Mutation Research 809 (2016) 1–15

Contents lists available at ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Standardized cell sources and recommendations for good cell culture practices in genotoxicity testing

E. Lorge[a], M.M. Moore[b], J. Clements[c], M. O'Donovan[d], M.D. Fellows[e], M. Honma[f], A. Kohara[g], S. Galloway[h], M.J. Armstrong[h], V. Thybaud[i], B. Gollapudi[j], M.J. Aardema[k], J.Y. Tanir[l,*]

[a] Servier Group, 45520, Gidy, France
[b] Ramboll Environ, Little Rock, AR, 72201, USA
[c] Covance Laboratories Ltd, Harrogate, HG3 1PY, UK
[d] O'Donovan GT Consulting Ltd., Epperstone, Nottingham, NG14 6AG, UK
[e] AstraZeneca, Drug Safety and Metabolism, Cambridge, CB4 0WG, UK
[f] National Institute of Health Sciences, Tokyo, Japan
[g] JCRB Cell Bank, National Institutes of Biomedical Innovation, Health and Nutrition, Osaka, Japan
[h] Merck Research Laboratories, W 45-316, West Point, PA 19486, USA
[i] Sanofi, 94400, Vitry sur Seine, France
[j] Exponent, Inc., 1910 St. Andrews St., Midland, MI 48640, USA
[k] Marilyn Aardema Consulting LLC, Fairfield, OH 45014, USA
[l] ILSI Health and Environmental Sciences Institute, Washington, DC 20005, USA

ARTICLE INFO

Article history:
Received 29 April 2016
Received in revised form 29 July 2016
Accepted 2 August 2016
Available online 3 August 2016

Keywords:
Genotoxicity
Cells
L5178Y TK$^{+/-}$
3.7.2C
TK6
CHO-WBL
CHL/IU

ABSTRACT

Good cell culture practice and characterization of the cell lines used are of critical importance in *in vitro* genotoxicity testing. The objective of this initiative was to make continuously available stocks of the characterized isolates of the most frequently used mammalian cell lines in genotoxicity testing anywhere in the world ('IVGT' cell lines). This project was organized under the auspices of the International Life Sciences Institute (ILSI) Health and Environmental Sciences Institute (HESI) Project Committee on the Relevance and Follow-up of Positive Results in In Vitro Genetic Toxicity (IVGT) Testing.

First, cell isolates were identified that are as close as possible to the isolate described in the initial publications reporting their use in genotoxicity testing. The depositors of these cell lines managed their characterization and their expansion for preparing continuously available stocks of these cells that are stored at the European Collection of Cell Cultures (ECACC, UK) and the Japanese Collection of Research Bioresources (JCRB, Japan). This publication describes how the four 'IVGT' cell lines, *i.e.* L5178Y TK$^{+/-}$ 3.7.2C, TK6, CHO-WBL and CHL/IU, were prepared for deposit at the ECACC and JCRB cell banks. Recommendations for handling these cell lines and monitoring their characteristics are also described. The growth characteristics of these cell lines (growth rates and cell cycles), their identity (karyotypes and genetic status) and ranges of background frequencies of select endpoints are also reported to help in the routine practice of genotoxicity testing using these cell lines.

1. Introduction

Good cell culture practices have already been shown to be of critical importance for the relevance of results obtained in cell systems [1–3]. The purpose of the current project was to make continuously available well-characterized and documented stocks of the main cell types used in genotoxicity testing. The project was organized under the auspices of the International Life Sciences Institute (ILSI) Health and Environmental Sciences Institute (HESI) Project Committee on the Relevance and Follow-up of Positive Results in In Vitro Genetic Toxicity (IVGT) Testing, now the Genetic Toxicology Technical Committee (GTTC). In addition, the authors describe the expected characteristics for the different cell types and give recommendations for their use.

* Corresponding author at: ILSI Health and Environmental Sciences Institute, 1156 Fifteenth St NW, Suite 200, Washington, DC 20005, USA.
E-mail address: jtanir@hesiglobal.org (J.Y. Tanir).

http://dx.doi.org/10.1016/j.mrgentox.2016.08.001

The first major issue to consider for practicing good quality genotoxicity testing is the source of the cell line, as the history/handling of the cells may influence their characteristics and, consequently, the results of the assays conducted with them. For example, data provided in a report on an International Workshop on Genotoxicity Testing (IWGT) [4], describe the marked change in modal number of certain CHO cells between passages 3 and 48. The CHO-WBL cell line described in the present paper has a modal number of 21, and its growth and cytogenetic characteristics have been shown to remain stable up to at least 32 passages from cloning when grown in the standardized conditions described here.

Therefore, the first objective was to prepare well-characterized and documented stocks of cells that would be easily available for any potential user in the different regions of the world. For this purpose, for each cell line considered, an isolate was identified that would be as close as possible to the isolate described in the initial publications, and the cells were characterized with the help of the depositors. This characterization provides the ultimate users with reference data on the performance of the cells (*e.g.*, growth properties, background levels of mutation/chromosome damage and karyotypic stability) that can be used for quality control when expanding and maintaining the cells. The expansion of the selected early source cells was then organized and they were stored in cell repositories to ensure the continuous availability of cells for use in genotoxicity testing from the same source and with the same characteristics in any part of the world.

Ampoules of cryopreserved cells are available at the European Collection of Cell Cultures (ECACC), Salisbury, UK (www.phe-culturecollections.org.uk/collections/ecacc.aspx) and at the Japanese Collection of Research Bioresources (JCRB) Cell Bank, Japan (www.cellbank.nibio.go.jp). Cells stored at ECACC are available through Sigma in the US. The cell lines are all tagged as 'IVGT' cell lines and their reference numbers are summarized in Table 1.

For day-to-day practice, in addition to good quality standards applied to any cell systems, the cells used for genotoxicity testing should demonstrate their ability to reliably quantify the occurrence of genetic damage. It is therefore essential to avoid "genetic drift" and to minimize selective pressure which could both affect key cell properties (*e.g.*, the rate of cell proliferation, and the background levels of mutation or chromosome damage), and as a result impact the results obtained using the test system to detect genotoxic events.

This paper makes recommendations on the appropriate use of cell cultures in genotoxicity testing, to help ensure good quality results, easy transferability of the techniques and appropriate reproducibility among laboratories. These recommendations should be of great utility for laboratories starting genotoxicity testing and they provide useful references also for experienced laboratories.

2. Results of a survey on the use of cell lines in genotoxicity testing and selection of the cells to be stored in the repository

First of all, a survey was organized to evaluate the needs for cell lines in genotoxicity testing and to have a better understanding of the practices in use in the genotoxicity laboratories. This survey was distributed by the EEMGS (European Environmental Mutagenesis and Genomics Society, named European Environmental Mutagen Society at the time of the survey), JPMA (Japan Pharmaceutical Manufacturers Association) and EMGS (Environmental Mutagenesis and Genomics Society, named Environmental Mutagen Society at the time of the survey) to their members in 2010. A total of 70 responses were obtained and are summarized in Figs. 1 and 2. Fig. 1 shows that the most widely used cells in genotoxicity research and testing are the mouse lymphoma L5178Y $TK^{+/-}$ 3.7.2C cells, mostly for the mouse lymphoma cell thymidine kinase (TK) mutation assay. The next most commonly used cells are TK6 human lymphoblastoid cells, mainly for the *in vitro* micronucleus assay and gene mutation tests. They are preferred by some laboratories because they are human-derived and have functional p53 in contrast to rodent cell lines that have defective p53 function [4]. However, it has been clearly shown, using a set of cell lines varying only in their p53 status, that the p53 status does not always correlate with the cell line sensitivity [5], and that the species' origin of the cells (human versus rodent) could also explain the differences in sensitivity [6]. Rodent fibroblastic cell lines, CHL-IU, CHO and V79 cells are usually used for both chromosome aberration and micronucleus cytogenetic tests, and for gene mutation tests. A well identified and characterized source of V79 cells could not be identified. Two different types of CHO cells are in use, *i.e.* CHO-WBL and CHO-K1. However, only the clonal origin of CHO-WBL cells is well known and recorded. Several sets of CHO-K1 cells with different histories exist, so that it has not been possible to identify an original isolate and a description of cell characteristics. Therefore it is recommended that CHO-WBL cells are preferred for use in genotoxicity testing, whenever possible. Hep G2 cells are less widely used and generally not included in the standard genotoxicity battery but are considered, for example, for investigative studies on the influence of metabolic activation on genotoxicity results because they are of human origin and somewhat metabolically competent. A few laboratories reported (as shown in Fig. 1) that cells such as Mutamouse® FE1, bronchial or lung cell lines are used for specific purposes. However they are not part of a standard battery of genotoxicity testing.

Fig. 2 shows that most users established their initial cell samples only once, from one or two vials, and had only rarely renewed the initial sample. This indicates that most laboratories participating in this survey expanded their own working stocks from a master-stock derived from the initial supplied sample(s).

Based on the results of the above survey and the ability to retrieve well-characterized cells, CHO-WBL and CHL hamster cells, L5178Y $TK^{+/-}$ 3.7.2C mouse lymphoma cells, and human TK6 cells were selected to be stored in the repositories and their characteristics are described in this paper.

3. Characterization and storage of cell lines in repositories for genotoxicity testing

Organizing the continuous availability of cell lines recommended for genotoxicity testing required several steps. The first as described above was to identify and select a good quality and well-characterized cell isolate for each of the most commonly used cell lines. Then, cells were provided to the cell repositories. The

Table 1
Summary of the references of the 'IVGT' cell lines.

Cell Line	Depositor	ECACC Accession Number	JCRB Accession Number
L5178Y $TK^{+/-}$ 3.7.2C (IVGT)	M. O' Donovan, AstraZeneca	12080201	JCRB0709.2
TK6 (IVGT)	Masamitsu Honma,NIHS, Japan	13051501	JCRB1435
CHO-WBL (IVGT)	S. Galloway, Merck, USA.	14043001	JCRB1702
CHL/IU (IVGT)	Arihiro Kohara, JCRB, Japan	14100201	JCRB0030