Studying the effect of oral vitamin D$_3$ supplementation on human gene expression

A systems biology approach

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Content

• Introduction
• Aim of the project
• Methods:
  - study design
  - systems biology steps and tools
• Results
• Discussion
• Conclusion
Introduction vitamin D

Deeb, K. K., et al. (2007)

Figure 1. Vitamin D metabolism

Nature Reviews | Cancer
**Genomic effect of vitamin D**

- Anti-proliferation
- Apoptosis
- Differentiation
- Anti-inflammation
- Immune regulation

Possible cross-talk

Chiang, K. C., et al. (2011)

**Non-genomic effect of Vitamin D**

1,25(OH)₂-vitamin D

mVDR

mVDR

Ca²⁺ channel

G protein

AC

Ca²⁺ channel

Possible cross-talk

Project aim

Study the effect of vitamin D induced gene expression on human process level using a systems biology approach.
Methods
**Study design**

**Pre**

- n=22

**Post**

- n=22

- 3 months

- 25 µg D₃

Gerke et al. 2014
Study

→ RNA isolation

→ Microarray
  Affymetrix human exon
  1.0 ST array

→ ArrayExpress: E-GEOD-56583

Alveolar macrophage
Results gene list

15,052 measured genes $\rightarrow$ 339 significantly differentially expressed

(-1.2 < fold change > 1.2 and P value < 0.05)
Results pathway analysis

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Z-Score</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptide GPCRs</td>
<td>3.22</td>
<td>Up: BRS3, CCR10, MC3R, SSTR4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Down: CCR1</td>
</tr>
<tr>
<td>GPCRs, Class A Rhodopsin-like</td>
<td>3.13</td>
<td>Up: BRS3, CCR10, GPR65, HTR55A, MC3R NPFFR1, OR1A2, P2RY10, SSTR4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Down: CCR1, CHRM3</td>
</tr>
<tr>
<td>Type II interferon signaling (IFNG)</td>
<td>2.97</td>
<td>Up: CXCL9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Down: HIST2H4A, PRKCD</td>
</tr>
<tr>
<td>TWEAK Signaling Pathway</td>
<td>2.50</td>
<td>Down: GSK3B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MAP3K7, RIPK1</td>
</tr>
<tr>
<td>ATM Signaling Pathway</td>
<td>2.45</td>
<td>Up: CDK1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Down: MDM4, RIPK1</td>
</tr>
<tr>
<td>IL-6 signaling pathway</td>
<td>2.45</td>
<td>Down: PRKCD, GSK3B, NCOA1</td>
</tr>
<tr>
<td>Toll-like receptor signaling pathway</td>
<td>2.42</td>
<td>Up: IFNA6, CXCL9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Down: AKT3, MAP3K7, RIPK1</td>
</tr>
<tr>
<td>Regulation of toll-like receptor</td>
<td>2.22</td>
<td>Up: IFNA6, CXCL9</td>
</tr>
<tr>
<td>signaling pathway</td>
<td></td>
<td>Down: AKT3, PEL11, RIPK1, MAP3K7</td>
</tr>
<tr>
<td>Cardiac Hypertrophic Response</td>
<td>2.09</td>
<td>Down: CAMK2, MAP3K7, GSK3B</td>
</tr>
<tr>
<td>Wnt Signaling Pathway Netpath</td>
<td>2.09</td>
<td>Up: AXIN2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Down: GSK3B, MAP3K7</td>
</tr>
<tr>
<td>Senescence and Autophagy</td>
<td>2.05</td>
<td>Up: SH3GLB1 PCNA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Down: GSK3B, FN1, ATG7, SH3GLB1</td>
</tr>
</tbody>
</table>

Minimum amount of significantly change genes: 3
Z Score >1.96

- WikiPathways collection
- Immune pathways **Blue**
Type II interferon signalling (IFNG)
Results vitamin D$_3$ network

129 genes
11 pathways
30 GO classes
Results vitamin D₃ network

Olfactory receptors (ORs)

Up: 16
Down: 1

G-protein coupled receptor protein signaling pathway
sensory perception of chemical stimulus
olfactory receptor activity

GPCRs_Class_A_Rhodopsin-like
Results vitamin D$_3$ network

- Vitamin D$_3$ affected expression of several ORs in alveolar macrophages
- Possible role of ORs in immune function of alveolar macrophage?
Results drug extension

- 76 drugs
  - 2 vitamins
  - 4 nutritional supplements
Results drug extension
Results drug extension

• Vitamin A and vitamin $K_3$

• synergistic or counteracting effects?
Results TF extension

86 TFs added
- 8 TFs

Altered gene expression
Results TF extension

- 5 immunomodulatory TFs

- Indirect effect of vitamin D on expression of immune genes?
Discussion

- Vitamin D deficient individuals as optimal study population.

- Change in vitamin D status of test subjects unknown.
Discussion

• Changes in gene expression of immune cells can also be due to other factors

• Changes in gene expression ≠ change in protein product
Conclusion

New hypotheses can be formulated e.g.:

- Vitamin D enhances innate immune function via increased olfactory receptors activity

- Vitamin D cooperates with Vitamin K\textsubscript{3} and Vitamin A to enhance immune function.

- Vitamin D enhances the innate immune system indirectly by affecting immune related TFs
Conclusion

• And many more........
Additional slides

• Will not be presented, Only used in case there are questions
Microarray technique

Pre vitamin D$_3$ supplementation

Experimental Sample

Post vitamin D$_3$ supplementation

Control Sample