Clinically relevant multidrug transporters are regulated by different microRNAs along the human intestine

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Site-specific mRNA and protein levels of transporters along the human intestine

Localisation of biopsies from organ donors

- Duodenum
- Jejunum
- Ileum
- Colon

targeted proteomics

small intestine

- PEPT1 50%
- ABCB1 8%
- ABCC2 10%
- ABCC3 7%
- ABCG2 4%
- OCT1 8%
- OATP2B1 6%
- OCT3 1%

colon

- OCT1 12%
- PEPT1 5%
- ABCB1 5%
- ABCC2 25%
- ABCC3 36%
- ABCG2 3%
- OATP2B1 12%
- OCT3 2%

Discrepancy between mRNA and protein levels


**Role of microRNAs?**

- **mRNA degradation**
  - microRNA
  - $\text{AAAA}$
  - $\text{AAAA}$

- **transcriptional repression**
  - microRNA
  - $\text{AAAA}$
  - ribosome

![Graphs showing ABCC3 mRNA and protein levels across different regions (D, J1, J2, I1, I2, C).](image-url)
Site-specific microRNA expression along the human intestine

Correlation microRNA/protein + in silico-target prediction

<table>
<thead>
<tr>
<th>correlation coefficients</th>
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<tbody>
<tr>
<td>ABCB1</td>
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<tr>
<td>miR-27a-3p</td>
</tr>
<tr>
<td>miR-100-5p</td>
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<tr>
<td>miR-192-5p</td>
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<tr>
<td>miR-193a-3p</td>
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<tr>
<td>miR-193b-3p</td>
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<tr>
<td>miR-215-3p</td>
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<tr>
<td>miR-365a-3p</td>
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<tr>
<td>miR-409-3p</td>
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<tr>
<td>miR-766-3p</td>
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</tbody>
</table>

Pearson correlation: p-value * < 0.05, ** < 0.01; n.s. = not significant
Verification of miR-192-5p / ABCC3 3′-UTR interaction

**Principle of reporter gene assay**

- **No binding of microRNA**
  - Protein binds to transporter 3′-UTR
  - Luciferase
  - Signal

- **Binding of microRNA**
  - Protein binds to microRNA
  - Transporter 3′-UTR
  - Luciferase
  - Signal

**microRNA-192-5p**

- **Empty vector**
  - Luciferase

- **Wild-type**
  - Luciferase

- **Mutated**
  - Luciferase
  - ABCC3 3′-UTR

Normalized relative reporter gene activity

Mann-Whitney-U test, p ** < 0.01
Verification of miR-27a-3p / miR-409-3p / ABCB1 3’-UTR interactions

Mann-Whitney-U test, $p$ *** $< 0.001$
Verification of miR-27a-3p / miR-193a-3p / miR-193b-3p / PEPT1 (SLC15A1) 3’-UTR interactions

Mann-Whitney-U test, p *** < 0.001
Combined effects of multiple microRNAs on *PEPT1 (SLC15A1)* 3′-UTR

Mann-Whitney-U test, p **< 0.01
Summary

The image depicts a diagram of the digestive tract, highlighting the expression levels of various proteins and miRNAs in different sections: Duodenum, Jejunum, and Ileum. The diagram includes columns for ABCC3, ABCB1, and PEPT1 proteins, each with corresponding miRNA levels (miR-192, miR-27a, miR-409, miR-27a, miR-193a, miR-193b).
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