FGF15 AND FGF19 INDUCE DISPARATE FGFR4-MEDIATED HEPATOCARCINOGENICITY IN-VITRO AND IN TWO MURINE MODELS: IMPLICATIONS FOR DRUG-ASSOCIATED CARCINOGENICITY RISK ASSESSMENTS

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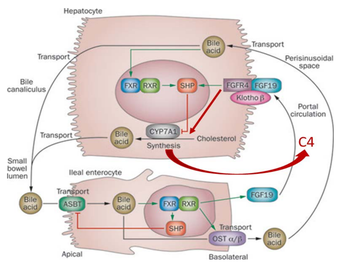
BACKGROUND

- The enteric endocrine hormone FGF19 is a key regulator of bile acid (BA) synthesis[1].
- FGF15 is the rodent ortholog of human FGF19 with ~50% amino acid homology.
- FGF19 and FGF15 are induced by agonists of Farnesoid X receptor (FXR) in human and mice, respectively[2-3].
- However, FGF19 produces hepatocellular carcinoma (HCC) tumors in transgenic mice in an FGFR4-dependent manner and has been linked to increased risk of post-resection recurrence in humans[4-8].
- Both FGF15 and FGF19 are potent inhibitors of Cyp7a1-mediated BA synthesis in rodent models but the carcinogenicity risk of FGF15 is not well characterized.
- We evaluated the hepatocarcinogenic potential of FGF15 and FGF19 head-to-head in leptin receptor-deficient (*db/db)* or diet-induced obese (DIO) mice for up to 24 weeks or 52 weeks of treatment, respectively.

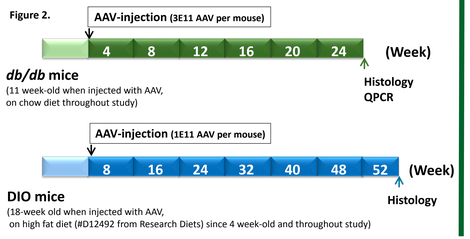


Adopted from Camilleri M. Nat Rev Gastro Hep. 9:173-184.

METHODS

- FGF15, FGF19, or control gene GFP were delivered to *db/db* or DIO mice via adeno-associated viral vector (AAV), which enables stable long-term transgene expression after a single tail vein injection.
  - AAV-GFP (control)
  - AAV-FGF19
  - AAV-FGF15
- *db/db* (#000642) and DIO mice (#380050) were purchased from Jackson Laboratories, *n* = 5-7 mice per group.
- Liver tissue was examined for tumors and liver weight at 24 weeks post-dose in *db/db* mice and 52 weeks post-dose DIO mice.
- Gene expression of markers of HCC-related risk and cellular proliferation were also evaluated by QPCR from liver tissue.

Figure 2.

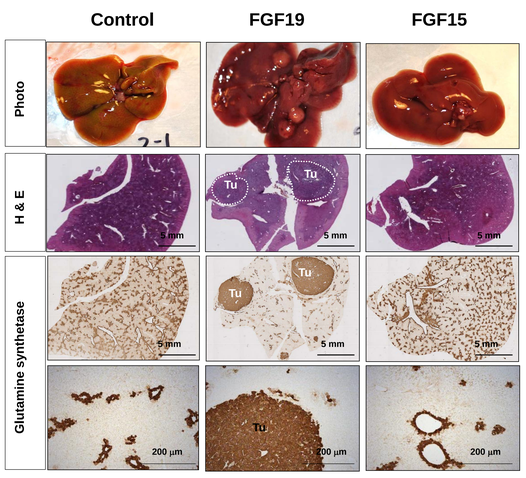


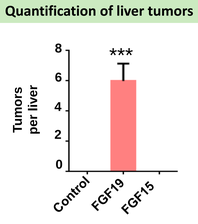
RESULTS

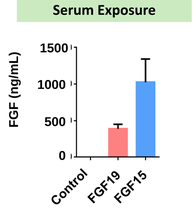
- AAV-based vectors provide a way to achieve long-lasting transgene expression without the inflammatory responses that are commonly associated with other viral delivery systems[9].
- We have previously evaluated multiple mouse strains for latency and robustness of FGF19-mediated liver tumor formation. Among several mouse strains tested, *db/db* mice exhibited the highest penetrance and shortest latency in tumor development following AAV-FGF19 delivery[10-11].

FGF15 is Non-tumorigenic in *db/db* Mouse Model

- *db/db* mice were euthanized 24 weeks after AAV delivery.
- Livers were examined macroscopically, and then fixed and embedded in paraffin and stained with H&E or anti-glutamine synthetase using DAB (Vector Laboratories) as substrates.
- Serum exposure of FGF19 was determined by enzyme-linked immunosorbent assay (Biovendor); Serum exposure of FGF15 was determined by enzyme-linked immunosorbent assay developed in-house using antibodies from Santa Cruz Biotechnology.

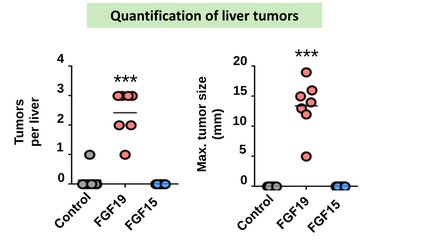


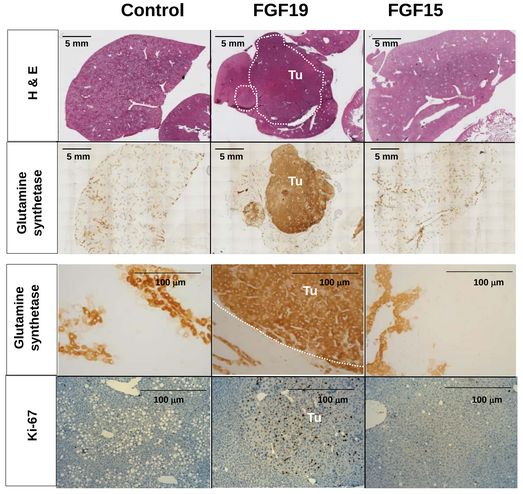


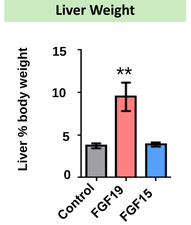


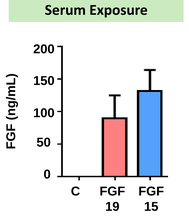
FGF15 is Non-tumorigenic in DIO Mouse Model

- DIO mice were euthanized 52 weeks after AAV delivery
- Livers were examined macroscopically, and then fixed and embedded in paraffin and stained with H&E or anti-glutamine synthetase using DAB (Vector Laboratories) as substrates.



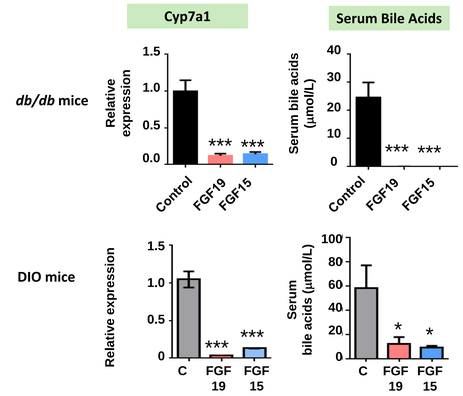






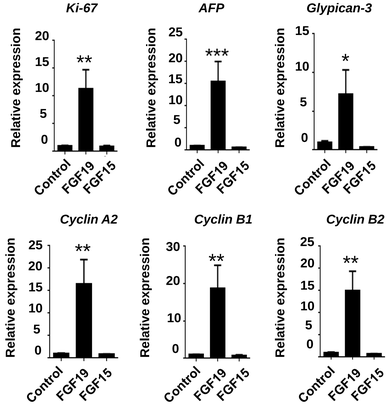
FGF15 Inhibits Bile Acid Synthesis in *db/db* and DIO Mice

- Hepatic Cyp7a1 expression was performed using quantitative RT-PCR method (QPCR) using premade primers from Life Technologies.
- Serum concentrations of total bile acids were measured using a 3α-hydroxysteroid dehydrogenase method (Diazyme)



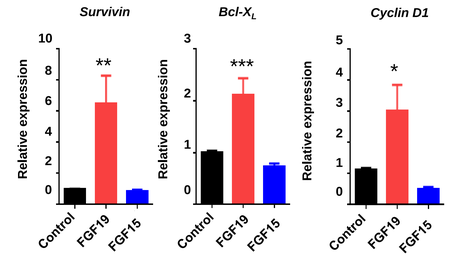
FGF19, but Not FGF15, Induces HCC-Related Gene Expression

- mRNA levels of proliferative marker Ki-67, HCC markers α-fetoprotein and Glypican-3, and cyclins critical for cell cycle progression and mitosis, were induced by FGF19, but not FGF15.



STAT3 Target Genes are Upregulated by FGF19, but not FGF15

- Anti-apoptotic genes, such as *Survivin, Bcl-x_L* and *Cyclin D1,* are direct downstream targets of Stat3 pathway activation
- Hepatic expression of *Survivin, Bcl-x_L* and *Cyclin D1* were assessed in *db/db* mice 24 weeks after AAV delivery.
- Gene expression was upregulated only by FGF19



CONCLUSIONS

- Human FGF19, but not murine FGF15, induces the formation of HCC in two independent mouse models.
- Both FGF19 and FGF15 inhibit hepatic expression of Cyp7a1 and decrease serum levels of total bile acids
- FGF19, but not FGF15, upregulates hepatic expression of genes related to HCC and the Stat3 pathway.
- As FGF19/FGF15 are primary downstream targets of FXR activation, the functional differences between mouse FGF15 and human FGF19 may restrict the relevance of mouse models for studying certain aspects of the FXR/FGF19/FGF15 pathway.

References:

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Disclosures

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- All authors are employees and stockholders of NGM Biopharmaceuticals, Inc.