Characterization of Two Novel Oncogenic FGFR2 Fusions Sensitive to the FGFR Selective Inhibitor Debio 1347 in Cholangiocarcinoma

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Abstract #689

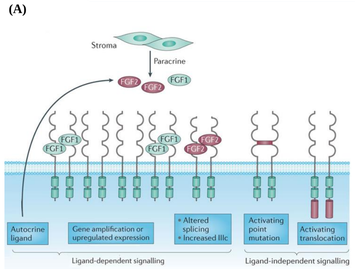
Summary

Dysregulation of the fibroblast growth factor receptor (FGFR) signaling pathway due to receptor overexpression, gene amplification, point mutations or fusions/chromosomal translocations is associated with cancer development and progression. FGFR gene fusions have recently been discovered in several cancers including bladder cancer, glioblastoma, lung squamous cell cancer and cholangiocarcinoma. Predominant gene fusion such as FGFR3-TACC3 (with different genomic breakpoints in the two genes) has been identified in bladder cancer whereas in cancers with a much more heterogeneously altered genomic landscape, e.g. cholangiocarcinoma, at least ten FGFR2 fusions each with a different fusion partner have been identified.

We herein report for the first time the discovery of two novel FGFR fusions in intra-hepatic cholangiocarcinoma patient samples. The new fusions contain an intact FGFR2 tyrosine kinase domain fused to a gene partner encoding at least one oligomerization domain, suggesting a mechanism of constitutive kinase activation through ligand-independent oligomerization similar to that described for other FGFR fusions.[1]

The selective FGFR inhibitor Debio 1347/CH5183284 significantly reduced cell proliferation in vitro and in vivo in models harboring either fusion suggesting that cholangiocarcinoma patients harboring these fusions could benefit from targeted FGFR inhibitors such as Debio 1347, currently investigated in a Phase I trial.

Background



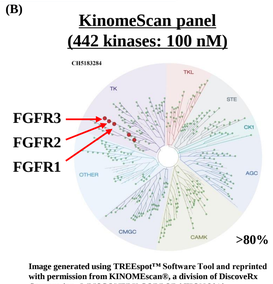


Figure 1. (A) Mechanisms of FGFR activation (adapted from Knowles et al., 2015[2]). **(B)** TREEspot™ Interaction Map for Debio 1347 tested at 100 nM (Kinome Scan panel, DiscoveRx[3]).

Methods

Identification of two novel FGFR2 fusions

Total RNA was extracted from two macro-dissected 10 µm thick sections of formalin-fixed, paraffin-embedded tissue from human cholangiocarcinoma biopsies using the Roche High Pure FFPET RNA Isolation Kit according to the manufacturer's instructions. DNA libraries were prepared starting from 500 ng input RNA using an ArcherDx (now Enzymatics Inc., Beverly, MA) FGFR Fusion Detection kit for IlluminaR according to the manufacturer's instructions. Bar-coded libraries were pooled at equimolar concentrations, loaded on an IlluminaR MiSeq desktop sequencer at 10 pM each and sequenced using the Illumina MiSeq v2 (300 cycles) reagent kit (MS-102-2002, Illumina Inc., San Diego, CA) and Nextera workflow chemistry. Presence of fusions was confirmed by traditional RT-PCR and validated by Sanger sequencing. CCDC147 refers to "coiled-coil domain containing 147" and VCL refers to "vinculin".

Establishment of stably expressing cell pools (Trenzyme GmbH, Konstanz, Germany)

Briefly, Rat2 parental cell line was transfected with 2µg of each plasmid DNA (pExoIN2-FGFR2_CCDC147 and pExoIN2-FGFR2_VCL) by electroporation (LONZA Nucleofector II Device/program [X-005], Solution R). At 24hours post-transfection, cells were subjected to 1.5µg/mL puromycin to derive stable expressor cell pools.

Soft-agar colony formation assay (Trenzyme GmbH, Konstanz, Germany)

Single cell suspensions were prepared using Accutase and diluted to ensure that appropriate cell numbers were seeded in 6 well dishes in 0.4% soft-agar top layer without selection antibiotic. Dishes were incubated in a 5% CO2 environment at 37° C for colony formation. After 21 days of incubation, colonies were fixed using 10% (v/v) acetic acid and 10% (v/v) methanol in H_2O and stained with crystal violet (0.01% (w/v) in H_2O).

Cell proliferation assay using FACS (Trenzyme GmbH, Konstanz, Germany)

24 hours after cell seeding (25'000 cells/well, either parental cells or cells expressing FGFR2-VCL or FGFR2-CCDC147 fusion proteins), Debio 1347 was added and the cultures were incubated for another 72 hours. At the end of the incubation period, cells were counted by FACS. IC_{50} values were calculated by Graphpad Prism 6 using sigmoidal response (variable slope) curve fit.

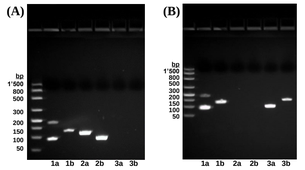
Mouse xenograft model (Proqinase GmbH, Freiburg, Germany)

Studies were approved by the local Ethics Committee for Animal Experimentation. Briefly, 1.10^6 or 5.10^6 tumor cells in 100µL PBS were injected subcutaneously (sc) into the right flank of female NMRI nude mice. Primary tumor sizes were determined twice weekly by calipering (manual caliper, OMC Fontana). Tumor sizes were calculated according to the formula W2xL/2 (L= length and W= the perpendicular width of the tumor, L > W). Debio 1347 was orally administered once a day for 14 consecutive days (30 or 60 mg/kg QD) in mice with established tumors.

Results

Identification of FGFR2-VCL and FGFR2-CCDC147, two novel FGFR2 fusions in cholangiocarcinoma patients

Two novel FGFR2 fusions with different fusion partners were identified in two biopsy samples from cholangiocarcinoma patients.



1a: FGFR2 wild-type (89 bp)
1b: FGFR2 wild-type (126 bp)
2a: FGFR2-VCL (114 bp)
2b: FGFR2-VCL (89 bp)
3a: FGFR2-CCDC147 (93 bp)
3b: FGFR2-CCDC147 (132 bp)

Figure 2. RT-PCR products from cDNA from two different patients, one harboring a FGFR2-VCL fusion gene **(A)** and the other a FGFR2-CCDC147 fusion gene **(B)**.

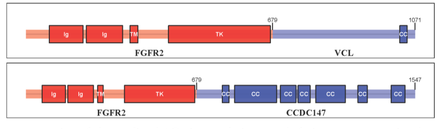


Figure 3. Schematic representation of the predicted FGFR gene fusions. Each fusion partner contains domains that facilitate dimerization – a proposed mechanism for the oncogenicity of these FGFR fusions.
Ig immunoglobulin extracellular domain; TM transmembrane domain; TK, tyrosine kinase domain; CC, coiled-coil domain.

FGFR2-VCL and FGFR2-CCDC147 fusions are oncogenic in vitro and sensitive to the FGFR selective inhibitor Debio 1347

Two novel FGFR2 fusions showed anchorage-independent growth and colony formation, indicating that they are both tumorigenic in vitro (Figure 4).

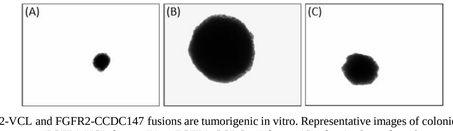
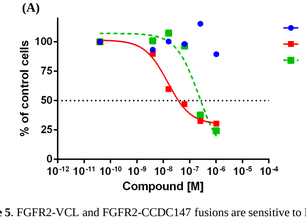


Figure 4. FGFR2-VCL and FGFR2-CCDC147 fusions are tumorigenic in vitro. Representative images of colonies from parental cells **(A)**, cells expressing FGFR2-VCL fusion **(B)** or FGFR2-CCDC147 fusion **(C)** after 21 days of incubation in soft agar.

The two novel FGFR2 fusions are sensitive to the FGFR selective inhibitor Debio 1347 whereas parental cells are not.



(B)

Cell line	Debio 1347 IC50 (nM)
Rat2 Parental	>1024
FGFR2-CCDC147	14.7
FGFR2-VCL	227

Figure 5. FGFR2-VCL and FGFR2-CCDC147 fusions are sensitive to Debio 1347 in vitro. IC_{50} curves **(A)** and calculated relative IC_{50} **(B)**.

FGFR2-VCL and FGFR2-CCDC147 fusions are oncogenic in vivo

In NMRI mice implanted with Rat2 parental cells (control), no primary tumor growth could be observed regardless of the quantity of cells implanted (Figure 6). In mice implanted with Rat2-FGFR2-CCDC147 cells, substantial tumor growth could be observed starting around day 28, and the animals implanted with a higher number of cells ($5x10^6$) exhibited faster tumor growth. After implantation of Rat2-FGFR2-VCL cells (Groups 5 and 6), tumor growth was observed starting around day 10. Owing to fast tumor growth, Group 5 ($5x10^6$ cells) had to be terminated for ethical reasons (tumor burden) on day 21, and Group 6 ($1x10^6$ cells) on day 28. Tumors expressing a FGFR2 fusion construct (FGFR2-VCL or FGFR2-CCDC147) were therefore shown to be tumorigenic in vivo in female NMRI nude mice. Animal weights of all groups increased continuously during the course of the study (not shown).

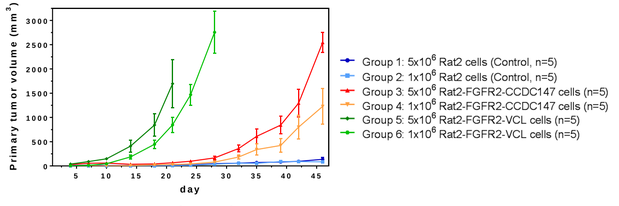


Figure 6. Primary tumor volumes. $5x10^6$ and $1x10^6$ Rat2 cells (Groups 1 and 2), Rat2-FGFR2-CCDC147 cells (Groups 3 and 4) and Rat2-FGFR2-VCL cells (Groups 5 and 6) were subcutaneously implanted into female NMRI nude mice on day 0. Data are displayed as means ± SEM.

FGFR2-CCDC147 fusion expressing tumors are sensitive to the FGFR selective inhibitor Debio 1347 in vivo

Debio 1347 induced tumor stasis in vivo in the FGFR2-CCDC147 expressing model at the two tested doses (p.o. daily, Figure 7A). Debio 1347 significantly inhibited tumor growth, whereas no significant effect was observed on body weight (Figure 7B)

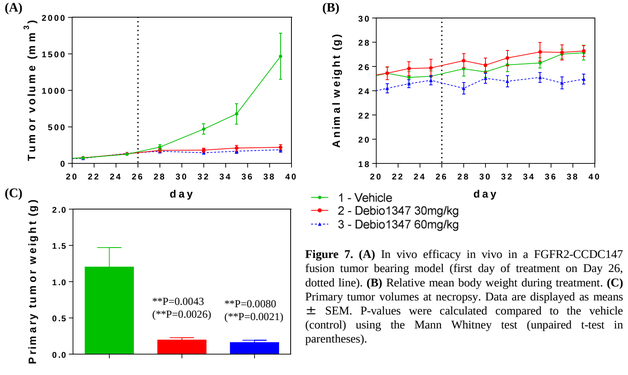


Figure 7. (A) In vivo efficacy in vivo in a FGFR2-CCDC147 fusion tumor bearing model (first day of treatment on Day 26, dotted line). **(B)** Relative mean body weight during treatment. **(C)** Primary tumor volumes at necropsy. Data are displayed as means ± SEM. P-values were calculated compared to the vehicle (control) using the Mann Whitney test (unpaired t-test in parentheses).

FGFR2-VCL fusion expressing tumors are sensitive to FGFR selective inhibitor Debio 1347 in vivo

Debio 1347 showed potent antitumor efficacy in vivo in the FGFR2-VCL expressing model (Figure 8). Debio 1347 inhibited tumor growth at the two tested doses (30 and 60 mg/kg p.o. daily, panel A), whereas no significant effect was observed on body weight (panel B). Tumor stasis was observed in the 60 mg/kg-treated group.

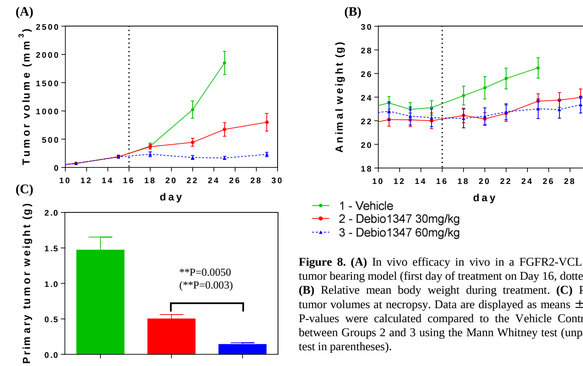


Figure 8. (A) In vivo efficacy in vivo in a FGFR2-VCL fusion tumor bearing model (first day of treatment on Day 16, dotted line). **(B)** Relative mean body weight during treatment. **(C)** Primary tumor volumes at necropsy. Data are displayed as means ± SEM. P-values were calculated compared to the Vehicle Control and between Groups 2 and 3 using the Mann Whitney test (unpaired t-test in parentheses).

Conclusions

- **We herein report for the first time the discovery of two novel FGFR fusions in intra hepatic cholangiocarcinoma patient samples. The new fusions FGFR2-VCL and FGFR2-CCDC147 both contain an intact FGFR2 tyrosine kinase domain fused to a gene partner encoding at least one oligomerization domain, suggesting a mechanism of ligand-independent constitutive kinase activation.**
- **Both fusions display oncogenic activities when introduced into Rat 2 cells in in vitro colony formation assays and were tumorigenic in vivo when implanted subcutaneously in NMRI nude mice.**
- **The selective FGFR inhibitor Debio 1347 significantly reduced cell proliferation in vitro and in vivo in models harboring the two fusions.**
- **Altogether, these results suggest that cholangioarcinoma patients harboring these fusions could benefit from targeted FGFR inhibitors such as Debio 1347/CH5183284, currently investigated in a Phase I trial in selected patients harboring FGFR genetic alterations (NCT01948297).**

Related Presentations

1. Mechanism of oncogenic signal activation by the novel fusion kinase FGFR3-BAIAP2L1 - Sunday, Apr 19, 2015, 1:00 PM - 5:00 PM - Abstract #123
2. Formulation switch and pharmacokinetics/pharmacodynamics of Debio 1347 (CH5183284), a novel FGFR inhibitor, in a first-in-human dose escalation trial in solid tumors patients - Monday, April 20, 2015, 8:00 AM - 12:00 AM - Abstract CT228

References

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(2) Knowles MA et al. Molecular biology of bladder cancer: new insights into pathogenesis and clinical diversity. Nat Rev Cancer. 2015 Jan;15(1):25-41
(3) Nakanishi Y et al. FGFR genetic alterations as a potential predictor of the sensitivity to CH5183284/Debio 1347, a selective FGFR inhibitor with a novel chemical scaffold. AACR 2014 abstract #2729

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