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online data

SUPPLEMENTARY ONLINE DATA

Farnesoid X receptor protects human and murine gastric epithelial cells against inflammation-induced damage

Fan LIAN*, Xiangbin XING*, Gang YUAN*, Claus SCHÄFER†, Sandra RAUSER‡, Axel WALCH‡, Christoph RÖCKEN§, Martin EBELING‖, Matthew B. WRIGHT‖, Roland M. SCHMID¶, Matthias P. A. EBERT** and Elke BURGERMEISTER**

*Department of Gastroenterology, The First Affiliated Hospital of Sun Yat-sen University, 510275 Guangzhou, China, †Department of Medicine II, Klinikum der Universität München, D-81377 Munich, Germany, ‡Institute of Pathology, Helmholtz Zentrum München, D-85764 Oberschleissheim, Germany, §Institute of Pathology, Christian Albrechts Universität, D-24105 Kiel, Germany, ‖F. Hoffmann-La Roche, CH-4070 Basel, Switzerland, ¶Department of Medicine II, Klinikum rechts der Isar, Technische Universität München, D-81675 Munich, Germany, and ** Department of Medicine II, Universitätsklinikum Mannheim der Universität Heidelberg, D-68167 Mannheim, Germany

Table S1 Primers and oligonucleotides

The primers were used for RT–PCR detecting human FXRα mRNA variants (GenBank® accession numbers NM_005123.2 and NT_029419.12). The oligonucleotides were used for ChIP and EMSA in the human genomic K13 locus (GenBank® accession number AF049259). E, exon; DBD, DNA-binding domain.

Gene	Amplicon	Sense	Antisense
FXRα1/2	605 bp	5′-AGGGCCTTGAAAGTCCATCT (E1)-3′	5′-AGCTCATCCCCTTTGATCCT (E4)-3′
ΔFXRα1/2	350 bp	5′-CATTCCCATTTACCTACCACAGA(E3)-3′	5′-AGCTCATCCCCTTTGATCCT (E4)-3′
FXRα3/4	404 bp	5′-GTAATGCAGTTTCAGGGGTTA(E3a) -3′	5′-AGCTCATCCCCTTTGATCCT (E4)-3′
FXRα total	113 bp	5′-GCATTACCAAAAACGCTGTG(DBD) -3′	5′-TCCCATCTCTTTGCATTTCC(DBD)-3′
K13	-500/+1	5′-GTGACCTTGCAAAGCACAGA-3′	5′-CCTCCATAGGGGCTGGTTAT-3′
K13	RARE1	5′-CGAGGGCTACGGTGACCTTGCAAA-3′	5′-TTTGCAAGGTCACCGTAGCCCTCG-3′
K13	RARE2-WT	5′-GTTCTAATACTGGGTGGGGCTCAG-3′	5′-CTGAGCCCCACCCAGTATTAGAAC-3′
K13	RARE2-MUT	5′-GTTCTAATGTATATTGGAATAAGG-3′	5′-CCTTATTCCAATATACATTAGAAC-3′
K13	RARE3	5′-CTTAAGTGGAGGTGAAACAGAATT-3′	5′-AATTCTGTTTCACCTCCACTTAAG-3′

Table S2 Bile-acid-regulated human genes in AGS cells identified by DNA microarray

Results are expressed as the fold change of mRNA expression in AGS/FXR compared with AGS/EV cells upon a 16 h stimulation with 100 μM CDCA. GO, gene ontology.

Gene name	Symbol	GenBank® accession number	GO-group	Change factor
Up-regulated genes				
Nuclear receptor subfamily 1, group H, member 4 (farnesoid X receptor)	NR1H4 FXR	NM_005123	Transcription	147
Organic solute transporter α	OST α	NM_152672	Metabolism	48
Keratin 13	KRT13	NM_002274	Epidermal development	42
Serpin peptidase inhibitor, clade B (ovalbumin), member 2	SERPINB2	NM_002575	Antiapoptosis	36
Keratin 6A/keratin 6C/keratin 6E	KRT6A/KRT6C/KRT6E	NM_005554/NM_058242/NM_173086	Ectoderm development	10
Keratin associated protein 2-1	KRTAP2-1	XM_927943	Structural constituent	10
TNFα-induced protein 8	TNFAIP8	NM_014350	Anti-apoptosis	7
Keratin, hair, basic, 1	KRTHB1	NM_002281	Epidermis development	6
Plakophilin 4	PKP4	NM_001005476	Cell adhesion	4
Catenin (cadherin-associated protein), α1, 102 kDa	CTNNA1	NM_001903	Cell adhesion	3
Down-regulated genes				
Progastricsin (pepsinogen C)	PGC	NM_002630	Proteolysis	0.4
Trypsinogen C	TRY6	NR_001296	Proteolysis	0.4
Matrix metallopeptidase 7 (matrilysin, uterine)	MMP7	NM_002423	Peptidoglycan metabolism	0.4
Matrix metallopeptidase 1 (interstitial collagenase)	MMP1	NM_002421	Peptidoglycan metabolism	0.3
Protease, serine, 1 (trypsin 1)	PRSS1	NM_002769	Proteolysis	0.2

To whom correspondence should be addressed (elke.burgermeister@lrz.tum.de).

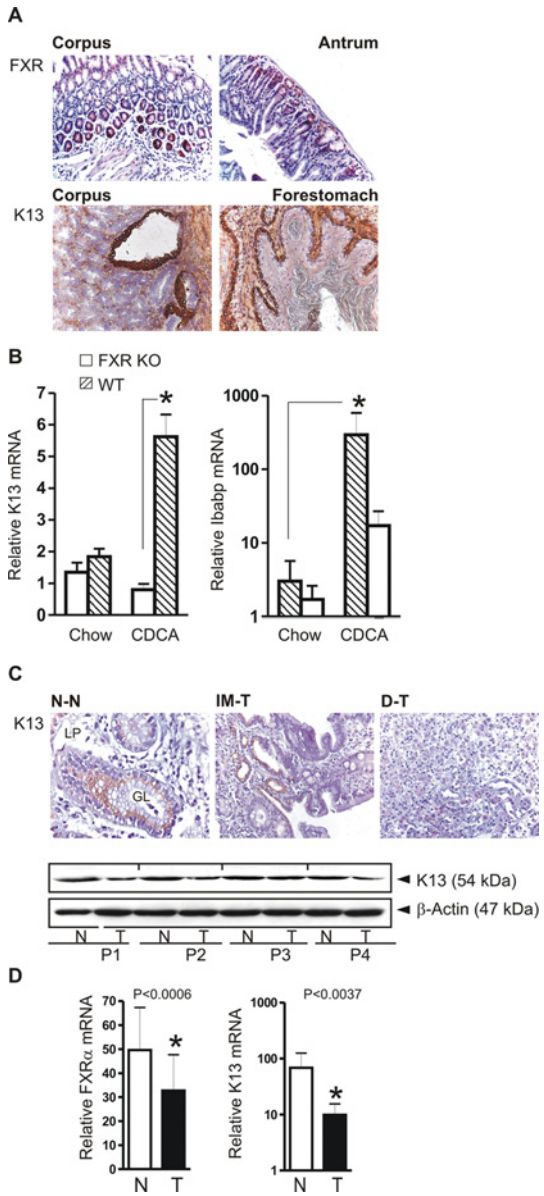

Figure S1 K13 is also regulated by FXR *in vivo*

(**A**) Upper panels: detection of FXR (Nr1h4/UniProtKB/Swiss-Prot number Q60641) by IHC in the corpus and antrum regions of stomachs from WT C57BL/6 mice using the rabbit polyclonal antiserum. Lower panels: detection of K13 (K1c13/UniProtKB/Swiss-Prot number P08730) by IHC in the mouse corpus and forestomach regions; magnification 100×. (**B**) Quantification of K13 mRNA (GenBank® accession number NM_010662.1) and the cognate FXR-target gene *Ibabp* in stomachs from WT and FXR-KO mice. Mice were fed on a chow diet with 1% (w/w) CDCA or a standard mouse chow (control) for 7 days respectively. Normalized C_T values from RT–qPCRs were calculated as fold changes ± S.E.M. ($n = 5$ per group and genotype). *$P <$ 0.05. (**C**) K13 (K1C13/UniProtKB/Swiss-Prot number P13646) protein is expressed in human GC specimens. Upper panel: detection of K13 by IHC in normal gastric glands (N-N), in I-T GC (I-T) and D-T GC (D-T) tissue. Magnification 200× and 400×. Lower panel: detection of K13 by WB in tissue lysates from tumour and paired non-tumour specimens of four different GC patients. (**D**) Quantification of total FXRα (GenBank® accession number NM_0012069) and K13 (GenBank® accession number NM_002274.3) mRNA in human GC tissue. Normalized C_T values from RT–qPCRs are fold change ± S.E.M. ($n = 30$ patients per group) in tumour tissue (T) compared with matched normal (N) tissue; *$P <$ 0.05 (Wilcoxon signed-rank test).

Table S3 Sequence alignment of a conserved hexameric element (RARE2) in genomic K13 loci

Predicted SP1 sites are marked by italics, functional RARE sites are marked by bold italics and are marked by underlined residues. Rev, reverse; Rev compl., reverse complemented DNA sequence.

M00762	RGG NCA A **A/GGG TCA** (PPAR, HNF4, COUP, RAR)
Rev compl.	**T/CCC AGT**
Rev	**ACT GGG/A**
CK13 Human	ATCTCAGGTCCC-GTTCTAAT **ACT *GGG*** *TGG GGC* TCAG (*SP1*)
CK13 Mouse	***GGTTCA***G-TCCC***TGTTCT***AAT **ACT GGG** CAG GGC TGGG (***RARE***)
CK13 Rat	TGTTCAG-TCCCTGTCCTAAT **ACT GGG** CAG GGC TGGG

Table S4 Histopathological analysis of gastric ulceration

WT and FXR-KO mice (C57BL/6) were treated for 24 h with Indo (35 mg/kg of body weight, intraperitoneal) or NaCl (0.9 %, intraperitoneal). Injury scores were assigned based on microscopical evaluation of H&E-stained paraffin sections comprising the gastric corpus and antrum regions.

Score	WT	KO	Description
3+	1	3	Severe gastritis, ulcerations and elongated erosions (>5 mm), and fibrinoid vascular necrosis
2+	2	3	Moderate discrete erosive gastritis (<5 mm)
1+	4	2	Superficial discrete erosive gastritis
0	2	0	Normal
Total *n*	9	8	Number of mice per genotype

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