Available online at www.sciencedirect.com
SCIENCE DIRECT®
Mutation Research 572 (2005) 45–57
Fundamental and Molecular Mechanisms of Mutagenesis
www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

Application of a two-stage Syrian hamster embryo cell transformation assay to cigarette smoke particulate matter

Damien Breheny [a], Haizhou Zhang [b], Eian D. Massey [a,*]

[a] *British American Tobacco, R&D Centre, Regents Park Road, Southampton SO15 8TL, UK*
[b] *Genetic Toxicology, Covance Laboratories Inc., 9200 Leesburg Pike, Vienna, VA 22182, USA*

Received 26 May 2004; received in revised form 7 December 2004; accepted 17 December 2004
Available online 29 January 2005

Abstract

The induction of transformation in Syrian hamster embryo (SHE) cells is a multifactorial process, in comparison to endpoints induced in in vitro genotoxicity assays such as Ames, mouse lymphoma and cytogenetics [Y. Berwald, L. Sachs, In vitro cell transformation with chemical carcinogens, Nature (London) 200 (1963) 1182–1184]. Furthermore, a number of non-genotoxic carcinogens and promoters such as clofibrate and diethylhexylphthalate, have been positively identified in this assay, while giving false negative results in traditional genotoxicity assays [H. Yamasaki, J. Ashby, M. Bignami, W. Jongen, K. Linnainmaa, R.F. Newbold, G. Nguyen-Ba, S. Parodi, E. Rivedal, D. Schiffmann, J.W.I.M. Simons, P. Vasseur, Nongenotoxic carcinogens: development of detection methods based on mechanisms: a European project, Mutat. Res. 353 (1996) 47–63]. A high concordance between results obtained in this assay when compared with rodent carcinogenesis bioassays has also been noted [R.J. Isfort, G.A. Kerckaert, R.A. LeBoeuf, Comparison of the standard and reduced pH Syrian hamster embryo (SHE) in vitro cell transformation assays to predict the carcinogenic potential of chemicals, Mutat. Res. 356 (1996) 11–63].

Carcinogenesis is known to be a multistage process, with agents potentially acting at each stage. Specifically, mouse skin painting experiments established that tumour induction could be mechanistically divided into two distinct phases, termed initiation and promotion. Initiation, is defined as the stage at which a normal cell is converted to a latent tumour cell, followed by promotion where the latent tumour cell progresses to a tumour [W.F. Friedwald, P. Rous, The initiating and promoting elements in tumour production: analysis of the effects of tar, benzpyrene and methylcholanthrene on rabbit skin, J. Exp. Med. 80 (1944) 101–125].

A protocol for the pH 6.7 SHE transformation assay has been developed which allows separation of cell transformation process into two phases, potentially analogous to initiation and promotion in vivo. This allows chemicals found to be positive in the traditional SHE cell transformation assay to be further classified as initiators or promoters.

Following validation with known initiators, benzo(a)pyrene and *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine and promoters, 12-*O*-tetradecanoyl-phorbol-13-acetate and phenobarbitone, the two-stage model was applied to cigarette smoke particulates which was found to act both at the initiation and promotion stage of cell transformation.

Keywords: Syrian hamster embryo (SHE) cells; In vitro; Cell transformation; Cigarette smoke; Initiation and promotion

* Corresponding author. Tel.: +44 2380 793715; fax: +44 2380 793076.
E-mail address: eian_massey@bat.com (E.D. Massey).

0027-5107/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.mrfmmm.2004.12.004

1. Introduction

Morphological cell transformation is the term used to describe the process by which normal cultured cells are altered in both their behaviour and growth characteristics. These alterations can manifest themselves phenotypically as changes in cell morphology and disorganised growth patterns, and in some cases, loss of anchorage independence [5]. The induction of morphological transformation in Syrian hamster embryo (SHE) cells was first reported by Berwald and Sachs in 1963 [1]. This endpoint is more general than those induced in traditional in vitro genotoxicity assays such as the *Salmonella typhimurium* mutation assay (Ames assay) and mouse lymphoma assay which detect mutational events, and the in vitro cytogenetics assays (e.g. micronucleus assay) which have structural and/or numerical chromosomal aberrations as their endpoints. This property may be of high predictive value, as carcinogenesis is a multistage process, resulting from a series of individual events which contribute to the eventual transformed phenotype. Changes in this endpoint may be indicative of the early stages in the carcinogenic process which may not be easily identifiable in other assay systems.

The SHE cell transformation assay is often capable of detecting promoters and carcinogens that are not picked up by tests for genotoxicity (e.g. clofibrate and diethylhexylphthalate), and is therefore considered a promising in vitro test for potential non-genotoxic carcinogens [2,6]. Its predictive ability is underlined by the high correlation between results from chemicals tested in the SHE assay and the traditional rodent bioassay [3]. This was further confirmed by the results from the International Life Sciences Institute Health and Environmental Sciences Institute (ILSI/HESI) Alternative Carcinogenicity Testing (ACT) programme [7], which included the SHE assay. In addition, since SHE cells retain a competent metabolic system, enabling the activation of procarcinogens to carcinogens, this negates the need for exogenous metabolic systems such as rat liver S9.

In the early years after its initial inception, the assay had to overcome some experimental difficulties with the SHE cells which made it problematic to perform. Many of these experimental difficulties were eliminated or reduced by using serum of high quality. Also, lowering the pH of 6.65–6.75 was said to improve performances [3].

The SHE cell transformation assay is the most established transformation assay utilising primary cells, in terms of predicting known rodent carcinogens, and currently the most sensitive and specific [6]. Transformation assays with immortalised cell lines have also been employed over the years, in particular assays using Balb/c 3T3 and C3H/10T1/2 cells [8,9]. In contrast to the SHE assay, cells are assessed for their ability to form foci of transformed cells in a confluent monolayer following treatment with a test agent. Therefore, these assays are considered to detect a later stage in cell transformation than the SHE cell transformation assay [8,9].

All three assays are similar in that they involve the use of rodent cells, which is a necessary compromise while human cell-based cell transformation assays are being developed [10,11]. The use of human cells has been hampered by the fact that they do not spontaneously immortalise, and need to be genetically altered to achieve an immortalised phenotype. Currently, two assay systems based on human cell lines are being developed [5]. These are the HaCaT keratinocyte cell transformation model [10] and the MSU-1 human fibroblast cell transformation model [11].

As cell transformation assays have developed over the years, it has become apparent that the transformation process can be divided up into at least two stages. It has been possible, therefore, to determine the stage in the transformation process at which a test agent exerts an effect. The first conclusive demonstration of two-stage transformation in a primary cell line was demonstrated in 1977 [12] in a study using rat embryo fibroblasts. Since then, the SHE cell assay [13–15] and the 3T3 and C3H/10T1/2 cell assays [16–19] have all been adapted to allow test agents to be evaluated for their effect at each of these two stages.

A similar two-stage process has been found to occur in carcinogenesis, where considerable knowledge has stemmed from mouse skin painting studies originally carried out in the 1920s and 1930s [20–22]. The terms ‘initiation’ and ‘promotion’ were adopted for these two stages of carcinogenesis [4], and these have also been applied to the two stages of cell transformation.

A 2001 OECD review of the performance of cell transformation assay systems has recommended the