Interferon signaling is associated with EGFR inhibitor resistance in chordoma

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Abstract #4865

ABSTRACT

- Target discovery studies have identified EGFR inhibition as a promising therapeutic strategy in chordoma, motivating Phase II clinical trials with afatinib (NCT03083678) and cetuximab (NCT05041127).
- EGFR is not mutated in chordoma, leaving the mechanisms associated with EGFR inhibitor (EGFRi) sensitivity and resistance unclear.
- We profiled a panel of 14 chordoma cell lines to categorize their sensitivity to afatinib, a second-generation covalent EGFRi with potency against the wild-type receptor, and looked for associations between response and gene expression to identify features associated with sensitivity or resistance.
- Proliferation assays revealed striking differential sensitivity of chordoma cell lines to afatinib: sensitive cell lines have Absolute EC50 values in the range of ~5-30 nM, whereas resistant cell lines have Absolute EC50 values greater than 1 μM.
- This differential sensitivity is mirrored in xenograft models, with tumor growth inhibition ranging from 0% in resistant models to ~100% in sensitive models. In matched cell line/xenograft models, afatinib pharmacotypes are conserved.
- Analysis of differentially-expressed genes in resistant versus sensitive cell lines reveals an association of interferon (IFN) signaling with afatinib resistance.
- Modulation of IFN signaling does not reverse sensitivity or resistance to afatinib, suggesting that it does not drive the resistant phenotype per se.
- Studies are ongoing to understand the relationship between EGFR and IFN signaling, which may be related to the activity or expression level of EGFR.

BACKGROUND

- Chordoma is a rare bone cancer of the skull base and spine that arises from remnants of the embryonic notochord.
- Disease incidence is 1 per million, with median survival from diagnosis of 8 years.
- Standard care is maximal surgical resection +/- radiation, which cures ~30% of patients.
- Chordoma is a relentless disease with a high rate of recurrence; most patients experience serial recurrences with progressively shorter disease-free intervals.
- There are no approved systemic therapies for the treatment of chordoma.
- Target discovery studies have nominated EGFR as an attractive therapeutic target in chordoma, but the molecular features associated with sensitivity and resistance to EGFR inhibition in chordoma remain unclear.

Figure 1. Chordoma is a bone cancer that forms in the skull base or spine.

RESULTS

Proliferation assay optimization

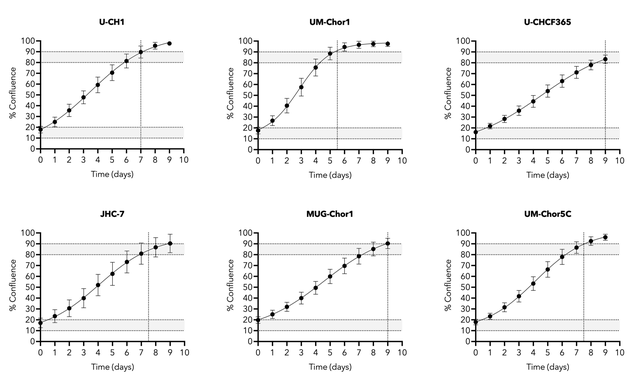

Figure 2. Optimization of proliferation assay parameters using Incucyte Live-Cell Analysis. Representative data is shown for six chordoma cell lines. Accurate interpretation of drug activity in proliferation assays requires optimization of seeding densities (10-20% confluence) and assay durations (endpoint 80-90% confluence). This is particularly important for drugs that may exert their effects through cytostatic mechanisms; untreated control cells must undergo 2-3 population doublings for an accurate determination of EC50.

Chordoma cell lines exhibit differential sensitivities to afatinib treatment

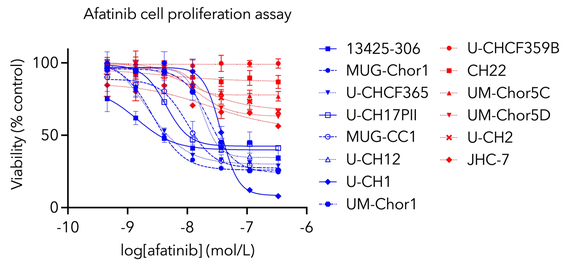

Figure 3. CellTiter-Glo proliferation assays of chordoma cell lines treated with afatinib reveal two distinct groups in terms of drug response. Sensitive cell lines are colored blue, whereas resistant cell lines are colored red. Assay endpoint for each cell line was time to 80-90% confluence for untreated cells. Experiments were repeated for each cell line at least once (N = 2-3 biological replicates) to ensure reproducibility; representative curves are shown.

Cell line	Abs EC50 (nM)	Response
13425-306	3	Sensitive
U-CHCF365	5	Sensitive
MUG-Chor1	8	Sensitive
U-CH17PII	11	Sensitive
MUG-CC1	14	Sensitive
U-CH12	27	Sensitive
UM-Chor1	23	Sensitive
U-CH1	33	Sensitive
U-CHCF359B	> 1000	Resistant
CH22	> 1000	Resistant
UM-Chor5C	> 1000	Resistant
UM-Chor5D	> 1000	Resistant
U-CH2	> 1000	Resistant
JHC-7	> 1000	Resistant

Table 1. Absolute EC50 values plotted for chordoma cell lines. This parameter is defined as the afatinib concentration that inhibits proliferation by 50% compared to control cells.

Differential gene expression analysis reveals an association between interferon signaling and afatinib resistance in chordoma

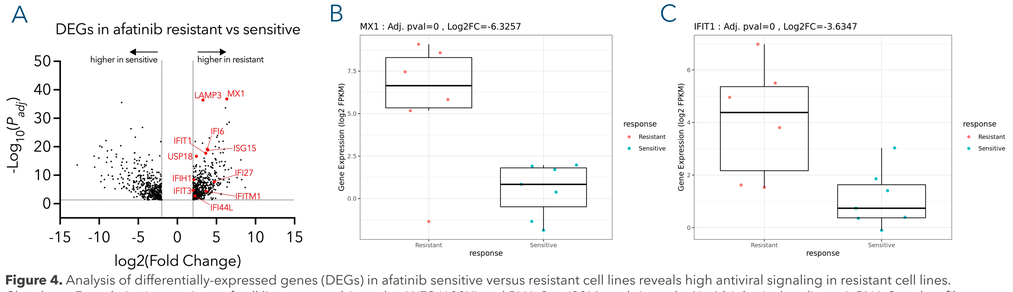

Figure 4. Analysis of differentially-expressed genes (DEGs) in afatinib sensitive versus resistant cell lines reveals high antiviral signaling in resistant cell lines. Chordoma Foundation's repository of cell lines were subjected to WES (100X) and RNA-Seq (80M reads/sample, N=4 biological replicates). RNA-Seq data files generated by the RSEM pipeline were used to perform differential expression analysis. RSEM gene-based count data were used to create a DESeq2 object and the full DESeq2 pipeline was run to generate an expression top table with FDR p-value adjustment. **(A)** Volcano plot of DEGs in afatinib resistant vs sensitive cell lines reveals higher expression of interferon-stimulated genes (ISGs) in resistant cell lines. Box plots for representative ISGs MX1 **(B)** and IFIT1 **(C)** in sensitive vs resistant cell lines. Tumor cell-intrinsic interferon (IFN) signaling has recently been reported for many cancer cell lines (H. Liu et al., *Nat Med*, 2019) and appears to be unusually high in chordoma cells (T. Sharifnia et al., *Nat Commun*, 2023). IFN signaling has been associated with adaptive resistance to EGFRi in lung cancer (K. Gong, *Nat Cancer*, 2020), underscoring its potential importance to EGFR inhibitor response in chordoma.

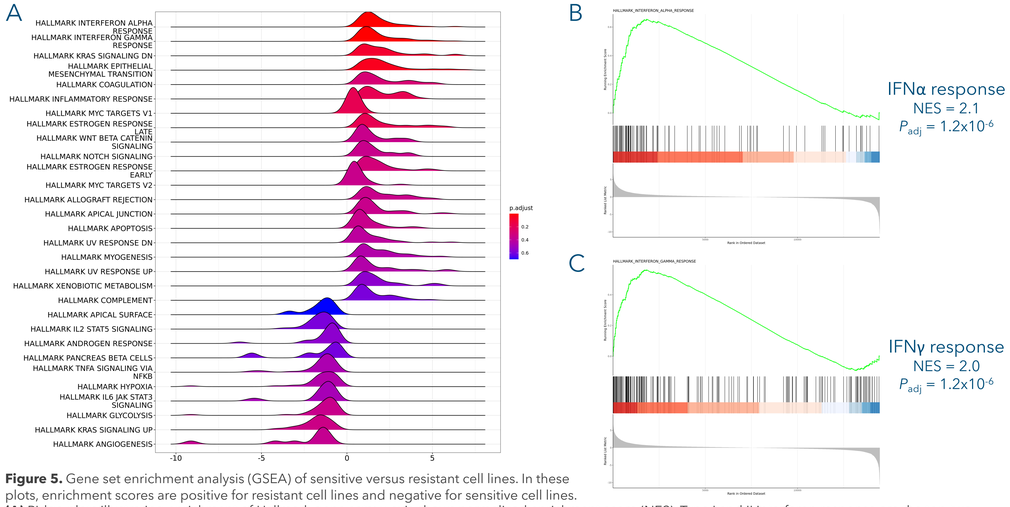

Figure 5. Gene set enrichment analysis (GSEA) of sensitive versus resistant cell lines. In these plots, enrichment scores are positive for resistant cell lines and negative for sensitive cell lines. **(A)** Ridge plots illustrating enrichment of Hallmark gene sets; x-axis shows normalized enrichment score (NES). Type I and II interferon response are the top two gene sets enriched in resistant cell lines. Individual GSEA plots for **(B)** Hallmark IFNα response and **(C)** Hallmark IFNγ response. The Hallmark IFNα response gene set was recently found to be enriched in a molecularly-defined subset of chordoma patient tumors (J. Bai et al., *Clin Cancer Res*, 2022).

Afatinib responses are conserved in chordoma xenograft models

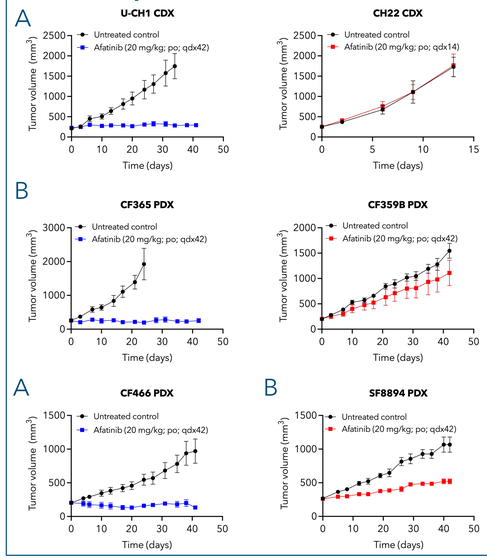

Figure 6. Afatinib responses are conserved in matched **(A)** Cell line-derived xenograft (CDX) and **(B)** Patient-derived xenograft (PDX) models (N = 5-7 mice/arm). (A) U-CH1 and CH22 are sensitive and resistant CDXs, respectively. (B) CF365 and CF359B are sensitive and resistant PDXs, respectively, corresponding to PDX-derived cell lines U-CHCF365 and U-CHCF359B. Refer to Figure 3 for *in vitro* data corresponding to matched cell lines. Cetuximab yields similar results in chordoma xenograft models (data not shown).

Figure 7. Further validation in chordoma PDX models. Afatinib sensitivity of **(A)** interferon "low" PDX model CF466 and **(B)** interferon "high" PDX model SF8894. N = 6-7 mice/arm.

IFN signaling does not directly drive afatinib resistance

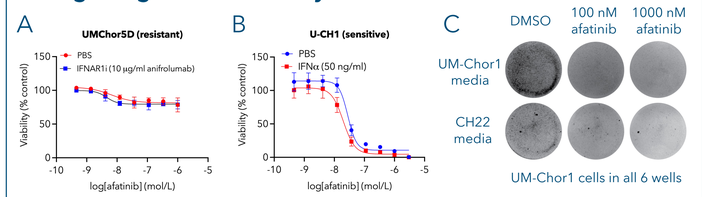

Figure 8. Modulating type I IFN signaling by inhibiting IFNAR1 in IFN-high resistant cells **(A)** or stimulating sensitive cells with IFNα **(B)** has no effect on afatinib response in chordoma cell lines. **(C)** Culturing sensitive UM-Chor1 cells for 7 days in IFN-high, afatinib-resistant CH22-conditioned media has no effect on afatinib response, however CH22-conditioned media alone inhibits the proliferation of UM-Chor1 cells.

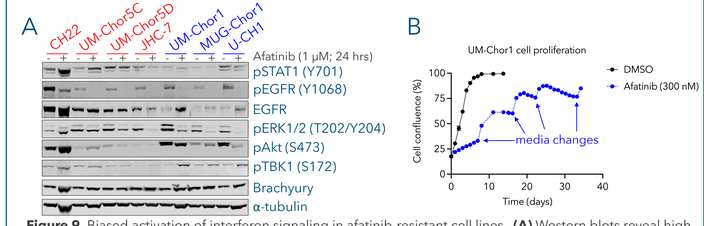

Figure 9. Biased activation of interferon signaling in afatinib-resistant cell lines. **(A)** Western blots reveal high IFN-induced phospho-STAT1 and elevated EGFR levels in resistant cell lines (colored red). Afatinib suppresses phospho-ERK1/2 in sensitive cell lines (colored blue) after 24 hrs of treatment. **(B)** Proliferation of UM-Chor1 cells treated with or without afatinib was monitored over time using Incucyte imaging. In afatinib-treated cells, sudden proliferative bursts were observed during media changes, suggesting the removal of a growth-inhibitory component (possibly IFN) that accumulates during long-term culture.

KEY FINDINGS

- Our data reveal a striking differential sensitivity of chordoma cell lines to afatinib, which was mirrored *in vivo* in xenograft models.
- Combining *in vitro* afatinib sensitivity data with gene expression analysis identified an enrichment of interferon (IFN) signaling in resistant cell lines.
- Despite its association with EGFRi resistance in lung cancer, IFN signaling does not appear to promote resistance in chordoma cell lines. Instead, it may serve as a biomarker to guide patient selection for EGFR-targeted therapy in chordoma.
- Studies are ongoing to understand the nature of the relationship between IFN signaling and EGFRi resistance.
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