resistance, whereas downregulation of DDR pathways is associated with chemosensitivity. When we further divided the CRC cohort into MSI-H and MSS/MSI-L groups, we found that in general, the relationship between DDR pathway ssGSEA scores and ln(IC50) values for cell lines in the MSS/MSI-L group was more consistent with that in the overall group than that in the MSS/MSI-L group (Figure 4B, Table S6). However, notably, the correlation

between the NHEJ pathway ssGSEA score and ln(IC50) for each drug in the MSS/MSI-L group was not statistically significant. The relationship between NHEJ pathway enrichment and drug sensitivity in the MSI-H group was more similar to that in the overall group (Figure 4C, Table S6), which further indicates the role of the NHEJ pathway in the high drug sensitivity of MSI-H CRC. We also analyzed the correlation between TCGA DNA damage-related drug sensitivity predictions and DDR pathway ssGSEA scores. We found significant correlations between sensitivity and ssGSEA scores for PRAD and UCEC (Figures S2 and S3). In PRAD, DDR pathway ssGSEA scores were negatively correlated with ln(IC50); in particular, upregulation of the FA and SSB pathways in the MSI-H group was negatively correlated with the ln(IC50) of camptothecin, which may explain the higher sensitivity to camptothecin in MSI-H PRAD compared to MSS/MSI-L PRAD. Similarly, there was a negative correlation between NHEJ pathway GSEA scores and bleomycin ln(IC50) values in UCEC; the upregulation of NHEJ in the MSI-H group may explain why the sensitivity to bleomycin is higher in MSI-H UCEC than in MSS/MSI-L UCEC. These findings suggest that DDR pathways can explain to some extent the increased sensitivity of certain tumors, such as UCEC and PRAD, to some DNA damage-related chemotherapeutic drugs. This is best exemplified by the role of the DDR pathways in the drug sensitivity of MSI-H CRC. In CRC, MSI-H status may mediate enhanced sensitivity to various chemotherapeutic drugs through downregulation of DDR pathways, particularly NHEJ.

### Positive circulation of CRC with increasing sensitivity to chemotherapeutic drugs

After determining that MSI-H status in CRC may enhance sensitivity to DDR-related chemotherapeutic drugs via downregulation of the NHEJ pathway, we further explored changes in the DDR pathway in CRC and chemosensitivity following treatment with chemotherapeutic drugs. We first divided the pancancer cell lines from the NCI-60 panel into treatment and control groups, calculated the DDR pathway ssGSEA scores, and analyzed differences in DDR pathway ssGSEA scores of pancancer cell lines before and after treatment with six different chemotherapeutic drugs. Cells were treated with the eight DDR-related chemotherapeutic agents (cisplatin, topotecan, 5-azacytidine, doxorubicin, and gemcitabine) in GEO: GSE116436, and in most cases all eight DDR pathways showed significantly greater enrichment in the treatment group vs. the control group (Figure 5A, Table S7, p value $< 0.05$, fold change $< 1$). In COAD, almost all DDR pathways were significantly downregulated following treatment with all drugs (Figure 5B, p value $< 0.05$); this trend was not significantly different among the overall, MSI-H, and MSS/MSI-L groups (Figures 5C and 5D), suggesting that this effect might not be related to MSI status. However, it is noteworthy that the NHEJ pathway in COAD was significantly downregulated following treatment with paclitaxel, cisplatin, topotecan, doxorubicin, and gemcitabine.

### DDR inhibitors affect the IC50 values of chemotherapeutic drugs in MSI-H COAD cells

We found that RKO and HCT116 cells were more sensitive to irinotecan and oxaliplatin than HT29 and SW620 cells. Therefore, we chose to study the effect of DDR pathway inhibitors on the IC50 values for irinotecan and oxaliplatin in COAD cells. First, we measured the IC50 values for UNC-2170, a DDR inhibitor, in four COAD strains treated for 24 h by CCK-8 assay. UNC-2170 alone did not significantly inhibit the growth of COAD cells. Therefore, to study the combined effect of DDR inhibitors and chemotherapy drugs in COAD cells, we selected two fixed concentrations of UNC-2170 (2 μg/mL and 20 μg/mL). In RKO cells, the addition of low (2 μg/mL) or high (20 μg/mL) doses of UNC-2170 increased sensitivity to irinotecan. The IC50 value of oxaliplatin when combined with the DDR inhibitor (20 μg/mL) was significantly lower than that in the other two treatment groups (oxaliplatin alone and oxaliplatin combined with the DDR inhibitor (2 μg/mL)) (Figure 6A, p value $< 0.05$). In HCT116 cells, a high dose of DDR inhibitor (20 μg/mL) increased sensitivity to oxaliplatin. We also found that with increasing concentrations of DDR inhibitor, the sensitivity of HCT116 cells to irinotecan increased gradually, and the IC50 values between groups were significantly different (Figure 6B, p value $< 0.05$). DDR inhibitors did not affect the sensitivity of HT29 cells to oxaliplatin. In contrast, when the DDR inhibitor was combined with irinotecan, the irinotecan IC50 was significantly lower than that in the irinotecan alone group, but there were no significant differences in the IC50 between the groups with different concentrations of DDR inhibitor (Figure 6C). For SW620 cells, DDR inhibitors did not affect sensitivity to chemotherapeutic drugs, and there was no significant difference in the IC50 between the treatment groups for oxaliplatin and irinotecan (Figure 6D).

## DISCUSSION

Although targeted therapies and immunotherapies for tumors are widely used,[3,4] they are typically used as adjuvant treatments since they are expensive, there are limitations regarding their use, and their

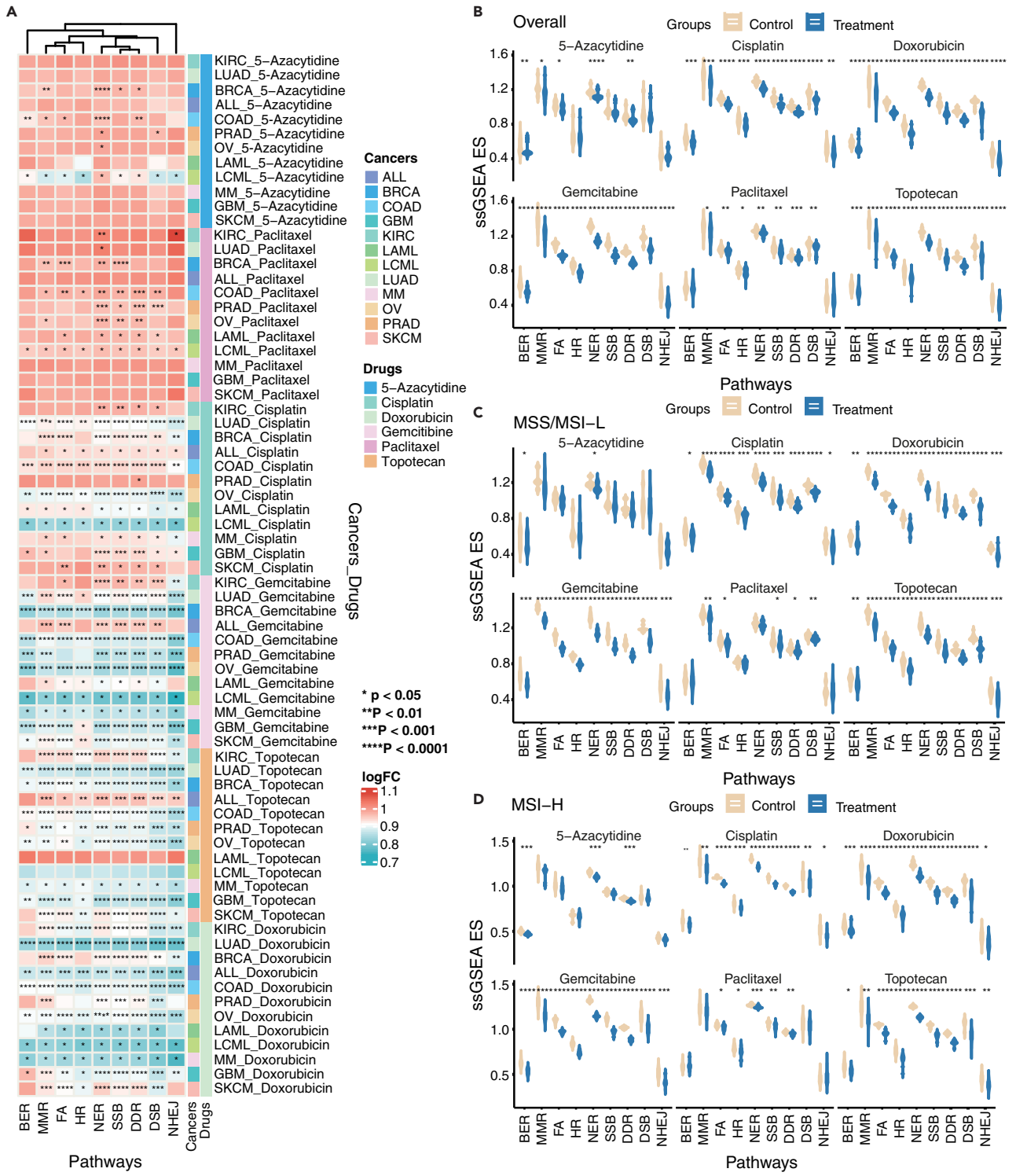


**Figure 5. Enrichment of tumors in the DDR pathway before and after drugs treatment**

(A) Heatmap of differential analysis of DDR pathway ssGSEA scores for NCI-60 pancancer cell lines before and after drug treatment, clustered by drug on the horizontal axis and FC results on the vertical axis.

(B–D) Violin plots of the differential analysis of DDR pathway ssGSEA scores in NCI-60 COAD cell lines before and after drug treatment. Three plots represent the results of the overall, MSS/MSI-L, and MSI-H groups (*p < 0.05; **p < 0.01; ***p < 0.001; and ****p < 0.0001; fold changes; Wilcoxon rank-sum test).

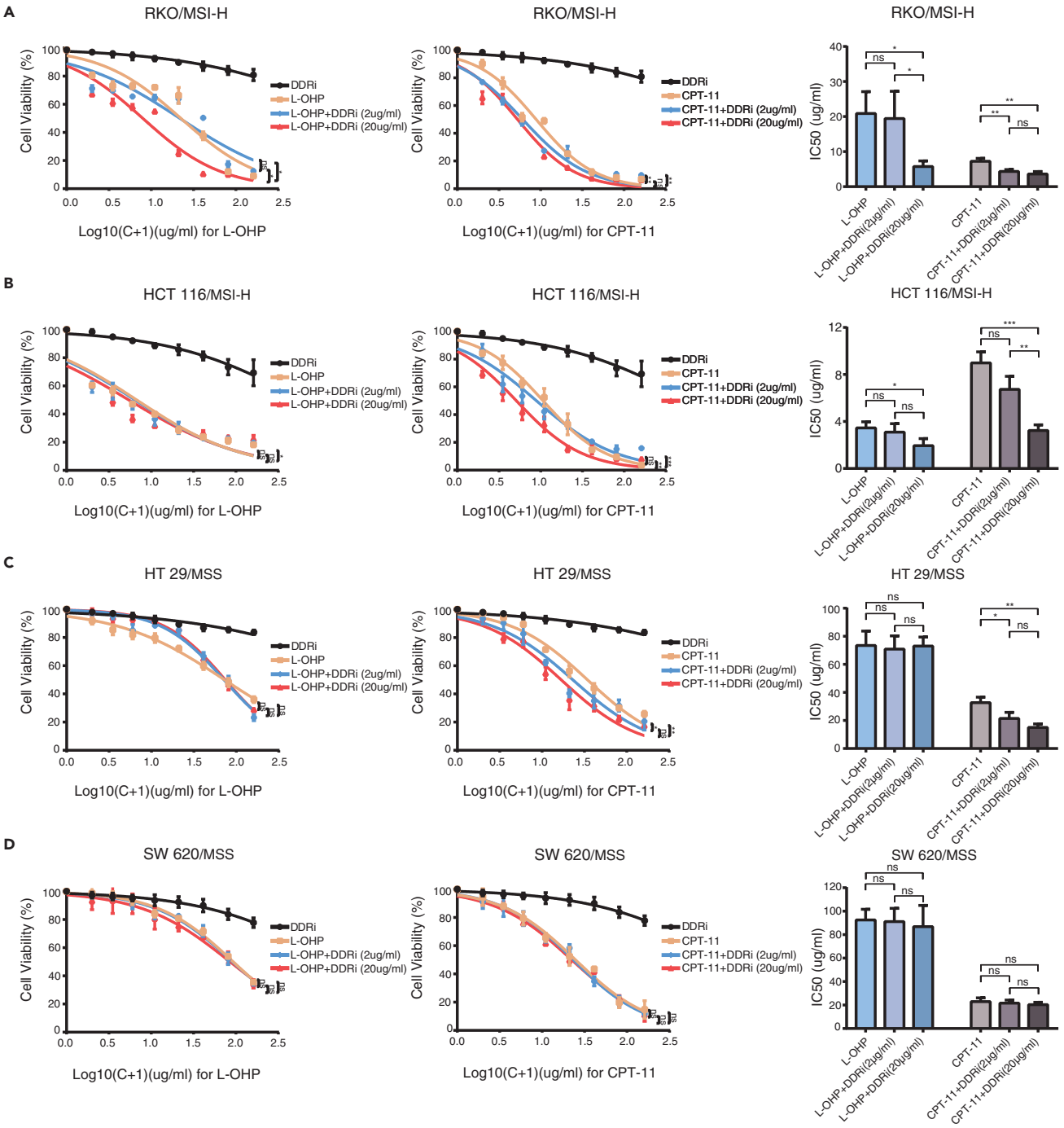


**Figure 6. Oxaliplatin and irinotecan IC50 curves for different COAD cell lines in combination with DDR inhibitors**

(A–D) show the results for RKO, HCT116, HT29, and SW620 cells, respectively (ns > 0.05; *p < 0.05; **p < 0.01; ***p < 0.001; and ****p < 0.0001).

efficacy is unclear. Therefore, chemotherapy remains the primary treatment modality for most intermediate to advanced tumors. However, the heterogeneity of the tumor genome often affects the response to chemotherapy. Thus, it is important to comprehensively understand the responses of various tumors to different chemotherapy drugs and to identify drug sensitivity prediction markers with real clinical application value in order to select appropriate drugs for patients and improve their prognoses. MSI, a biomarker with various functions, has been mostly used in studies of the response of a specific tumor type to

individual chemotherapeutic drugs, while research on MSI as a biomarker for pancancer chemotherapeutic responses is lacking. Therefore, we analyzed the differences in the sensitivity to numerous chemotherapy drugs for various MSI-H and MSS/MSI-L tumors. We found that most MSI-H tumors were significantly more sensitive to chemotherapeutic agents than MSS/MSI-L tumors in most of the significant results, including drug response prediction analysis. We also confirmed the value of MSI status in predicting the CRC response to chemotherapy by meta-analysis. When we further studied the mechanism by which MSI status may affect chemosensitivity, we identified a close relationship between MSI status and the DDR pathway. In particular, MSI-H status in CRC may mediate enhanced sensitivity to multiple chemotherapeutic agents, including 5-FU, oxaliplatin, and irinotecan, through downregulation of the NHEJ pathway. Additionally, the DDR pathway differentially affected the prognosis of different tumors, and in CRC, the NHEJ pathway was a protective factor. We also observed that many cancer cell lines, including CRC cell lines, showed significant downregulation of DDR pathways following treatment with chemotherapeutic agents, and in COAD, this downregulation was directly proven to contribute to increased chemosensitivity.

DNA damage occurs due to endogenous factors such as replication errors and exogenous factors such as chemotherapy.[34] The main types of damage are base or pentose damage, base mismatches, single- and double-strand DNA breaks (SSB and DSB), and covalent cross-linking of DNA.[35] Different chemotherapeutic agents can cause different types of damage, e.g., cisplatin or alkylating agents such as cyclophosphamide can cause cross-linking of DNA and proteins,[36,37] and topoisomerase inhibitors such as irinotecan can cause DNA strand breaks.[38] However, multiple types of damage often coexist. For instance, DNA cross-linking due to platinum-based chemotherapy can lead to base mismatches and DNA breaks.[39] This DNA damage can lead to genomic instability, which may lead to cellular carcinogenesis[40] or, in severe cases, cell death; DSBs are the most lethal type of damage.[41] However, the DDR network can repair this DNA damage, and even if it cannot be repaired, it can avoid the transmission of this error to the offspring cells by blocking the cell cycle and initiating the apoptosis system.[42]

There are six DDR pathways at the molecular level, HR, NHEJ, MMR, BER, NER, and FA, which repair different types of DNA damage and maintain genomic stability under physiological conditions.[43] Alterations in these pathways often accompany cell carcinogenesis,[44] but the pathways that are aberrantly regulated differ among tumor types, which is consistent with the results we obtained (Figures 3A and S1). For example, Aurora A Kinase (*AURKA*) is upregulated in CRC dependent on *TP53* downregulation, which leads to downregulation of many DDR genes, such as Rad3-related protein (*ATR*), replication protein A1 (*RPA1*), X-ray repair cross complementing 1 (*XRCC1*), and *NHEJ1*, thereby mediating downregulation of pathways, such as NER, FA, and NHEJ and enhancing the chemosensitivity of CRC.[45] In contrast, mutation of the speckle-type POZ (pox virus and zinc finger protein) protein (*SPOP*) in PRAD leads to functional upregulation of the HR pathway through upregulation of key factors of the HR pathway, such as RAD51 recombinase (*RAD51*) and checkpoint kinase 1 (*CHK1*), mediating chemoresistance in PRAD.[46]

The HR pathway mainly repairs DSBs. It uses homologous sister chromatids as templates for error-free repair with high fidelity.[47] Among all DDR pathways, the relationship between the HR pathway and chemotherapy has been studied the most. (1) In terms of chemotherapy prediction, various methods for predicting tumor chemotherapy sensitivity, such as the HRD score,[48] HRD elect,[49] and HR score,[50] have been developed thus far for a variety of tumors, such as OV and triple-negative breast cancer (TNBC).[51] However, few previous studies have found a significant relationship between HR and chemotherapy sensitivity in colorectal cancer, which is consistent with the results we obtained (Figure 4). (2) In terms of overcoming chemoresistance, HR-targeted poly (ADP-ribose) polymerase inhibitors (PARPis) have been clinically successful in increasing chemosensitivity in a variety of tumors.[35,52] PARPis are usually used after chemotherapy because the HR pathway is usually activated by chemotherapy-mediated DNA damage.[53] Inhibiting the function of HR after its activation can greatly reduce the ability of cancer cells to repair DNA damage, thus causing a greater killing effect on cancer cells. This is why our experiments on COAD cells with DDR inhibitors alone did not show a significant effect on tumor growth inhibition (Figure 6). However, chemotherapeutic agents in turn can promote the sensitivity of cancer cells to PARPis. For example, paclitaxel can reduce the activity of the HR pathway by inhibiting CDK1 expression, similar to our findings on the NHEJ pathway (Figure 5). Inhibition of HR pathway activity in turn blocks BRCA1 phosphorylation in OV, thereby enhancing PARPi sensitivity.[54] In addition to PARPis, previous studies have developed a number

of new promising radiotherapy sensitizers targeting the HR pathway. For example, ATMis enhance the sensitivity of OV to platinum-based chemotherapy.[47] Hydroxygenkwanin (*HGK*) increases the sensitivity of hepatocellular carcinoma to doxorubicin by downregulating *RAD51*.[55] Interferon-regulatory factor-1 can bind to the promoter of *RAD51* to inhibit its expression in gastric cancer cells to reverse chemoresistance.[56] (3) In terms of chemotherapy combination with immunotherapy, it has been shown that TNBC with HRD has higher levels of lymphocytic infiltration and that immune checkpoint blockade therapy is an option for this subtype despite the presence of chemoresistance (a more specific analysis of immune infiltration is presented later).[57]

The NHEJ pathway also primarily repairs DSBs, and this process is error-prone due to the need to trim DNA ends before ligation and the fact that it does not rely on homologous DNA as a template.[58] However, the NHEJ pathway can ligate almost any type of DSB end.[59] Downregulation of the NHEJ pathway was found to be generally associated with increased chemotherapy sensitivity in CRC,[60] OV,[61] and BRCA.[62] MSI-H status in CRC may mediate hypersensitivity to multiple chemotherapeutic agents via downregulation of the NHEJ pathway. Because we observed downregulation of the NHEJ pathway in the MSI-H group of CRC, which is consistent with some previous studies,[63,64] this process may be related to ultraviolet irradiation resistance-associated gene (*UVRAG*)-shifting mutations caused by MSI-H status, and these mutations mediate downregulation of NHEJ.[65,66] The drugs used in these previous studies (5-FU, oxaliplatin, and platinum analogs such as cisplatin and irinotecan), are associated with DNA damage response pathways and could thus improve sensitivity to chemotherapeutic drugs.[33,67] In other studies, the highly conserved protein heat shock protein 110 (*HSP110*), which is only expressed under physical and environmental stress, was shown to be mutated in MSI-H CRC,[25,68] and this mutation directly prevents its migration to the nucleus to interact with Ku70/80 and other DNA repair proteins involved in the NHEJ pathway[69,70](p11). Either way, MSI-H CRC may have heightened sensitivity to multiple chemotherapeutic drugs related to DNA damage responses due to downregulation of the NHEJ pathway. Likewise, in our findings, the NHEJ pathway in MSI-H OV was downregulated and chemosensitivity was increased compared to that in MSS/MSI-L OV. Previous work suggests that this is due to downregulation of Ku70, which prevents OV cells from recognizing damaged DNA ends via the NHEJ pathway and thus mediates increased chemosensitivity.[71] This mechanism may explain the high sensitivity of MSI-H OV to 5-FU and oxaliplatin. In addition, the downregulation of Ku70 may also promote MSI through Ku70 binding protein 5 (Kub5)-Hera (K-H)/RPRD1B.[72]

The MMR pathway, which repairs mismatched base pairs,[73] is a post-replication repair pathway and is seen as a special form of base excision repair.[74] MMR distinguishes between parent and offspring strands so that only the wrong nucleotides in the daughter strand are excised, while the otherwise normal nucleotides in the parent strand are retained.[75] MMR deficiency (dMMR) is strongly associated with increased chemosensitivity in tumors, such as bladder[76] and lung cancer.[77] Most studies on dMMR and cancer focus on its association with CRC. However, the relationship between MMR and chemotherapy sensitivity in CRC is highly controversial. As mentioned previously, MSI-H status and dMMR are related. Many previous studies have suggested that MSI-H status and dMMR make CRC more sensitive to chemotherapy.[57–61] However, a number of studies have also found that dMMR has little impact on the prognosis of CRC patients after chemotherapy,[14,62,63] or is even associated with chemoresistance.[64–72] This is similar to the paradox we encountered when studying the association of MSI status and MMR with chemotherapy sensitivity (Figures 1 and 4). Sensitivity may be determined by the combined effect of factors, such as intestinal flora[78] and tumor immune infiltration.[78] More specific mechanisms need to be further investigated.

The BER pathway corrects small-scale base damage that does not significantly distort the helix structure of DNA.[79] BER can be activated by various changes in base deamination, oxidation, methylation, deletion (deacylation), etc.[80] BER has been less studied in relation to tumor chemotherapy sensitivity. The main studies on BER in this context show that it is induced by some herbal medicines to increase the sensitivity to chemotherapy or PARPis. For example, berberine can inhibit BER by downregulating XRCC1, thus increasing the sensitivity of BRCA to cisplatin, camptothecin and methyl methanesulfonate.[81] Curcumin can downregulate both short patch (SP) and long patch (LP) BER components in cancer cells to increase the cytotoxicity of PARPi.[82]

The NER pathway differs from the BER pathway in that it does not recognize specific damage but rather the distortion of the DNA double helix structure caused by the damage.[83] NER primarily repairs DNA damage

that is regional to the chromosomal structure. Similar to observations for MMR, the relationship between NER and chemotherapy sensitivity in various tumors is also controversial. For example, in CRC, upregulation of *ERCC1* mediates resistance to oxaliplatin by enhancing NER,[84] but high expression of *XPF* mediates sensitivity to cisplatin by upregulating NER.[85] In non-small cell lung cancer (NSCLC), high expression of *XPF* mediates resistance to cisplatin by upregulating NER,[86] but upregulation of hsa_circ_0001946 increases the sensitivity to cisplatin by enhancing NER.[87] This may be because the related genes are important regulators of both DDR and oxidative stress, and their downregulation would lead to both failure to repair the damage caused by chemotherapy due to DDR deficiency and suppression of the hypoxic damage caused by chemotherapy.[88]

The FA pathway mainly repairs DNA crosslinks[89] and involves NER, MMR, and HR proteins; the leading and lagging strands of DNA are processed sequentially in different ways during the repair process.[90] Thus, the FA pathway is usually activated together with other DDR pathways to reduce the sensitivity to chemotherapy. For example, aberrant activation of glioma-associated oncogene 1 (GLI1) in breast cancer mediates HR through transcriptional upregulation of FANCD2 expression and its focal formation and thus mediates drug resistance together.[91] Downregulation of the FA pathway is also closely related to increased chemosensitivity in head and neck squamous cell carcinoma (HNSCC),[92] bladder cancer,[93] and lung cancer,[94] and is an emerging and popular target for chemosensitizer development. For example, planispine A[95] and *Centipeda minima*[94] can enhance cisplatin sensitivity by inhibiting FA through downregulation of *FANCD2*.

Considering the DDR pathway as a whole, although defects in each DDR pathway are associated with enhanced chemotherapy sensitivity, the most prominent DDR pathways mediating the sensitivity to various DNA-damaging chemotherapeutic agents in different cancers differ somewhat due to their specific targets, similar to the general summary of our results (Figure 4). For example, the sensitivity of CRC to the topoisomerase inhibitors camptothecin,[63] topotecan,[96] and irinotecan,[97] which cause DNA strand breaks, correlates with the NHEJ pathway. These chemotherapeutic agents, who cause DNA damage to induce cell death, may also act as chemosensitizers themselves. For example, gemcitabine may inhibit HR by inhibiting Rad51 to increase the sensitivity of the DNA cross-linking agents' actinomycin C and epirubicin.[98] Our studies also showed that DDR pathways, especially the NHEJ pathway, were significantly downregulated in CRC following treatment with chemotherapeutic drugs, which was consistent with previous studies.[63] Moreover, this finding suggested that in CRC, there might be a positive cycle of increasing sensitivity to chemotherapeutic drugs related to the DNA damage response; that is, MSI-H CRC is associated with downregulation of the NHEJ pathway, which leads to increased sensitivity to chemotherapeutic agents, and the damage caused by chemotherapy in turn leads to further downregulation of the NHEJ pathway, resulting in a cycle of increasing chemosensitivity. This may be due to upregulation of the miR-191 and its inhibition of RCC2 expression,[99,100] which interfere with its interaction with Ku86, which combines with Ku70, thus inhibiting the repair of chemotherapy-induced DNA damage by NHEJ and mediating CRC's high sensitivity to chemotherapy.[101,102] This finding provides new insight into the role of MSI status and DDR pathways in drug-treated CRC and may provide new targets for combating secondary drug resistance in tumors; these findings could be used to improve the effectiveness of chemotherapy in cancer patients.[103] DDR pathways such as NHEJ are potential new targets for combating chemoresistance.[104]

Moreover, these DDR pathways are not necessarily uniformly up- or downregulated in a sample, which is influenced by many factors overall, and the differences in the alterations of each pathway in different tumors we obtained in our study reflect this phenomenon (Figure 5). For instance, high expression of integrins in TNBC upregulates HR and downregulates NHEJ because NHEJ is more error-prone than HR, so overall, the genome becomes relatively more stable and more resistant to chemotherapy.[105] In addition, GSEA DDR pathway enrichment in the two groups of PRAD may be due to DDR pathway mutations.[106] Although this mechanism is different from that in OV and CRC, it nonetheless leads to high sensitivity to various chemotherapeutic drugs in PRAD.[107,108] For UCEC, the relationship between MSI-H status and enhanced drug sensitivity may be due to upregulation of the HR pathway, which is consistent with our GSEA results. Therefore, MSI-H status may affect the DDR pathways differently in different tumor types, and DDR pathways may have different effects on chemosensitivity. However, the relationship between chemotherapy sensitivity and prognosis is not always consistent. In our Cox regression analysis, for some tumors, all DDR pathways were significant protective factors, but for other tumors, the DDR

pathways were risk factors[109,110](Figure 3C). There are two main reasons for this. (1) In the Cox regression analysis of CRC, the protective effect of the NHEJ pathway on patient prognosis does not conflict with the decreased chemotherapy sensitivity associated with NHEJ enrichment. Similar to other DDR pathways, the NHEJ pathway can inhibit tumor progression[111]; this reflects the dual nature of DDR pathways in cancer, which can both inhibit tumor development and promote chemotherapy resistance. (2) If the specific gene downregulated is the one that governs cell-cycle arrest and apoptosis in the DDR network, then downregulation of that gene would, on one hand, allow cells to escape the cell cycle checkpoint and continue to proliferate without undergoing apoptosis, and, on the other hand, decrease the ability of cells to repair chemotherapy-mediated DNA damage. As *PTEN* mediates DSB repair through the HR pathway, its downregulation enhances mitomycin C sensitivity. Moreover, *PTEN* downregulation and persistent activation of *AKT* keep *CHK1* in the cytoplasm, which impairs the G2/M phase checkpoint after radiotherapy, resulting in the inability to block the cell cycle in G2/M phase and thus initiate HR for DSB repair. Therefore, *PTEN* downregulation is associated with a poorer prognosis.[112]

However, in the era of comprehensive personalized therapy, we have also examined the relationship between MSI status and immunotherapy to leverage this biomarker to improve patient prognosis.[113] We found that patients with MSI-H CRC had higher levels of tumor immune infiltration and better response to immune checkpoint inhibitors (ICIs) than those with MSS CRC. Previous studies also revealed that dMMR, which can also be interpreted as MSI-H status, affects the repair of DNA damage caused by chemotherapy, resulting in increased sensitivity to chemotherapy. On the other hand, it also upregulates CXCL10-mediated cGAS/STING and type 1 IFN signaling pathways, thereby recruiting and activating a large number of $CD8^+$ tumor-infiltrating lymphocytes, making MSI-H CRCs more sensitive to ICIs.[57] Thus, patients with CRC of MSI-H have high sensitivity to both ICIs and chemotherapy. Moreover, clinical studies found no significant difference in the effect of the two treatment types on the survival of CRC patients,[114] while ICIs resulted in fewer post-treatment adverse effects than chemotherapy.[115] Further studies found that combining ICIs and chemotherapy is a better option than monotherapy because conventional chemotherapeutic agents can upregulate *CCL5* and *CXCL10* in all CRC types, which can enhance immune infiltration and enhance the response to ICI treatment.[57]

### Limitations of the study

The present study nonetheless has limitations. First, due to a limited sample size, we were not able to study the correlation between DDR pathways and DNA damage-related chemosensitivity in OV, and PRAD and UCEC as the samples were not divided into MSI-H and MSS/MSI-L groups. In addition, the relationship between MSI status, DDR pathway enrichment, and drug resistance in tumors other than CRC was not analyzed in detail, and there was no *in vitro* verification of our findings. Second, the positive cycle of increasing chemosensitivity in MSI-H CRC after drug treatment was not directly verified in molecular mechanism studies and related animal experiments *in vivo*. Third, we did not explore cases of decreased drug sensitivity associated with MSI-H status and subsequent drug treatment, and future research in this area will help us to understand the role of MSI status in drug responses.

## STAR★METHODS

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## SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.107045.

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## AUTHOR CONTRIBUTIONS

Conceptualization: P.L., J.Z., and Q.C.; Formal analysis: T.Y., A.L., Z.Q.. Experiment: S.H.. Resources: T.Y., J.Z., and P.L. Software: T.Y., C.Z., and A.L. Supervision: P.L., J.Z., and Q.C. Visualization: T.Y., C.Z., A.L., and S.H. Writing – Original Draft: T.Y., C.Z., and A.L.. Writing – Review & Editing: All authors. All authors contributed to the article and approved the submitted version.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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145. Meta-Analysis with R.

## STAR★METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| **Chemicals, peptides, and recombinant proteins** | | |
| Irinotecan (CPT-11) | The Central Laboratory of Zhujiang Hospital, Southern Medical University | N/A |
| 5-Fluorouracil (5-FU) | The Central Laboratory of Zhujiang Hospital, Southern Medical University | N/A |
| Oxaliplatin (L-OHP) | The Central Laboratory of Zhujiang Hospital, Southern Medical University | N/A |
| Cisplatin (CDDP) | The Central Laboratory of Zhujiang Hospital, Southern Medical University | N/A |
| Adriamycin (ADM) | The Central Laboratory of Zhujiang Hospital, Southern Medical University | N/A |
| UNC-2170 | The Central Laboratory of Zhujiang Hospital, Southern Medical University | N/A |
| **Critical commercial assays** | | |
| Cell Counting Kit-8 (CCK-8) assay | Dojindo, Kumamoto, Japan | Cat#CK04 |
| **Deposited data** | | |
| Geonomics of Drug Sensitivity in Cancer Database | Yang et al.[32] | https://www.cancerrxgene.org/; RRID:SCR_022717 |
| Cell Model Passport | N/A | https://cellmodelpassports.sanger.ac.uk/ |
| UCSC Xena | Goldman et al.[116] | https://xena.ucsc.edu/; RRID:SCR_018938 |
| National Cancer Institute (NCI) Developmental Therapeutics Program Tumor Repository | N/A | https://dtp.cancer.gov/discovery_development/nci-60/cell_list.htm; RRID:SCR_011403 |
| NCBI Gene Expression Omnibus (GEO) | N/A | https://www.ncbi.nlm.nih.gov/geo/; RRID:SCR_005012 |
| The Cancer Genome Atlas (TCGA) Database | N/A | https://www.cancer.gov/ccg/research/genome-sequencing/tcga; RRID:SCR_003193 |
| Molecular Signatures Database(MSigDB) of the Broad Institute | N/A | https://www.gsea-msigdb.org/gsea/msigdb/; RRID:SCR_016863 |
| Catalog of Somatic Mutations in Cancer (COSMIC) database | N/A | https://cancer.sanger.ac.uk/cosmic; RRID:SCR_002260 |
| The MANTIS score of cancers | Bonneville et al.[117] | N/A |
| **Experimental models: Cell lines** | | |
| RKO | The Central Laboratory of Zhujiang Hospital, Southern Medical University | RRID:CVCL_0504 |
| HCT116 | The Central Laboratory of Zhujiang Hospital, Southern Medical University | RRID:CVCL_1R01 |
| HT29 | The Central Laboratory of Zhujiang Hospital, Southern Medical University | RRID:CVCL_A8EZ |
| SW620 | The Central Laboratory of Zhujiang Hospital, Southern Medical University | RRID:CVCL_0547 |
| **Software and algorithms** | | |
| R version 4.1.2 | https://www.r-project.org/ | RRID:SCR_001905 |
| GraphPad Prism software version 7.0 | https://www.graphpad.com/features | RRID:SCR_002798 |

(*Continued on next page*)

*Continued*

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| pRRophetic version 0.5 | Geeleher et al.[118] | N/A |
| ComplexHeatmap version 2.8.0 | Gu et al.[119] | RRID:SCR_017270 |
| clusterProfiler version 4.0.5 | Yu et al.[120] | RRID:SCR_016884 |
| GSVA version 1.42.0 | N/A | RRID:SCR_021058 |
| limma version 3.48.3 | N/A | RRID:SCR_010943 |
| survival version 3.4–0 | Lin et al.[121] | RRID:SCR_021137 |
| meta version 5.5–0 | | RRID:SCR_019055 |
| metafor version 3.4–0 | Viechtbauer et al.[122] | RRID:SCR_003450 |
| forestplot version 3.1.1 | | N/A |
| Other | | |
| Resource website for codes and supplemental tables | This study | https://github.com/yetaojun/MSI_chemotherapy |

## RESOURCE AVAILABILITY

### Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Peng Luo (luopeng@smu.edu.cn).

### Materials availability

This study did not generate new unique reagents.

### Data and code availability

- The data which are used in this study and publicly available are collected from the GDSC database, Cell Model Passport, UCSC Xena[116] (https://xenabrowser.net), National Cancer Institute (NCI) Developmental Therapeutics Program Tumor Repository (https://dtp.cancer.gov/discovery_development/nci-60/cell_list.htm), NCBI Gene Expression Omnibus (GEO), Molecular Signatures Database (MSigDB) of the Broad Institute and Catalog of Somatic Mutations in Cancer (COSMIC) database. The MANTIS score[117] was used to categorize samples as MSI-H or MSS/MSI-L and for MSI status correlation analysis of the pancancer samples.
- Codes used to obtain the expression data from the sequencing data (fastq), result figures, and supplementary tables are available at https://github.com/yetaojun/MSI_chemotherapy.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

## EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

### Cell line

The human COAD cell lines RKO (RRID:CVCL_0504), HCT116 (RRID:CVCL_1R01), HT29 (RRID:CVCL_A8EZ) and SW620 (RRID:CVCL_0547) were provided by the Central Laboratory of Zhujiang Hospital, Southern Medical University. All four cell lines were cultured at 37°C in a 5% CO2 incubator. SW620 and HCT116 cells were cultured in RPMI-1640 medium (KeyGEN BioTECH, Jiangsu, China) supplemented with 10% fetal bovine serum (FBS, Procell Life Science & Technology Co., Ltd., Wuhan, China) and penicillin/streptomycin antibiotics. RKO and HT29 cells were cultured in DMEM (KeyGEN BioTECH, Jiangsu, China) supplemented with 10% fetal bovine serum and penicillin/streptomycin antibiotics.

## METHOD DETAILS

### Data sources

To study the sensitivity of pancancer cells with different MSI statuses to different chemotherapeutic drugs, we downloaded phenotype and drug sensitivity data for pancancer cell lines treated with different drugs from the GDSC database; data included the MSI status of each cell line and ln(IC50) values

(version: 25Feb20). The IC50 indicates the half-maximal inhibitory concentration of a given drug. Cell lines were divided into two groups based on MSI status: MSI-H and MSS/MSI-L. COAD, rectum adenocarcinoma (READ), colorectal carcinoma, and caecum adenocarcinoma were combined into the CRC category for the purpose of this study. Twenty-three commonly used chemotherapeutic drugs were analyzed in the dataset (Table S1), and the expression data (log[TPM +1]) collected from Cell Model Passport were used for subsequent analysis; TPM stands for transcript per million in this context.

We also downloaded TCGA pancancer RNA-seq expression data in which log2(FPKM-UQ + 1) was transformed into log2(TPM +1), and basic phenotypic data and survival data (TCGA program cohort: GDC pancancer [PANCAN]) from UCSC Xena[116] (https://xenabrowser.net); FPKM-UQ stands for fragments per kilobase of transcript per million mapped reads upper quartile in this context. After genes were annotated with Xena's annotation file (gencode.v22.annotation.gene), expression and phenotypic data for colon cancer (COAD) and rectal cancer (READ) from the GDC TCGA database were combined to form the TCGA-CRC dataset for analysis. The MANTIS score,[117] which is used to predict the MSI status of cancer, was used to categorize samples as MSI-H or MSS/MSI-L and for MSI status correlation analysis of the pancancer samples. Samples with MANTIS scores $\geq$0.4 were defined as MSI-H, and samples with MANTIS scores <0.4 were considered MSS/MSI-L.

Cell line data from the NCI-60 cancer cell line panel were obtained from the National Cancer Institute (NCI) Developmental Therapeutics Program Tumor Repository (https://dtp.cancer.gov/discovery_development/nci-60/cell_list.htm). We downloaded the cell line data (GEO: GSE116436[123]) before and after drug treatment from the NCBI Gene Expression Omnibus (GEO) database for further analysis. The gene symbol annotation of Robust Multiarray Average (RMA) standardized chip data, which records the gene expression of NCI-60 cell lines after 0,2,6, and 24 h of treatment with 15 anticancer drugs (only 6 of which were used in our analysis), was based on the GPL571 platform. The cell lines from GEO: GSE116436 with a dosage of 0 nM drug were regarded as baseline/control groups, and those treated with 10-150000nM were regarded as treatment groups. IC50 data in NCI-60 (https://wiki.nci.nih.gov/display/NCIDTPdata/NCI-60+Growth+Inhibition+Data) were used to analyze differences in MSI status (MSI status information for cell lines was obtained from the GDSC database), and 28 chemotherapeutic drugs were selected for our analysis.

In addition, we downloaded 25 CRC RNA-seq datasets that contain MSI status annotation information and has both MSI-H and MSS/MSI-L CRC cell lines from the GEO database (GEO: GSE103340,[124] GEO: GSE11543,[125] GEO: GSE13067, GEO: GSE13294, GEO: GSE143985,[126] GEO: GSE146889, GEO: GSE156915,[127] GEO: GSE18088,[128] GEO: GSE185055,[129] GEO: GSE2138,[130] GEO: GSE24550, GEO: GSE24551,[131] GEO: GSE24795,[132] GEO: GSE25071,[133] GEO: GSE26682, GEO: GSE27544,[134] GEO: GSE29638,[135] GEO: GSE30378, GEO: GSE35566,[136] GEO: GSE35896,[137] GEO: GSE39084,[138] GEO: GSE41258,[139] GEO: GSE4459,[140] GEO: GSE75315,[141] GEO: GSE92921[142]). They were used to calculate the ssGSEA score, which was used to further divide samples into two groups for the assessment of chemosensitivity. These data will eventually be used in a meta-analysis for analysis of variance and correlation analysis.

Among all the samples, only "primary tumor" and "metastatic" samples were used for analysis.

### Prediction of clinical chemotherapeutic response

We used the R package pRRophetic[118] to predict chemotherapeutic drug sensitivity for the MSI-H and MSS/MSI-L groups in the TCGA pancancer dataset; the predicted log(IC50) value was calculated by ridge regression analysis and was positively correlated with drug sensitivity. The R package ComplexHeatmap[119] was used to visualize the variance analysis results of pancancer multidrug sensitivity data.

### GSEA and ssGSEA

GSEA was performed by the R package clusterProfiler.[120] The DDR gene set used for GSEA analysis and ssGSEA score calculation was from the Molecular Signatures Database[143] (MSigDB) of the Broad Institute (Table S2), and p values <0.05 were considered to indicate significance. ssGSEA scores were calculated by the R package GSVA. All RNA-seq data used to calculate ssGSEA scores were standardized by log2(TPM +1). A Gaussian distribution was used for nonparametric estimation of the expression levels between samples using the cumulative distribution function in ssGSEA. The R package limma was used to search for differentially expressed genes and pathways.

### Survival analysis

We selected the cut-off values based on the best results of the Cox regression analysis, in which the ssGSEA scores of each DDR pathway obtained by ssGSEA for each tumor were used as the continuous variable.[144] The ssGSEA scores were converted into binary variables of ("high" and "low"), and then univariate Cox regression analysis was performed using the R package survival.[121] Log10(HR) was used to represent the relationship between DDR pathways and prognosis in pancancer; log10(HR) > 0 denotes risk factors, and log10(HR) < 0 denotes protective factors.

### Cell culture and reagents

The human COAD cell lines RKO, HCT116, HT29 and SW620 were provided by the Central Laboratory of Zhujiang Hospital, Southern Medical University. All four cell lines were cultured at 37°C in a 5% CO2 incubator. SW620 and HCT116 cells were cultured in RPMI-1640 medium (KeyGEN BioTECH, Jiangsu, China) supplemented with 10% fetal bovine serum (FBS, Procell Life Science & Technology Co., Ltd., Wuhan, China) and penicillin/streptomycin antibiotics. RKO and HT29 cells were cultured in DMEM (KeyGEN BioTECH, Jiangsu, China) supplemented with 10% fetal bovine serum and penicillin/streptomycin antibiotics. The Catalog of Somatic Mutations in Cancer (COSMIC) database (https://cancer.sanger.ac.uk/cosmic) was used to determine the MSI status of the four COAD cell lines. RKO and HCT116 cells showed MSI-H status, while HT29 and SW620 cells had MSS status. 53BP1 is a methyllysine (Kme)-binding protein that plays a key role in the DDR pathway. UNC-2170 (HY-115531, MCE, New Jersey, USA) is a functional and active fragment ligand of 53BP1 that can block the DDR by selectively binding to 53BP1.

### Cell counting Kit-8 (CCK-8) assay and IC50 determination

A CCK-8 assay was used to determine the IC50 values of different chemotherapy drugs, including irinotecan (CPT-11), 5-fluorouracil (5-FU), oxaliplatin (L-OHP), cisplatin (CDDP), adriamycin (ADM) and UNC-2170, in the four COAD cell lines. RKO, HCT116, HT29 and SW620 cells in the logarithmic growth phase were plated in 96-well cell culture plates (10,000 cells in 100 μL per well). After 12 h, concentration gradients (0, 1, 2.5, 5, 10, 20, 40, 80, 160 μg/mL) of irinotecan, 5-FU, oxaliplatin, cisplatin, doxorubicin and UNC-2170 were added to the cells. After 24 h of drug treatment (or 48 h of 5-FU treatment), 10 μL CCK-8 solution (Dojindo, Kumamoto, Japan) was added to each well. The plates were incubated in a 37°C cell incubator for 2 h and then transferred to a microplate reader (BioTek SYNERGY H1, USA) to measure absorbance (OD) at 450 nm. There were five replicates for each drug concentration, and the average OD was used to calculate the cell survival rate. The cell survival rate was calculated using the following formula: cell survival rate = [OD (drug) - OD (blank)]/[OD (control) - OD (blank)] * 100%. A dose-response curve was generated by calculating the cell inhibition rate at different drug concentrations, and IC50 values for each drug were obtained. To determine the effect of combining a DDR inhibitor with chemotherapeutic drugs on the chemotherapeutic drug IC50, we added the same dose of UNC-2170 to wells containing different concentrations of chemotherapeutic drugs and used the CCK8 assay to verify changes in the chemotherapeutic drug IC50. The IC50 values were calculated by GraphPad Prism (version 7.0) software, and the IC50 values for different groups were compared by an unpaired t test.

## QUANTIFICATION AND STATISTICAL ANALYSIS

The Wilcoxon rank-sum test (Mann–Whitney U test) was used to analyze differences between two groups of independent samples that did not satisfy the normal distribution condition. Spearman rank correlation was used to detect the correlation between ssGSEA scores in the DDR pathway and ln(IC50) values of chemotherapeutic drugs in CRC. Meta-analysis was performed and forest plots were generated with the R packages "meta",[145] "metafor"[122] and "forestplot" respectively. All statistical analyses were two-tailed. p values <0.05 were considered to indicate statistical significance. Except for the IC50 value calculation, all statistical analyses and visualization steps were conducted in R software (RRID:SCR_001905, version 4.1.2).