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Supplemental Material

Human CYP2A13 and CYP2F1 Mediate Naphthalene Toxicity in the Lung and Nasal Mucosa of CYP2A13/2F1-Humanized Mice

Lei Li, Sarah Carratt, Matthew Hartog, Nataliia Kovalchuk, Kunzhi Jia, Yanan Wang, Qing-Yu Zhang, Patricia Edwards, Laura Van Winkle, and Xinxin Ding

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Methods Development for Detection of CYP2F1 Protein Using A Probe Peptide

To achieve optimal sensitivity and selectivity for identification and quantification of CYP2F1, a probe peptide to represent the target protein was selected using a combination of *in silico* tryptic digestion and database similarity search. Firstly, *in silico* tryptic digestion was performed on the amino acid sequence of CYP2F1 (NP_000765.2) using Protein Prospector, with a setting of 0 for missed cleavages, yielding a list of potential surrogate peptides for CYP2F1. Candidate probe peptides were chosen from this list based on the biochemical criteria described previously (Kamiie et al., 2008), and compared against the Swissprot database (blast for *Spodoptera frugiperda, human and mouse*) using the Basic Local Alignment search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/) to rule out nonspecific peptides. Secondly, tryptic digests of recombinant CYP2F1 protein (in Sf9 cell microsomes, with unknown CYP2F1 amount) was analyzed using LC-MS/MS Q1 multiple ion scan to ensure that doubly charged ions of the potential surrogate peptides selected by the *in silico* process were present and readily detectable. Then, MS/MS spectra were obtained for these peptides, to confirm their similarity with data in the MS-product database (http://prospector.ucsf.edu/prospector/cgi-bin/msform.cgi?form=msproduct). The most abundant peptide, EALVDQGEEFSGR, was selected for use in subsequent CYP2F1 identification and quantification analysis. The MS/MS spectra obtained in the enhanced product ion (EPI) scan mode, and the spectra for the probe peptide EALVDQGEEFSGR and its isotope-labeled internal standard EALVDQGEEFSG[$R^{13}C^{15}N$] are shown in Figure S1A. Identity of the tryptic probe peptide was further confirmed by co-elution with authentic standards and matching of MS/MS spectra.

The LC-MS/MS system has been described in the manuscript. For initial identification of CYP2F1 probe peptide, the tryptic digest of CYP2F1-expressing Sf9 cell microsomes was

analyzed. The mobile phase consisted of solvent A [5% acetonitrile (ACN), 95% H_2O, 0.1 % formic acid] and solvent B (95% ACN, 5% H_2O, 0.1% formic acid). The samples were eluted at a flow rate of 0.2 ml/min, with 100%A for 2 min, followed by linear increases from 0%B to 60%B between 2 and 30 min, and then 60%B for another 10 min. The mass spectrometer was set for the Q1 multiple ions scan mode, followed by EPI scanning to identify the CYP2F1 probe peptide as described above.

REFERENCES

Kamiie J, Ohtsuki S, Iwase R, Ohmine K, Katsukura Y, Yanai K, Sekine Y, Uchida Y, Ito S and Terasaki T (2008) Quantitative atlas of membrane transporter proteins: development and application of a highly sensitive simultaneous LC/MS/MS method combined with novel in-silico peptide selection criteria. Pharmaceutical research 25:1469-1483.

Supplementary Figures

Figure S1. LC-MS/MS analysis of CYP2F1 probe peptide. (A) MS^2 product-ion spectra of unlabeled (left) and stable isotope-labeled probe peptide (right) for CYP2F1. Each peptide (0.1 nmol) was injected into the LC-MS/MS, and MS^2 enhanced product ion scan analyses were performed. The characteristic y series ions are shown. **(B)** Typical LC-MS chromatograms for detection of CYP2F1 probe peptide in microsomal samples. CYP2F1-expressing Sf9 cells microsomes (100 µg), and OM and lung microsomes (100 µg) from 2A13/2F1-TG$^{(+/+)}$ mice, were digested, spiked with 1 pmol of stable isotope-labeled peptide (internal standard) and injected into the LC-MS/MS system for analysis. The representative parent/product ion pairs of m/z 718.8/781.4 for probe peptide and 724.4/791.4 for internal standard (not shown) were monitored for quantitative analysis.

Figure S2. Plasma levels of NA and NA-GSH at various times after NA inhalation. Male, 2-month old *Cyp2abfgs*-null, CYP2A13/2F1-humanized, and WT mice were exposed to NA vapor (10 ppm) for 4 h. Plasma was obtained at various times after termination of exposure, for determination of the levels of NA (left) and NA-GSH (right), as described in *Materials and Methods*. Data represent means ± S.D. (n = 4). $^{**}p < 0.01$, $^{*}p < 0.05$, compared to WT mice (One-way ANOVA, followed by Tukey's multiple comparisons test).

Figure S3. BAL fluid constituent analysis. Two-month old male *Cyp2abfgs*-null, CYP2A13/2F1-humanized, and WT mice were exposed to FA or NA vapor (10 ppm) for 4 h. BAL was performed for individual mouse at 2 h or 20 h after the termination of inhalation exposure, as

described in *Materials and Methods*. Three cytotoxicity markers, total cell counts, protein release, and LDH release, were measured in the BAL fluid. Data represent means ± S.D. (n = 3~6). *$p < 0.05$, **$p < 0.01$, compared to corresponding mice exposed to FA (basal level) (One-way ANOVA, followed by Tukey's multiple comparisons test).

Fig. S1.

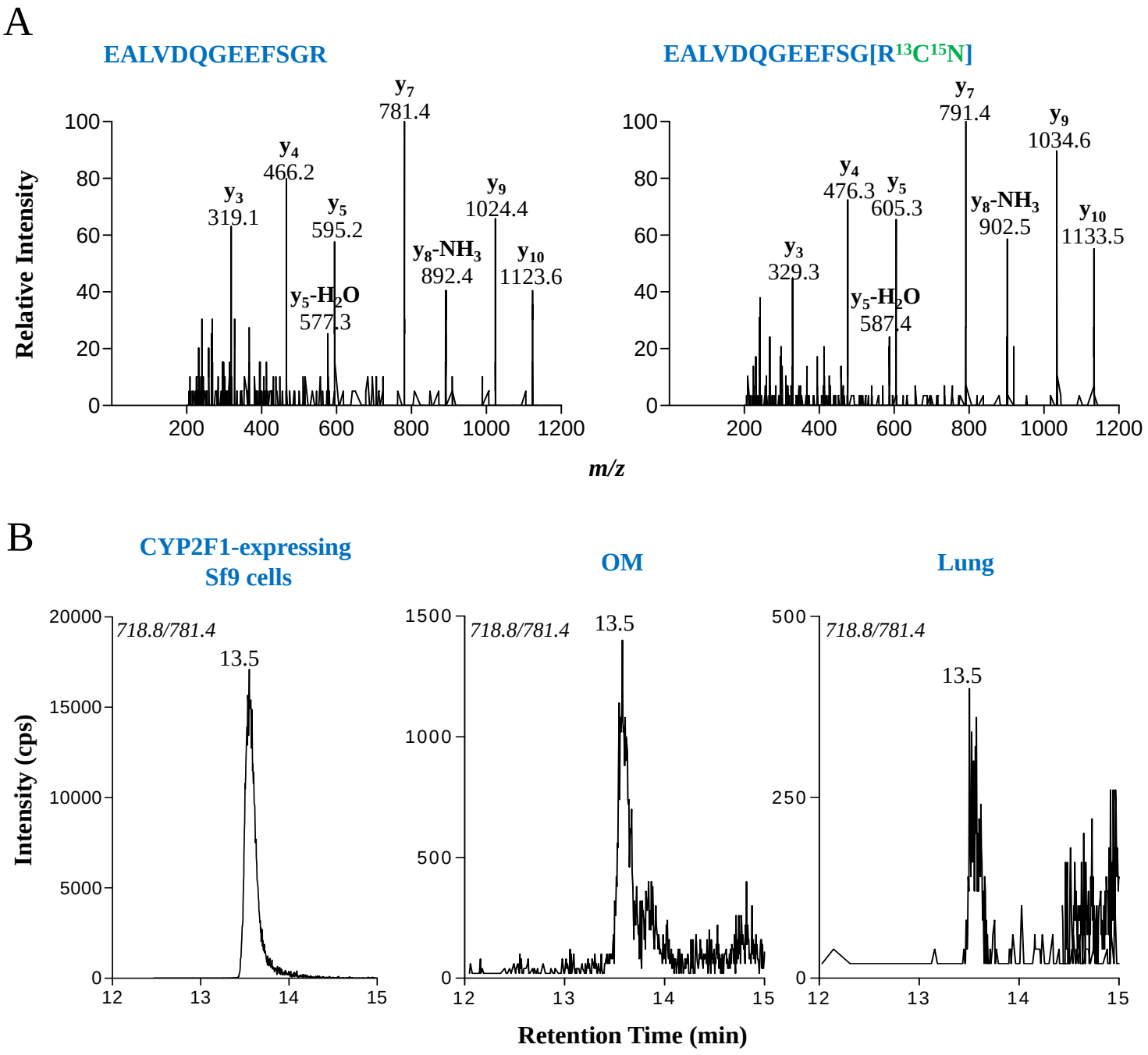

A
EALVDQGEEFSGR
EALVDQGEEFSG[R13C15N]
Relative Intensity
y3 319.1
y4 466.2
y5 595.2
y5-H2O 577.3
y7 781.4
y8-NH3 892.4
y9 1024.4
y10 1123.6
y3 329.3
y4 476.3
y5 605.3
y5-H2O 587.4
y7 791.4
y8-NH3 902.5
y9 1034.6
y10 1133.5
m/z
B
CYP2F1-expressing Sf9 cells
OM
Lung
Intensity (cps)
718.8/781.4
13.5
Retention Time (min)

Fig. S2.

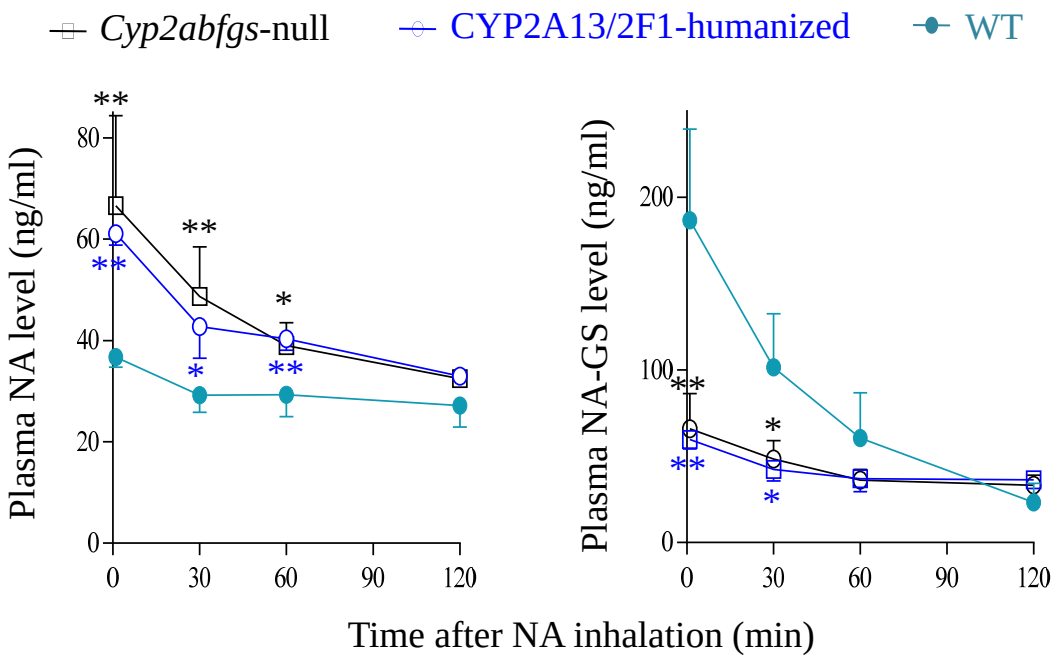
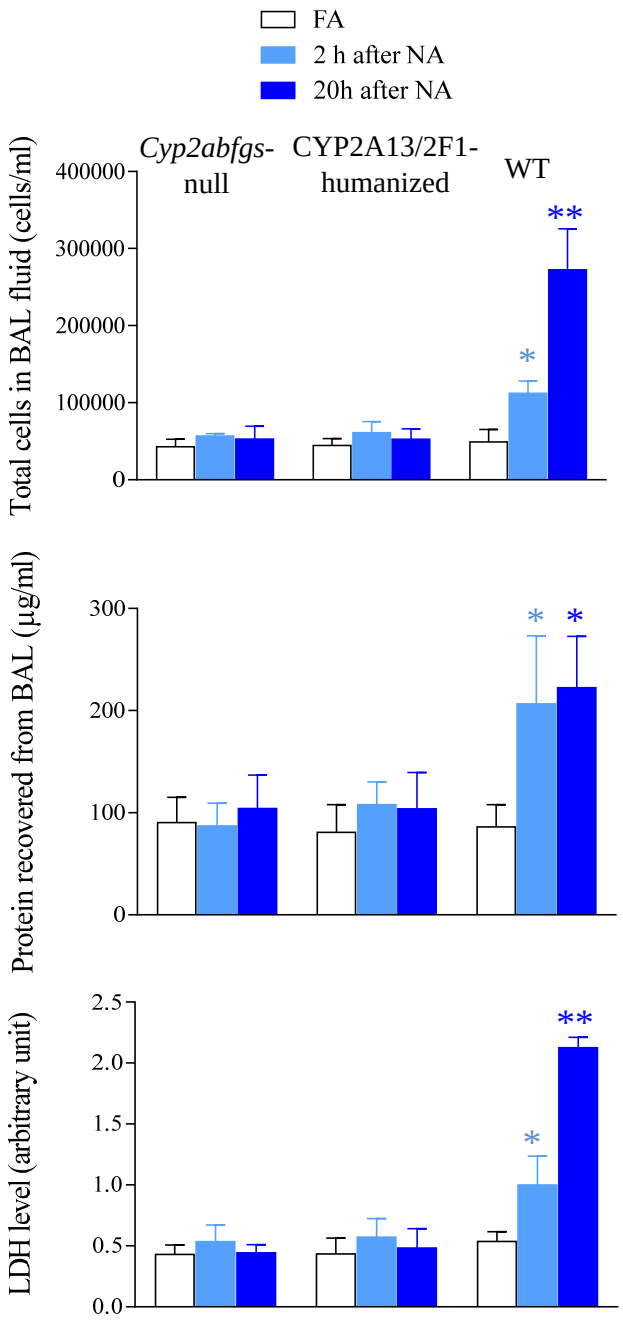

Cyp2abfgs-null
CYP2A13/2F1-humanized
WT
Plasma NA level (ng/ml)
Plasma NA-GS level (ng/ml)
Time after NA inhalation (min)
0
30
60
90
120
20
40
60
80
100
200
**
*

Fig. S3.


FA
2 h after NA
20h after NA
Cyp2abfgs-null
CYP2A13/2F1-humanized
WT
Total cells in BAL fluid (cells/ml)
400000
300000
200000
100000
0
**
*
Protein recovered from BAL (μg/ml)
300
200
100
0
*
*
LDH level (arbitrary unit)
2.5
2.0
1.5
1.0
0.5
0.0
**
*