Trends in Pharmacological Sciences

CellPress

Opinion

Transgenic Mouse Models Transferred into the Test Tube: New Perspectives for Developmental Toxicity Testing *In Vitro*?

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Despite our increasing understanding of molecular mechanisms controlling embryogenesis, the identification and characterization of teratogenic substances still heavily relies on animal testing. Embryonic development depends on cell-autonomous and non-autonomous processes including spatiotemporally regulated extracellular signaling activities. These have been elucidated in transgenic mouse models harboring easily detectable reporter genes under the control of evolutionarily conserved signaling cascades. We propose combining these transgenic mouse models and cells derived thereof with existing alternative toxicological testing strategies. This would enable the plausibility of *in vitro* data to be verified in light of *in vivo* data and, ultimately, facilitate regulatory acceptance of *in vitro* test methods.

Developmental Toxicity Testing Strategies

A total of 11 million animals were used in Europe in 2011 for experimental purposes, including 1 million for toxicological risk assessment [1]. Most of the animals are used in biomedical research and a significant number can be attributed to the development and use of transgenic animals that play a pivotal role in the characterization of human diseases [2]. In addition, it is expected that current chemical legislation in Europe [i.e., Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH)] might lead to a significant increase in animal experimentation, with estimates ranging from 4 million to up to 54 million additional vertebrates needed [3,4]. Testing for teratogenic activities according to Organization for Economic Cooperation and Development (OECD) guidelines, particularly the prenatal development toxicity assay [5], the reproduction/developmental toxicity screening test [6], and the combined repeated dose toxicity assay with the reproduction/developmental toxicity screening test [7], already require high numbers of animals. Accordingly, the analysis of potential developmental toxicity constituted over 40% of all testing proposals submitted to the European Chemicals Agency by 2013 [8].

In past years various approaches have been developed to address embryonic toxicity *in vitro*. In 2004 the murine embryonic stem cell test (EST) was validated by the European Center for the Validation of Alternative Methods (ECVAM) [9]. The EST uses the following three independent end points to classify chemical substances: (i) the IC_{50} for somatic 3T3 cells; (ii) the IC_{50} for embryonic D3 cells; and (iii) the ID_{50} for the inhibition of the capacity of D3 cells to differentiate

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Transgenic mouse models are ubiquitously used to understand signaling events during development and modern reporter gene approaches enable *in vivo* detection and quantification.

Disturbances during developmental processes or cell differentiation cause spatiotemporal alterations of signaling events, which therefore can be used to predict teratogenic effects of substances.

Utilizing recent advantages in embryonic and induced pluripotent stem cell generation and cultivation, transgenic cells carrying *in vivo*-characterized reporter constructs can be produced.

A plethora of *in vitro* assays has been developed for toxicological testing and can be advanced using the aforementioned transgenic cells to increase the information content and relevance of *in vitro* tests.

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spontaneously into beating cardiomyocytes [10]. However, more cell types of the developing embryo need to be addressed [11] and therefore protocols for the differentiation of embryonic stem cells into neurons [12], bone cells [13,14], and endothelial cells [15] have been developed.

In parallel, the ToxCast program of the US Environmental Protection Agency (EPA) [16] and the Tox21 consortium [17] focus on high-throughput and high-content analysis of multiple *in vitro* assays with diverse end points to predict the *in vivo* toxicity of the chemical compounds under consideration. Thousands of chemicals are evaluated in hundreds of assays to generate toxicity profiles and to identify molecular and cellular pathways that mediate toxicity [18]. Using these data, Kleinstreuer *et al.* [19] were able to develop an *in silico* model to predict a chemical's impact on vascular development. Establishing the zebrafish as a model for developmental toxicity testing, one strategy uses phenotypic analysis of maturing embryos by utilizing high-content imaging methods [20,21]. In a second approach, modern sequencing and microarray methods are used to gather mechanistic information that needs to be analyzed and correlated with phenotypes [22] to identify toxicity pathways mediated by the tested compounds. These approaches are likely to be instrumental in the future in predicting teratogenic activities of chemicals.

Processes during development and differentiation are orchestrated by a few highly conserved, essential cellular signaling cascades and minute alterations may have strong impacts on the developing organism. The underlying mechanisms have been well characterized and, especially, transgenic mouse models have been used to uncover signaling activities *in vivo*. We recently showed that teratogenic effects can be monitored *in vitro* when analyzing reporter gene activities that represent specific cellular signaling pathways [23,24]. Here we elaborate on the future potential to combine toxicological testing methods with transgenic mouse models and cells derived thereof (Figure 1).

Transgenic Mice: General Aspects and Toxicity Testing

In basic and biomedical research, high-content and high-throughput approaches already play an important role in the characterization of biological processes and the detection of druggable new lead structures [25–27]. Since these methods are particularly suited to unraveling molecular mechanisms and regulatory networks that mediate physiological, pathological, or toxicological processes, they have also entered the field of pharmacology and toxicology [28]. Despite these developments, our knowledge of molecular mechanisms and biological functions, including embryonic development, remains highly dependent on the study of genetically modified mouse models. Since the dawn of modern molecular techniques that enabled the generation of transgenic mice by pronuclear injection [29] and specific genomic manipulations by homologous recombination [30], thousands of mouse strains have been generated that either mimic specific aspects of human diseases or facilitate the analysis of the function of single genes as well as complex signaling pathways. With the more recent development of the CRISPR/Cas technique, a new era might already have begun, with an enormous additional number of modified mouse lines to be expected in the years ahead [31]. The Jackson Laboratory, which supplies the largest compilation of transgenic animal models, already comprises over 7000 different lines of humanized mouse models ranging over diverse disease models. In contrast to the importance and widespread use of genetically modified mouse strains, only a few are applied to the toxicological assessment of chemicals [32]. To date only the transgenic rodent mutation assay created to detect chemical mutagens [33] has received regulatory acceptance. Another well-established, although not validated, mouse model expresses luciferase (Luc) under the control of an estrogen response element (ERE) promoter, thereby allowing the investigation of estrogen receptor-dependent transcriptional activity *in vivo* through optical imaging in living animals [34]. This mouse model enables the quantification and specific profiling of the acute or prolonged endocrine disruptor activity of suspected compounds *in vivo* [35,36], thereby proving the strengths and advantages of transgenic models over conventional animal testing.