Life Sciences 158 (2016) 137–160

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Growing knowledge of using embryonic stem cells as a novel tool in developmental risk assessment of environmental toxicants

Mohammad Amin Rezvanfar, Mahshid Hodjat, Mohammad Abdollahi *

Department of Toxicology and Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Endocrinology & Metabolism Research Institute, Tehran University of Medical Sciences, Tehran 1417614411, Iran

ARTICLE INFO

Article history:
Received 18 January 2016
Received in revised form 27 April 2016
Accepted 17 May 2016
Available online 18 May 2016

Keywords:
Environmental toxicants
Developmental toxicity
Embryonic stem cells
Embryotoxicity
Review

ABSTRACT

Developmental toxicology is an important area of novel toxicology. In recent years, there have been big concerns toward the increasing exposure to pharmaceutical agents, food additives, pesticides, occupational toxicants, and environmental pollutants, as well as their possible association with all aspects of male or female-mediated transient or permanent defects in progeny. Therefore, it is of great importance to look for new predictive models to evaluate environmental toxicants before they can harm the human health and embryo development. In this regard, new cell-based in vitro screening models have been developed and validated in predictive toxicology to minimize assay costs and animal usage.

Stem cell-based models have been increasingly applied for predicting the toxicity of chemicals. One of the most promising existing in vitro developmental toxicity tests is the validated embryonic stem cell test (EST) which employs marine or human embryonic stem cells to assess the potential of chemicals embryotoxicity. These cells are very suitable for embryotoxicity assessment as they have been demonstrated to specify cellular developmental processes during early embryogenesis and gene expression patterns of differentiation to functionally competent specialized cell types.

The present paper aimed at criticizing the human and experimental evidence for developmental toxic effects of environmental toxicants based on ESCs models. Accordingly, pesticides, heavy metals, plasticizers, nanomaterials and some solvents have been considered as the main evaluated environmental toxicants inducing developmental toxicity. At the end, current challenges, pros and cons of using ESCs as an alternative validated in vitro model for specific developmental toxicity screening are discussed.

1. Introduction

Environmental toxicants are generally defined as any organic or inorganic substances, compounds, or agents in the environment that are potentially harmful to either human health or ecosystem. These toxicants include a large number of hazardous chemicals, physical and biological substances such as chlorine-containing compounds used in pesticides, industrial toxic substances, heavy metals (e.g., lead, cadmium, mercury, and selenium), radioactive elements, acids, and petroleum hydrocarbons, as well as various forms of energy (e.g., noise, radiation, heat).

Toxicants are surrounding our environment at home, in the workplace, and in our community via air, soil, water, and food. Each year, million tons of toxicants are released into the environment that can have a deleterious effect on human health. They have been implicated in a variety of pathologies including organ specific defects, cancers, behavioral disorders, and reproductive problems [1,2]. However, only <1% of their effects have been thoroughly studied in terms of toxicity [3]. It has been reported that of approximately 87,000 chemicals registered for commercial use in the United States, only 10% have been tested for their potential negative effects, mostly due to inability to predict their toxicity and little knowledge on their developmental and reproductive toxicities [4–6].

It is notable that, during the last century, the pattern of disease among children has been shifted from infectious disease to the so called multifactorial-origin disease such as asthma, childhood cancer, and neurodevelopmental and congenital defects [7–10]. The estimation of what percentage of these changes results from the environmental toxicants is still unknown.

Accordingly, a growing concern has been raised about the consequences of adverse effects of environmental toxicants on the reproductive system, germ cells, fertility, and fetal development. Industrialization of societies and lifestyle changes has gradually added a variety of environmental risk factors that lead to disorders, diseases, and cancers in various organs of the body. Exposing to cosmetics containing harmful chemicals such as lead, mercury, cadmium, etc.; contaminated drinking water with harmful substances such as arsenic, benzene, chromium, etc.; contaminated water and soil with pesticides and chemical fertilizers,

* Corresponding author.
E-mail addresses: Mohammad@TUMS.Ac.Ir, Mohammad.Abdollahi@UToronto.Ca (M. Abdollahi).

http://dx.doi.org/10.1016/j.lfs.2016.05.027
0024-3205/

and consequently contaminated crops, using various steroidal drugs and hormones in livestock and poultry breeding industry, as well as their residues in meat or dairy products which are excreted in milk; increasing application of preservatives in the food industry (food additives); daily contact with household cleaning reagents and a variety of harmful radiation such as X-rays and ultraviolet radiation in the workplace and in life; and the waves emitted from mobile phones and telecommunication antennas, all are factors that can directly or indirectly cause deleterious effects on fertility or on developmental and differentiation potential of growing fetus. On the other hand, there is a fundamental difference between recognized number of environmental substances and the number of substances that have been toxicologically studied.

In fact, scientists have encountered many difficulties in finding the cause and effect relationship between environmental toxicants and disease. The inefficiency of traditional toxicological tests and time latency of toxic responses are the major reasons for difficulties in monitoring the harmful health effects of toxicants. During the last decades, multiple in vivo *and* in vitro models, as well as epidemiological and computerized modeling techniques (in silico) have been employed to assess the strength and mechanism of toxicants which induce adverse effects. However, these approaches are still not very precise and have many limitations. The most common used in vitro models for toxicity testing are based on cell lines and primary cells isolated from different tissues. In these models, any changes following the exposure to toxicants in gene expression pattern, cell cytotoxicity, metabolism, and enzyme kinetics are examined. The primary disadvantage of such in vitro experimental studies is that they represent the effect of toxicants on cells derived from a single donor with low predictive value for the human population. It is also concerning that the cell culture may not possess genotypic or phenotypic characterization of the original tissue. In this case, the isolated cells might undergo some changes in their morphology, gene expression pattern, and function that differ from their original state [11,12].

For a long time, in vivo animal models have been considered as a gold standard of toxicology studies. For industrial chemicals and pesticides as environmental toxicants, the rat limb bud micro-mass (MM) tests and the post-implantation rat whole-embryo culture (WEC) test, prenatal developmental, combined repeat dose, one-generation, two-generation, reproductive and developmental toxicity screening tests should be carried out to determine their effect on particular animal species and finally on human.

However, these models involve many restrictions that hinder their application for the future toxicity evaluation purposes. The major problem is that the experimental toxicology in animals might not accurately predict the possible effect on human and the tests might not account for human response in terms of variability and susceptibility toward toxicants. On the other hand, conducting animal studies is more labor intensive, costly and time-consuming and requires experimenting on a large number of animals which raises ethical and economic problems [13]. Moreover, studying the effects of a mixture of chemical compounds on animal models is particularly so costly and time consuming. Adding to these limitations, it is difficult to observe the chronic toxicity resulted from the exposure to chemicals over a short period of time. In fact, the effects of environmental toxicants could be observed after months or years which restrict the use of in vivo models, particularly to examine a large amount of chemicals introduced to the market. Finally, studying the dose-response relationship on animal models and its relevance to human is complex and might result in many inaccuracies.

Therefore, considering the economical and ethical concerns of conventional toxicity testing, and according to the advantages of using in vitro models, including the non-independency to animal studies, shortening the time needed, small amount of a chemical needed for testing, and the ability to evaluate a variety of chemicals, many researchers have, recently, focused on improving the in vitro toxicology models.

Using stem cells has emerged as a new promising strategy in the field of toxicology. Embryonic stem cells (ESCs) are undifferentiated immature cells with a potential to differentiate into different specified cell types. They are primarily separated from the inner cell mass (ICM) of blastocyst-stage of embryos. Following treatment with particular substances they can be isolated from cell aggregates called embryoid bodies (EBs) [14], consisting progenitor cells of all three primary embryonic germ layers. Embryonic stem cell test (EST) indicates the effect of environmental toxicants through either direct adding of chemicals to stem cells followed by evaluating several endpoints (e.g., cell death, proliferation, survival, growth, morphology, etc.) or alternatively by using conditions that alter the progression of ESCs to specific differentiated cells, as well as monitoring the effect of toxicants on EBs and their differentiation into various cell types such as cardiomyocytes and neurons. Therefore, three strategies have been considered for the purpose of using stem cells in toxicological assays: pre- and post-implantation developments and differentiated cells of the embryos (Fig. 1). For the preimplantation strategy, embryonic stem cells can be exposed directly to the chemicals. In the post-implantation development model, instead of ESCs, the chemical is added to the EBs which can be carried out at any time of their formation (before or after EB formation or at both times). For the last strategy, the impacts of chemicals are investigated after differentiation of the EBs into specific cell types, such as cardiomyocytes or neurons (Fig. 1). The partially differentiated cells then can be fully differentiated to enable comparison of several endpoints in fetal and adult counterparts.

Based on the successful predictive results of EST, in 2004, the European Centre for the Validation of Alternative Methods (ECVAM) reported the scientific validation of this screening assay, as an in vitro toxicology alternative method [15]. Using EST, many chemicals were successfully classified into strongly embryotoxic, weakly embryotoxic and non-embryotoxic reagents based on their toxic severity.

Having laid the ground, this review aimed to summarize and discuss the studies that have been conducted during the past 14 years to predict the developmental toxic effects of some environmental toxicants using embryonic stem cells. In order to fulfill this aim, a systematic literature review was carried out to finally identify the possible knowledge gaps in future toxicology studies using EST.

2. Search and selection strategy

In order to gather comprehensive body of literature, the authors searched Scopus, PubMed, and Google Scholar to identify reliable articles published in English language. The main key words used in searching were “human ESCs”, “monkey ESCs” and “mouse ESCs” along with “environmental toxicants”, “pesticides”, “heavy metals”, and “solvents”. No limitations were considered regarding the type or date of publication. Moreover, supplementary articles were extracted from the reference lists of the reviewed publications. Studies that used adult stem cells, cell lines, or whole embryos, as well as reviews and non-original articles were excluded from our search. Findings were summarized in different tables according to the type of toxicants. Data tables were prepared based on toxicant concentration, exposure time, type of ESCs, as well as assessed biomarkers. Meanwhile, the potential mechanisms of the action of toxicants were fully discussed.

3. Results and discussion

According to the present literature review, there were about 47 published reports after the year 2000 that related to the screening of environmental toxicants by embryonic stem cell tests. All studies were divided into five separate categories and corresponding tables based on the primary outcomes. In this regard, Table 1 indicates pesticides (gathered from 8 studies); Table 2 indicates heavy metals (gathered from 13 studies); Table 3 indicates plasticizers (gathered from 13 studies); Table 4 indicates nanomaterials (gathered from 5 studies); and Table 5 indicates other environmental toxicants (gathered from 13 studies). In 8 of the studies, more than one toxicant of different