Transcriptomic-based biomarker for detection of genotoxicants

Commonly used acronym: *GENOMARK*

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Organisation

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SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Basic Research
Type of method	In vitro - Ex vivo
Specify the type of cells/tissues/organs	HepaRG

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 HepaRGTM 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

A novel genotoxin-specific qPCR array based on the metabolically competent human HepaRG™ cell line as a rapid and reliable tool for improved in vitro hazard assessment. Gamze Ates, Birgit Mertens, Anja Heymans, Luc Verschaeve, Dimiter Milushev, Philippe Vanparys, Nancy H. C. Roosens, Sigrid C. J. De Keersmaecker, Vera Rogiers, Tatyana Y. Doktorova.

Associated documents

Ates2018_Article_ANovelGenotoxin-specificQPCRAr.pdf

Links

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

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