Mutation Research 773 (2017) 91–103

Contents lists available at ScienceDirect

Mutation Research/Reviews in Mutation Research

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

Review

Transient and stable vector transfection: Pitfalls, off-target effects, artifacts

Aleksei A. Stepanenko[a,*], Henry H. Heng[b]

[a] Laboratory of Biosynthesis of Nucleic Acids, Department of Functional Genomics, Institute of Molecular Biology and Genetics, Zabolotnogo Str. 150, Kyiv 03680, Ukraine
[b] Center for Molecular Medicine and Genetics and Department of Pathology, Wayne State University School of Medicine, Detroit, MI 48201, United states

ARTICLE INFO

Article history:
Received 30 April 2016
Received in revised form 9 May 2017
Accepted 13 May 2017
Available online 16 May 2017

Keywords:
Aneuploidy
Chromosomal instability
Geneticin/G418
Puromycin
Tumor heterogeneity
Zeocin

ABSTRACT

Transient and stable vector transfections have played important roles in illustrating the function of specific genes/proteins. The general assumption is that such a platform could effectively link a given gene/protein to gained phenotypes, revealing the mechanism of how a gene works. However, in reality, increased studies have surprisingly noticed some unexpected results. In this review, we demonstrate that an assumption that empty vector-transfected cells preserve the cytogenetic and phenotypic characteristics, and represent the adequate control in transfection experiments is not universally valid. A DNA vector, a transfection reagent, expression of an antibiotic resistance (trans)gene, expression of a reporter (trans)gene, and selection by acute/chronic antibiotic treatment may evoke cellular responses that affect the biochemical processes under investigation. We exemplify a number of studies, which reported obvious genomic, transcriptomic and phenotypic changes of tumor cells after transient/stable transfection of an empty vector. To further address the common mechanisms of these unexpected findings, we will apply the genome theory of somatic evolution to explain stress-mediated system dynamics and the limitations of predicting the system behavior solely based on targeted genes. We conceptualize that the diverse experimental manipulations (e.g., transgene overexpression, gene knock out/down, chemical treatments, acute changes in culture conditions, etc.) may act as a system stress, promoting intensive genome-level alterations (chromosomal instability, CIN), epigenetic and phenotypic alterations, which are beyond the function of manipulated genes. Such analysis calls for more attention on the reduced specificities of gene-focused methodologies.

Contents

Abbreviations: CIN, chromosomal instability; GFP, green fluorescent protein; Pac, the puromycin N-acetyltransferase; Sh ble, Streptoalloteichus hindustanus bleomycin resistance gene; Neo, aminoglycoside 3′-phosphotransferase gene.

* Corresponding author.
E-mail addresses: a.a.stepanenko@gmail.com (A.A. Stepanenko), hheng@med.wayne.edu (H.H. Heng).

http://dx.doi.org/10.1016/j.mrrev.2017.05.002
1383-5742/

1. Introduction

A transient (plasmid DNA is transiently maintained in the nucleus) or stable (plasmid DNA is integrated into the genome of the host cells) transfection of recombinant vector DNA with the insertion of the recombinant transgene of interest into the cultured cell lines is a prevailing tool of choice for molecular and cellular investigation of gene/protein functions, regulation, and oncogenic/tumor suppressive properties. To evaluate the functions and effects of an ectopically expressed transgene, an empty vector-transfected control cell line (a negative control, which does not contain a transgene of interest but may contain a reporter (trans)gene: e.g., GFP or luciferase) is routinely generated. It is widely believed that transfection of an empty vector DNA has negligible effect on cells or the putative effects are similar in empty vector-transfected and transgene-transfected cells due to the use of the same plasmid backbone, transfection reagent, transfection protocol, selection conditions, etc. In such experimental settings, molecular, genetic and phenotypic changes observed in transgene-transfected cells but not empty vector-transfected control cells are taken for granted to result from the activities of transgene itself. However, a series of molecular manipulations will likely impact the biological system, leading to various non-specific effects. For example, the nonspecificity of the plasmid DNA illegitimate integration into the host genome, varying multiplicity of introduced copies of plasmid and randomness of chromosomal aberrations during stable transfection experiments may contribute to phenotypic differences between empty vector-transfected and transgene-transfected cells. Furthermore, a variety of artifacts and side effects, resulting from the sequences within the plasmid vector backbone, application of cytotoxic transfection chemical reagents (liposome- and non-liposome based), overexpression of antibiotic resistance (trans)gene (e.g., *Pac*, puromycin N-acetyltransferase gene or *Neo*, aminoglycoside 3′-phosphotransferase gene), overexpression of a reporter (trans)gene (e.g., *GFP*, *luciferase*), and cytotoxic antibiotic treatment (e.g., G418/geneticin, puromycin, hygromycin, and zeocin) were documented. Finally, the procedure of single-cell cloning of stable transfectants may also contribute to phenotypic differences between empty vector-transfected and transgene-transfected cells due to single-cell genomic and phenotypic heterogeneity of cultured cells. The phenotype changes not relevant to the biology of the transgene of interest may mask mechanisms underlying the action of the transgene and/or complicate the downstream data analysis and interpretation.

Unfortunately, the scientific community has not appreciated yet the full spectrum of potential artifacts of recombinant DNA transfection. The results on unexpected effects in plasmid transfection experiments are often either underreported by authors or ignored by readers. To our best knowledge, the only systematic review addressing pitfalls and artifacts of plasmid transfection was published almost three decades ago [1]. For comparison, a number of publications of off-target activity and artifacts of short interfering RNA (siRNA) or short hairpin RNA (shRNA) in mammalian cells is overwhelming. In this review, we demonstrate that an assumption that empty vector-transfected cells preserve the cytogenetic and phenotypic characteristics, and represent the adequate control in transfection experiments is not universally valid. A DNA vector, a transfection reagent, expression of an antibiotic resistance (trans)gene, expression of a reporter (trans)gene, and selection by acute/chronic antibiotic treatment may evoke cellular responses that affect the biochemical processes under investigation. We exemplify a number of studies, which reported obvious genomic, transcriptomic and phenotypic changes of tumor cells after transient/stable transfection of an empty vector. In summary, we conceptualize from the grounds of the genome theory of somatic evolution that the diverse experimental manipulations (e.g., transgene overexpression, gene knock out/down, chemical treatments, acute changes in culture conditions, etc.) may act as a system stress, promoting intensive genome-level alterations (chromosomal instability, CIN), epigenetic, transcriptomic, proteomic, metabolomic and, consequently, phenotypic alterations, which are beyond the function of manipulated genes. Such analysis not only cautions the interpretation of transfection data, but also suggests the importance of monitoring genomic dynamics of recombinant DNA-transfected cells.

2. Pitfalls, off-target effects and artifacts of the procedure of transient/stable vector transfection

2.1. Transfection reagents: not as innocent as one would like

Many transfection methods have been developed, which are broadly classified into biologically (e.g., virus-mediated transfection, also known as transduction), chemically (e.g., calcium phosphate, cationic polymer, cationic lipid), and physically (e.g., electroporation, magnetofection, sonoporation, and phototransfection) mediated methods (reviewed in [2]). Here we focus on the most popular chemical approaches. A plethora of liposome- and non-liposome based commercially available transfection reagents is routinely employed for plasmid delivery due to their easy use and applicability to many different cell lines. However, most of transfection reagents have cytotoxic effects on the cells, especially when transfection reagent/plasmid amounts are not optimized for a given cell line. Although this fact is generally accepted, there were scarce efforts to analyze side effects of transfection reagents treatment per se. A systematic comparison of cytotoxicity of multiple transfection reagents is also rare. Using four different transfection reagents (FuGENE HD, Lipofectamine 2000, Effectene, and Lipofectamine LTX with Plus Reagent), Jacobsen et al. transfected MCF7 breast cancer cells with pM1-*SEAP* vector (expressing the secreted embryonic alkaline phosphatase, a reporter widely used to study promoter activity or gene expression) or pM1 empty vector without the reporter *SEAP* gene insert. The transfected MCF7 cells were compared with the non-transfected parental cells to identify potential off-target transcriptional effects by transcriptome profiling [3]. Lipofectamine 2000 affected the largest number of transcripts, followed by Effectene and then Lipofectamine LTX with Plus Reagent. FuGENE HD produced the fewest total number of differentially expressed transcripts. Thus, transfection reagents differed in the extent to which they perturbed transcriptome (e.g., >10-fold difference in the number of differentially expressed transcripts between Lipofectamine 2000 and FuGENE HD). In general, each reagent had a unique combination of transcripts that were differentially expressed. However, expression of eighty genes was found to be influenced by all four transfection reagents. A Gene Ontology enrichment analysis of this set of genes demonstrated