Expression and Induction of Xenobiotic Metabolism Genes in the StrataTest® Human Skin Model

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Abstract

Human in vivo skin models have shown broad utility for toxicological testing due to their similarity to the in vivo state and ability to evaluate compounds as dosed in actual use or exposure. Importantly, keratinocytes and in vitro skin models are metabolically competent. Numerous studies have shown the induction of phase I and phase II metabolic enzymes in keratinocytes and skin models. In the present study, the metabolic competency of the StrataTest® human skin model was investigated by evaluating the expression and induction of genes involved in Phase I and Phase II metabolism. The StrataTest full-thickness human skin model, containing both epithelial and dermal components, faithfully recapitulates many of the biological characteristics of human skin. The model is generated using NIKS keratinocytes, a clinically tested and consented non-tumorigenic, pathogen-free human keratinocyte progenitor. To confirm that NIKS keratinocytes provide appropriate metabolic capacity, gene expression profiles of phase I and II enzymes from NIKS and primary keratinocytes were compared. Differences in expression of two-fold or greater between NIKS and primary keratinocytes were considered significant. Concordance for expression of 51 Phase I and Phase II metabolic enzymes in NIKS and primary human keratinocytes was 98%. RT-PCR and qPCR were then employed to evaluate baseline gene expression and induction after exposure to xenobiotic agents, in NIKS keratinocytes and the three-dimensional human skin model. cytochrome p450 T1A1 (CYP1A1) induction was confirmed in NIKS keratinocytes after exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The skin model CYP1A1 and CYP1B1 were weakly expressed constitutively, while N-acetyltransferase 1A (NAT1) displayed more constitutive expression. Upon exposure to 3-methylcholanthrene (3MC) CYP1A1 and CYP1B1 expression was strongly induced. These results show that similar to other in vitro skin models, StrataTest skin tissues express genes critical to xenobiotic metabolism, further demonstrating the utility of this model for toxicological testing applications.

The StrataTest Human Skin Model

StrataTest is a full-thickness human skin model containing both epidermal and dermal components. The fully-stratified epidermal compartment is composed of NIKS keratinocytes. The dermal compartment is composed of normal human dermal fibroblasts. RNA was extracted from triplicate subconfluent monolayer cultures of NIKS and primary keratinocytes. Gene expression profiling and analysis was performed by Nimblegen (Madison, WI) using the 20041100 Human 60k microarray platform. Differences in expression (increases or decreases) between NIKS and primary keratinocytes of two-fold or greater were considered significant. Concordance for expression of 51 Phase I and Phase II metabolic enzymes in NIKS and primary human keratinocytes was 98%.

1. Phase I and II Metabolic Gene Expression Comparison between NIKS and Primary Keratinocytes.

<table>
<thead>
<tr>
<th>Phase I Metabolic Enzymes</th>
<th>Gene Name</th>
<th>Symbol</th>
<th>Fold Expression</th>
<th>NIKS/Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I Metabolic Enzymes</td>
<td>NADH dehydrogenase 1, mitochondrial NDH1</td>
<td>NDH1</td>
<td>0.734</td>
<td>0.734</td>
</tr>
<tr>
<td>Phase I Metabolic Enzymes</td>
<td>NADH dehydrogenase 2, mitochondrial NDH2</td>
<td>NDH2</td>
<td>0.750</td>
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<tr>
<td>Phase I Metabolic Enzymes</td>
<td>NADH dehydrogenase 3, mitochondrial NDH3</td>
<td>NDH3</td>
<td>0.750</td>
<td>0.750</td>
</tr>
<tr>
<td>Phase I Metabolic Enzymes</td>
<td>NADH dehydrogenase 4, mitochondrial NDH4</td>
<td>NDH4</td>
<td>0.750</td>
<td>0.750</td>
</tr>
</tbody>
</table>

2. Induction of CYP1A1 in NIKS Keratinocytes Following Exposure to TCDD.

Relative CYP1A1 Expression

NIKS keratinocytes were incubated for 24h with medium containing 10 μM TCDD in a final concentration of 0.1% dimethyl sulfoxide (DMSO) or 0.1% DMSO alone as a negative control. Cultures were then harvested and RNA was analyzed by qPCR amplification using CYP1A1-specific primer pairs. Data were calculated after threshold cycle (Ct) was determined. Relative expression was calculated using the ΔΔCt method.

3. Terminal Differentiation of NIKS Keratinocytes Generates StrataTest, a Fully-Stratified, Multi-layered Human Skin Tissue.

Histological analysis (hematoxylin and eosin staining) confirmed appropriate tissue architecture formation with distinct basal, spinous, granular, and cornified layers characteristic of stratified squamous epithelia. GAPDH served as a loading control.

4. Viability and Barrier Function Analysis of StrataTest Skin Tissue Demonstrates Lot-to-Lot Consistency.

StrataTest skin tissues were incubated for 24h with medium containing 5 μM or 10 μM 3MC in 0.1% DMSO or 0.1% DMSO alone. Tissues were then harvested for gene expression analysis. RNA was then isolated and subjected to qPCR amplification using CYP1A1-specific primer pairs. Each individual experiment CYP1A1 expression levels were normalized and fold induction was determined by comparing TCDD-exposed level to that found in DMSO-treated cultures. Data (mean ± SD) are from a single experiment performed in triplicate and are representative of at least 3 independent experiments. (p<0.0001 ANOVA)

5. CYP1A1, CYP1B1, and NAT1 Expression in the StrataTest® Human Skin Model Following Exposure to 3MC.

Conclusions

- The StrataTest human skin model displays the physical and physiological characteristics of native human skin.
- Low lot-to-lot variability between independent batches of StrataTest skin tissue ensures consistent performance in a range of research and screening applications.
- NIKS keratinocytes and the full-thickness StrataTest human skin model have the capacity for metabolic activation of xenobiotic compounds.

These results demonstrate the similarity of the StrataTest human skin model to native human skin and further support use of this reproducible and reliable model for toxicological screening applications.

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References

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