EWSR1-FLI1-Associated Cohesin and DNA Damage Response Dependencies Identified by Pan-Cancer CRISPR Profiling in Ewing Sarcoma

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Significance

Ewing sarcoma (EwS) is a highly aggressive cancer caused by EWSR1 fusion oncoproteins and currently lacks widely effective targeted treatments. Although specific genetic vulnerabilities have been identified, there is no consistent method to rank EwS weaknesses using uniform functional screening data or to compare them with other cancers. Previous research indicates that EwS depends on fusion-driven transcriptional programs, chromatin structure, and responses to replication stress, but the degree of their specificity across different cancer types remains uncertain. We hypothesize that EwS contains measurable, subtype-specific CRISPR dependencies that can be identified systematically through cross-cancer comparisons and careful filtering of universally essential genes.

Innovation

This study creates the first functional dependency atlas for EwS using harmonized DepMap CRISPR Chronos data. By integrating pan-cancer comparisons, sarcoma-specific analyses, and empirical pan-essential gene filtering, we offer a reproducible framework for ranking EwS vulnerabilities based on statistical significance, effect size, and biological coherence. The approach goes beyond single-gene observations to identify coordinated dependency modules, providing a mechanistic map of EWSR1-FLI1–imposed cellular requirements and a solid foundation for therapeutic prioritization.

Approach

DepMap CRISPR Chronos gene effect profiles were combined with model metadata to define a sarcoma cohort and assign EwS subtype labels. EwS-specific dependencies were identified by comparing gene effect distributions in EwS cell lines (n = 24) versus all non-sarcoma cancer cell lines using Wilcoxon rank-sum testing with Benjamini–Hochberg false discovery rate (FDR) correction. Empirical pan-essential genes were excluded based on overall dependency patterns across the entire CRISPR matrix. Genes were ranked based on three criteria: (1) statistical significance (FDR < 0.05), (2) subtype specificity (Δ = mean EwS – mean rest ≤ –0.25), and (3) level of dependency (mean EwS gene effect ≤ –0.5). Replication was evaluated through within-sarcoma comparisons and leave-one-out resampling.

Results

After essential pan-filtering, the pan-cancer comparison identified 32 EwS-selective dependencies that meet predefined thresholds. The top vulnerabilities included FLI1 (Δ = –0.699, FDR < 0.0001), TRIM8 (Δ = –0.515), and cohesin regulators STAG1 (Δ = –0.445) and NIPBL. Additional actionable dependencies involved CDK4, IGF1R, and IRS2, along with DNA damage response components RNF168 and RAD1. Thirteen dependencies were confirmed in sarcoma-only analyses, and nine core genes (FLI1, TRIM8, STAG1, NIPBL, CDK4, IGF1R, IRS2, RNF168, RAD1) remained significant across all leave-one-out iterations. Integrated pathway analysis uncovered coordinated modules involving cohesin-mediated chromatin architecture, DNA damage response, and proliferative signaling.

Discussion

This study creates a reproducible framework for identifying EwS-specific CRISPR vulnerabilities and shows that EwS has a shared dependency structure driven by EWSR1-FLI1. The ranked vulnerability map emphasizes biologically relevant, drug-related targets and offers a systematic foundation for studying mechanisms and developing combination therapies. This atlas also introduces a scalable method for expanding dependency mapping to genomically stratified sarcoma groups and future translational research.

References

1. Li M, Chen CW. Epigenetic and Transcriptional Signaling in Ewing Sarcoma—Disease Etiology and Therapeutic Opportunities. Biomedicines. 2022 Jun 5;10(6):1325. doi:10.3390/biomedicines10061325 PubMed PMID: 35740349; PubMed Central PMCID: PMC9219675.

Gene	Selectivity (EwS vs non-sarcoma), Δ	Adjusted P (FDR), EwS vs non-sarcoma	Mean dependency in EwS	Mean dependency in non-sarcoma	Selectivity (EwS vs other sarcomas), Δ	Adjusted P (FDR), EwS vs other sarcomas	Mean dependency in other sarcomas	Replicates within sarcoma (FDR<0.05)
FLI1	-0.699	<0.0001	-0.780	-0.081	-0.772	0.0001	-0.008	Yes
TRIM8	-0.515	<0.0001	-0.579	-0.064	-0.498	0.0002	-0.081	Yes
STAG1	-0.445	<0.0001	-0.555	-0.109	-0.409	0.0023	-0.146	Yes
GLRX3	-0.428	<0.0001	-0.783	-0.355	-0.296	0.0521	-0.487	No
CDK4	-0.602	<0.0001	-1.127	-0.525	-0.515	0.0193	-0.612	Yes
RNF168	-0.363	<0.0001	-0.791	-0.428	-0.318	0.0129	-0.473	Yes
RAD1	-0.278	<0.0001	-0.746	-0.468	-0.240	0.0387	-0.506	Yes
NIPBL	-0.282	<0.0001	-0.547	-0.265	-0.331	0.0007	-0.217	Yes
ZEB2	-0.252	<0.0001	-0.516	-0.264	-0.178	0.1310	-0.338	No
IRS2	-0.394	<0.0001	-0.705	-0.311	-0.404	0.0129	-0.302	Yes
DDX19A	-0.318	<0.0001	-0.603	-0.285	-0.273	0.0981	-0.331	No
IGF1R	-0.318	<0.0001	-0.574	-0.256	-0.299	0.0296	-0.275	Yes
FURIN	-0.329	0.0001	-0.506	-0.176	-0.284	0.0530	-0.222	No
CDIN1	-0.257	0.0001	-0.515	-0.258	-0.241	0.0296	-0.273	Yes
CHMP4B	-0.495	0.0003	-1.423	-0.928	-0.127	0.5390	-1.296	No
ARHGAP29	-0.320	0.0004	-0.631	-0.311	-0.152	0.3750	-0.479	No
FANCD2	-0.270	0.0007	-0.705	-0.435	-0.251	0.0953	-0.454	No
HNRNPDL	-0.285	0.0008	-0.546	-0.261	-0.237	0.1310	-0.309	No
BUB1B	-0.294	0.0009	-1.006	-0.712	-0.195	0.3280	-0.811	No
BRIP1	-0.256	0.0010	-0.715	-0.459	-0.193	0.1560	-0.521	No
COPS7A	-0.285	0.0011	-0.659	-0.374	-0.300	0.0530	-0.359	No
RINT1	-0.286	0.0014	-0.706	-0.420	-0.141	0.3280	-0.566	No
NARS1	-0.259	0.0016	-0.836	-0.577	-0.251	0.1290	-0.585	No
CDK1	-0.288	0.0022	-1.388	-1.100	-0.162	0.3750	-1.226	No
ARPC1B	-0.320	0.0022	-0.703	-0.383	-0.319	0.0193	-0.384	Yes

Table 1. EwS-selective genetic dependencies identified by pan-cancer CRISPR fitness profiling and within-sarcoma replication.

Chronos gene effect scores were derived from DepMap genome-scale CRISPR knockout screens. Selectivity was defined as the difference in mean Chronos score between EwS and the comparator cohort, with more negative values indicating greater dependency in EwS. EwS cell lines (n=24) were compared first against non-sarcoma cell lines (n=1,032) and then against other sarcoma subtypes (n=30). Genes meeting predefined thresholds (FDR < 0.05, effect size ≤ −0.25, mean EwS Chronos ≤ −0.5) in the pan-cancer comparison were retained. Within-sarcoma replication was defined as FDR < 0.05 in the EwS versus other sarcoma comparison. Δ, difference in mean Chronos score; FDR, Benjamini-Hochberg adjusted p-value.

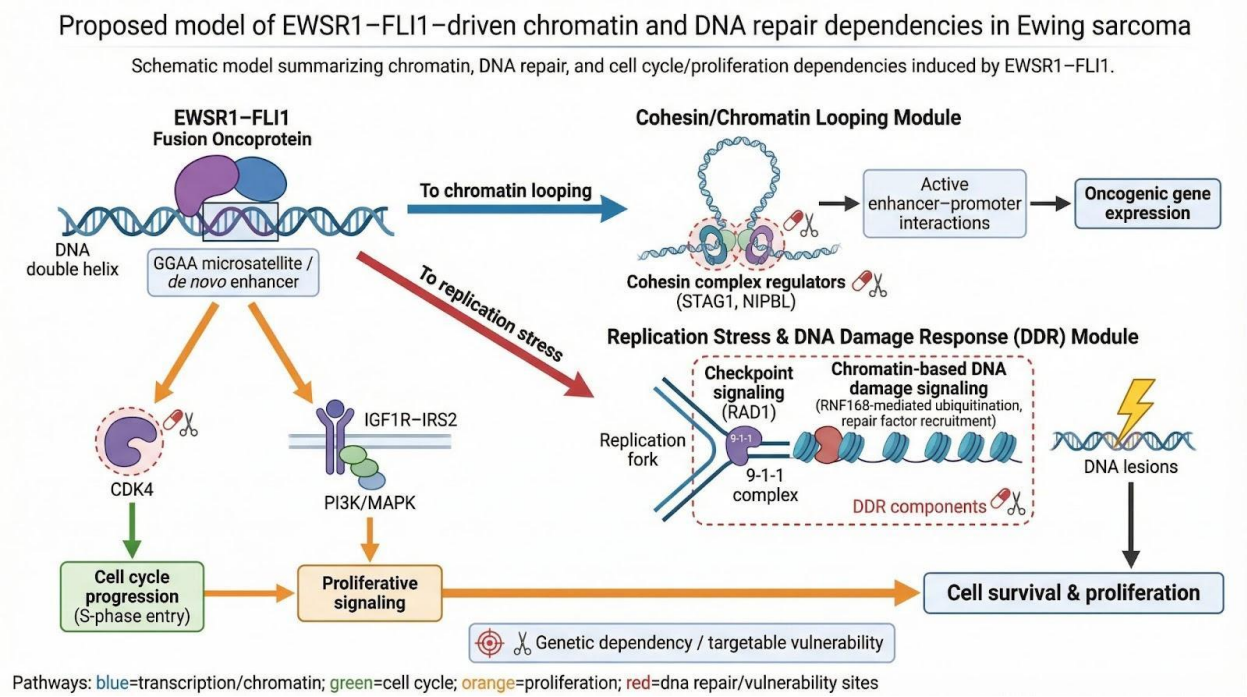

Figure 1. Proposed model of EWSR1-FLI1-driven chromatin and DNA repair dependencies in Ewing sarcoma
EWSR1-FLI1 binds GGAA microsatellites to establish de novo enhancer activity and oncogenic transcription, which requires cohesin-mediated chromatin looping regulated by STAG1 and NIPBL. Fusion-driven hypertranscription induces replication stress and DNA lesions, creating dependence on DNA damage response pathways including RAD1-mediated checkpoint signaling and RNF168-dependent chromatin-based repair factor recruitment. Downstream proliferation is supported by CDK4-driven cell-cycle progression and IGF1R-IRS2 mitogenic signaling. These convergent dependencies define a dual requirement for chromatin architectural maintenance and genomic stress buffering in Ewing sarcoma and highlight cohesin regulators, DNA damage response components, and CDK4 as genetically defined vulnerabilities.