Transcriptomic-based biomarker for detection of genotoxicants

Commonly used acronym: GENOMARK
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SCOPE OF THE METHOD

<table>
<thead>
<tr>
<th>The Method relates to</th>
<th>Human health</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Method is situated in</td>
<td>Basic Research</td>
</tr>
<tr>
<td>Type of method</td>
<td>In vitro - Ex vivo</td>
</tr>
<tr>
<td>This method makes use of</td>
<td>Human derived cells / tissues / organs</td>
</tr>
<tr>
<td>Specify the type of cells/tissues/organs</td>
<td>HepaRG</td>
</tr>
</tbody>
</table>

DESCRIPTION

Method keywords
- genotoxicity
- gene expression biomarker
- MTT assay
- qPCR array
Toxicogenomics
human-derived metabolically competent cells

**Scientific area keywords**

in vitro toxicology
genotoxicity
transcriptomics
hazard assessment

**Method description**

The GENOMARK tool in metabolically competent human HepaRG cells is based on the transcriptomic results obtained with 12 genotoxic (*in vivo* positive results) and 12 non-genotoxic (*in vivo* negative results) reference compounds. Genotoxic compounds were selected to cover different mechanisms of action including bulky adduct formation, DNA alkylation, cross-linking, radical generation causing DNA strand breaks, inhibition of tubulin polymerization and base analogues. The GENOMARK biomarker includes 84 genes (+ 5 housekeeping genes) of which the expression can be assessed in an easy-to-handle qPCR array. Briefly, after cultivation for 7 days, cells are exposed for 72 hours to the test compound at the IC10 concentration as determined by an MTT test (cytotoxicity formazan-forming test). Next, RNA is isolated and transcribed to cDNA which is applied on the qPCR pre-spotted plate to investigate the expression of the 84 genes included in the GENOMARK tool. Gene expression results are analyzed in R (software environment for statistical computing and graphics). All experiments are performed in triplicate with different batches of HepaRG™ cells to ensure reproducibility.

**Lab equipment**

- Standard equipment for working with cell cultures;
- Vacuum system;
- Water bath ;
- Nanodrop spectrophotometer ;
- Centrifuge ;
- Vortex mixer ;
- Shaker for microtiter plate ;
- Bio-rad CFX qPCR instrument ;
- PCR cycler ;
- R software ;
- GraphPad software ;
- Cold rack (-20°C) ;
- Automatic cell counter or microscope with cell count chamber.

**Method status**

Internally validated
Published in peer reviewed journal

**PROS, CONS & FUTURE POTENTIAL**

**Advantages**

- Selected genes represent different pathways involved in the DNA damage response ;
- All pro-genotoxins included as reference or test compound were correctly classified.

**Challenges**

- No standalone test, but an important part of an integrated testing strategy ;
- Further validation needed ;
- Only one dose is tested, problems with unstable compounds that might result in an unreliable IC10 value (introducing an IC10 control in the main test).

**Modifications**
- Faster procedure to determine IC10 concentration;
- Higher throughput methodology to investigate gene expression.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References


Associated documents

Ates2018_Article_ANovelGenotoxin-specificQPCRAr.pdf

Links

Short presentation GENOMARK at the ASCCT congress
Short presentation GENOMARK at the EUROTOX congress

PARTNERS AND COLLABORATIONS