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(54) **REAGENTS AND METHODS FOR USING HUMAN EMBRYONIC STEM CELLS TO EVALUATE TOXICITY OF PHARMACEUTICAL COMPOUNDS AND OTHER CHEMICALS**

(75) Inventor: **Gabriela G. Cezar**, Middleton, WI (US)

(73) Assignee: **Wisconsin Alumni Research Foundation**, Madison, WI (US)

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(51) **Int. Cl.**
C12N 5/00 (2006.01)

(52) **U.S. Cl.**
USPC **435/325**; 435/366; 435/375

(58) **Field of Classification Search**
USPC 435/325, 366, 375
See application file for complete search history.

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(57) **ABSTRACT**

The invention provides biomarker profiles of cellular metabolites and methods for screening chemical compounds including pharmaceutical agents, lead and candidate drug compounds and other chemicals using human embryonic stem cells (hESC) or lineage-specific cells produced therefrom. The inventive methods are useful for testing toxicity, particularly developmental toxicity and detecting teratogenic effects of such chemical compounds.

48 Claims, 13 Drawing Sheets

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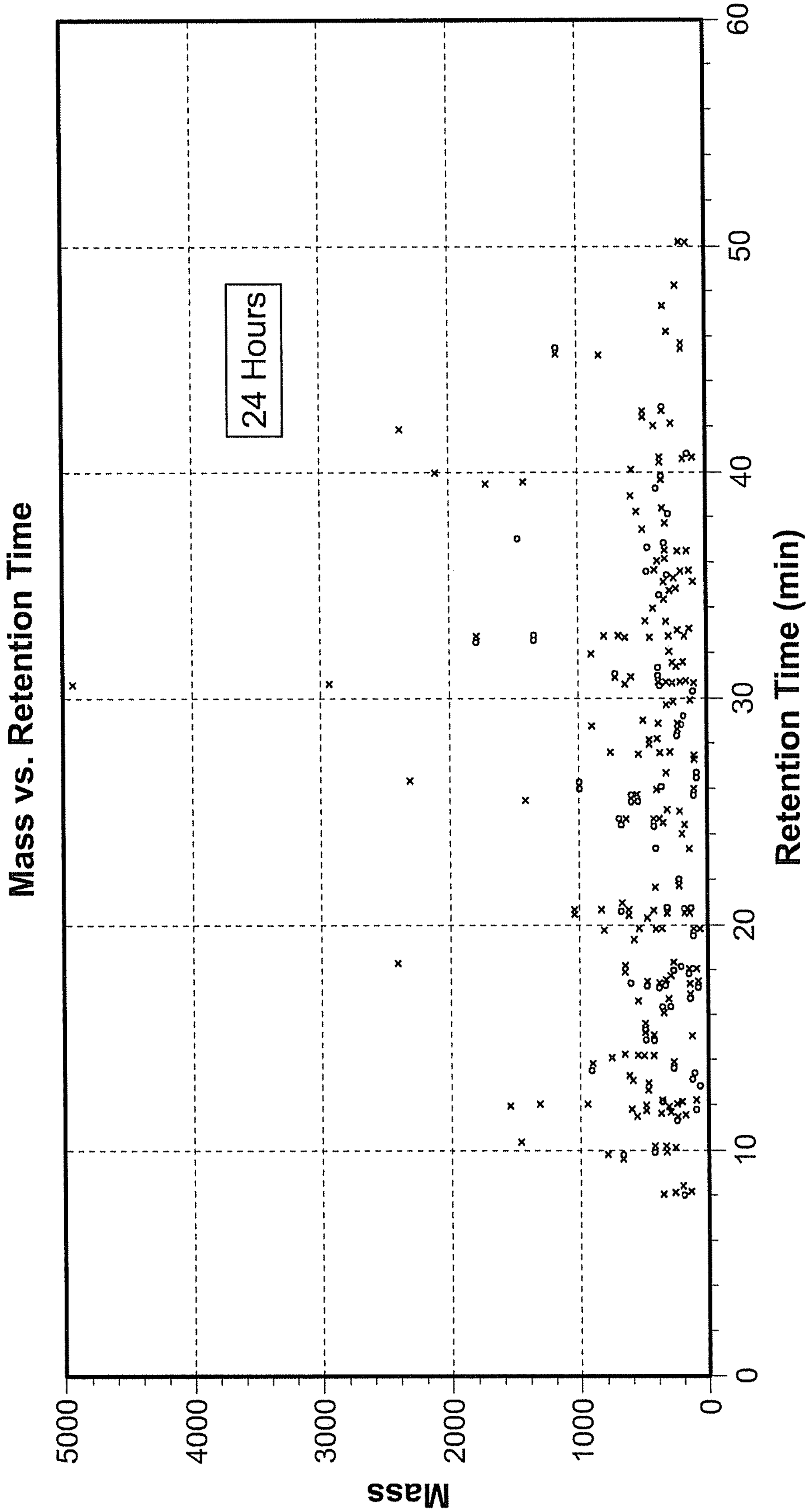
Mass vs. Retention Time
24 Hours
Mass
0
1000
2000
3000
4000
5000
Retention Time (min)
0
10
20
30
40
50
60

FIG. 1A

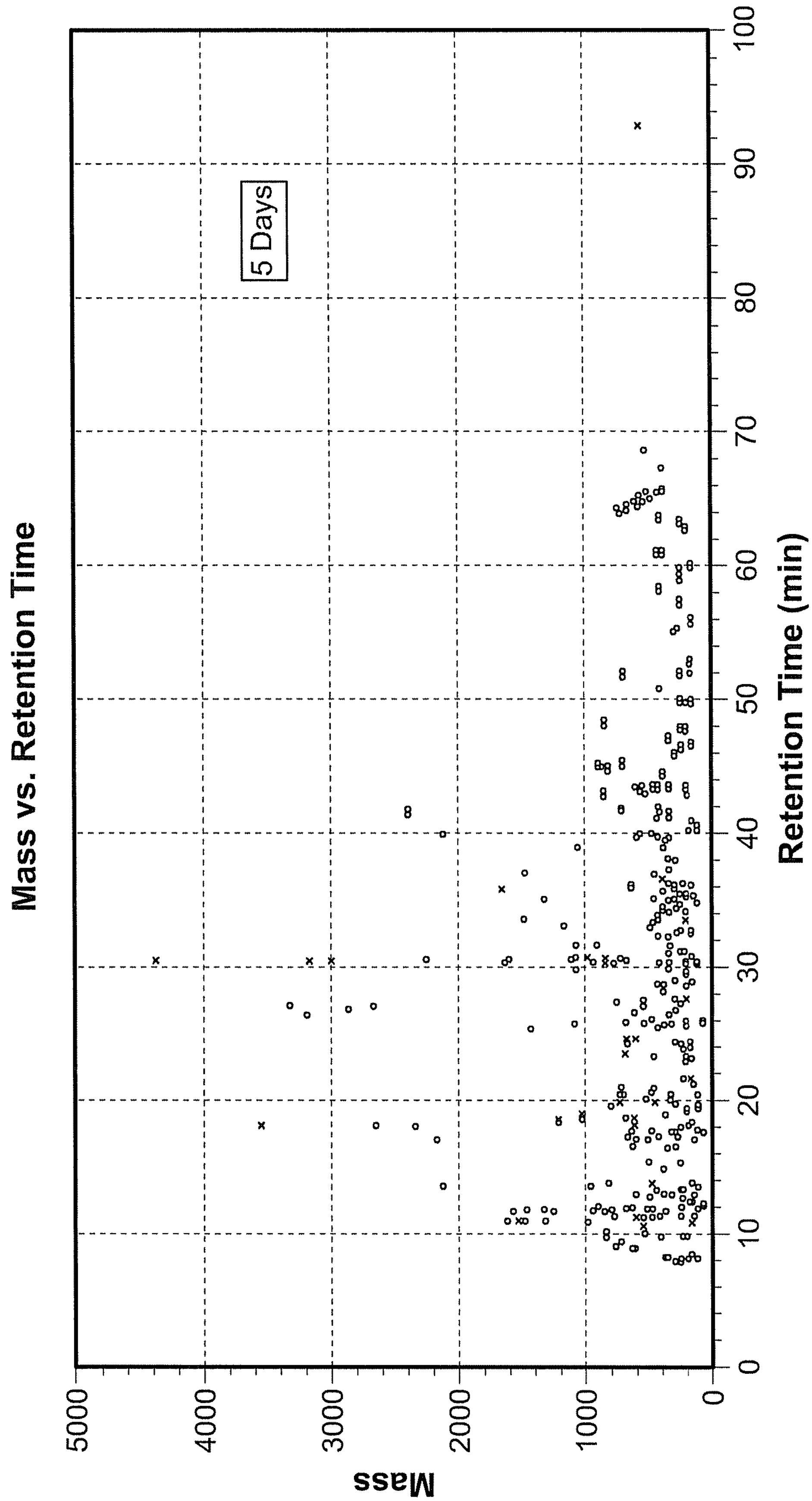
Mass vs. Retention Time
5 Days
Mass
5000
4000
3000
2000
1000
0
Retention Time (min)
0
10
20
30
40
50
60
70
80
90
100

FIG. 1B

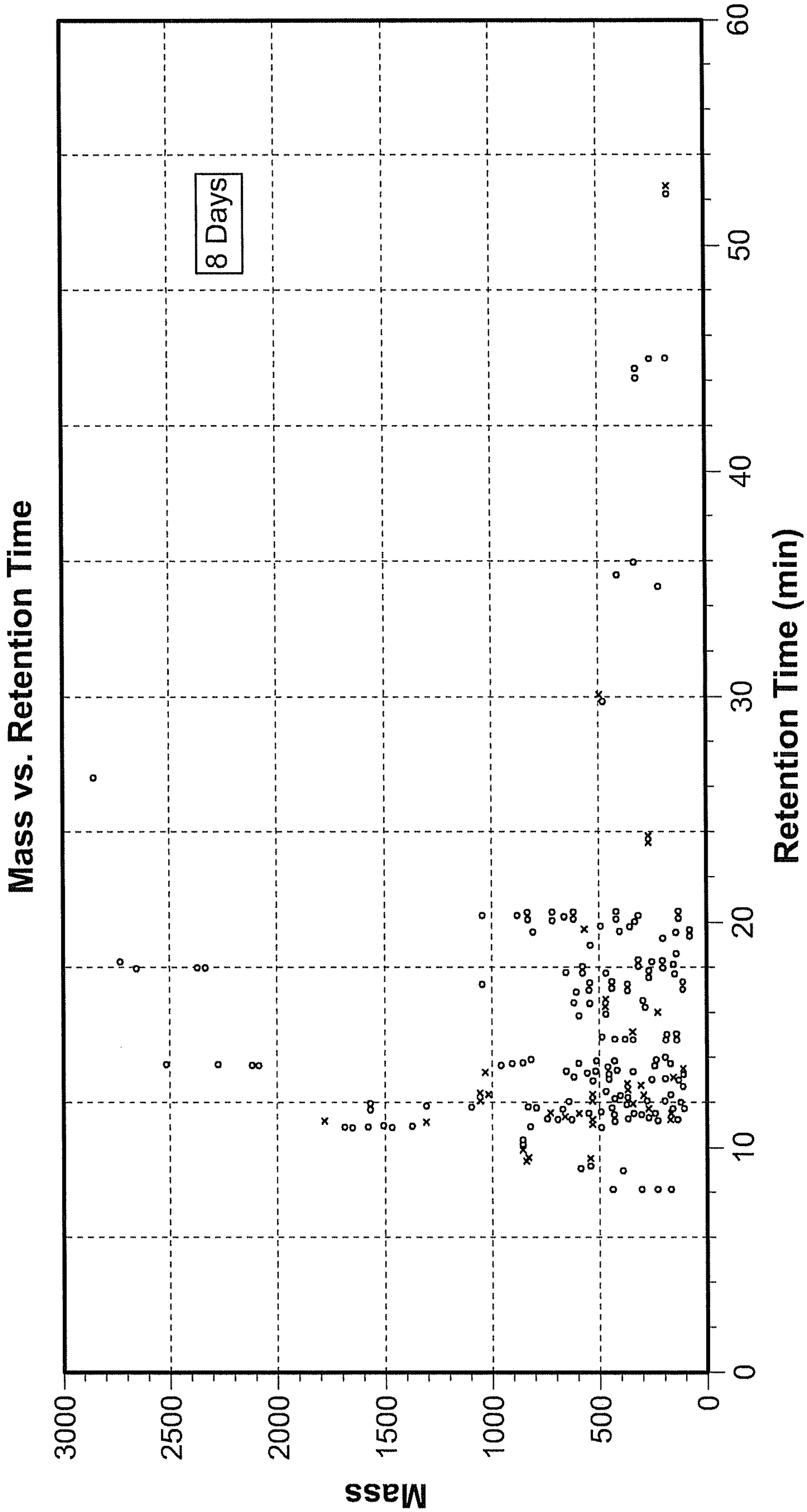
Mass vs. Retention Time
8 Days
Mass
3000
2500
2000
1500
1000
500
0
Retention Time (min)
0
10
20
30
40
50
60

FIG. 1C

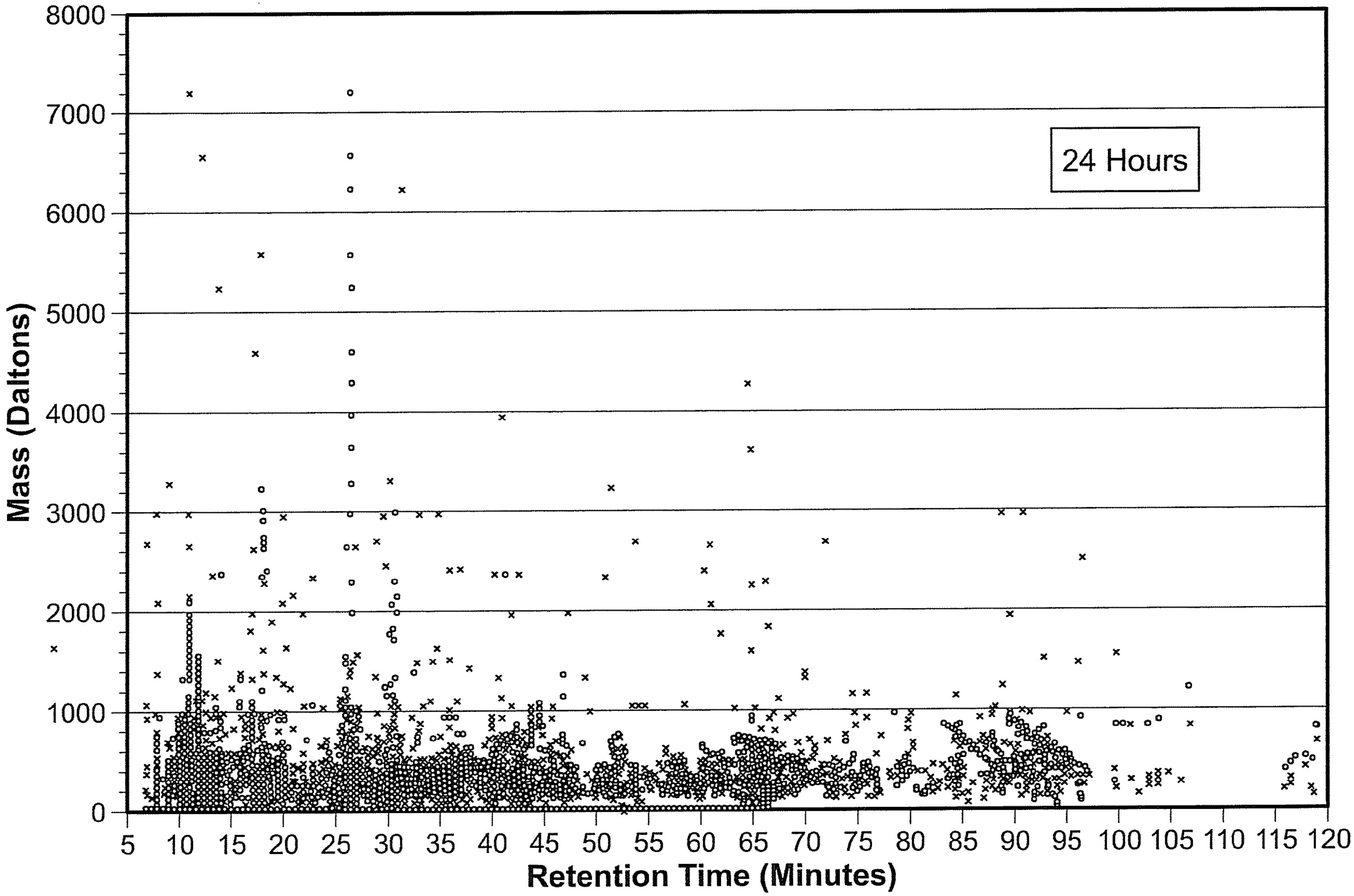
24 Hours
Mass (Daltons)
Retention Time (Minutes)
0
1000
2000
3000
4000
5000
6000
7000
8000
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120

FIG. 2A

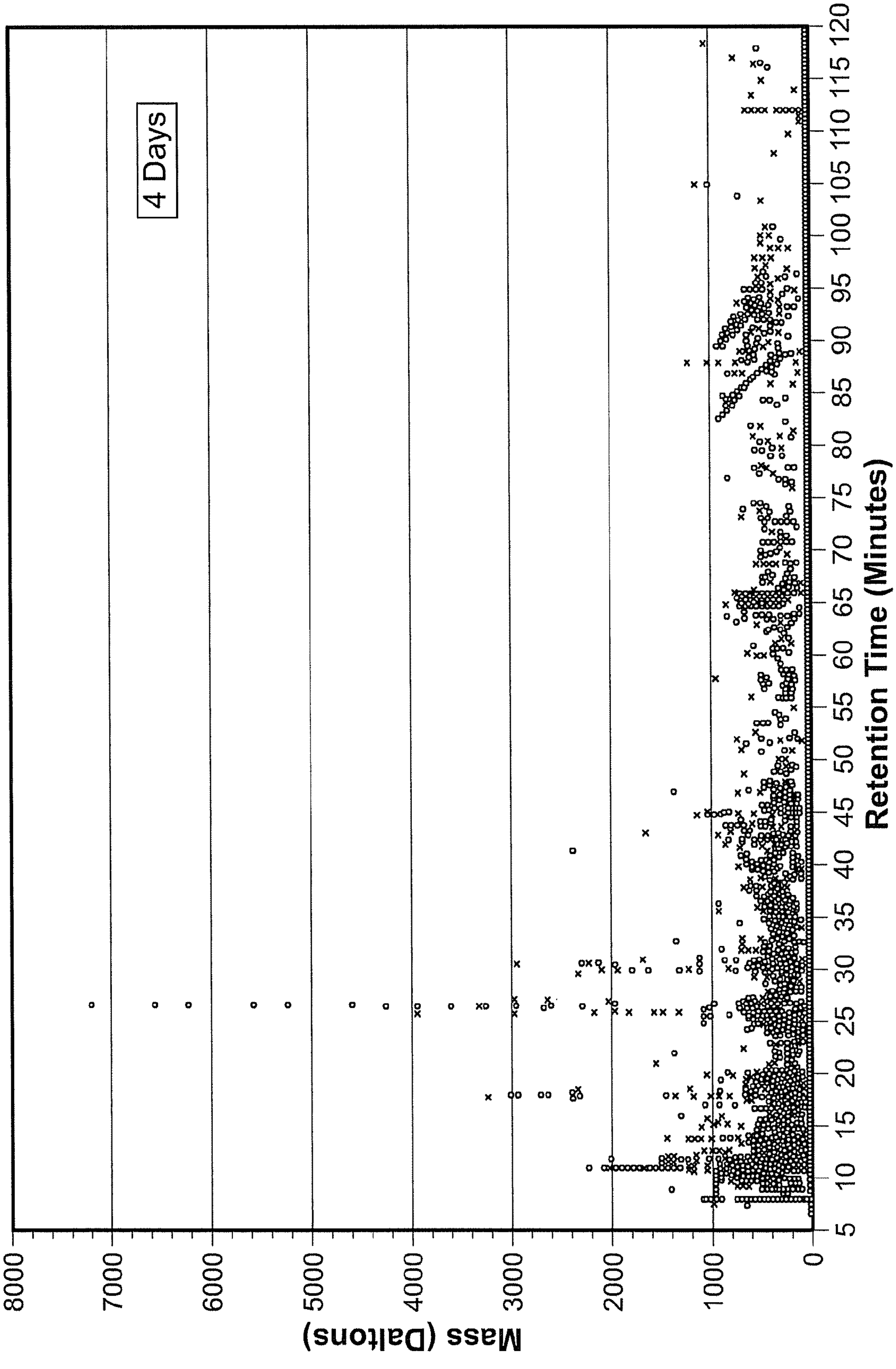
4 Days
Mass (Daltons)
0
1000
2000
3000
4000
5000
6000
7000
8000
Retention Time (Minutes)
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120

FIG. 2B

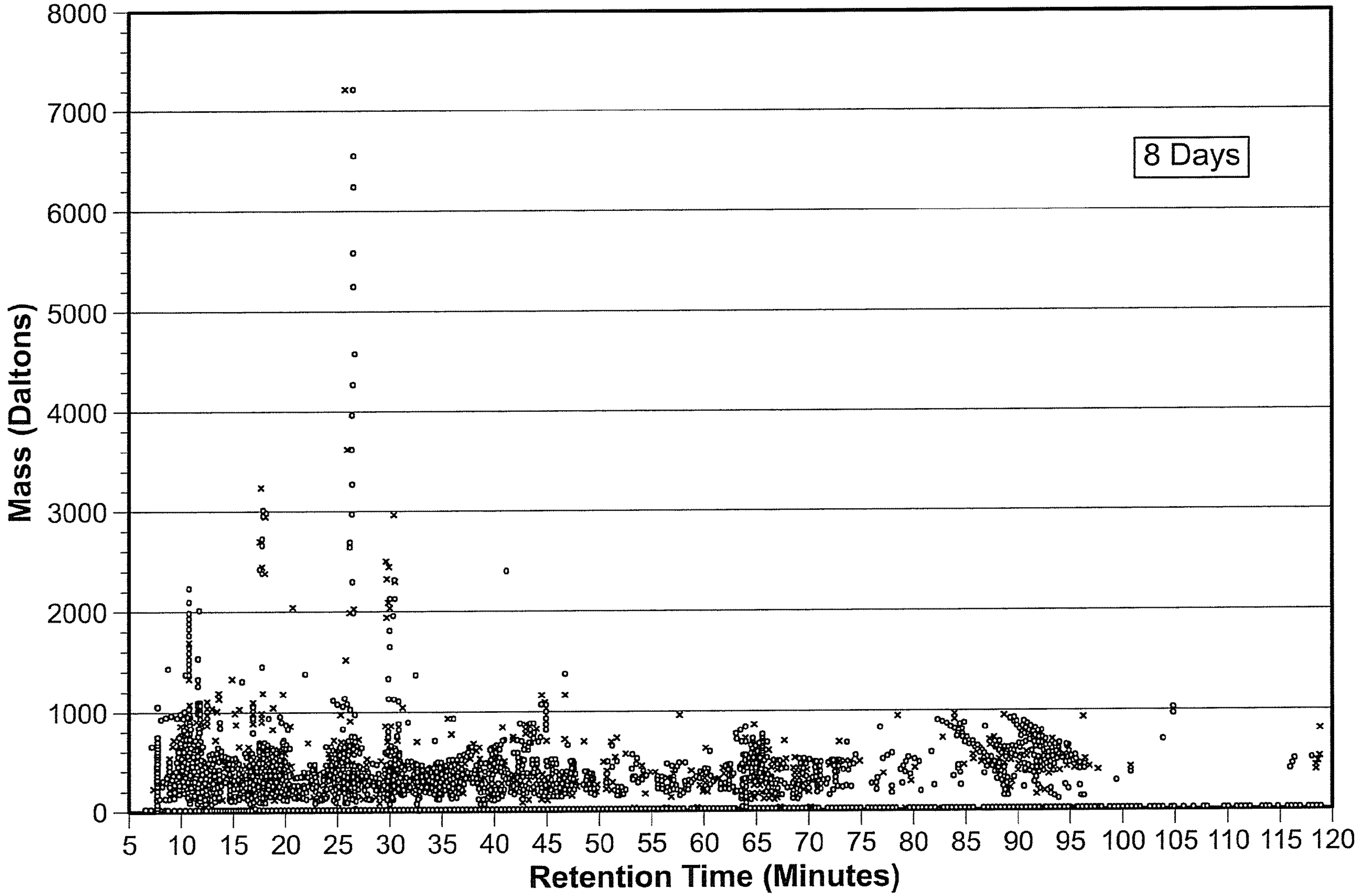
8 Days
Mass (Daltons)
8000
7000
6000
5000
4000
3000
2000
1000
0
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120
Retention Time (Minutes)

FIG. 2C

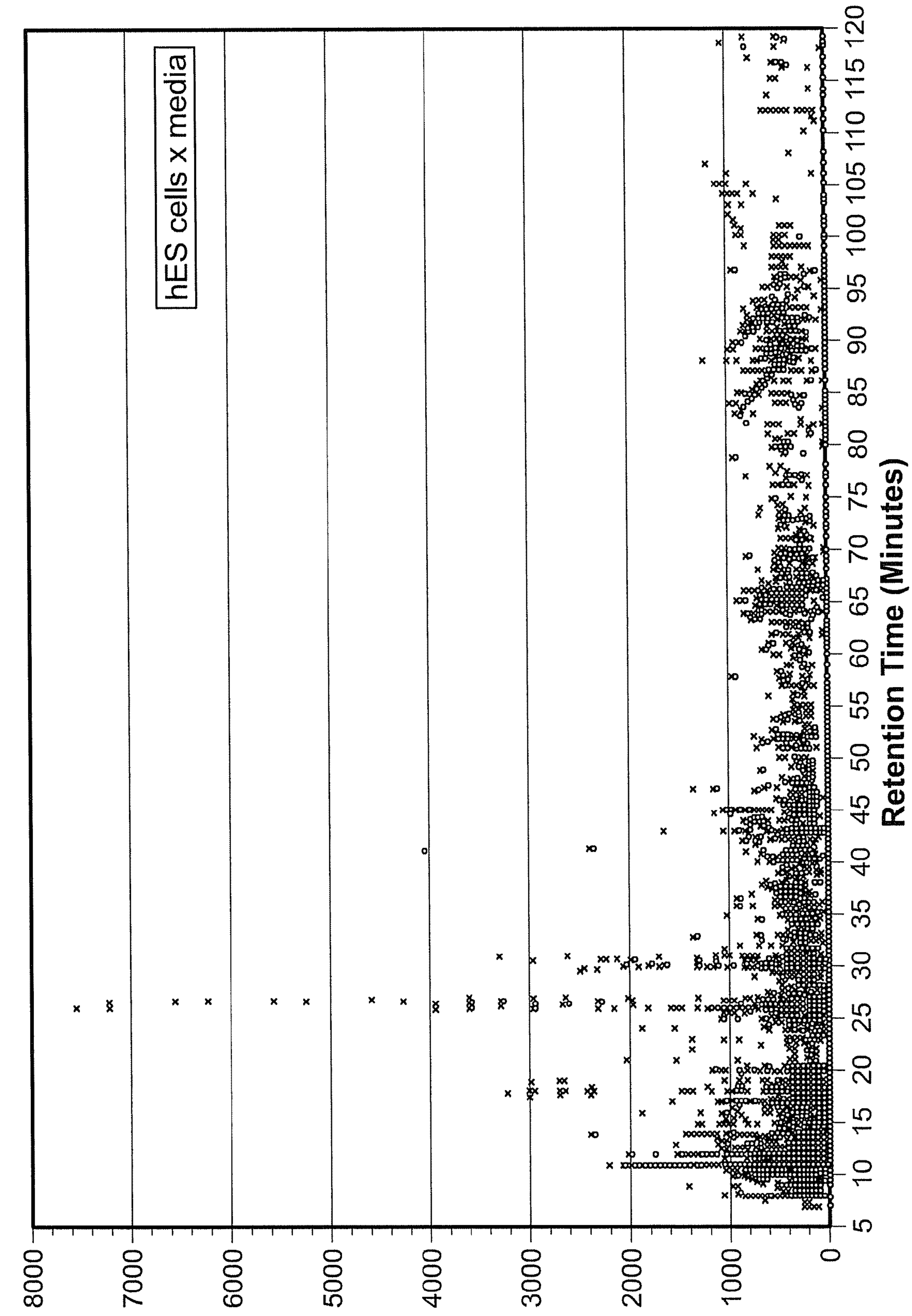
hES cells x media
8000
7000
6000
5000
4000
3000
2000
1000
0
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120
Retention Time (Minutes)

FIG. 2D

A
B
C
D

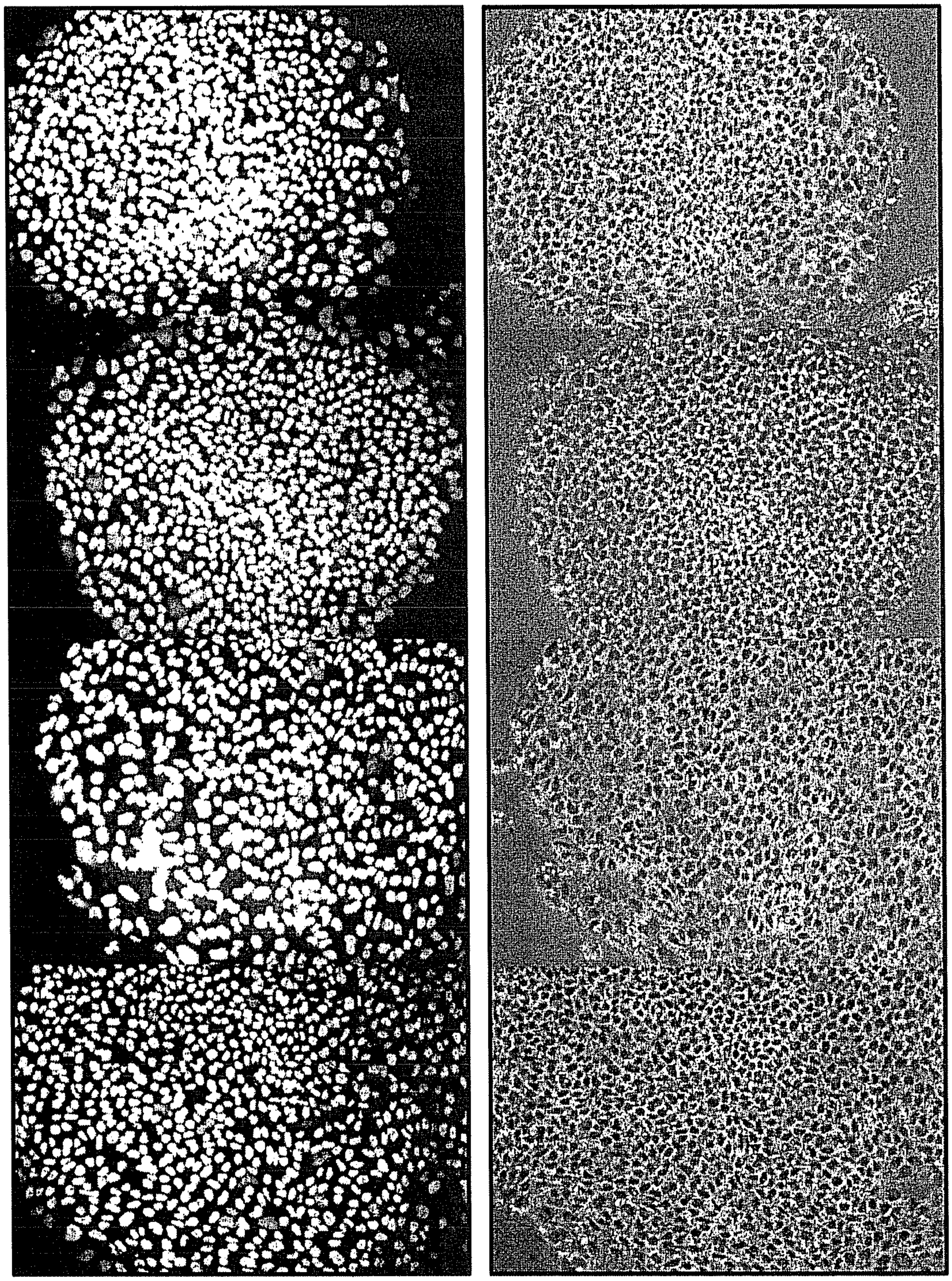

FIG. 3

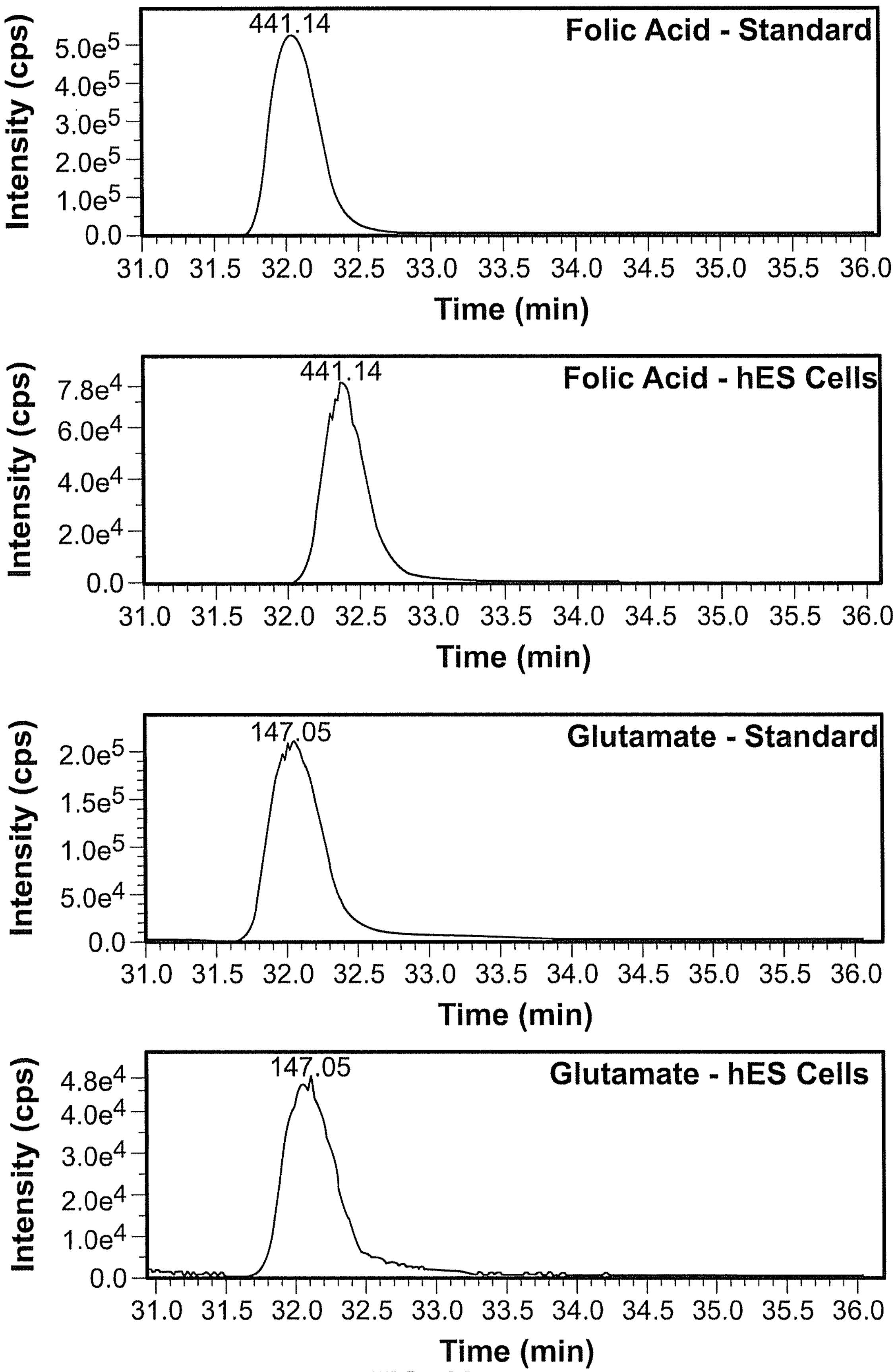
441.14
Folic Acid - Standard
Intensity (cps)
5.0e5
4.0e5
3.0e5
2.0e5
1.0e5
0.0
31.0 31.5 32.0 32.5 33.0 33.5 34.0 34.5 35.0 35.5 36.0
Time (min)
441.14
Folic Acid - hES Cells
Intensity (cps)
7.8e4
6.0e4
4.0e4
2.0e4
0.0
31.0 31.5 32.0 32.5 33.0 33.5 34.0 34.5 35.0 35.5 36.0
Time (min)
147.05
Glutamate - Standard
Intensity (cps)
2.0e5
1.5e5
1.0e5
5.0e4
0.0
31.0 31.5 32.0 32.5 33.0 33.5 34.0 34.5 35.0 35.5 36.0
Time (min)
147.05
Glutamate - hES Cells
Intensity (cps)
4.8e4
4.0e4
3.0e4
2.0e4
1.0e4
0.0
31.0 31.5 32.0 32.5 33.0 33.5 34.0 34.5 35.0 35.5 36.0
Time (min)

FIG. 4A

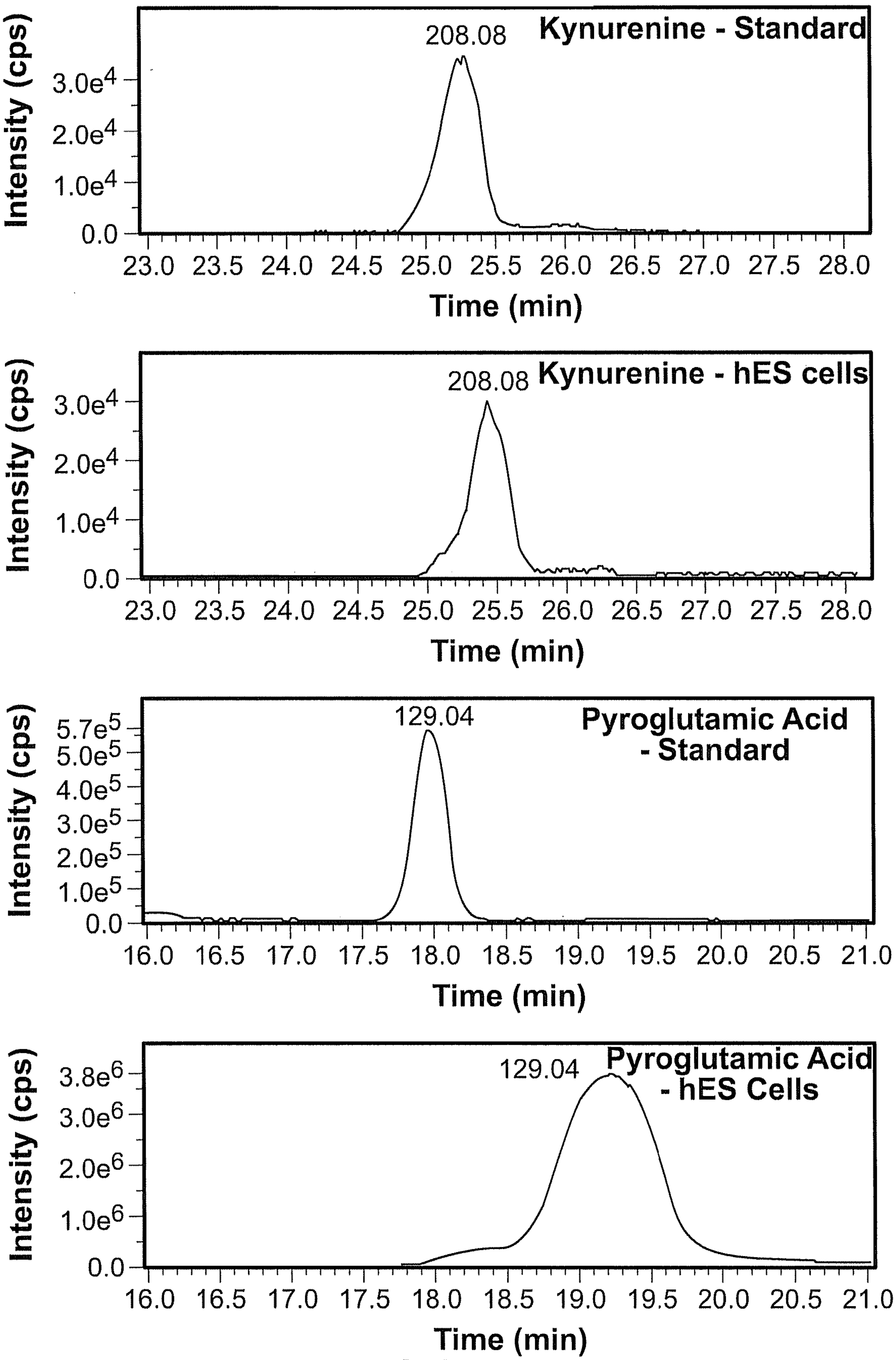
Kynurenine - Standard
208.08
Intensity (cps)
3.0e4
2.0e4
1.0e4
0.0
23.0 23.5 24.0 24.5 25.0 25.5 26.0 26.5 27.0 27.5 28.0
Time (min)
Kynurenine - hES cells
208.08
Intensity (cps)
3.0e4
2.0e4
1.0e4
0.0
23.0 23.5 24.0 24.5 25.0 25.5 26.0 26.5 27.0 27.5 28.0
Time (min)
Pyroglutamic Acid - Standard
129.04
Intensity (cps)
5.7e5
5.0e5
4.0e5
3.0e5
2.0e5
1.0e5
0.0
16.0 16.5 17.0 17.5 18.0 18.5 19.0 19.5 20.0 20.5 21.0
Time (min)
Pyroglutamic Acid - hES Cells
129.04
Intensity (cps)
3.8e6
3.0e6
2.0e6
1.0e6
0.0
16.0 16.5 17.0 17.5 18.0 18.5 19.0 19.5 20.0 20.5 21.0
Time (min)

FIG. 4B

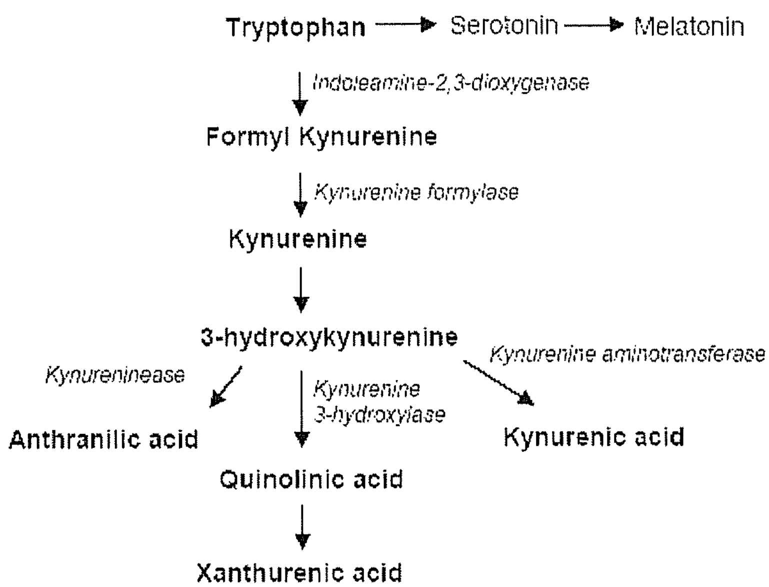
Tryptophan
Serotonin
Melatonin
Indoleamine-2,3-dioxygenase
Formyl Kynurenine
Kynurenine formylase
Kynurenine
3-hydroxykynurenine
Kynureninease
Kynurenine 3-hydroxylase
Kynurenine aminotransferase
Anthranilic acid
Quinolinic acid
Kynurenic acid
Xanthurenic acid

FIG. 5

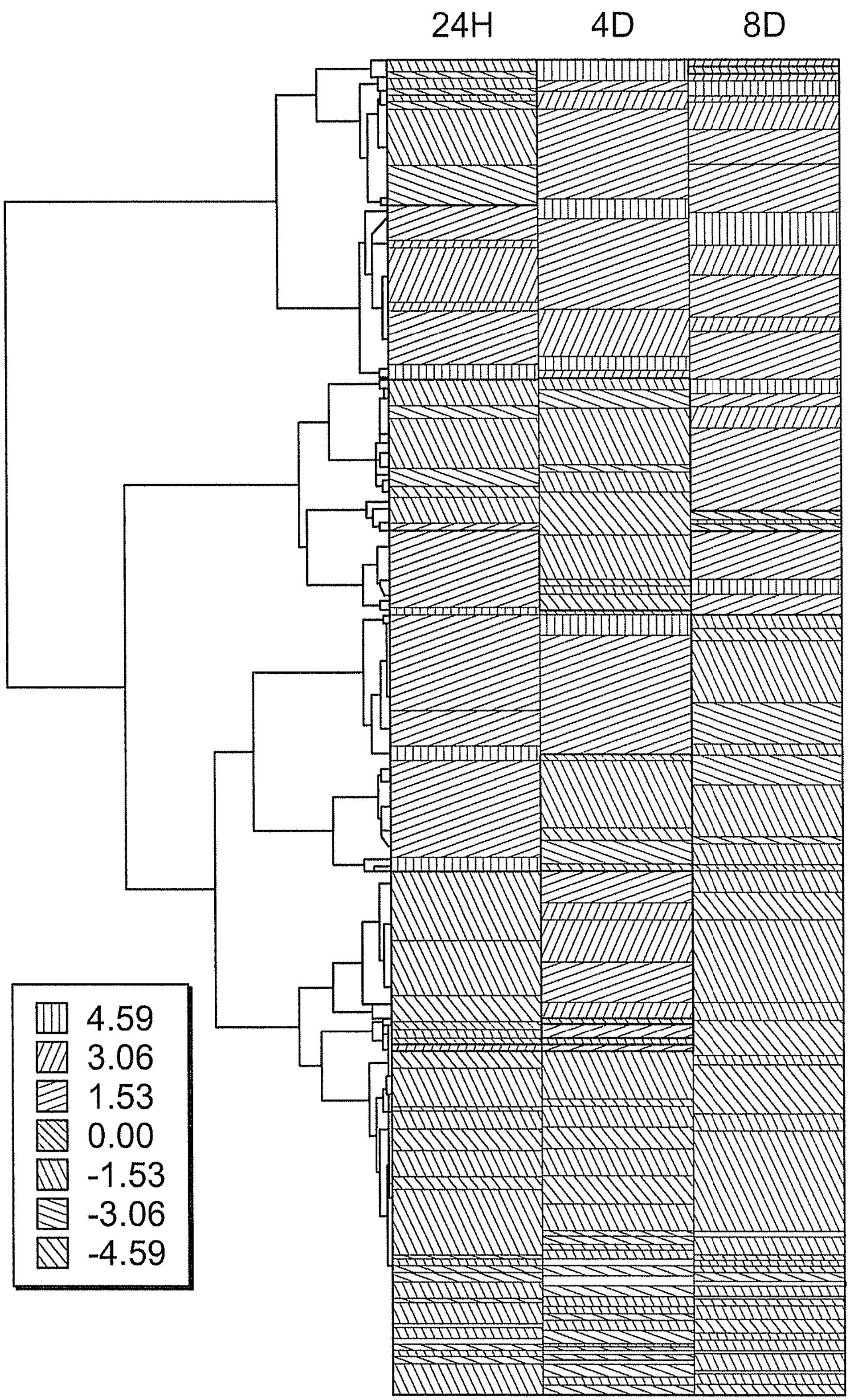

FIG. 6

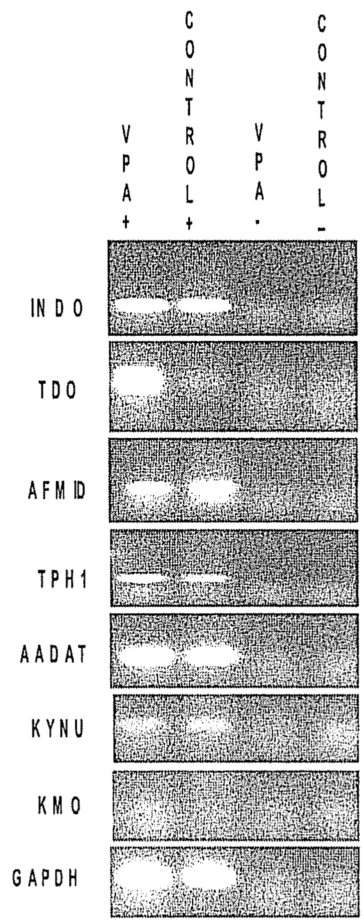
VPA+
CONTROL+
VPA-
CONTROL-
INDO
TDO
AFMID
TPH1
AADAT
KYNU
KMO
GAPDH

FIG. 7

REAGENTS AND METHODS FOR USING HUMAN EMBRYONIC STEM CELLS TO EVALUATE TOXICITY OF PHARMACEUTICAL COMPOUNDS AND OTHER CHEMICALS

This application claims the priority benefit of U.S. provisional patent applications, Ser. Nos. 60/790,647, filed Apr. 10, 2006, and 60/822,163, filed Aug. 11, 2006, the entirety of which are incorporated by reference herein.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention provides methods for toxicological screening of pharmaceuticals and other chemical compounds. The invention specifically provides reagents that are human embryonic stem cells (hESC) or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, as well as methods for using these cells to detect developmental toxicity or teratogenic effects of pharmaceutical compounds and other chemicals. More particularly, the invention provides an in vitro means for analyzing toxicity of compounds predictive of their toxicity during human development. Candidate predictive biomarkers for toxic or teratogenic effects are also identified and provided herein.

2. Background of Invention

Birth defects are a major cause of infant morbidity in the United States, affecting 1 in every 33 infants born (Brent & Beckman, 1990, *Bull NY Acad Med* 66: 123-63; Rosano et al., 2000, *J Epidemiology Community Health* 54:660-66), or approximately 125,000 newborns per year. It is understood that developmental toxicity can cause birth defects, and can generate embryonic lethality, intrauterine growth restriction (IUGR), dysmorphogenesis (such as skeletal malformations), and functional toxicity, which can lead to cognitive disorders such as autism. There is an increasing concern about the role that chemical exposure can play in the onset of these disorders. Indeed, it is estimated that 5% to 10% of all birth defects are caused by in utero exposure to known teratogenic agents (Beckman & Brent, 1984, *Annu Rev Pharmacol* 24: 483-500).

Concern exists that chemical exposure may be playing a significant and preventable role in producing birth defects (Claudio et al., 2001, *Environm Health Perspect* 109: A254-A261). This concern has been difficult to evaluate, however, since the art has lacked a robust and efficient model for testing developmental toxicity for the more than 80,000 chemicals in the market, plus the new 2,000 compounds introduced annually (General Accounting Office (GAO), 1994, *Toxic Substances Control Act: Preliminary Observations on Legislative Changes to Make TSCA More Effective*, Testimony, Jul. 13, 1994, GAO/T-RCED-94-263). Fewer than 5% of these compounds have been tested for reproductive outcomes and even fewer for developmental toxicity (Environmental Protective Agency (EPA), 1998, *Chemical Hazard Data Availability Study*, Office of Pollution Prevention and Toxins). Although some attempts have been made to use animal model systems to assess toxicity (Piersma, 2004, *Toxicology Letters* 149:147-53), inherent differences in the sensitivity of humans in utero have limited the predictive usefulness of such models. Development of a human-based cell model system would have an enormous impact in drug development and risk assessment of chemicals.

Toxicity, particularly developmental toxicity, is also a major obstacle in the progression of compounds through the drug development process. Currently, toxicity testing is conducted on animal models as a means to predict adverse effects of compound exposure, particularly on development and organogenesis in human embryos and fetuses. The most prevalent models that contribute to FDA approval of investigational new drugs are whole animal studies in rabbits and rats (Piersma, 2004, *Toxicology Letters* 149: 147-53). In vivo studies rely on administration of compounds to pregnant animals at different stages of pregnancy and embryonic/fetal development (first week of gestation, organogenesis stage and full gestation length). However, these in vivo animal models are limited by a lack of robustness between animal and human responses to chemical compounds during development. Species differences are often manifested in trends such as dose sensitivity and pharmacokinetic processing of compounds. At present, animal models are only 50% efficient in predicting human developmental response to compounds (Greaves et al., 2004, *Nat Rev Drug Discov* 3:226-36). Thus, human-directed predictive in vitro models present an opportunity to reduce the costs of new drug development and enable safer drugs.

In vitro models have been employed in the drug industry for over 20 years (Huuskonen, 2005, *Toxicology & Applied Pharm* 207:S495-S500). Many of the current in vitro assays involve differentiation models using primary cell cultures or immortalized cells lines (Huuskonen, 2005, *Toxicology & Applied Pharm* 207:S495-S500). Unfortunately, these models differ significantly from their in vivo counterparts in their ability to accurately assess development toxicity. In particular, the ECVAM initiative (European Center for Validation of Alternative Methods) has used mouse embryonic stem cells as a screening system for predictive developmental toxicology. The embryonic stem cell test (EST) has shown very promising results, with a 78% statistically significant correlation to in vivo studies, and the test was able to differentiate strong teratogens from moderate/weak or non-embryotoxic compounds (Spielmann et al., 1997, *In Vitro Toxicology* 10:119-27). This model is limited in part because toxicological endpoints are defined only for compounds that impair cardiac differentiation. This model also fails to account for interspecies developmental differences between mice and humans, and so does not fully address the need in the art for human-specific model systems.

Thus there remains a need in this art for a human-specific in vitro method for reliably determining developmental toxicity in pharmaceutical agents and other chemical compounds. There also is a need in the art to better understand human development and its perturbation by toxins and other developmental disrupting agents, to assist clinical management of acquired congenital disorders and the many diseases that share these biochemical pathways, such as cancer.

The present invention provides for the assessment of a plurality of small molecules, preferably secreted or excreted by hES cells or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, and is determined and correlated with health and disease or insult state. Similar analyses have been applied to other biological systems in the art (Want et al., 2005 *Chem Bio Chem* 6: 1941-51), providing biomarkers of disease or toxic responses that can be detected in biological fluids (Sabatine et al., 2005 *Circulation* 112:3868-875).

SUMMARY OF THE INVENTION

The present invention provides reagents and methods for in vitro screening of toxicity and teratogenicity of pharmaceutical and non-pharmaceutical chemicals using undifferentiated human embryonic stem cells (hESC) or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells. The invention provides human-specific in vitro methods for reliably determining toxicity, particularly developmental toxicity and teratogenicity, of pharmaceuticals and other chemical compounds using human embryonic stem cells (hESCs) or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells. As provided herein, hESCs or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, are useful for assessing toxic effects of chemical compounds, particularly said toxic and teratogenic effects on human development, thus overcoming the limitations associated with interspecies animal models. In particular, the invention demonstrates that metabolite profiles of hES cells or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells are altered in response to known disruptors of human development.

The invention shows that the hESC metabolome is a source of human biomarkers for disease and toxic response. In particular embodiments, exposure of hESC to valproate induced significant changes in different metabolic pathways, consistent with its known activity as a human teratogen. In other embodiments, hESC exposure to varying levels of ethanol induced significant alterations in metabolic pathways consistent with alcohol's known effects on fetal development.

In one aspect, the invention provides methods for using undifferentiated pluripotent human embryonic stem cells (hESC) or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, for in vitro evaluation. In the inventive methods, undifferentiated hESCs or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells are exposed to test compounds, preferably at concentrations reflective of in vivo levels or at levels found in maternal circulation. Further embodiments of this aspect of the invention provide for determination of the capacity of the test compound to induce differentiation of pluripotent hESC into particular cell types. In other embodiments, the inventive methods are provided using pluripotent, non-lineage restricted cells. The benefit of utilizing pluripotent stem cells is they permit analysis of global toxic response(s) and are isolated from the physiological target of developmental toxicity, i.e. the human embryo. In addition, because these cells have not differentiated into a specific lineage, the potential for false negatives is reduced. In yet further embodiments are provided methods using hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, for assessing toxicity and particularly developmental toxicity and teratogenicity.

In another aspect the invention provides methods for identifying predictive biomarkers of toxic responses to chemical compounds, particularly pharmaceutical and non-pharmaceutical chemicals, and particularly to known teratogens. In embodiments of this aspect, a dynamic set representative of a plurality of cellular metabolites, preferably secreted or excreted by hES cells or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, is determined and correlated with health and disease or toxic insult state. Cellular metabolites according to this aspect of the invention generally range from about 10 to about 1500 Daltons, more particularly from about 100 to about 1000 Daltons, and include but are not limited to compounds such as sugars, organic acids, amino acids, fatty acids and signaling low-molecular weight compounds. Said biomarker profiles are diagnostic for toxicity of chemical compounds, particularly pharmaceutical and non-pharmaceutical chemicals, that participate in and reveal functional mechanisms of cellular response to pathological or toxic chemical insult, thus serving as biomarkers of disease or toxic response that can be detected in biological fluids. In particularly preferred embodiments of this aspect of the invention, these biomarkers are useful for identifying active (or activated) metabolic pathways following molecular changes predicted, inter alia, by other methods (such as transcriptomics and proteomics).

The invention thus also provides biomarker and pluralities of biomarkers, in some instances associated with metabolites from particular metabolic pathways, that are indicative of toxic or teratogenic insult. Said markers as provided by the invention are used to identify toxic and teratogenic insult, and in particular embodiments are used to characterize the amount or extent of said insult by being correlated with the amount or extent of the particular biomarker or plurality of biomarkers detected in cell culture media. In particular embodiments, said plurality of biomarkers provide a diagnostic pattern of toxic or teratogenic insult, more particularly identifying one or a multiplicity of specific metabolic pathways comprising metabolites detected after toxic or teratogenic insult.

The present invention is advantageous compared with inter alia the ECVAM mouse model because toxicity testing and biomarker identification are performed with human cells, specifically human embryonic stem cells (hESC). Human embryonic stem cells are able to recapitulate mammalian organogenesis in vitro (Reubinoff et al., 2000, *Nature Biotechnology* 18:399-404; He et al., 2003, *Circ Res* 93:32-9; Zeng et al., 2004, *Stem Cells* 22:925-40; Lee et al., 2000, *Mol Genet Metab* 86:257-68; Yan et al., 2005, *Stem Cells* 22:781-90) because they are pluripotent and self-renewing cells. Thus, hESCs can reveal mechanisms of toxicity, particularly developmental toxicity, and identify developmental pathways that are particularly sensitive to chemicals during early human development. The "human for human" embryonic model provided by the inventive methods disclosed herein permits a better understanding of the pathways associated with developmental toxicity, as this is a system developed directly from the target organism, as well as being a more accurate and sensitive assay for toxic or teratogenic insult in human development.

The methods of the invention provide further advantages in identifying important biomarkers for toxicity and teratogenicity by functional screening of hESCs or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells. These biomarkers advantageously identify metabolic and cellular pathways and mechanisms of toxicity, particularly developmental toxicity. Importantly, these biomarkers may also assist in the evaluation of toxic effects of chemicals on the developing human embryo.

In yet another aspect of the invention, differentially-detected secreted or excreted cellular products identified by methods of the invention include those associated with neurodevelopmental disorders and alterations in associated metabolic pathways, and include but are not limited to kynurenine, glutamate, pyroglutamic acid, 8-methoxykynurenate, N'-formylkynurenine 5-hydroxytryptophan, N-acetyl-D-tryptophan and other metabolites in the tryptophan and glutamate metabolic pathways.

Functional toxicity in post-natal life can be predicted using hESC since differentiated cells with critical in vivo properties can be generated in vitro. hESCs can be used to produce lineage-specific cells, including lineage-specific stem cells, precursor cells and terminally-differentiated cells, providing therein enriched populations of cells typically present in vivo in mixtures of different cell types comprising tissues. The invention thus provides methods for using hESCs to produce said enriched and developmental stage-specific populations of cells for toxicity screening of chemical compounds, particularly drugs, drug lead compounds and candidate compounds in drug development, to identify human-specific toxicities of said chemical compounds. These aspects of the methods of the invention are advantageous over art-recognized in vitro and in vivo animal model systems.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other objects and features of this invention will be better understood from the following detailed description taken in conjunction with the drawing wherein:

FIGS. **1**A through **1**C are profiles of secreted cellular metabolite biomarkers produced after contacting hESCs with 1 mM valproate. These profiles were produced using liquid chromatography/electrospray ionization-time of flight (TOF) mass spectrometry (LC/ESI-TOF-MS) after treating the cells with valproate for 24 hours (FIG. **1**A), four days (FIG. **1**B) and eight days (FIG. **1**C). Secreted small molecules from treated (blue) and untreated (red) human embryonic stem cells were measured.

FIGS. **2**A through **2**D are profiles of secreted/excreted cellular metabolite biomarkers produced after contacting hESCs with 1 mM valproate. These profiles were produced using liquid chromatography/electrospray ionization time of flight mass spectrometry (LC/ESI-TOF-MS) after treating cells with valproate for 24 hours (FIG. **2**A), four days (FIG. **2**B), eight days (FIG. **2**C), and comparative metabolic profiling of hES cells (blue) and conditioned media (yellow) (FIG. **2**D).

FIGS. **3**A through **3**D are photomicrographs of cellular morphology showing the pluripotent embryonic stem cells following extended culture. The marker Oct-4 was retained in a similar manner as untreated controls (FIG. **3**A=5 days valproate, FIG. **3**B=5 days control, FIG. **3**C-**8** days valproate, FIG. **3**D-**8** days control).

FIG. **4** shows the results of comparative mass spectrometry in the presence of chemical standards confirming the chemical identity of folic acid (exact mass 441.14), pyroglutamic acid (exact mass 129.04), glutamate (exact neutral mass 147.05) and kynurenine (exact mass 208.08).

FIG. **5** represents the kynurenine metabolism pathway of tryptophan in humans (Wang et al., 2006*, J Biol Chem* 281: 22021-22028, published electronically on Jun. 5, 2006).

FIG. **6** illustrates a hierarchical clustering of fold-change differences from 22,573 unique masses and is representative of multiple independent experiments in which hESCs and neural precursors produced from hESCs were treated with 1 mM valproate. Non-embryonic cells (human fibroblasts) were used as controls (data not shown). Positive fold changes are red, negative fold changes are green, and missing data is grey.

FIG. **7** shows the relative expression of enzymes in the kynurenine and serotonin synthesis pathways in hES cells. INDO, indoleamine 2,3 dioxygenase, TDO or TDO2, tryptophan 2,3-dioxygenase. (TDO2 was upregulated in valproate-treated hES cells in comparison to controls.) AFMID, arylformamidase, TPH1, tryptophan hydroxylase, AADAT, aminoadipate aminotransferase, KYNU, kynunreninase, GAPDH, glyceraldehyde 3-phosphate dehydrogenase, housekeeping control gene. KMO, kynurenine 3-monooxygenase, was not expressed in valproate-treated cells or controls.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The invention provides reagents, including human embryonic stem cells (hESC) or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells produced therefrom, for assessing developmental toxicity using the human embryonic stem cell metabolome. Human embryonic stem cells are pluripotent, self-renewing cells isolated directly from preimplantation human embryos that recapitulate organogenesis in vitro. Lineage-specific precursor cells are derived from hES cells and have entered a specific cellular lineage, but yet remain multipotent with regard to cell type within that specific lineage. For example, neural precursors have committed to neural differentiation but yet remain unrestricted as to its neural cell type. Also within the scope of the inventive methods are terminally-differentiated cell types, such as neurons. Biochemical pathways of human development and disease are active in hESCs and or hESC-derived lineage-specific cells, because they recapitulate differentiation into functional somatic cells. Disruption of these pathways during development contributes to disorders such as neural tube defects (NTDs) and cognitive impairment. Environmental agents, namely chemicals or drugs, participate in the ontogenesis of certain acquired congenital disorders. The question of which pathways during early human development are particularly susceptible to the effects of the environment remains unsolved.

The metabolome, defined as the total dynamic set of cellular metabolites present in cells, is a product of health or disease/insult states. Metabolomics is particularly sensitive to environmental effects in comparison to other "omic" areas of study, such as genomics and proteomics. Cellular metabolites include but are not limited to sugars, organic acids, amino acids and fatty acids, particularly those species secreted or excreted from cells, that participate in functional mechanisms of cellular response to pathological or chemical insult. These cellular metabolites serve as biomarkers of disease or toxic response and can be detected in biological fluids (Soga et al., 2006*, J Biol Chem* 281:16768-78; Zhao et al., 2006*, Birth Defects Res A Clin Mol Teratol* 76:230-6), including hESC culture media. Importantly, metabolomic profiling may confirm functional changes that are often predicted by transcriptomics and proteomics.

However, because it was known that hESCs are highly sensitive to the culture microenvironment (Levenstein et al., 2005*, Stem Cells* 24: 568-574; Li et al., 2005*, Biotechnol Bioeng* 91:688-698.), their application as a source of predictive biomarkers in response to chemical compounds, including toxins, teratogens and particularly pharmaceutical agents, drug lead compounds and candidate compounds in drug development, and their usefulness in establishing in vitro models of disease and development was uncertain, inter alia because those of skill in the art could anticipate that exposure to an exogenous chemicals could be highly detrimental to survival of hES cells and preclude obtaining useful information from them. This concern has turned out not to be justified.

As used herein, the term "human embryonic stem cells (hESCs)" is intended to include undifferentiated stem cells originally derived from the inner cell mass of developing blastocysts, and specifically pluripotent, undifferentiated human stem cells and partially-differentiated cell types thereof (e.g., downstream progenitors of differentiating hESC). As provided herein, in vitro cultures of hESC are pluripotent and not immortalized, and can be induced to produce lineage-specific cells and differentiated cell types using methods well-established in the art. In preferred embodiments, hESCs useful in the practice of the methods of this invention are derived from preimplantation blastocysts as described by Thomson et al., in co-owned U.S. Pat. No. 6,200,806. Multiple hESC cell lines are currently available in US and UK stem cell banks.

The terms "stem cell progenitor," "lineage-specific cell," "hESC derived cell" and "differentiated cell" as used herein are intended to encompass lineage-specific cells that are differentiated from hES cells such that the cells have committed to a specific lineage of diminished potency. In some embodiments, these lineage-specific precursor cells remain undifferentiated with regard to final cell type. For example, neuronal stem cells are derived from hESCs and have differentiated enough to commit to neuronal lineage. However, the neuronal precursor retains 'stemness' in that it retains the potential to develop into any type of neuronal cell. Additional cell types include terminally-differentiated cells derived from hESCs or lineage-specific precursor cells, for example neural cells.

The term "cellular metabolite" as used herein refers to any small molecule secreted and/or excreted by a hESC or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, produced therefrom. In preferred embodiments, cellular metabolites include but are not limited to sugars, organic acids, amino acids, fatty acids, hormones, vitamins, oligopeptides (less than about 100 amino acids in length), as well as ionic fragments thereof. Cells may also be lysed in order to measure cellular products present within the cell. In particular, said cellular metabolites are from about 10 to about 3600 Daltons in molecular weight, more particularly about 10 to about 1500 Daltons, and yet more particularly from about 100 to about 1000 Daltons.

hESCs are cultured according to the methods of the invention using standard methods of cell culture well-known in the art, including, for example those methods disclosed in Ludwig et al. (2006, Feeder-independent culture of human embryonic stem cells, *Nat Methods* 3: 637-46.). In preferred embodiments, hESCs are cultured in the absence of a feeder cell layer during the practice of the inventive methods; however, hESCs may be cultured on feeder cell layer prior to the practice of the methods of this invention.

The term "administering" as used herein refers to contacting in vitro cultures of hESCs or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells produced therefrom with a toxic, teratogenic, or test chemical compound. In a preferred embodiment the dosage of the compound is administered in an amount equivalent to levels achieved or achievable in vivo, for example, in maternal circulation.

The phrases "identifying cellular metabolites that are differentially produced" or "detecting alterations in the cells or alternations in cell activity" as used herein include but are not limited to comparisons of treated hES cells or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, to untreated (control) cells (i.e., cells cultured in the presence (treated) or absence (untreated) of a toxic, teratogenic, or test chemical compound). Detection or measurement of variations in cellular metabolites, excreted or secreted therefrom, between treated and untreated cells is included in this definition. In a preferred embodiment, alterations in cells or cell activity are measured by determining a profile of changes in cellular metabolites having a molecular weight of less than 3000 Daltons, more particularly between 10 and 1500 Daltons, and even more particularly between 100 and 1000 Daltons, in a treated versus untreated cell as illustrated in FIGS. **1**A through **1**C.

The term "correlating" as used herein refers to the positive correlation or matching of alterations in cellular metabolites including but not limited to sugars, organic acids, amino acids, fatty acids, and low molecular weight compounds excreted or secreted from hES cells or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, to an in vivo toxic response. The screened cellular metabolites can be involved in a wide range of biochemical pathways in the cells and related to a variety of biological activities including, but not limited to inflammation, anti-inflammatory response, vasodilation, neuroprotection, oxidative stress, antioxidant activity, DNA replication and cell cycle control, methylation, and biosynthesis of, inter alia, nucleotides, carbohydrates, amino acids and lipids, among others. Alterations in specific subsets of cellular metabolites can correspond to a particular metabolic or developmental pathway and thus reveal effects of a test compound on in vivo development.

The term "physical separation method" as used herein refers to any method known to those with skill in the art sufficient to produce a profile of changes and differences in small molecules produced in hESCs or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, contacted with a toxic, teratogenic or test chemical compound according to the methods of this invention. In a preferred embodiment, physical separation methods permit detection of cellular metabolites including but not limited to sugars, organic acids, amino acids, fatty acids, hormones, vitamins, and oligopeptides, as well as ionic fragments thereof and low molecular weight compounds (preferably with a molecular weight less than 3000 Daltons, more particularly between 10 and 1500 Daltons, and even more particularly between 100 and 1000 Daltons). In particular embodiments, this analysis is performed by liquid chromatography/electrospray ionization time of flight mass spectrometry (LC/ESI-TOF-MS), however it will be understood that cellular metabolites as set forth herein can be detected using alternative spectrometry methods or other methods known in the art for analyzing these types of cellular compounds in this size range.

Data for statistical analysis were extracted from chromatograms (spectra of mass signals) using the Agilent Mass Hunter software (Product No. G3297AA, Agilent Technologies, Inc., Santa Clara, Calif.); it will be understood that alternative statistical analysis methods can be used. Masses were binned together if they were within 10 ppm and eluted within a 2 minutes retention time window. A binned mass was considered to be the same molecule across different LC/ESI-TOF-MS analyses (referred to herein as an "exact mass," which will be understood to be ±10 ppm). Binning of the data is required for statistical analysis and comparison of masses across the entire experiment. If multiple peaks with the same mass at the same retention time within a single sample were detected by Mass Hunter, they were averaged to assist data analysis. Masses lacking a natural isotopic distribution or with a signal-to-noise ratio of less than 3 were removed from the data prior to analysis. One of skill in the art will appreciate that the results from this assay provide relative values that are assessed according to annotated values within 10 ppm to provide an identity for the molecular weight detected. Thus, a mass shift within 10 ppm is considered consistent with determining the identity of a specific cellular metabolite annotated known in the art due to differences in ionization source and instrumentation, e.g. between different experiments or using different instruments.

As used herein, a mass was considered to be the same across LC/ESI-TOF-MS runs using a simple algorithm that first sorts the data by mass and retention time. After sorting, a compound was considered unique if it had a retention time difference of less than or equal to three minutes and a mass difference less than or equal the weighted formula (0.000011× mass). If a series of measurements fit this definition it was considered to be from the same compound. If either the mass or the retention time varied by more than the limits listed above it was considered to be a different compound and given a new unique designation.

Significance tests were determined by performing ANOVAs on the log base 2 transformed abundance values of unique compounds present in treated and untreated media at each time point. A randomized complete block design was used with the ANOVA model including the effects of treatment, experiments, and a residual term, with the following formula: $\mathrm{Log}_2(\mathrm{abundance}_{tb})=\mathrm{treatment}_t+\mathrm{experiment}_b+\mathrm{error}_{tb}$.

Missing data were omitted from the test changing the degrees of freedom rather than assuming the missing data were absent. This assumption was made because the extensive filtering performed by the Mass Hunter software may miss or filter certain peaks because they are below a certain abundance threshold and not zero. The ANOVA F-test was considered significant if its p-value was less than 0.05. Fold changes were calculated using the least squared means for a given time and treatment.

The term "biomarker" as used herein refers to cellular metabolites that exhibit significant alterations between treated and untreated controls. In preferred embodiments, biomarkers are identified as set forth above, by methods including LC/ESI-TOF-MS. Metabolomic biomarkers are identified by their unique molecular mass and consistency with which the marker is detected in response to a particular toxic, teratogenic or test chemical compound; thus the actual identity of the underlying compound that corresponds to the biomarker is not required for the practice of this invention. Alternatively, certain biomarkers can be identified by, for example, gene expression analysis, including real-time PCR, RT-PCR, Northern analysis, and in situ hybridization, but these will not generally fall within the definition of the term "cellular metabolites" as set forth herein.

The basal metabolome of undifferentiated hESCs served as a collection of biochemical signatures of functional pathways that are relevant for sternness and self-renewal. Metabolite profiling was conducted on excreted or secreted cellular metabolites as opposed to intracellular compounds. Ultimately, biomarkers discovered in vitro are expected to be useful for analyzing in vivo biofluids such as serum, amniotic fluid and urine, complex mixtures of extracellular biomolecules. This is advantageous over invasive procedures such as tissue biopsies because small molecules in biofluids can be detected non-invasively (in contrast to intracellular compounds). In addition, processing cellular supernatant for mass spectrometry is more robust and less laborious than cellular extracts. However, cellular extracts (from, for example, lysed cells) can be utilized in the methods of the invention.

The term "biomarker profile" as used herein refers to a plurality of biomarkers identified by the inventive methods. Biomarker profiles according to the invention can provide a molecular "fingerprint of the toxic and teratogenic effects of a test compound and convey what cellular metabolites, specifically excreted and secreted cellular metabolites, were significantly altered following test compound administration to hESCs or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells. In these embodiments, each of the plurality of biomarkers is characterized and identified by its unique molecular mass and consistency with which the biomarker is detected in response to a particular toxic, teratogenic or test chemical compound; thus the actual identity of the underlying compound that corresponds to the biomarker is not required for the practice of this invention.

The term "biomarker portfolio" as used herein refers to a collection of individual biomarker profiles. The biomarker portfolios may be used as references to compare biomarker profiles from novel or unknown compounds. Biomarker portfolios can be used for identifying common pathways, particularly metabolic or developmental pathways, of toxic or teratogenic response.

These results set forth herein demonstrated that human embryonic stem cell metabolomics, and metabolomics from hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, can be used in biomarker discovery and pathway identification. Metabolomics detected small molecules secreted by hESCs or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, produced therefrom and the identified biomarkers can be used for at least two purposes: first, to determine specific metabolic or developmental pathways that respond to or are affected by toxin or teratogen exposure, particularly said pathways utilized or affected during early development that are sensitive to toxic, teratogenic or test chemical compounds that are developmental disruptors and participate in the ontogenesis of birth defects; and second, to provide cellular metabolites that can be measured in biofluids to assist management and diagnosis of toxic exposure, birth defects or other disease.

A biomarker portfolio from hESCs or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, produced therefrom can also serve as a high throughput screening tool in preclinical phases of drug discovery. In addition, this approach can be used to detect detrimental effects of environmental (heavy metals, industrial waste products) and nutritional chemicals (such as alcohol) on human development. Ultimately, the methods of this invention utilizing the hESC metabolome or the metabolome of or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, can assist pharmaceutical, biotechnology and environmental agencies on decision-making towards development of compounds and critical doses for human exposure. The integration of chemical biology to embryonic stem cell technology also offers unique opportunities to strengthen understanding of human development and disease. Metabolomics of cells differentiated from hESC should serve similar roles and be useful for elucidating mechanisms of toxicity and disease with greater sensitivity for particular cell or tissue types, and in a human-specific manner. For example, key metabolic pathways, including as set forth herein folate, glutamate and tryptophan synthesis and degradation, may be differentially disrupted in earlier versus later stages of human development. In addition, metabolite profiles of neural precursor cells or neuronal cell populations can reveal biomarkers of neurodevelopmental disorders in target cell types. The association of metabolomics to stem cell biology can inform the mechanisms of action of folic acid and neural tube defects in the early human embryo.

Biomarker portfolios produced using the hESC-dependent and hESC-derived lineage-specific cell-dependent methods of this invention can also be used in high throughput screening methods for preclinical assessment of drug candidates and lead compounds in drug discovery. This aspect of the inventive methods produces minimal impact on industry resources in comparison to current developmental toxicology models, since implementation of this technology does not require experimental animals. The resulting positive impact on productivity enables research teams in the pharmaceutical industry to select and advance compounds into exploratory development with greater confidence and decreased risk of encountering adverse developmental effects.

The term "developmental pathway" as used herein refers to developmental or metabolic pathways in embryonic and fetal development.

"Supernatant" as used herein may include but is not limited to extracellular media, co-cultured media, cells, or a solution of fractionated or lysed cells.

Cellular metabolite profiles obtained from analysis of toxins, teratogens, alcohol, and test chemical compounds can be used to compose a library of biomarker portfolios. These portfolios can then be used as a reference for toxicological analysis of unknown chemical compounds. A similar strategy has been validated as a means to determine cellular changes that arise in response to chemicals in non-hESC systems (Daston & Nacliff, 2005, *Reprod Toxicolog* 19:381-94; Fella et al., 2005, *Proteomics* 5:1914-21). Metabolic profiles of novel compounds can be compared to known biomarker portfolios to identify common mechanisms of toxic response. This approach can reveal functional markers of toxic response, which serve as screening molecules that are shared at least in part as a consequence of exposure to various different toxic and teratogenic compounds. Such hESC-derived small molecules can be used as measurable mediators of toxic response that refine or replace costly and complex screening systems (such as in vivo animal models) and have the additional advantage of being specific for human cells and human metabolic and developmental pathways.

EXAMPLES

The Examples which follow are illustrative of specific embodiments of the invention, and various uses thereof. They are set forth for explanatory purposes only, and are not to be taken as limiting the invention.

Example 1

Developmental Toxicology Screening

To demonstrate the efficacy of hESCs as a model system for developmental toxicity testing, hESCs were treated with a known teratogen, valproate (VPA). Valproate is a common mood stabilizer and anti-convulsant drug with clinical indications in epilepsy and bipolar disorder (Williams et al., 2001, *Dev Med Child Neuro* 43:202-6) that has been associated with developmental abnormalities (Meador et al., 2006, *Neurology* 67: 407-412). The mechanism by which valproate produces developmental defects, however, is not fully understood, despite the increased susceptibility of the nervous system (Bjerkedal et al., 1982, *Lance* 2:109: Wyszynski et al., 2005, *Neurology* 64:961-5; Rasalam et al., 2005, *Dev Med Child Neuro* 47:551-555). Exposure to valproate results in a pronounced increase in spina bifida and neural tube defects (NTDs; Bjerkedal et al., 1982, *Lancet* 2:109) at ten-to-twenty times that of the general population, as well as cognitive disorders such as autism (Adab et al., 2004, *J Neurol Neurosurg Psychiatry* 75:1575-83). However, since VPA is an anticonvulsant drug with clinical indications in epilepsy and

bipolar disorder (Williams et al., 2001, *Dev Med Child Neurol* 43:202-06), treatment generally must be sustained throughout pregnancy.

Folic acid supplementation prior to pregnancy reduces the incidence of spina bifida by 70% (Shaw et al., 1995, *Epidemiolog* 6:219-226) although its precise mechanism of action is unknown. In addition, homocysteine and glutathione have also been implicated in NTDs (Zhao et al., 2006, *Birth Defects Res A Clin Mol Teratol* 76:230-6). Thus, metabolite profiles of folate and related pathways were candidates for changes in response to valproate. In the results set forth herein, folic acid was significantly increased (by 16%) in the extracellular media of hES cells treated with valproate (p=0.022 at eight days, Table 3 and FIG. **4**) but not its derivative dihydrofolate. Since mammalian cells do not synthesize folic acid, valproate may act by interfering with cellular uptake of folic acid.

Exposure of hESCs was performed as follows. H1 hESC (passage 41) were cultured on Matrigel (BD Scientific, San Jose, Calif.) in the absence of a feeder layer. hESCs were maintained in conditioned medium (CM) collected from mouse embryonic fibroblasts (MEFs) (80% DMEM/F12, Invitrogen, Carlsbad, Calif.) and 20% KNOCKOUT serum replacement (Invitrogen) supplemented with 1 mM L-glutamine (Invitrogen), 1% MEM non-essential amino acids (Invitrogen), and 0.1 mM 2-mercaptoethanol (Sigma, Chemical Co., St. Louis, Mo.). Prior to feeding hESCs, the culture medium was supplemented with 4 ng/mL human recombinant basic fibroblast growth factor (Invitrogen). hESCs were passaged when the wells were ~80% confluent. To passage, hESCs were incubated in a 1 mg/mL dispase (Invitrogen)/DMEM/F12 solution for 7-10 minutes at 37° C. After this treatment hESCs were washed and seeded on fresh Matrigel coated plates. In parallel studies disclosed herein, H1 and H9 cells were cultured in defined medium known as TeSR (Ludwig et al., 2006, Id.).

H1 and H9 (equivalent to NIH code WA01/WA09) hESC were treated with valproate (VPA) (22 μM and 1 mM) (Sigma # P4543) according to the procedure outlined below; each experiment involved three separate VPA treatments, and each treatment group had a parallel control group with a total of six 6-well culture dishes (Nunc, Naperville, Ill.) per experiment (two 6-well culture dishes per treatment). Treatment 1 (labeled 24 H) exposed hESC cells to 1 mM VPA (Sigma) for 24 hours followed by collection of supernatant and cell pellets. In a second treatment group (labeled 4 D), hESC cells were exposed to 22 μM or 1 mM VPA for 4 days and harvested on day 4. In a third treatment group (labeled extended culture, EC), hESC cells received 22 μM or 1 mM VPA for 4 days followed by culture in standard hESC media for an additional four days. For this group, cells and supernatant were harvested on day eight.

To assess the effects of teratogenic VPA treatment on hESCs, the treated cells were analyzed as set forth below to determine changes in a total dynamic set of small molecules present in cells according to health and disease or insult states. Small molecules including but not limited to sugars, organic acids, amino acids, fatty acids, hormones, vitamins, oligopeptides (less than about 100 amino acids in length), as well as ionic fragments thereof and signaling low molecular weight compounds were known to participate in and reveal functional mechanisms of cellular response to pathological or chemical insult. These analyses were also used to identify active pathways following molecular changes predicted by other analyses including for example transcriptomics and proteomics.

Supernatant from VPA-treated and control hESCs were subjected to liquid chromatography and electrospray ionization time of flight mass spectrometry (LC/ESI-TOF-MS) to assess changes and differences in small molecules (as defined herein) produced by the cells in the presence and absence of VPA treatment. Supernatant was collected from control and treated plates of hESCs at 24 H, 4 D, and 8 D, and CM was collected as a "no treatment" control. The supernatant and media were stored at −80° C. until preparation for mass spectrometry analysis. For analysis, samples were prepared in a 20% Acetonitrile (Fisher Scientific Co., Pittsburgh, Pa.) solution (comprising 500 μL of supernatant, 400 μL acetonitrile and 1.1 mL distilled water) and centrifuged through a Millipore 3 kDa Centricon column (Millipore, Billerica, Mass.) for 3 hours at 4575×g to remove proteins. The flow-through was retained for analysis, as it contains small molecules free of high molecular weight compounds such as proteins. In each analysis, three replicates for each sample were injected into a 2.1×200 mm C18 column using a 90 minute gradient from 5% Acetonitrile, 95% Water, 0.1% Formic Acid to 100% Acetonitrile, 0.1% Formic Acid at a flow rate of 40 μL/min. ESI-TOF-MS (TOF) was performed on the flow-through using an Agilent ESI-TOF mass spectrometer. Data was collected from 100-3600 m/z, and particularly in the 0-1500 m/z range. The raw data was analyzed to identify the separated small molecules using a computer compilation and analysis program (Mass Hunter) provided by the manufacturer and according to manufacturer's instructions (Agilent; statistical analyses were performed as described above in the Detailed Description and Preferred Embodiments. This analysis generated lists of retention time/accurate mass pair feature. Another program (Mass Profiler, Agilent) was used to compare multiple run sets to find ion intensity changes of features that changed between the sample conditions. Significance tests were determined by performing ANOVAs on the log base 2 transformed abundance values of unique compounds present in treated and untreated media at each time point.

The plurality of small molecules identified using these methods were then compared with exact mass and retention time from ESI-TOF-MS using public databases (for example, at http://metlin.scripps.edu., www.nist.gov/srd/chemistry.htm; http://www.metabolomics.ca/). Mass spectrometry analysis also included predicted chemical structures of small molecules based upon exact mass, although currently-available public databases do not in every instance include matching small molecules due to database limitations. In addition, more comprehensive private databases are available for comparative analysis, such as the NIST/EPA/NIH Mass Spectral Library: 05. NIST ASCII Version.

The results of these analyses are shown in FIGS. **1**A through **1**C and FIG. **2**A through **2**C. In FIGS. **1**A through **1**C each feature on the plot corresponds to a small molecule with specific exact mass and retention time. The plots summarize significant differences found between treated (blue) and untreated (red) groups at different time points. As shown in the Figure, at 24 hours (24 H) there was consistent down-regulation of the secreted biomolecules in treated (blue) cells in comparison to untreated (red) controls. At four days (4 D) and eight days (EC), treated (blue) cells secreted a higher number of small molecules in comparison to untreated cells (red); said small molecules were thus considered as candidate biomarkers. In particular, metabolites from the folate pathway, including tetrahydrofolate (exact mass 444) and dihydrofolate (exact mass 441) were detected. These findings were considered significant, since they show for the first time that hESCs contacted with a known teratogen (VPA) that causes a birth defect (spina bifida) respond by up-regulating a metabolic pathway that produces a compound (folate) known to ameliorate the effects of the teratogen when administered to a woman bearing a developing embryo or fetus.

Further, the results shown in FIGS. **1**A through **1**C revealed approximately 40 small molecules that were absent in treated groups, suggesting that multiple cellular pathways were "silenced" in response to VPA at 24 hours in comparison to untreated controls. At four and eight days after treatment, however, multiple candidate biomarkers were upregulated in treated versus untreated human embryonic stem cells; these results are shown in Table 1. Candidate biomarkers were identified as small molecules showing a change in treated versus untreated cells measured to be at least a two-fold difference. In many instances, these small molecules are absent or detected at very low concentrations in untreated human embryonic stem cells.

These studies demonstrated that the claimed methods for assessing developmental toxicity and the identification of biomarkers using hESCs provided robust information on changes in small molecule content of cells in response to being contacted with a known teratogen, VPA. The results concerning a compound (VPA) that is involved in the etiology of spina bifida and neural tube defects (NTDs) (Bjerkedal et al., 1982, *Lancet* 2:109) when exposed to a developing human conceptus are particularly striking. The results shown here indicated a marked increase (2 to 8 fold) in key metabolites of the folate pathway (dihydrofolic acid, tetrahydrofolic acid, S-adenosylmethionine) following treatment with VPA (in comparison to untreated cells). These methods were reproducible, having been repeated with consistent results obtained in three independent studies using hESCs and on non-embryonic cells (human fibroblasts) as controls (data not shown), and suggested a heretofore unknown adaptive response of the fetus to the chemical/environmental insult and identified sensitive markers for said insult(s).

The mechanism for VPA developmental defects, however, is not fully understood despite the fact that the nervous system is particularly sensitive to its effects (Bjerkedal et al., 1982, *Lancet* 2:109; Narita et al., 2000, *Pediatric Res* 52:576-79; Rasalam et al., 2005, *Dev Med Child Neurol* 47:551-55). Folic acid supplementation prior to pregnancy prevents the incidence of spina bifida by 70% (Shaw et al., 1995, *Epidemiolog* 6:219-226), although the exact mechanism of action is also unknown. The information obtained herein can be used to elucidate mechanisms of action of folic acid and neural tube defects in the early human embryo. These methods can also be applied to other known teratogens, such as retinoic acid, warfarin, and thalidomide (Franks et al., 2004, *Lancet* 363:1802-11) to validate the predictive ability of hESCs using the methods of the invention.

Table 1: Candidate small molecules (biomarkers) of developmental toxicity detected in undifferentiated human embryonic stem cells treated with 1 mM valproate in comparison to untreated controls.

TABLE 1

Candidate small molecules (biomarkers) of developmental toxicity detected in undifferentiated human embryonic stem cells treated with 1 mM valproate in comparison to untreated controls.
Change in VPA Treated hESCs in comparison to untreated controls

Exact mass	RT	24 Hours	4 Days	8 Days	Candidate Biomarker
355.066	16		UP		SAM S-ADENOSYLMETHIONINAMINE
355.12	30		UP		SAM
381.1574	12		UP	UP	GLUTATHIONE
398.21	39	DOWN	UP		SAM OXOBUTANOATE
441.8831	12			UP	DIHYDROFOLIC ACID
444.1729	17		UP	UP	TETRAHYDROFOLIC ACID
472.16	17	ZERO	UP	UP	TETRAHYDROFOLATE
612.15	17	DOWN	DOWN	UP	GLUTATHIONE OXIDIZED

RT = retention time
Small molecule detection was conducted with LC/ESI-TOF-MS in triplicate samples of supernatant processed independently.

As discussed above, metabolite profiles were determined at 24 hours, four days and eight days after valproate treatment. At four days after treatment, multiple candidate biomarkers were upregulated in treated versus untreated human embryonic stem cells (shown in FIGS. **2**A through **2**C). In addition to the results set forth above regarding increased levels of certain metabolites, multiple metabolite peaks were down-regulated in response to valproate at 24 hours in comparison to untreated controls (FIGS. **2**A through **2**C).

hESCs were cultured in conditioned media from mouse embryonic fibroblasts, which generated 1277 of the 3241 measured compounds. Many metabolites in human development and disease are likely present in conditioned media from mouse embryonic fibroblasts due to common metabolic pathways. Rigorous investigation is required to validate candidate biomarkers that are not exclusive to hESCs and are also present in the media.

Example 2

Gene Expression Analysis

The efficacy of the analysis shown in Example 1 was confirmed by gene expression studies, wherein changes in gene expression were observed following VPA treatment of hESCs. VPA treatment was not detrimental to hESCs, which remained viable for multiple passages following teratogen exposure, thus enabling gene expression analysis to be performed.

Treated and control H1/NIH code WA01 hES cells (passage 41) were analyzed by real-time PCR, and each treatment group was paired with a corresponding control group that received the standard growth media combination of CM+bFGF without VPA. In these studies, total cellular RNA was extracted from cells harvested at 24 hours (24 H), 4 days (4 D) and 8 days (EC) using the RNA Easy Kit (Qiagen, Valencia, Calif.) according to the manufacturer's instructions.

Expression levels of candidate test genes and a housekeeping gene (Beta-2-microglobulin) were evaluated by quantitative real-time PCR using a DNA Engine-Opticon 2 Detection System (MJ Research, Watertown, Mass.). The housekeeping gene acts as an internal control for normalization of RNA levels. The primers used for real-time PCR reactions were designed using Beacon Designer software (Premier Biosoft International, Palo Alto, Calif.). RNA was reverse transcribed using iScript cDNA Synthesis kit (Bio-Rad, Hercules, Calif.), wherein each cDNA synthesis reaction (20 μL) included 4 μL of 5× iScript reaction mix, 1 μL of iScript reverse transcriptase, and 2 μL of RNA. PCR was performed on cDNA in PCR reaction mixtures (25 μL) each containing 12.5 μL of Supermix (contains dNTPs, Taq DNA polymerase, SYBR Green I, and fluorescein), 250 nM forward primer, 250 nM reverse primer, and 1.6 μL RT-PCR products. Melting curve analysis and agarose gel electrophoresis were performed after real-time PCR reaction to monitor PCR specificity, wherein PCR products were detected with SYBR Green I using the iQ SYBR Green Supermix kit (Bio-Rad).

Quantifying the relative expression of real-time PCR was performed using the 2-ΔΔCt method (Livak & Schmittgen, 2001, *Methods* 25:402-8), and a general linear model was employed to fit the expression data. The PROC GLM procedure in SAS (version 8.2; SAS Institute, Cary, N.C.) was used to estimate least squares means in expression between treated and untreated hESCs and $P<0.05$ was considered statistically significant

Real-time PCR was conducted on samples from 24 hours (24 H), 4 days (4 D) and 8 days (EC) after VPA treatment to investigate expression levels of epigenetic regulators (such as DNA methyltransferase-1, DNMT-1, BMI-1, EED) and critical transcription factors responsible for embryonic patterning and neurodevelopment (RUNX2, BMP7, FGF8, CBX2, GLI3, SSH and SP8) in human embryonic stem cells. These experiments showed hESCs treated with VPA were subject to marked changes in their transcriptional activity following teratogen treatment. VPA induced overall marked (2 to 30 fold) downregulation of transcription levels as early as 24 hours after exposure in all genes tested (with the exception of DNMT-1 and Shh). At 4 days after treatment, however, expression of the ubiquitous DNA methyltransferase-1 was almost abolished, and sonic hedgehog, which is absolutely critical for neurogenesis (Ye et al., 1998, *Cell* 93:755-66), was down-regulated five-fold in comparison to untreated controls. At 8 days after VPA treatment, the majority of the genes were upregulated in comparison to untreated controls.

These results embodied two major implications for developmental toxicology. First, VPA induced persistent changes in key epigenetic modulators that also participate in differentiation of other tissues, such as DNMT-1 and the polycomb family member EED. Second, the effects of teratogens persisted in hESCs during critical stages of neurogenesis and organogenesis. For example, genes whose expression was affected as shown herein (including sonic hedgehog and FGF-8) are known to be master regulators of differentiation of serotonergic neurons in the brain (Ye et al., 1998, *Cell* 93:755-66). Of particular notice is the fact that DNMT-1 expression is almost abolished at four days after treatment. In vivo, disruption of this enzyme is lethal to embryos, since it is the major maintenance methyltransferase during DNA replication (Li et al., 1992, *Cell* 69:915-26).

Following teratogen exposure, temporal-specific alterations in developmental gene expression were observed. Developmental genes differ in their susceptibility to teratogens at different times. This indication may be critical to understanding specificity of epigenetic disruptors on certain organs or tissues. RUNX2, for example, is a transcriptional activator of bone development (Napierala et al., 2005, *Mol Genet Metab* 86:257-68), and is more sensitive to VPA-mediated up-regulation at very early or late stages following exposure. Real-time PCR results from hESCs disclosed herein were correlated to previous findings in vivo in mice (Okada et al., 2004, *Birth Defects Res A Clin Mol Teratol* 70:870-879) and rats (Miyazaki et al., 2005, *Int J Devl Neuroscience* 23:287-97). In these animal studies, VPA inhibited the expression of Polycomb genes, Eed, Bmil and Cbx2 and induced downregulation of Shh while FGF8 levels remained unchanged. The results shown here at four days following VPA treatment (Table 2) were consistent with these observations using other developmental model systems.

TABLE 2

1 mM VPA treatment resulted in marked changes in the expression of epigenetic regulators and developmental genes that are critical for embryonic patterning and differentiation of neurons.

Gene	1-24 H control	2-24 H VPA treated	3-4 D control	4-4 D VPA treated	5-EC control	6-EC VPA treated
BMI-1	0.543	0.252	1.651	0.112	1.020	1.071
DNMT1	0.664	0.624	1.742	0.002	0.731	1.124
EED	0.757	0.342	1.501	0.185	1.381	1.769
H19	0.207	0.006	0.144	0.846	10.660	2.756
RUNX2	0.325	1.769	5.198	4.020	1.177	2.434
BMP7	0.511	0.093	0.397	0.342	0.731	1.664
FGF8	2.544	0.801	0.384	0.314	0.16	0.837
CBX2	1.113	0.245	1.221	1.1881	0.81	1.946
GLI3	0.202	0.015	0.016	0.774	0.950	1.918
Shh	0.562	0.562	2.772	0.533	1.369	—
SP8	0.49	0.235	0.25	1.703	2.132	5.808

Gene expression levels are relative to a housekeeping gene (target gene/Beta-2-Microglobulin).

Example 3

Human Embryonic Stem Cell Metabolome: Metabolite Profiles Following Teratogen Exposure

Exposure of hES cells to the teratogen valproate induced significant changes in different metabolic pathways, including pathways important during pregnancy and development. An alternative metabolic pathway activated during pregnancy are shown in FIG. **5**, wherein tryptophan is converted to kynurenine. To investigate this aspect of the invention, hESCs were cultured as described in Example 1, and the procedure for valproate treatment was performed as described therein. Treatment 1 (24 hours) exposed hES cells to 22 μM valproate for 24 hours followed by collection of supernatant and cell pellets. In the second treatment group (4 days), hES cells were exposed to 22 μM valproate for 4 days and harvested on day 4. In the third treatment or extended culture (EC, 8 days), hES cells received valproate for 4 days followed by culture in standard hES cell media for an additional four days. Cells and supernatant were harvested on day eight. Each treatment had a parallel control group with a total of six 6-well culture dishes per experiment (two 6-well culture dishes per treatment).

Metabolome analysis was performed as described in Example 1 (Wu and McAllister, 2003, *J Mass Spectrom* 38:1043-53). Complex mixtures were separated by liquid chromatography (LC) prior to electrospray ionization (ESI) time of flight (TOF) mass spectrometry according to the methods described in this Example and Example 1. Mass Hunter (Agilent) software was applied to deconvolute the data and determine the abundance of each mass. Data were extracted from the entire mass spectrum using the m/z range of 0 to 1500 and the top 2 million most abundant mass peaks from each sample were used for data deconvolution. The minimum signal-to-noise ratio was set to 5. The masses with a minimum relative abundance greater than 0.1% were exported from the Mass Hunter software and used for further analysis.

hESCs treated with 22 μM valproate resulted in 3,241 detected mass signals 42 injections. Of the total of 3,241 mass signals detected in these experiments, 1,963 compounds were measured solely in hES cells and 1,278 compounds were also present in conditioned media; 443 of these were only measured in 1 of 42 injections. 110 compounds (3%) had statistically-significant differences in at least one time point in valproate-treated hESCs compared with control. Fold changes as high as seven- to thirteen-fold were measured after valproate treatment, but these mass signals exhibited high variability across experiments. Representative masses identified following treatment of cells with 1 mM and 22 μM VPA are summarized in Tables 3 and 4, respectively. Several peaks (1,963) were detected in hES cells but not in conditioned media. One of these small molecules was kynurenine, a compound produced by an alternative tryptophan metabolic pathway, activated during pregnancy and immune response. The levels of kynurenine increased by 44% (p value=0.004 at four days, Table 5) following valproate treatment. Kynurenine was detected exclusively in hES cells and absent in conditioned media. The chemical identity of this peak was confirmed by comparative mass spectrometry in the presence of the chemical standard (FIG. **4**).

The results of these experiments suggested that kynurenine is a candidate biomarker for neurodevelopmental disorders, in particular those originated by exposure of the human embryo to anti-epileptic drugs such as VPA (Ornoy et al., 2006, *Reproductive Toxicol* 21:399-409). Strikingly, recent studies have suggested that kynurenine metabolism may be a novel target for the mechanism of action of anti-epileptic drugs (Kocki et al., 2006, *Eur J Pharmacol* 542:147-51). Cognitive and behavioral disorders are known adverse effects of antiepileptic exposure during pregnancy. Tryptophan is the precursor of serotonin, a key neurotransmitter in the pathogenesis of these and other diseases, such as depression. In addition, increased plasma levels of kynurenine have been linked to postpartum depression (Kohl et al., 2005, *J Affect Disord* 86:135-42). The alteration in tryptophan metabolism detected herein is a means for examining novel mechanisms in pathogenesis of serotonin-related behavioral disorders such as autism (Chugani, 2004, *Ment Retard Dev Disabil Res Rev* 10:112-116). An increase in kynurenine levels during development may reduce the bioavailability of tryptophan and consequently serotonin, leading to cognitive dysfunction.

Glutamate and pyroglutamic acid were also elevated in hESCs treated with valproate. Glutamate and pyroglutamic acid were elevated in response to valproate (20% and 27%, respectively), although only pyroglutamic acid exhibited statistically significant changes (p=0.021 at 4 days, FIGS. **3**A through 3D). Glutathione (GSH) is metabolized by gamma-glutamyltranspeptidase into glutamate, a neurotransmitter of NMDA receptors, and cysteinylglycine (Cys-Gly). Glutathione (exact neutral mass 612.15) and S-adenosyl-homocysteine (exact neutral mass 384.12) were detected at very low levels in comparison to other mass signals (data not shown). For these experiments for low level detection, small molecules were identified by comparative ESI-TOF-MS with chemical standards that were "spiked" into conditioned media at different concentrations and used to confirm neutral exact masses and retention times of experimental mass signals (FIGS. **3**A through **3**D). Neutral exact masses and/or empirical chemical formulas generated by ESI-TOF-MS were searched in public databases (including, for example, metlin.scripps.edu., www.nist.gov/srd/chemistry.htm, www-.metabolomics.ca) for candidate compounds.

These results suggested that valproate affects the glutamate synthesis pathway in the developing human embryo. The affinity of anti-epileptic drugs towards glutamate targets has been previously suggested (Rogawski and Loscher, 2004, *Nat Rev Neurosci* 2004 5:553-64). Abnormal levels of glutamate metabolites were measured in maternal serum and amniotic fluid of pregnant women whose infants were diagnosed spina bifida (Groenen et al., 2004, *Eur J Obstet Gynecol Reprod Biol.;* 112:16-23) with nuclear magnetic resonance (NMR). The levels of glutamine and hydroxyproline were significantly higher in NTDs, and as a result the hESC methods provided herein provide a robust resource to model in vivo alterations of development.

Table 5. Changes in metabolic profiles of four compounds in hES cells treated with valproate versus untreated controls at 24 hours (24 h), 4 days (4 D), and eight days (8 D) after treatment.

TABLE 5

Changes in metabolic profiles of four compounds in hES cells treated with valproate versus untreated controls at 24 hours (24 h), 4 days (4 D), and eight days (8 D) after treatment.

Molecule	24 h P-value	24 h fold	4 D P-value	4 D fold	8 D P-value	8 D fold	Mass	RT
Pyroglutamic acid	0.242	57% decrease	**0.021**	27% increase	0.917	3% decrease	129.0426	19.9
Folic acid	0.638	3% increase	0.626	4% increase	**0.022**	16% increase	441.1395	32.7
Glutamate	0.969	1% increase	0.108	24% increase	0.651	10% increase	147.0535	20.0
Kynurenine	0.087	29% increase	**0.004**	44% increase	N.D.	N.D.	208.0850	25.9

RT = retention time

Fold changes are represented as percent difference of the least squared means of valproate treated and untreated hES cells. p-values were determined by ANOVA. The mass is the average neutral mass detected by ESI-TOF-MS and the RT is the average retention time the molecule eluted at. P-values less than 0.05 are in bold.

The amino acid tryptophan (TRP) is a precursor of the neurotransmitter serotonin, a key mediator of numerous CNS disorders, such as depression, neurodegeneration and cognitive impairment. Tryptophan catabolism into kynurenic acid is an alternative route for tryptophan metabolism (FIG. **5**), that is activated in specific circumstances such as inflammatory response or pregnancy. Up-regulation of the kynurenine pathway is correlated with psychosis in adult diseases such as schizophrenia and bipolar disorder, an indication that increased levels of pathway intermediates may trigger psychotic features (Miller et al., 2006, *Brain Res* 16:25-37). Significantly, metabolism using the kynurenine pathway is accompanied by decreased tryptophan metabolism using the serotonin pathway (in the absence of exogenous tryptophan, an essential amino acid not synthesized by mammals including man). An increase in kynurenine levels during development can reduce the bioavailability of tryptophan and consequently serotonin, leading to cognitive dysfunction.

In addition, kynurenic acid (KYNA), one of the end products of this tryptophan metabolic pathway, is an antagonist of glutamate neurotransmission and N-methyl-D-aspartate (NMDA) receptors. Recent studies have demonstrated that kynurenic acid is a druggable target via its role in the activation of the previously orphan GPCR receptor GPR35 (Wang et al., 2006, *J Biol Chem* 281:22021-8). Quinolinic acid (QUIN), another end product of the pathway (FIG. **5**), and 3-hydroxy-kynurenine, an intermediate, act as neurotoxicants (Guillemin et al., 2005, *J Neuroinflammation* 26:16; Chiarugui et al., 2001, *J Neurochem* 77:1310-8). QUIN is involved in the pathogenesis of Alzheimer's disease where its neurotoxicity may be involved in increased inflammation and in convulsions by interacting with the N-methyl-D-aspartate (NMDA) receptor complex, a type of glutamate receptor (Guillemin et al., 2002, *J Neuroinflammation* 26:16; Nemeth et al., 2005, *Curr Neurovasc Res* 2:249-60). Kynurenin (KYN), another pathway intermediate, is synthesized in the brain and is transported across the blood-brain barrier (Nemeth et al., 2005, *Curr Neurovasc Res* 2:249-60). KYN is metabolized to the neurotoxic quinolinic acid (QUIN) and the neuroprotective kynurenic acid (KYNA) (FIG. **5**). Increased serum levels of KYN have been correlated to clinical manifestation of depression with different etiologies, such as postpartum disorder (Kohl et al., 2005, *J Affect Disord* 86:135-42) and interferon-alpha treatment (Capuron et al., 2003, *Biol Psychiatry* 54:906-14).

Exposure of hES cells to valproate, a disruptor of human development, induced significant changes in different metabolic pathways, including the production of kynurenine (ex-

Example 4

Kynurenine: Biomarker for Diagnosis and Treatment of Developmental Toxicity and CNS Disorders

Kynurenine was shown in Example 3 to be detected in valproate-treated hES cells. Kynurenine (along with glutamate and pyroglutamic acid) was differentially produced in valproate-treated human embryonic stem cells (hES) versus controls. Kynurenine is a novel biomarker useful for the identification of neurodevelopmental disorders in infants and in vitro developmental toxicity of chemicals. This example describes the identification of biomarkers for neurodevelopmental disorders, including cellular products differentially produced in teratogen-treated hESCs.

act neutral mass 208.08), which was significantly upregulated in response to valproate as detected by liquid chromatography electrospray ionization time of flight mass spectrometry (LC/ESI-TOF-MS) as described in Example 4. Additionally, novel chemical entities, having exact neutral masses of 328.058, 336.163, 343.080, were detected and are not yet catalogued in public databases.

When neural precursors derived from hESCs were exposed to 1 mM valproate, a marked decrease in both serotonin (176.0946) and indoleacetaldehyde (159.0689), a downstream sub-product of serotonin generated by monoaminoxoidase activity (MAO) was observed (Table 6). Glutamate and pyroglutamic acid or hydroxyproline (p=0.021) were also elevated in hES cells treated with valproate. These results suggest that valproate affects the glutamate synthesis pathway in the developing human embryo. This finding emulates in vivo neurophysiology, where compounds from the kynurenine pathway modulate activity at NMDA glutamate receptors and produce epileptic phenotypes, including seizures (Perkins and Stone, 1982, *Brain Res* 247:184-187.).

As a consequence of the identification of kynurenine herein, chemical inhibitors of kynurenine synthesis can be used as novel therapeutics in mood disorders; for example, small molecules that antagonize indoleamine 2,3-dioxygenase (IDO) or kynurenine formylase activities, which converts tryptophan (TRP) into kynurenine (KYN). Inhibition of TRP catabolism to KYN can be used to ameliorate disease symptoms in cognitive and neurodegenerative disorders by increasing serotonin levels, via elevated synthesis of this neurotransmitter or reduced depletion through the kynurenine pathway.

Collectively, the metabolite changes detected in hES cells in response to valproate converge functionally towards folate, kynurenine and glutamate pathways. FIG. **6** illustrates the hierarchical clustering of the fold change differences from 22,573 unique masses. Changes in the above-mentioned pathways were consistent and reproducible in multiple independent studies of 1 mM VPA treated hESCs, and neural precursors produced from hESCs (FIG. **6**).

Example 5

Gene Expression Analysis of Kynurenine Pathway

The efficacy of the analysis in Example 4 was confirmed by gene expression studies, wherein changes in gene expression were observed following VPA treatment of hESC. Valproate treatment of human embryonic stem cells induced a marked upregulation in the small molecule kynurenine, an intermediate metabolite in the catabolism of tryptophan. Tryptophan is the precursor of the neurotransmitter serotonin (5HT). Thus, whether expression of enzymes in the metabolism of tryptophan to kynurenine and its opposite route, serotonin synthesis, was altered in human embryonic stem cells was investigated to examine the mechanistic properties of the kynurenine pathway and its response to valproate.

Human embryonic stem cells treated with 1 mM valproate and untreated controls were harvested at four days after treatment and stored at −80° C. prior to RNA isolation using RNeasy (Qiagen). 5 μg of RNA templates were reverse transcribed and amplified (QIAGEN OneStep RT-PCR) according to the manufacturer's instructions using primers designed for transcribed human sequences of the following genes: INDO, indoleamine 2,3 dioxygenase, TDO or TDO2, tryptophan 2,3-dioxygenase, AFMID, arylformamidase, TPH1, tryptophan hydroxylase the rate-limiting enzyme in serotonin biosynthesis, AADAT, aminoadipate aminotransferase, KYNU, kynunreninase, KMO, kynurenine 3-monooxygenase, GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

The results of this study showed that the majority of enzymes in the kynurenine pathway and serotonin synthesis were expressed in hES cells at four days after treatment of hES cells with 1 mM valproate (FIG. **7**). Indoleamine 2,3 dioxygenase INDO, catabolizes tryptophan into the kynurenine pathway, and produces kynurenine as an end product. The expression of tryptophan 2,3 dioxygenase (TDO or TDO2) was also examined. TDO2, like INDO, catalyzes the first step in the kynurenine pathway. These data suggested that TDO2 expression was upregulated in hES cells treated with valproate in comparison to untreated controls. The rate limiting enzyme in 5HT synthesis, TPH1, was also expressed in hES cells (FIG. **7**). Expression of these enzymes supported the conclusion that hES cells recapitulate metabolic pathways of tryptophan catabolism and serotonin synthesis. Interestingly, VPA induced pronounced expression of rate-limiting enzymes in this pathway.

Example 6

Developmental Toxicology Screening for Prenatal Alcohol Exposure

To identify differentially secreted metabolites in response to alcohol, as well as the pathways involved in fetal alcohol syndrome, human embryonic stem cells were treated with 0, 0.1 and 0.3% ethanol for four days followed by LC/ESI-TOF mass spectrometry according to the general methods described above for valproate in Example 1. Extracellular media was collected and processed at 24 hours and four days after treatment, and 49,481 mass signals were detected following three technical replications. Of the 49,481 mass signals, 1,860 compounds were significantly different ($p<0.05$) in at least one treatment and had a significant time change (<1 or >1). (Table 7). Binned masses were annotated in silico by querying the neutral masses in several different databases. These databases included Metlin, Biological Magnetic Resonance Data Bank (BMRB), NIST Chemistry WebBook, and the Human Metabolome Database. A mass was considered identified when its neutral mass was within 10 ppm of a known compound annotated in one of the databases listed above.

The putative kynurenine compound (measured exact neutral mass 208.0816) was upregulated three-fold at day four, but not 24 hours, in both treatments (0.1%, p=0.001 and 0.3% p=0.002, respectively). Another putative metabolite in the kynurenine pathway, 8-methoxykynurenate (219.0532) was also upregulated at four days in response to both 0.1% and 0.3% alcohol treatment ($p<0.05$). The analysis also detected a significant downregulation of 5-hydroxy-L-tryptophan (220.0848) at four days following 0.3% alcohol treatment ($p<0.05$) in comparison to untreated controls. 5-hydroxy-L-tryptophan is the only intermediate metabolite between tryptophan and serotonin and its synthesis is mediated by tryptophan hydroxylase, the rate limiting enzyme in serotonin synthesis. These results suggest that alcohol exposure during human development can affect serotonin bioavailability due to upregulation of tryptophan catabolism into kynurenines. In addition, alcohol exposure induced significant changes in metabolic pathways and small molecules involved in neural development such as glutamate, gabapentin, adrenaline and glutathione.

Example 7

Developmental Toxicology Screening of Neuronal Precursor Cells

Metabolomic assessment of teratogens on embryonic development is not limited exclusively to hESCs. The methods of the invention are also useful with other progenitor stem cells, including lineage-restricted stem cells such as neural precursor cells. To illustrate the efficacy of toxicology screening on lineage-specific stem cells, neuronal precursors derived from hESCs were treated with 1 mM valproate according to the methods described in Example 1.

Approximately 135 compounds were differentially secreted in VPA-treated neuronal precursors versus control. (See Table 6). The results of this study illustrated that the methods of the invention reveal alterations in the metabolic profile of lineage-specific stem cells in response to teratogen exposure.

The results disclosed herein are set forth in the following tables

TABLE 3

Cellular metabolites measured in human embryonic stem cells treated with 1 mM of valproate

EXP	RT	roundMASS	time	trt	Fold	Probt	annotation.1	annotation.2
1 mM VPA	8.31910526	103.056358	4 D	1 mM VPA	1.987286671	0.01734377	gamma-Aminobutryic acid	
1 mM VPA	6.78779869	103.098349	4 D	1 mM VPA	2.143101233	0.00047552	2-Aminoisobutyric acid	
1 mM VPA	8.39854546	113.082093	4 D	1 mM VPA	16.4054129	0.01342684	1-Pyrroline-5-carboxylic acid	
1 mM VPA	11.7534444	120.043328	4 D	1 mM VPA	1.355758298	0.03951319	3,4-Dihydroxybutyric acid	
1 mM VPA	85.7330833	121.088708	24 H	1 mM VPA	10.30519572	0.02442001	Phenylethylamine	
1 mM VPA	7.307128	122.071261	4 D	1 mM VPA	−2.2989897	0.01870947	Unknown	
1 mM VPA	38.1047857	129.070626	8 D	1 mM VPA	3.023878137	0.03988213	2-Ketobutyric acid; 2-Oxobutyric acid; alpha-Ketobutyric acid; alpha-Ketobutyrate	
1 mM VPA	14.2761702	136.038753	8 D	1 mM VPA	2.696709281	0.00083818	Hypoxanthine	Allopurinol
1 mM VPA	31.3610896	141.114252	8 D	1 mM VPA	1.865419366	0.02273706	Unknown	
1 mM VPA	43.9201842	143.095482	8 D	1 mM VPA	2.064797071	0.03120757	1-Aminocyclohexanecarboxylic acid	
1 mM VPA	51.283453	144.113677	24 H	1 mM VPA	4.820891632	0.02775836	Caprylic acid	Valproic acid
1 mM VPA	51.283453	144.113677	4 D	1 mM VPA	8.26720694	0.0011089	Caprylic acid	Valproic acid
1 mM VPA	16.307931	147.068314	4 D	1 mM VPA	−2.05380612	0.03872229	3-Methyloxindole	
1 mM VPA	16.307931	147.068314	24 H	1 mM VPA	−1.80875876	0.037812	3-Methyloxindole	
1 mM VPA	22.5095926	153.079352	4 D	1 mM VPA	−2.65737163	0.01268393	Dopamine	
1 mM VPA	5.36288806	155.068364	8 D	1 mM VPA	−1.34957018	0.0476914	L-Histidine	
1 mM VPA	20.6395854	155.072941	4 D	1 mM VPA	3.82298622	0.00676609	L-Histidine	
1 mM VPA	14.2071091	160.060653	8 D	1 mM VPA	12.348809	1.23E-06	Unknown	
1 mM VPA	14.3670392	161.081539	8 D	1 mM VPA	4.777314979	0.0001252	Unknown	
1 mM VPA	44.8165285	162.067353	4 D	1 mM VPA	1.640573238	0.01289447	Unknown	
1 mM VPA	31.5154611	162.124096	24 H	1 mM VPA	1.911228139	0.01933211	Unknown	
1 mM VPA	31.3624074	165.079533	8 D	1 mM VPA	1.705269784	0.00206584	4-(3-Pyridyl)-butanoic acid	
1 mM VPA	32.0426154	167.094942	8 D	1 mM VPA	−2.20640862	0.01874726	Methyldopamine	
1 mM VPA	20.0098065	173.083484	4 D	1 mM VPA	5.458861144	0.00198632	2-Oxoarginine	
1 mM VPA	15.3496532	177.082617	8 D	1 mM VPA	2.397781171	0.01291216	Unknown	
1 mM VPA	24.1314286	179.094351	4 D	1 mM VPA	1.851635336	0.03464009	Salsolinol	Homophenylalanine
1 mM VPA	21.8046482	187.064183	8 D	1 mM VPA	2.839427352	0.00590774	Unknown	
1 mM VPA	21.8046482	187.064183	4 D	1 mM VPA	3.356831218	1.24E-05	Unknown	
1 mM VPA	23.317	187.08492	4 D	1 mM VPA	3.781608467	0.03987705	6-Acetamido-3-oxohexanoate	

TABLE 3-continued

Cellular metabolites measured in human embryonic stem cells treated with 1 mM of valproate								
EXP	RT	roundMASS	time	trt	Fold	Probt	annotation.1	annotation.2
1 mM VPA	27.7865556	189.042631	8 D	1 mM VPA	2.865724657	0.02214848	Kynurenic acid	
1 mM VPA	61.5462473	196.090684	4 D	1 mM VPA	3.519815968	0.01102697	Unknown	
1 mM VPA	24.0551398	197.105003	4 D	1 mM VPA	2.231478645	0.00076838	L-Metanephrine	
1 mM VPA	20.0989286	197.175657	8 D	1 mM VPA	4.237754463	0.00034728	Unknown	
1 mM VPA	73.9582188	198.16015	4 D	1 mM VPA	2.039619449	0.01232495	5-Dodecenoic acid	
1 mM VPA	29.2935714	201.100569	4 D	1 mM VPA	5.966976107	0.00328699	Unknown	
1 mM VPA	28.6036393	203.115212	8 D	1 mM VPA	1.872544495	0.00040874	L-Glutamic acid n-butyl ester	Acetylcarnitine
1 mM VPA	9.07926923	209.06985	8 D	1 mM VPA	−12.4054314	0.01848957	4-Carboxyphenylglycine	
1 mM VPA	48.3434453	213.079468	8 D	1 mM VPA	2.512458907	0.02116099	Unknown	
1 mM VPA	44.6001887	214.064259	4 D	1 mM VPA	1.446032522	0.02767084	Unknown	
1 mM VPA	44.6001887	214.064259	8 D	1 mM VPA	1.783609761	0.00082206	Unknown	
1 mM VPA	69.6687917	214.064356	24 H	1 mM VPA	1.316493137	0.0171064	Unknown	
1 mM VPA	18.7504371	216.094569	4 D	1 mM VPA	2.349086763	0.01043169	Unknown	
1 mM VPA	30.5773235	216.100485	4 D	1 mM VPA	2.050108123	0.04358571	Unknown	
1 mM VPA	6.39474737	218.076897	4 D	1 mM VPA	1.737243521	0.02003307	Unknown	
1 mM VPA	6.68071429	219.144891	24 H	1 mM VPA	−1.58008262	0.02066251	Unknown	
1 mM VPA	10.5609423	220.085746	4 D	1 mM VPA	−2.39827983	1.63E−06	5-Hydroxytryptophan	
1 mM VPA	53.8814512	222.078039	4 D	1 mM VPA	2.882259036	0.02550855	Unknown	
1 mM VPA	73.0997018	223.049411	8 D	1 mM VPA	−4.31392173	0.04819747	7,8-Dihydro-7,8-dihydroxykynurenate	
1 mM VPA	6.45641791	229.095757	8 D	1 mM VPA	2.4471457	0.00094873	Malonylcarnitine	
1 mM VPA	23.7927189	229.095855	4 D	1 mM VPA	2.057653416	0.00038761	Malonylcarnitine	
1 mM VPA	55.5371429	229.145042	4 D	1 mM VPA	2.032140286	0.00236036	Unknown	
1 mM VPA	19.9783175	229.164555	8 D	1 mM VPA	3.779774064	1.34E−08	Unknown	
1 mM VPA	32.6065714	229.201979	4 D	1 mM VPA	3.088058322	0.00439209	Unknown	
1 mM VPA	9.79255	230.080163	4 D	1 mM VPA	−1.383766	0.02197836	Unknown	
1 mM VPA	7.80846914	233.123128	8 D	1 mM VPA	6.784300156	0.00043647	Unknown	
1 mM VPA	14.3537949	236.080213	4 D	1 mM VPA	−3.35334287	0.00032832	N'-Formylkynurenine	
1 mM VPA	45.2867439	238.12327	8 D	1 mM VPA	3.718961983	0.01466842	2-Amino-3-methylbutyric acid	
1 mM VPA	12.1171264	244.109135	4 D	1 mM VPA	1.659329044	0.01962434	Unknown	
1 mM VPA	12.127642	245.119507	4 D	1 mM VPA	2.061936638	0.02383097	Unknown	
1 mM VPA	19.8036863	246.100428	4 D	1 mM VPA	4.924577653	0.02636633	N-Acetyl-D-tryptophan	
1 mM VPA	8.947	247.140975	8 D	1 mM VPA	2.877468231	0.01777412	Unknown	
1 mM VPA	19.7590488	247.173942	8 D	1 mM VPA	3.071620539	3.15E−06	Unknown	
1 mM VPA	48.7837308	248.191881	4 D	1 mM VPA	2.692786782	0.00724013	Unknown	
1 mM VPA	8.00665714	249.119037	4 D	1 mM VPA	1.948008537	0.01865508	Unknown	
1 mM VPA	53.1723271	256.1093	8 D	1 mM VPA	3.068428571	0.00024804	D-2-Amino-3-hydroxybutyric acid	gamma-Amino-beta-hydroxybutyric acid
1 mM VPA	23.22678	257.099256	8 D	1 mM VPA	2.601240877	0.00033049	5-Methylcytidine	

TABLE 3-continued

Cellular metabolites measured in human embryonic stem cells treated with 1 mM of valproate								
EXP	RT	roundMASS	time	trt	Fold	Probt	annotation.1	annotation.2
1 mM VPA	5.57045	257.891738	24 H	1 mM VPA	−1.17015529	0.01640996	Unknown	
1 mM VPA	7.08067539	258.019715	24 H	1 mM VPA	1.315125063	0.02206679	Unknown	
1 mM VPA	22.8759189	258.121153	4 D	1 mM VPA	1.510263204	0.01588551	Unknown	
1 mM VPA	28.8676889	258.133722	24 H	1 mM VPA	5.578974665	0.03000729	Unknown	
1 mM VPA	47.6525584	258.133727	4 D	1 mM VPA	−1.91733177	0.01442461	Unknown	
1 mM VPA	18.7499759	259.11867	4 D	1 mM VPA	1.504620863	0.02037249	N-(gamma-L-Glutamyl)amino-D-proline	
1 mM VPA	13.2221957	260.083648	8 D	1 mM VPA	2.984522231	0.02760558	Unknown	
1 mM VPA	22.373	264.109282	4 D	1 mM VPA	−1.74811488	0.01791457	Acetyl-N-formyl-5-methoxykynurenamine	
1 mM VPA	58.8093824	265.132541	8 D	1 mM VPA	2.363623094	0.03359569	(2R,3S)-rel-2,3-dihydroxy--Butanoic acid	
1 mM VPA	27.779593	271.112691	4 D	1 mM VPA	1.685060044	0.03867385	Unknown	
1 mM VPA	24.7259575	272.124009	4 D	1 mM VPA	2.036511555	0.02960407	Unknown	
1 mM VPA	44.0582051	272.168272	8 D	1 mM VPA	2.056227653	0.00325332	Unknown	
1 mM VPA	41.65612	272.211662	8 D	1 mM VPA	−5.12943527	0.00643051	3-Oxo-delta1-steroid	
1 mM VPA	39.378469	273.105953	4 D	1 mM VPA	2.674742484	0.00030929	Unknown	
1 mM VPA	8.93373171	276.136639	4 D	1 mM VPA	5.215118375	0.0006752	Unknown	
1 mM VPA	67.6599775	280.237772	24 H	1 mM VPA	2.086232575	0.04410145	Linoleic acid	Octadecadienoic acid
1 mM VPA	14.2211017	281.125398	8 D	1 mM VPA	8.170361997	1.56E−06	1-Methyladenosine	
1 mM VPA	71.5568571	282.225649	4 D	1 mM VPA	4.282045127	0.00101203	Unknown	
1 mM VPA	72.7757434	282.253397	8 D	1 mM VPA	−1.86257691	0.03054583	Oleic acid	Elaidic acid
1 mM VPA	59.4208378	284.19613	4 D	1 mM VPA	5.253576839	0.0351483	Unknown	
1 mM VPA	5.70108824	284.980449	4 D	1 mM VPA	1.366608495	0.03819988	Unknown	
1 mM VPA	6.9018125	285.140063	4 D	1 mM VPA	15.96344365	0.00293691	Unknown	
1 mM VPA	64.3362162	286.186749	4 D	1 mM VPA	2.524154118	0.04701735	N-Acetyl-leucyl-leucine	
1 mM VPA	64.3362162	286.186749	24 H	1 mM VPA	2.577549261	0.00532464	N-Acetyl-leucyl-leucine	
1 mM VPA	75.3938769	288.263273	4 D	1 mM VPA	2.556553115	0.0003234	Unknown	
1 mM VPA	26.6263806	289.137569	8 D	1 mM VPA	7.105814367	0.00077408	Unknown	
1 mM VPA	15.0419293	289.139413	8 D	1 mM VPA	3.953690666	0.00671585	Unknown	
1 mM VPA	15.8950204	295.128678	8 D	1 mM VPA	2.286752138	1.23E−06	N6,N6-Dimethyladenosine	
1 mM VPA	6.02850649	301.172858	4 D	1 mM VPA	5.302600282	0.00165331	Unknown	
1 mM VPA	59.5394364	301.222733	4 D	1 mM VPA	4.091131755	0.01328711	Unknown	
1 mM VPA	72.4551579	304.237816	8 D	1 mM VPA	−5.09223853	0.00158305	Arachidonic acid	
1 mM VPA	44.6849231	305.936181	8 D	1 mM VPA	2.13864941	0.00372138	3-Iodo-4-hydroxyphenylpyruvate	
1 mM VPA	7.93489796	306.092269	24 H	1 mM VPA	−2.59907813	0.00116532	Unknown	
1 mM VPA	22.339	306.121765	24 H	1 mM VPA	2.666042908	0.00540921	Z-Gly-Pro; Z-Gly-Pro-OH	
1 mM VPA	59.461	306.180711	4 D	1 mM VPA	2.390810858	0.01473431		
1 mM VPA	12.9788342	307.161748	8 D	1 mM VPA	5.76411852	0.00135244	Unknown	
1 mM VPA	4.76167568	308.158497	8 D	1 mM VPA	3.212062578	7.02E−05	Unknown	
1 mM VPA	7.58973529	316.131974	4 D	1 mM VPA	1.861931503	0.01887819	Unknown	

TABLE 3-continued

Cellular metabolites measured in human embryonic stem cells treated with 1 mM of valproate

EXP	RT	roundMASS	time	trt	Fold	Probt	annotation.1	annotation.2
1 mM VPA	66.9950694	316.200989	4 D	1 mM VPA	2.178145003	0.00392399	Gibberellin A12 aldehyde	
1 mM VPA	62.665	319.244008	24 H	1 mM VPA	3.088914632	0.0457215	Unknown	
1 mM VPA	19.019754	320.137541	4 D	1 mM VPA	2.083198045	0.04299189	Unknown	
1 mM VPA	67.8541343	320.230187	4 D	1 mM VPA	1.784846494	0.03271491	Unknown	
1 mM VPA	67.8541343	320.230187	24 H	1 mM VPA	1.981647012	0.01061379	Unknown	
1 mM VPA	10.672	321.168775	24 H	1 mM VPA	2.153375627	0.00361195	Unknown	
1 mM VPA	35.4656491	324.169472	4 D	1 mM VPA	2.684214566	0.00585101	Unknown	
1 mM VPA	63.932859	326.0008	24 H	1 mM VPA	1.479797739	0.00340947	Unknown	
1 mM VPA	63.932859	326.0008	4 D	1 mM VPA	1.541142217	0.01010729	Unknown	
1 mM VPA	62.4897344	328.242558	4 D	1 mM VPA	1.831213495	0.03531113	Docosahexaenoic acid	
1 mM VPA	55.092	329.001202	24 H	1 mM VPA	1.889887032	0.01315641	Unknown	
1 mM VPA	6.02840404	330.105879	8 D	1 mM VPA	4.856779538	0.01414085	Unknown	
1 mM VPA	12.8257065	330.153322	4 D	1 mM VPA	−1.46094311	0.02278769	Unknown	
1 mM VPA	47.63185	330.240548	4 D	1 mM VPA	2.777910272	0.01585359	Unknown	
1 mM VPA	67.7267647	330.242694	4 D	1 mM VPA	3.168939244	0.02399703	Unknown	
1 mM VPA	9.01253333	331.103633	8 D	1 mM VPA	5.010657754	0.00879812	Unknown	
1 mM VPA	18.8430244	334.151446	24 H	1 mM VPA	7.598422851	0.04853637	Unknown	
1 mM VPA	4.05694118	336.031706	4 D	1 mM VPA	−23.1557728	5.36E−05	Unknown	
1 mM VPA	6.7701658	336.15353	4 D	1 mM VPA	2.062222503	0.01789166	Unknown	
1 mM VPA	54.915974	347.982073	4 D	1 mM VPA	16.74896451	0.02976287	Unknown	
1 mM VPA	45.6079091	348.203076	4 D	1 mM VPA	3.375263185	0.00190076	Unknown	
1 mM VPA	6.04629167	349.134979	8 D	1 mM VPA	1.449645356	0.02177991	Unknown	
1 mM VPA	67.3780816	352.221576	24 H	1 mM VPA	2.329951622	0.01190993	Prostaglandin	
1 mM VPA	22.8247245	353.157641	4 D	1 mM VPA	3.01822425	0.00974537	2-Keto-3-Methylvaleric acid	
1 mM VPA	19.103773	353.158931	4 D	1 mM VPA	2.134354771	0.00286811	Unknown	
1 mM VPA	29.94892	355.242828	4 D	1 mM VPA	3.751584361	0.01212028	Unknown	
1 mM VPA	15.1070313	356.156972	4 D	1 mM VPA	5.181249294	0.00024122	I-Glutamic-gamma-semialdehyde	
1 mM VPA	59.1895507	358.229755	4 D	1 mM VPA	4.389936283	0.00450968	Unknown	
1 mM VPA	7.78296	359.071286	8 D	1 mM VPA	3.504721971	0.02819084	Unknown	
1 mM VPA	27.8286847	359.198793	4 D	1 mM VPA	2.67566964	0.04513681	Unknown	
1 mM VPA	7.67294845	362.15214	24 H	1 mM VPA	3.677690313	0.01613594	Aminohexanoic acid	
1 mM VPA	6.16412	364.18324	4 D	1 mM VPA	4.422922613	0.01590116	Unknown	
1 mM VPA	19.3098372	364.185514	8 D	1 mM VPA	2.529233091	0.00135633	Gibberellin A44	
1 mM	53.9854054	366.239292	4 D	1 mM	6.176116644	3.03E−05	3b-Allotetrahydrocortisol	
1 mM VPA	17.7238836	372.188649	4 D	1 mM VPA	3.32395304	2.43E−07	Ornithine	
1 mM VPA	11.4481111	374.168865	8 D	1 mM VPA	3.707636994	0.00715743	Unknown	
1 mM VPA	13.8172619	374.207426	8 D	1 mM VPA	2.50185816	0.03397986	Unknown	
1 mM VPA	17.6750468	388.183189	8 D	1 mM VPA	3.124878291	0.00060074	Malic acid	Diglycolic acid

TABLE 3-continued

Cellular metabolites measured in human embryonic stem cells treated with 1 mM of valproate

EXP	RT	roundMASS	time	trt	Fold	Probt	annotation.1	annotation.2
1 mM VPA	21.3150329	392.209899	4 D	1 mM VPA	2.402938958	0.02146723	Unknown	
1 mM VPA	79.7277263	404.258123	4 D	1 mM VPA	2.231633324	0.02122436	7a,12a-Dihydroxy-3-oxo-4-cholenoic acid	
1 mM VPA	24.7042427	407.206755	4 D	1 mM VPA	2.564362115	0.03457095	Unknown	
1 mM VPA	16.9907536	408.172332	8 D	1 mM VPA	1.47549599	0.01779219	4-Hydroxyphenylacetaldehyde;	
1 mM VPA	16.9907536	408.172332	4 D	1 mM VPA	1.84894204	0.00218606	4-Hydroxyphenylacetaldehyde;	
1 mM VPA	29.02944	411.227331	4 D	1 mM VPA	3.412904392	0.00663705	Gln His Lys	
1 mM VPA	31.0764706	411.788303	4 D	1 mM VPA	4.812211329	0.01959219	Unknown	
1 mM VPA	9.74277941	412.191819	8 D	1 mM VPA	4.778970957	0.02280244	Unknown	
1 mM VPA	24.0870602	416.213608	8 D	1 mM VPA	1.904483779	0.00013882	Unknown	
1 mM VPA	13.7165	420.160178	4 D	1 mM VPA	4.500233939	0.04166145	Unknown	
1 mM VPA	27.4410904	421.219685	4 D	1 mM VPA	2.92999865	0.01453378	Unknown	
1 mM VPA	84.9993429	424.278966	4 D	1 mM VPA	2.302178983	0.02079967	1b,3a,7a,12a-Tetrahydroxy-5b-cholanoic acid	
1 mM VPA	84.9993429	424.278966	24 H	1 mM VPA	2.318995467	0.00016902	1b,3a,7a,12a-Tetrahydroxy-5b-cholanoic acid	
1 mM VPA	54.5975206	429.099036	4 D	1 mM VPA	3.524698852	2.05E−05	Unknown	
1 mM VPA	25.5684706	430.183077	4 D	1 mM VPA	1.148698355	0.00012401	Unknown	
1 mM VPA	47.39725	432.071275	4 D	1 mM VPA	2.645059178	0.01175067	Unknown	
1 mM VPA	14.5288987	445.217914	8 D	1 mM VPA	2.820595921	0.00824753	Unknown	
1 mM VPA	7.5585	445.286693	4 D	1 mM VPA	−1.13705339	0.04453994	Unknown	
1 mM VPA	19.9357624	455.226453	8 D	1 mM VPA	2.768491323	0.0146344	Adipate	
1 mM VPA	84.7522195	470.350048	4 D	1 mM VPA	3.767741534	0.00027941	Unknown	
1 mM VPA	8.479	471.146232	24 H	1 mM VPA	2.569343893	0.02598742	10-Formyldihydrofolate	
1 mM VPA	22.7650396	471.202698	4 D	1 mM VPA	2.257302866	1.30E−06	Unknown	
1 mM VPA	15.4262845	491.253192	8 D	1 mM VPA	4.916392167	0.00321994	Unknown	
1 mM VPA	60.5202059	493.458959	4 D	1 mM VPA	2.789487333	0.00131052	Unknown	
1 mM VPA	44.8878444	502.216027	4 D	1 mM VPA	1.922921676	0.00968802	Unknown	
1 mM VPA	14.5675854	502.227438	8 D	1 mM VPA	3.101787817	0.00862582	Unknown	
1 mM VPA	31.0262667	504.2848	4 D	1 mM VPA	5.119489655	7.48E−05	Unknown	
1 mM VPA	17.4495833	516.244788	4 D	1 mM VPA	2.722628233	0.00035163	Unknown	
1 mM VPA	44.14925	527.321492	8 D	1 mM VPA	2.213454933	0.00740287	Unknown	
1 mM VPA	70.8363171	528.362659	4 D	1 mM VPA	2.941801698	0.03554601	Unknown	
1 mM VPA	74.25245	530.344375	24 H	1 mM VPA	3.680750602	0.03848341	Unknown	
1 mM VPA	9.15167857	532.249475	8 D	1 mM VPA	7.170133597	0.00736475	Unknown	
1 mM VPA	29.9863488	535.254098	8 D	1 mM VPA	6.049014001	0.00097203	Unknown	
1 mM VPA	87.9394	535.392963	4 D	1 mM VPA	1.80125196	0.03183737	Unknown	
1 mM VPA	8.02928261	549.20135	8 D	1 mM VPA	11.08087574	0.00035145	Unknown	
1 mM VPA	19.5000458	550.228302	4 D	1 mM VPA	2.35969435	0.00064579	Unknown	
1 mM VPA	24.7983934	551.248871	24 H	1 mM VPA	1.396388132	0.01890342	Unknown	
1 mM VPA	74.3730889	552.326244	24 H	1 mM VPA	1.885569072	0.031072	Lithocholate 3-O-glucuronide	

TABLE 3-continued

Cellular metabolites measured in human embryonic stem cells treated with 1 mM of valproate								
EXP	RT	roundMASS	time	trt	Fold	Probt	annotation.1	annotation.2
1 mM VPA	75.3072222	561.322428	4 D	1 mM VPA	5.181249294	0.00798648	Unknown	
1 mM VPA	14.1881491	565.230107	4 D	1 mM VPA	2.651300141	0.04312251	Unknown	
1 mM VPA	5.97658065	574.262774	8 D	1 mM VPA	2.982867719	0.00682514	Unknown	
1 mM VPA	70.4439429	594.37144	4 D	1 mM VPA	2.591881931	0.04970674	2-Hydroxyadenine	
1 mM VPA	15.895551	598.283549	8 D	1 mM VPA	4.074717385	0.00446383	2-Hydroxyadenine	
1 mM VPA	76.5242159	599.574322	8 D	1 mM VPA	2.569165805	2.63E−05	Unknown	
1 mM VPA	76.2647	600.576755	4 D	1 mM VPA	1.998614186	0.00464652	Unknown	
1 mM VPA	76.2647	600.576755	8 D	1 mM VPA	2.690920931	0.00059418	Unknown	
1 mM VPA	79.7894576	613.589997	8 D	1 mM VPA	3.099853425	0.01314731	Unknown	
1 mM VPA	8.59329167	658.254492	8 D	1 mM VPA	13.32441233	0.02367066	Unknown	
1 mM VPA	60.8503	688.51026	4 D	1 mM VPA	3.690969971	0.00301918	Unknown	
1 mM VPA	69.7080715	690.409258	4 D	1 mM VPA	3.89061979	0.001728	Unknown	
1 mM VPA	65.32996	738.583348	4 D	1 mM VPA	3.877159268	0.00021681	Unknown	
1 mM VPA	69.7792917	810.640892	4 D	1 mM VPA	3.934008296	0.00141534	Unknown	
1 mM VPA	21.6729286	921.002586	24 H	1 mM VPA	10.91318268	0.00761215	Unknown	
1 mM VPA	5.86856	1007.84992	4 D	1 mM VPA	23.49041018	0.04324009	3-Dehydrocarnitine	

TABLE 4

Cellular metabolites produced in hESCs treated with 22 μM valproate								
cpdID	RT	MASSavg	time	_trt	Fold	P-value	Compound 1	Compound2
77	28.21	99.0681	4 days	VPA	−1.81	**0.020**	N-Methyl-2-pyrrolidinone	
103	12.00	103.0991	4 days	VPA	−2.24	**0.028**	Gamma-Aminobutryic acid	2-Aminoisobutyric acid
141	34.03	113.0840	4 days	VPA	−1.43	**0.013**	Unknown	
189	12.08	119.0473	8 days	VPA	1.22	**0.040**	4-Amino-3-hydroxybutanoate	
210	96.44	120.0436	4 days	VPA	−4.22	**0.006**	3,4-Dihydroxybutyric acid	
263	19.93	129.0426	4 days	VPA	−1.27	**0.021**	Pyroglutamic acid	1-Pyrroline-4-hydroxy-2-carboxylate
323	29.83	134.0939	4 days	VPA	−1.43	**0.034**	Unknown	
329	16.96	136.0384	24 hours	VPA	−2.09	**0.038**	Hypoxanthine	Allopurinol
343	12.98	141.0412	4 days	VPA	−1.24	**0.011**	1,4,4,6-Tetrahydro-6-oxonicotinate	2-Aminomuconate semialdehyde
362	11.40	141.9381	4 days	VPA	1.19	**0.034**	Unknown	
396	11.97	146.0683	4 days	VPA	−1.02	**0.002**	Glutamine	
413	44.84	148.0638	8 days	VPA	−2.72	**0.004**	Unknown	
444	12.37	144.0687	8 days	VPA	−1.42	**0.014**	Unknown	
449	20.19	146.0066	8 days	VPA	−1.24	**0.002**	2,4-dicarboxylic acid	
496	30.40	161.0688	8 days	VPA	−1.23	**0.019**	4-Methyl-L-glutamate	2,2'-Iminodipropanoate
431	24.83	164.4009	4 days	VPA	−1.17	**0.049**	Unknown	
603	72.83	173.9844	8 days	VPA	−1.80	**0.017**	Unknown	
604	20.34	174.0160	4 days	VPA	−1.36	**0.003**	cis-Aconitate	Dehydroascorbate
636	42.18	178.0994	8 days	VPA	1.47	0.002	Phenylvaleric acid	
646	24.00	181.0740	4 days	VPA	−1.11	**0.004**	Salsolinol	Homophenylalanine
671	24.90	187.0609	4 days	VPA	−1.40	**0.018**	Unknown	
674	36.08	187.0973	4 days	VPA	2.14	**0.042**	Unknown	
812	29.93	204.0899	8 days	VPA	1.08	**0.034**	L-Tryptophan	
843	24.94	208.0840	4 days	VPA	−1.14	**0.004**	Kynurenine	Formyl-4-hydroxykynurenamine
893	44.64	214.1680	8 days	VPA	−2.11	0.005	Fenamic acid	
1089	47.40	242.0808	8 days	VPA	1.87	0.02	Unknown	
1104	7.98	243.9760	4 days	VPA	1.92	0.032	Unknown	
1282	44.30	274.0947	8 days	VPA	1.78	0.019	3-Oxo-delta4-steroid	
1447	43.40	300.2784	8 days	VPA	−2.22	**0.033**	Unknown	
1440	27.94	314.2032	4 day	VPA	−1.12	**0.012**	Unknown	
1637	24.91	330.1480	4 day	VPA	−1.42	**0.004**	Unknown	

TABLE 4-continued

Cellular metabolites produced in hESCs treated with 22 μM valproate								
cpdID	RT	MASSavg	time	__trt	Fold	P-value	Compound 1	Compound2
1684	11.94	336.1634	4 days	VPA	−1.13	**0.023**	Unknown	
1691	34.87	338.0974	4 days	VPA	1.74	**0.033**	Unknown	
1776	39.67	342.1130	4 day	VPA	1.46	**0.044**	Unknown	
1816	24.40	348.1139	8 days	VPA	2.56	0.025	Unknown	
1838	11.00	361.9194	4 days	VPA	−1.08	**0.004**	Unknown	
1948	12.00	384.1664	8 days	VPA	1.38	**0.018**	Unknown	
1949	14.98	387.1498	4 days	VPA	−1.26	**0.001**	Unknown	
2084	64.24	414.2934	4 days	VPA	−1.20	**0.031**	Unknown	
2131	88.14	426.2983	4 days	VPA	1.85	0.022	Cholanoic acid	
2134	74.20	427.1200	8 day	VPA	−1.88	**0.044**	Unknown	
2138	26.91	428.2423	8 day	VPA	1.70	**0.003**	Unknown	
2144	34.14	431.2733	4 days	VPA	−1.24	**0.041**	Unknown	
2186	32.68	441.1394	8 days	VPA	−1.16	**0.022**	Folate	Folic acid
2191	64.67	442.2934	8 days	VPA	1.79	**0.001**	Unknown	
2214	92.89	440.3448	8 days	VPA	−1.84	**0.037**	Unknown	
2233	12.00	444.0841	8 days	VPA	−1.30	**0.041**	Unknown	
2244	64.41	449.3198	24 hours	VPA	−1.78	**0.024**	Unknown	
2291	87.74	470.3249	4 days	VPA	−0.18	**0.002**	Unknown	
2244	30.91	467.2631	8 days	VPA	1.69	**0.004**	Unknown	
743	13.81	197.0186	8 day	VPA	−1.39	**0.016**	Unknown	
636	42.18	178.0994	8 days		1.47	**0.010**	Unknown	

TABLE 6

Cellular metabolites measured in neural precursors derived from hESells treated with 1 mM of valproate							
EXP	RT	roundMASS	time	trt	Fold	annotation.1	annotation.2
NS 1 mM VPA	36.648	102.0322438	2 d	NS 1 mM VPA	−2.23119	2-Ketobutyric acid	Acetoacetic acid
NS 1 mM VPA	36.648	102.0322438	4 d	NS 1 mM VPA	−1.83846	2-Ketobutyric acid	Acetoacetic acid
NS 1 mM VPA	9.33225	119.958645	4 d	NS 1 mM VPA	6.98086	Unknown	
NS 1 mM VPA	12.2841	121.0621387	4 d	NS 1 mM VPA	3.502341	Unknown	
NS 1 mM VPA	24.10558	125.0838833	2 d	NS 1 mM VPA	1.79316	Unknown	
NS 1 mM VPA	30.43985	125.08394	2 d	NS 1 mM VPA	1.529012	1-Methylhistamine	
NS 1 mM VPA	30.43985	125.08394	4 d	NS 1 mM VPA	1.576622	1-Methylhistamine	
NS 1 mM VPA	23.33772	129.0573222	2 d	NS 1 mM VPA	1.543375	Pyroglutamic acid	
NS 1 mM VPA	23.33772	129.0573222	4 d	NS 1 mM VPA	1.663008	Pyroglutamic acid	
NS 1 mM VPA	12.13216	131.0941359	4 d	NS 1 mM VPA	1.577796	L-Isoleucine	Aminocaproic acid
NS 1 mM VPA	12.13216	131.0941359	2 d	NS 1 mM VPA	2.474877	L-Isoleucine	Aminocaproic acid
NS 1 mM VPA	8.881211	136.0366263	2 d	NS 1 mM VPA	2.287439	Erythronic acid	Erythronic acid
NS 1 mM VPA	8.881211	136.0366263	4 d	NS 1 mM VPA	2.653537	Erythronic acid	Erythronic acid
NS 1 mM VPA	12.00967	136.0376917	2 d	NS 1 mM VPA	2.346054	Erythronic acid	Erythronic acid
NS 1 mM VPA	12.00967	136.0376917	4 d	NS 1 mM VPA	2.914393	Erythronic acid	Erythronic acid
NS 1 mM VPA	3.9669	138.04396	2 d	NS 1 mM VPA	1.521407	Urocanic acid	Nicotinamide N-oxide
NS 1 mM VPA	3.9669	138.04396	4 d	NS 1 mM VPA	2.642558	Urocanic acid	Nicotinamide N-oxide
NS 1 mM VPA	4.28225	141.9392625	2 d	NS 1 mM VPA	1.077336	5,10-Methylenetetrahydrofolate	
NS 1 mM VPA	4.28225	141.9392625	4 d	NS 1 mM VPA	1.111532	5,10-Methylenetetrahydrofolate	
NS 1 mM VPA	23.33784	143.0734947	2 d	NS 1 mM VPA	1.728349	Unknown	
NS 1 mM VPA	23.33784	143.0734947	4 d	NS 1 mM VPA	1.986515	Unknown	
NS 1 mM VPA	55.5845	144.1153313	4 d	NS 1 mM VPA	10.83178	Caprylic acid	Valproic acid

TABLE 6-continued

Cellular metabolites measured in neural precursors derived from hESells treated with 1 mM of valproate							
EXP	RT	roundMASS	time	trt	Fold	annotation.1	annotation.2
NS 1 mM VPA	55.5845	144.1153313	2 d	NS 1 mM VPA	11.64535	Caprylic acid	Valproic acid
NS 1 mM VPA	5.609182	145.1572818	2 d	NS 1 mM VPA	1.00993	Spermidine	
NS 1 mM VPA	5.609182	145.1572818	4 d	NS 1 mM VPA	1.117314	Spermidine	
NS 1 mM VPA	33.6357	148.03738	4 d	NS 1 mM VPA	−5.75294	Citramalic acid	Hydroxyglutaric acid
NS 1 mM VPA	33.6357	148.03738	2 d	NS 1 mM VPA	−1.49356	Citramalic acid	Hydroxyglutaric acid
NS 1 mM VPA	62.42614	152.1201762	4 d	NS 1 mM VPA	2.50602	Unknown	
NS 1 mM VPA	62.42614	152.1201762	2 d	NS 1 mM VPA	3.371426	Unknown	
NS 1 mM VPA	8.862333	158.0177333	2 d	NS 1 mM VPA	1.596402	Unknown	
NS 1 mM VPA	8.862333	158.0177333	4 d	NS 1 mM VPA	2.236076	Unknown	
NS 1 mM VPA	8.269857	158.1374571	4 d	NS 1 mM VPA	3.106829	Unknown	
NS 1 mM VPA	8.269857	158.1374571	2 d	NS 1 mM VPA	3.626498	Unknown	
NS 1 mM VPA	10.07033	159.0688667	2 d	NS 1 mM VPA	−2.87026	Indoleacetaldehyde	
NS 1 mM VPA	10.07033	159.0688667	4 d	NS 1 mM VPA	−1.35298	Indoleacetaldehyde	
NS 1 mM VPA	12.85888	161.0509118	2 d	NS 1 mM VPA	2.601443	Unknown	
NS 1 mM VPA	12.85888	161.0509118	4 d	NS 1 mM VPA	5.136057	Unknown	
NS 1 mM VPA	6.713565	166.0840609	4 d	NS 1 mM VPA	33.80296	Unknown	
NS 1 mM VPA	6.713565	166.0840609	2 d	NS 1 mM VPA	90.97629	Unknown	
NS 1 mM VPA	23.58909	168.0687909	2 d	NS 1 mM VPA	4.649885	Unknown	
NS 1 mM VPA	23.58909	168.0687909	4 d	NS 1 mM VPA	5.02165	Unknown	
NS 1 mM VPA	31.08471	171.1250706	4 d	NS 1 mM VPA	1.626943	Unknown	
NS 1 mM VPA	62.57554	172.1454	4 d	NS 1 mM VPA	1.745543	Capric acid	Decanoic acid
NS 1 mM VPA	62.57554	172.1454	2 d	NS 1 mM VPA	1.8794	Caprica cid	Decanoic acid
NS 1 mM VPA	20.68721	175.0830857	2 d	NS 1 mM VPA	1.153935	N-Carboxyethyl-gamma-aminobutyric acid	
NS 1 mM VPA	20.68721	175.0830857	4 d	NS 1 mM VPA	2.294392	N-Carboxyethyl-gamma-aminobutyric acid	
NS 1 mM VPA	41.71109	176.0946	2 d	NS 1 mM VPA	−1.60014	Serotonin	
NS 1 mM VPA	41.71109	176.0946	4 d	NS 1 mM VPA	−1.23797	Serotonin	
NS 1 mM VPA	25.29	177.0469231	2 d	NS 1 mM VPA	3.379693	N-Formyl-L-methionine	
NS 1 mM VPA	25.29	177.0469231	4 d	NS 1 mM VPA	3.82485	N-Formyl-L-methionine	
NS 1 mM VPA	26.75621	177.0789684	2 d	NS 1 mM VPA	1.26513	5-Hydroxytryptophol	
NS 1 mM VPA	26.75621	177.0789684	4 d	NS 1 mM VPA	1.423219	5-Hydroxytryptophol	
NS 1 mM VPA	8.503333	177.113375	2 d	NS 1 mM VPA	−6.74695	Unknown	
NS 1 mM VPA	8.503333	177.113375	4 d	NS 1 mM VPA	−2.88736	Unknown	
NS 1 mM VPA	27.53982	179.0938118	2 d	NS 1 mM VPA	−2.71897	Salsolinol	Homophenylalanine
NS 1 mM VPA	27.53982	179.0938118	4 d	NS 1 mM VPA	−1.71231	Salsolinol	Homophenylalanine
NS 1 mM VPA	55.15089	179.0949632	4 d	NS 1 mM VPA	−1.64525	Salsolinol	Homophenylalanine
NS 1 mM VPA	55.15089	179.0949632	2 d	NS 1 mM VPA	−1.39458	Salsolinol	Homophenylalanine
NS 1 mM VPA	37.08443	185.1406571	4 d	NS 1 mM VPA	−2.39254	Unknown	
NS 1 mM VPA	23.33726	187.0635211	2 d	NS 1 mM VPA	1.674013	Unknown	

TABLE 6-continued

Cellular metabolites measured in neural precursors derived from hESells treated with 1 mM of valproate

EXP	RT	roundMASS	time	trt	Fold	annotation.1	annotation.2
NS 1 mM VPA	23.33726	187.0635211	4 d	NS 1 mM VPA	1.921765	Unknown	
NS 1 mM VPA	28.77111	187.1206333	2 d	NS 1 mM VPA	2.457813	8-Amino-7-oxononanoic acid	
NS 1 mM VPA	28.77111	187.1206333	4 d	NS 1 mM VPA	3.559361	8-Amino-7-oxononanoic acid	
NS 1 mM VPA	62.50765	190.1720118	4 d	NS 1 mM VPA	1.758553	Unknown	
NS 1 mM VPA	62.50765	190.1720118	2 d	NS 1 mM VPA	1.940004	Unknown	
NS 1 mM VPA	7.850167	196.0933333	4 d	NS 1 mM VPA	1.937281	Unknown	
NS 1 mM VPA	7.850167	196.0933333	2 d	NS 1 mM VPA	6.390957	Unknown	
NS 1 mM VPA	45.36418	197.1060727	2 d	NS 1 mM VPA	1.280472	L-Metanephrine	
NS 1 mM VPA	45.36418	197.1060727	4 d	NS 1 mM VPA	1.62879	L-Metanephrine	
NS 1 mM VPA	8.363925	199.0952975	2 d	NS 1 mM VPA	10.44025	Unknown	
NS 1 mM VPA	8.363925	199.0952975	4 d	NS 1 mM VPA	10.88407	Unknown	
NS 1 mM VPA	22.22829	206.06375	4 d	NS 1 mM VPA	2.263839	Unknown	
NS 1 mM VPA	22.22829	206.06375	2 d	NS 1 mM VPA	4.317383	Unknown	
NS 1 mM VPA	62.46678	208.1829217	4 d	NS 1 mM VPA	2.052914	Unknown	
NS 1 mM VPA	62.46678	208.1829217	2 d	NS 1 mM VPA	2.734885	Unknown	
NS 1 mM VPA	9.2636	211.0349075	4 d	NS 1 mM VPA	1.516795	Creatine phosphate	
NS 1 mM VPA	9.2636	211.0349075	2 d	NS 1 mM VPA	1.893074	Creatine phosphate	
NS 1 mM VPA	44.11333	212.1400167	4 d	NS 1 mM VPA	−9.35257	Unknown	
NS 1 mM VPA	44.11333	212.1400167	2 d	NS 1 mM VPA	−6.85493	Unknown	
NS 1 mM VPA	7.746115	217.1048885	2 d	NS 1 mM VPA	2.916422	N-a-Acetylcitrulline	
NS 1 mM VPA	7.746115	217.1048885	4 d	NS 1 mM VPA	23.86569	N-a-Acetylcitrulline	
NS 1 mM VPA	22.26729	217.1307097	2 d	NS 1 mM VPA	2.122093	Propionylcarnitine	
NS 1 mM VPA	22.26729	217.1307097	4 d	NS 1 mM VPA	2.236406	Propionylcarnitine	
NS 1 mM VPA	16.1278	220.0841	2 d	NS 1 mM VPA	−1.25214	5-Hydroxytryptophan	5-Hydroxy-L-tryptophan
NS 1 mM VPA	16.1278	220.0841	4 d	NS 1 mM VPA	1.215413	5-Hydroxytryptophan	5-Hydroxy-L-tryptophan
NS 1 mM VPA	9.809786	220.0845	2 d	NS 1 mM VPA	−1.06102	5-Hydroxytryptophan	5-Hydroxy-L-tryptophan
NS 1 mM VPA	9.809786	220.0845	4 d	NS 1 mM VPA	1.371126	5-Hydroxytryptophan	5-Hydroxy-L-tryptophan
NS 1 mM VPA	12.15958	220.0845895	2 d	NS 1 mM VPA	−1.54275	5-Hydroxytryptophan	5-Hydroxy-L-tryptophan
NS 1 mM VPA	12.15958	220.0845895	4 d	NS 1 mM VPA	1.162644	5-Hydroxytryptophan	5-Hydroxy-L-tryptophan
NS 1 mM VPA	8.4172	223.92951	2 d	NS 1 mM VPA	−3.24844	Unknown	
NS 1 mM VPA	8.4172	223.92951	4 d	NS 1 mM VPA	−2.85631	Unknown	
NS 1 mM VPA	22.009	225.62685	4 d	NS 1 mM VPA	2.716119	Unknown	
NS 1 mM VPA	22.009	225.62685	2 d	NS 1 mM VPA	3.854852	Unknown	
NS 1 mM VPA	10.0963	227.01938	4 d	NS 1 mM VPA	1.631698	L-Glutamic acid 5-phosphate	
NS 1 mM VPA	6.0771	227.09052	4 d	NS 1 mM VPA	−5.41292	Deoxycytidine	
NS 1 mM VPA	6.0771	227.09052	2 d	NS 1 mM VPA	−2.98012	Deoxycytidine	
NS 1 mM VPA	14.51476	228.05894	4 d	NS 1 mM VPA	3.339111	Unknown	
NS 1 mM VPA	14.51476	228.05894	2 d	NS 1 mM VPA	6.425869	Unknown	

TABLE 6-continued

Cellular metabolites measured in neural precursors derived from hESells treated with 1 mM of valproate

EXP	RT	roundMASS	time	trt	Fold	annotation.1	annotation.2
NS 1 mM VPA	67.13919	230.1515667	4 d	NS 1 mM VPA	−5.02528	Dodecanedioic acid	
NS 1 mM VPA	67.13919	230.1515667	2 d	NS 1 mM VPA	−2.98776	Dodecanedioic acid	
NS 1 mM VPA	19.28286	234.1010143	2 d	NS 1 mM VPA	−1.25569	5-Methoxytryptophan	
NS 1 mM VPA	19.28286	234.1010143	4 d	NS 1 mM VPA	−1.18869	5-Methoxytryptophan	
NS 1 mM VPA	10.51438	236.0815625	4 d	NS 1 mM VPA	1.367658	N'-Formylkynurenine	
NS 1 mM VPA	17.826	238.0864167	2 d	NS 1 mM VPA	5.397657	Propanoic acid	
NS 1 mM VPA	17.826	238.0864167	4 d	NS 1 mM VPA	5.703771	Propanoic acid	
NS 1 mM VPA	7.8115	239.087425	4 d	NS 1 mM VPA	1.950568	Unknown	
NS 1 mM VPA	7.8115	239.087425	2 d	NS 1 mM VPA	30.57925	Unknown	
NS 1 mM VPA	42.05716	246.1469838	4 d	NS 1 mM VPA	−2.28801	3-Hydroxydodecanedioic acid	
NS 1 mM VPA	42.05716	246.1469838	2 d	NS 1 mM VPA	−1.71537	3-Hydroxydodecanedioic acid	
NS 1 mM VPA	30.669	256.09664	4 d	NS 1 mM VPA	1.692487	Aryl beta-D-glucoside	
NS 1 mM VPA	30.669	256.09664	2 d	NS 1 mM VPA	1.966122	Aryl beta-D-glucoside	
NS 1 mM VPA	59.82681	256.1080938	2 d	NS 1 mM VPA	2.962348	Unknown	
NS 1 mM VPA	59.82681	256.1080938	4 d	NS 1 mM VPA	3.845884	Unknown	
NS 1 mM VPA	69.57173	258.18226	4 d	NS 1 mM VPA	4.075358	Tetradecanedioic acid	
NS 1 mM VPA	69.57173	258.18226	2 d	NS 1 mM VPA	5.744182	Tetradecanedioic acid	
NS 1 mM VPA	22.0274	264.11209	2 d	NS 1 mM VPA	1.230997	Acetyl-N-formyl-5-methoxykynurenamine	
NS 1 mM VPA	22.0274	264.11209	4 d	NS 1 mM VPA	1.338241	Acetyl-N-formyl-5-methoxykynurenamine	
NS 1 mM VPA	11.94583	268.0806	4 d	NS 1 mM VPA	3.240011	3-Deoxy-D-glycero-D-galacto-2-nonulosonic acid	
NS 1 mM VPA	11.94583	268.0806	2 d	NS 1 mM VPA	3.329815	3-Deoxy-D-glycero-D-galacto-2-nonulosonic acid	
NS 1 mM VPA	20.58271	270.1203286	2 d	NS 1 mM VPA	1.808333	L-gamma-Glutamyl-L-hypoglycin;	
NS 1 mM VPA	20.58271	270.1203286	4 d	NS 1 mM VPA	1.890677	L-gamma-Glutamyl-L-hypoglycin;	
NS 1 mM VPA	56.5351	272.08566	4 d	NS 1 mM VPA	1.178071	5-S-Cysteinyldopamine	
NS 1 mM VPA	56.5351	272.08566	2 d	NS 1 mM VPA	1.718998	5-S-Cysteinyldopamine	
NS 1 mM VPA	64.11407	278.0251024	4 d	NS 1 mM VPA	5.17024	Unknown	
NS 1 mM VPA	64.11407	278.0251024	2 d	NS 1 mM VPA	6.667641	Unknown	
NS 1 mM VPA	28.90879	290.1501643	4 d	NS 1 mM VPA	3.329013	Unknown	
NS 1 mM VPA	28.90879	290.1501643	2 d	NS 1 mM VPA	8.490919	Unknown	
NS 1 mM VPA	26.42889	295.1063913	2 d	NS 1 mM VPA	7.265582	Unknown	
NS 1 mM VPA	26.42889	295.1063913	4 d	NS 1 mM VPA	8.896581	Unknown	
NS 1 mM VPA	73.28533	315.2406111	4 d	NS 1 mM VPA	2.599877	Decanoylcarnitine	
NS 1 mM VPA	73.28533	315.2406111	2 d	NS 1 mM VPA	3.899691	Decanoylcarnitine	
NS 1 mM VPA	77.38144	318.2193688	4 d	NS 1 mM VPA	1.932263	Leukotriene A4	
NS 1 mM VPA	77.38144	318.2193688	2 d	NS 1 mM VPA	2.342543	Leukotriene A4	
NS 1 mM VPA	41.33024	324.1144588	4 d	NS 1 mM VPA	3.317923	Acetohexamide	
NS 1 mM VPA	41.33024	324.1144588	2 d	NS 1 mM VPA	3.713445	Acetohexamide	
NS 1 mM VPA	33.60576	330.1013765	4 d	NS 1 mM VPA	2.25561	Unknown	

TABLE 6-continued

Cellular metabolites measured in neural precursors derived from hESells treated with 1 mM of valproate

EXP	RT	roundMASS	time	trt	Fold	annotation.1	annotation.2
NS 1 mM VPA	33.60576	330.1013765	2 d	NS 1 mM VPA	2.310447	Unknown	
NS 1 mM VPA	35.06233	331.1049867	4 d	NS 1 mM VPA	2.719086	Unknown	
NS 1 mM VPA	35.06233	331.1049867	2 d	NS 1 mM VPA	3.041317	Unknown	
NS 1 mM VPA	52.557	349.22592	4 d	NS 1 mM VPA	2.41993	Unknown	
NS 1 mM VPA	52.557	349.22592	2 d	NS 1 mM VPA	6.652089	Unknown	
NS 1 mM VPA	53.57858	350.2096	4 d	NS 1 mM VPA	1.508076	Prostaglandin E3	
NS 1 mM VPA	53.57858	350.2096	2 d	NS 1 mM VPA	1.612834	Prostaglandin E3	
NS 1 mM VPA	65.76353	356.2702895	4 d	NS 1 mM VPA	2.05875	Tetracosahexaenoic acid	
NS 1 mM VPA	65.76353	356.2702895	2 d	NS 1 mM VPA	2.405825	Tetracosahexaenoic acid	
NS 1 mM VPA	81.79271	369.2880824	4 d	NS 1 mM VPA	6.636435	cis-5-Tetradecenoylcarnitine	
NS 1 mM VPA	81.79271	369.2880824	2 d	NS 1 mM VPA	9.965816	cis-5-Tetradecenoylcarnitine	
NS 1 mM VPA	27.34076	374.1222235	4 d	NS 1 mM VPA	2.300022	Unknown	
NS 1 mM VPA	27.34076	374.1222235	2 d	NS 1 mM VPA	2.889783	Unknown	
NS 1 mM VPA	8.881	380.164	4 d	NS 1 mM VPA	2.519949	Unknown	
NS 1 mM VPA	8.881	380.164	2 d	NS 1 mM VPA	2.633667	Unknown	
NS 1 mM VPA	22.46583	385.1025667	4 d	NS 1 mM VPA	1.45497	S-Inosyl-L-homocysteine	
NS 1 mM VPA	22.46583	385.1025667	2 d	NS 1 mM VPA	1.533705	S-Inosyl-L-homocysteine	
NS 1 mM VPA	68.31689	386.23145	4 d	NS 1 mM VPA	6.386163	1-tridecanoyl-sn-glycero-3-phosphate	
NS 1 mM VPA	68.31689	386.23145	2 d	NS 1 mM VPA	7.117538	1-tridecanoyl-sn-glycero-3-phosphate	
NS 1 mM VPA	89.1946	390.27608	2 d	NS 1 mM VPA	1.682855	7-Hydroxy-3-oxocholanoic acid	
NS 1 mM VPA	89.1946	390.27608	4 d	NS 1 mM VPA	3.296512	7-Hydroxy-3-oxocholanoic acid	
NS 1 mM VPA	26.42904	394.2127308	2 d	NS 1 mM VPA	2.670186	Unknown	
NS 1 mM VPA	26.42904	394.2127308	4 d	NS 1 mM VPA	3.50273	unknown	
NS 1 mM VPA	76.06971	398.2439857	4 d	NS 1 mM VPA	2.845315	Unknown	
NS 1 mM VPA	76.06971	398.2439857	2 d	NS 1 mM VPA	3.609136	Unknown	
NS 1 mM VPA	33.53038	399.2100125	2 d	NS 1 mM VPA	6.345173	unknown	
NS 1 mM VPA	33.53038	399.2100125	4 d	NS 1 mM VPA	8.777833	unknown	
NS 1 mM VPA	8.9305	406.1058875	4 d	NS 1 mM VPA	1.976577	unknown	
NS 1 mM VPA	8.9305	406.1058875	2 d	NS 1 mM VPA	2.00634	unknown	
NS 1 mM VPA	65.76447	409.3155632	4 d	NS 1 mM VPA	4.662331	Unknown	
NS 1 mM VPA	65.76447	409.3155632	2 d	NS 1 mM VPA	5.930421	Unknown	
NS 1 mM VPA	18.93053	416.0834333	4 d	NS 1 mM VPA	3.527468	Unknown	
NS 1 mM VPA	18.93053	416.0834333	2 d	NS 1 mM VPA	4.202864	Unknown	
NS 1 mM VPA	8.6127	416.20208	2 d	NS 1 mM VPA	3.29783	Lactone	
NS 1 mM VPA	8.6127	416.20208	4 d	NS 1 mM VPA	3.495266	Lactone	
NS 1 mM VPA	14.963	420.05275	2 d	NS 1 mM VPA	−3.434	Unknown	
NS 1 mM VPA	14.963	420.05275	4 d	NS 1 mM VPA	−3.09195	Unknown	
NS 1 mM VPA	62.93221	427.1025357	2 d	NS 1 mM VPA	3.882014	Unknown	

TABLE 6-continued

Cellular metabolites measured in neural precursors derived from hESells treated with 1 mM of valproate

EXP	RT	roundMASS	time	trt	Fold	annotation.1	annotation.2
NS 1 mM VPA	62.93221	427.1025357	4 d	NS 1 mM VPA	6.045912	Unknown	
NS 1 mM VPA	33.59771	430.1185143	2 d	NS 1 mM VPA	3.469797	N-Ethylmaleimide-S-glutathione	
NS 1 mM VPA	33.59771	430.1185143	4 d	NS 1 mM VPA	3.98295	N-Ethylmaleimide-S-glutathione	
NS 1 mM VPA	24.9718	434.19845	4 d	NS 1 mM VPA	3.713915	Unknown	
NS 1 mM VPA	24.9718	434.19845	2 d	NS 1 mM VPA	3.76882	Unknown	
NS 1 mM VPA	23.07	434.1985875	2 d	NS 1 mM VPA	2.416021	Unknown	
NS 1 mM VPA	23.07	434.1985875	4 d	NS 1 mM VPA	3.44524	Unknown	
NS 1 mM VPA	37.33089	438.1460579	2 d	NS 1 mM VPA	2.829002	Unknown	
NS 1 mM VPA	37.33089	438.1460579	4 d	NS 1 mM VPA	3.253909	Unknown	
NS 1 mM VPA	5.410909	441.9424	4 d	NS 1 mM VPA	−3.5345	Unknown	
NS 1 mM VPA	5.410909	441.9424	2 d	NS 1 mM VPA	−2.45689	Unknown	
NS 1 mM VPA	34.58505	443.2362947	2 d	NS 1 mM VPA	2.648862	Unknown	
NS 1 mM VPA	34.58505	443.2362947	4 d	NS 1 mM VPA	3.427563	Unknown	
NS 1 mM VPA	33.85267	445.1694	2 d	NS 1 mM VPA	−1.31487	Tetrahydrofolic acid	Tetrahydrofolate
NS 1 mM VPA	33.85267	445.1694	4 d	NS 1 mM VPA	−1.04073	Tetrahydrofolic acid	Tetrahydrofolate
NS 1 mM VPA	38.63717	449.1638333	2 d	NS 1 mM VPA	3.04704	Unknown	
NS 1 mM VPA	38.63717	449.1638333	4 d	NS 1 mM VPA	4.206864	Unknown	
NS 1 mM VPA	21.9772	456.2448	4 d	NS 1 mM VPA	4.145738	unknown	
NS 1 mM VPA	21.9772	456.2448	2 d	NS 1 mM VPA	8.390862	unknown	
NS 1 mM VPA	42.40369	467.1731077	2 d	NS 1 mM VPA	−10.3323	Unknown	
NS 1 mM VPA	42.40369	467.1731077	4 d	NS 1 mM VPA	−4.42263	Unknown	
NS 1 mM VPA	23.41189	474.1090778	4 d	NS 1 mM VPA	2.094815	Unknown	
NS 1 mM VPA	23.41189	474.1090778	2 d	NS 1 mM VPA	2.928466	Unknown	
NS 1 mM VPA	22.666	482.1554563	4 d	NS 1 mM VPA	2.075721	Unknown	
NS 1 mM VPA	22.666	482.1554563	2 d	NS 1 mM VPA	2.519832	Unknown	
NS 1 mM VPA	75.55014	493.3252405	4 d	NS 1 mM VPA	2.09415	1-(9E-hexadecenoyl)-sn-glycero-3-phosphocholine	
NS 1 mM VPA	75.55014	493.3252405	2 d	NS 1 mM VPA	2.34894	1-(9E-hexadecenoyl)-sn-glycero-3-	phosphocholine
NS 1 mM VPA	37.34533	506.1856556	2 d	NS 1 mM VPA	2.031088	Unknown	
NS 1 mM VPA	37.34533	506.1856556	4 d	NS 1 mM VPA	2.405509	unknown	
NS 1 mM VPA	23.67792	514.162208	2 d	NS 1 mM VPA	2.2744	unknown	
NS 1 mM VPA	23.67792	514.162208	4 d	NS 1 mM VPA	3.27837	unknown	
NS 1 mM VPA	36.44195	514.2430667	4 d	NS 1 mM VPA	4.332807	Unknown	
NS 1 mM VPA	36.44195	514.2430667	2 d	NS 1 mM VPA	4.565629	Unknown	
NS 1 mM VPA	41.29621	527.3552786	2 d	NS 1 mM VPA	9.44893	Unknown	
NS 1 mM VPA	41.29621	527.3552786	4 d	NS 1 mM VPA	12.3251	Unknown	
NS 1 mM VPA	21.94381	534.2784846	4 d	NS 1 mM VPA	2.818876	unknown	
NS 1 mM VPA	21.94381	534.2784846	2 d	NS 1 mM VPA	2.912693	unknown	
NS 1 mM VPA	26.05061	546.3146929	2 d	NS 1 mM VPA	1.574871	Unknown	

TABLE 6-continued

Cellular metabolites measured in neural precursors derived from hESells treated with 1 mM of valproate							
EXP	RT	roundMASS	time	trt	Fold	annotation.1	annotation.2
NS 1 mM VPA	26.05061	546.3146929	4 d	NS 1 mM VPA	3.130802	Unknown	
NS 1 mM VPA	25.92929	556.13945	4 d	NS 1 mM VPA	4.837329	unknown	
NS 1 mM VPA	25.92929	556.13945	2 d	NS 1 mM VPA	5.293236	unknown	
NS 1 mM VPA	9.093727	575.1451545	4 d	NS 1 mM VPA	2.795937	Unknown	
NS 1 mM VPA	9.093727	575.1451545	2 d	NS 1 mM VPA	4.232054	Unknown	
NS 1 mM VPA	36.92372	575.3159222	2 d	NS 1 mM VPA	2.816601	Unknown	
NS 1 mM VPA	36.92372	575.3159222	4 d	NS 1 mM VPA	3.446719	unknown	
NS 1 mM VPA	80.9297	583.44194	2 d	NS 1 mM VPA	1.503812	Unknown	
NS 1 mM VPA	80.9297	583.44194	4 d	NS 1 mM VPA	4.532225	Unknown	
NS 1 mM VPA	4.824	594.2327	4 d	NS 1 mM VPA	2.212473	Unknown	
NS 1 mM VPA	4.824	594.2327	2 d	NS 1 mM VPA	4.279664	Unknown	
NS 1 mM VPA	41.278	632.232825	2 d	NS 1 mM VPA	3.171377	Unknown	
NS 1 mM VPA	41.278	632.232825	4 d	NS 1 mM VPA	4.764468	Unknown	
NS 1 mM VPA	14.97571	659.1506941	4 d	NS 1 mM VPA	3.054219	Unknown	
NS 1 mM VPA	23.39707	660.1513786	2 d	NS 1 mM VPA	3.542683	Unknown	
NS 1 mM VPA	23.39707	660.1513786	4 d	NS 1 mM VPA	4.100844	Unknown	
NS 1 mM VPA	14.884	682.2853	2 d	NS 1 mM VPA	2.823221	Unknown	
NS 1 mM VPA	14.884	682.2853	4 d	NS 1 mM VPA	5.153298	Unknown	
NS 1 mM VPA	33.64471	822.2805429	2 d	NS 1 mM VPA	2.668941	Unknown	
NS 1 mM VPA	33.64471	822.2805429	4 d	NS 1 mM VPA	3.816104	Unknown	
NS 1 mM VPA	83.24742	907.54555	4 d	NS 1 mM VPA	1.507945	Unknown	
NS 1 mM VPA	83.24742	907.54555	2 d	NS 1 mM VPA	1.586431	Unknown	
NS 1 mM VPA	31.5316	908.22015	2 d	NS 1 mM VPA	1.640148	Unknown	
NS 1 mM VPA	31.5316	908.22015	4 d	NS 1 mM VPA	1.998983	Unknown	
NS 1 mM VPA	33.44333	1028.3246	2 d	NS 1 mM VPA	5.833998	Unknown	
NS 1 mM VPA	33.44333	1028.3246	4 d	NS 1 mM VPA	8.654592	Unknown	
NS 1 mM VPA	4.649125	1291.75965	2 d	NS 1 mM VPA	1.739338	beta-D-Glucosyl-1,4-N-acetyl-D-glucosaminyldiphosphoundecaprenol	
NS 1 mM VPA	4.649125	1291.75965	4 d	NS 1 mM VPA	1.793695	beta-D-Glucosyl-1,4-N-acetyl-D-glucosaminyldiphosphoundecaprenol	

TABLE 7

Cellular metabolites measured in hES cells treated with alcohol							
Experiment	Retention time	Mass	Time	Fold	p-value	Compound 1	Compound 2
ETOH 0.1	15.48433	99.0689	4 D	1.434154	0.034571	N-Methyl-2-pyrrolidinone	
ETOH 0.1	52.01225	99.1043	4 D	2.703447	0.012638	Unknown	
ETOH 0.1	13.40565	120.2112	4 D	4.847027	0.029776	Unknown	
ETOH 0.1	16.73904	129.0452	24 H	1.502328	0.002871	3,4-Dihydroxybutyric acid	
ETOH 0.1	88.64043	130.9541	24 H	1.631614	0.046779	Unknown	
ETOH 0.1	22.22892	131.0746	24 H	−1.85703	0.037466	3-Methylindole	
ETOH 0.1	14.35336	131.076	4 D	3.62907	0.014778	Unknown	
ETOH 0.1	3.958833	148.0052	24 H	−1.94841	0.034059	Unknown	

TABLE 7-continued

Cellular metabolites measured in hES cells treated with alcohol							
Experiment	Retention time	Mass	Time	Fold	p-value	Compound 1	Compound 2
ETOH 0.1	52.88652	148.016	4 D	−2.44409	0.047122	2-Oxo-4-methylthiobutanoic acid	
ETOH 0.1	19.18355	168.0434	4 D	−1.46785	0.019426	Homogentisic acid	Vanillic acid
ETOH 0.1	26.70635	171.1244	24 H	2.376107	0.008413	GABA analogue	
ETOH 0.1	22.17997	187.1343	24 H	1.48782	0.045452	(+/−)-2-(4'-Isobutylphenyl)propionitrile	
ETOH 0.1	46.31086	187.1348	4 D	2.329144	4.21E−05	(+/−)-2-(4'-Isobutylphenyl)propionitrile	
ETOH 0.1	5.935143	194.073	4 D	−1.38924	0.003217	Phenanthrene-9,10-oxide	
ETOH 0.1	31.19917	195.124	4 D	−2.22499	0.004386	Benzenemethanol, 2-(2-aminopropoxy)-3-methyl-	a-[1-(ethylamino)ethyl]-p-hydroxy-Benzyl alcohol
ETOH 0.1	38.99212	195.1253	4 D	2.158606	0.00953	Benzenemethanol, 2-(2-aminopropoxy)-3-methyl-	a-[1-(ethylamino)ethyl]-p-hydroxy-Benzyl alcohol
ETOH 0.1	48.37093	197.1769	4 D	−3.11904	0.016197	Unknown	
ETOH 0.1	9.675726	203.1138	4 D	−1.70728	0.023636	Acetylcarnitine	L-Glutamic acid n-butyl ester
ETOH 0.1	6.747279	205.1304	4 D	−1.2578	0.005318	Pantothenol	dimethylbutanamide
ETOH 0.1	36.18938	210.0922	4 D	1.864256	0.040527	3-(2,5-Dimethoxy phenylpropionic acid	
ETOH 0.1	24.52067	229.0949	24 H	−2.33044	0.008877	Malonylcarnitine	
ETOH 0.1	17.7027	243.1089	4 D	−2.18071	0.016141	Unknown	
ETOH 0.1	64.66999	266.1613	24 H	−1.51898	0.047652	Unknown	
ETOH 0.1	42.3656	268.2487	4 D	−1.54971	0.015019	Unknown	
ETOH 0.1	4.86619	271.9364	24 H	2.629339	0.04245	Unknown	
ETOH 0.1	43.99398	272.16	24 H	1.929598	0.032191	Unknown	
ETOH 0.1	43.99398	272.16	4 D	2.186768	0.003018	Unknown	
ETOH 0.1	63.00428	285.2285	24 H	4.002774	0.018095	Unknown	
ETOH 0.1	37.97297	292.1862	4 D	2.239381	0.0496	Unknown	
ETOH 0.1	4.014175	293.9773	4 D	1.938848	0.036775	Unknown	
ETOH 0.1	6.160071	294.0957	24 H	1.269183	0.005089	Unknown	
ETOH 0.1	8.967061	295.1521	4 D	1.513407	0.042025	Unknown	
ETOH 0.1	71.55535	296.2308	24 H	3.545035	0.003758	Unknown	
ETOH 0.1	50.75387	298.174	4 D	−3.00237	0.045113	Unknown	
ETOH 0.1	18.88505	300.1147	4 D	2.686262	0.023485	Unknown	
ETOH 0.1	17.18696	300.1656	24 H	1.548853	0.036582	Unknown	
ETOH 0.1	7.719471	301.1345	4 D	6.648828	0.030822	Unknown	
ETOH 0.1	15.22357	312.1341	4 D	−2.01503	0.041156	Unknown	
ETOH 0.1	26.51229	315.6732	4 D	2.014609	0.010998	Unknown	
ETOH 0.1	18.82373	325.2711	4 D	−1.75625	0.013853	Unknown	
ETOH 0.1	20.94557	325.2714	4 D	−2.07426	0.00333	Unknown	
ETOH 0.1	8.542672	337.2012	24 H	1.402499	0.000372	Unknown	
ETOH 0.1	3.85935	353.2765	4 D	2.622059	0.002006	Unknown	
ETOH 0.1	25.16993	357.1781	4 D	2.217294	0.001346	Unknown	
ETOH 0.1	24.01428	359.1532	4 D	1.535704	0.033	Unknown	
ETOH 0.1	18.50245	360.1321	4 D	2.11023	0.004465	Unknown	
ETOH 0.1	83.72506	362.2787	24 H	2.819814	0.04795	Unknown	
ETOH 0.1	83.72506	362.2787	4 D	2.916423	0.023844	Unknown	
ETOH 0.1	27.98054	368.2122	4 D	1.69537	0.02565	Unknown	
ETOH 0.1	20.76168	379.1771	24 H	−1.72285	0.021135	Unknown	
ETOH 0.1	20.76168	379.1771	4 D	1.539861	0.019592	Unknown	
ETOH 0.1	15.20829	383.1721	4 D	1.914543	0.043581	Unknown	
ETOH 0.1	23.38956	384.2127	4 D	−2.55567	0.025704	Unknown	
ETOH 0.1	51.65871	386.1724	4 D	5.308852	0.032774	(+)-Eudesmin	
ETOH 0.1	19.97914	387.0812	4 D	−1.97698	0.024641	Unknown	
ETOH 0.1	19.97914	387.0812	24 H	1.739051	0.018391	Unknown	
ETOH 0.1	17.53242	388.1815	24 H	−1.44894	0.012322	Unknown	
ETOH 0.1	46.346	388.2349	4 D	1.946524	0.002762	Unknown	
ETOH 0.1	15.90129	393.1889	24 H	−1.43098	0.022977	Unknown	
ETOH 0.1	6.259963	396.1687	24 H	1.717607	0.047952	Unknown	
ETOH 0.1	51.66325	403.1978	4 D	3.11601	0.048224	Unknown	
ETOH 0.1	30.70733	405.2001	4 D	2.668076	0.001676	Unknown	
ETOH 0.1	16.21743	408.1636	4 D	1.965641	0.028858	Unknown	
ETOH 0.1	21.14975	417.2386	4 D	−1.97972	0.007183	Unknown	
ETOH 0.1	33.09057	417.2338	4 D	2.032563	0.016902	Unknown	
ETOH 0.1	26.77212	420.1862	4 D	3.282511	0.030236	Unknown	
ETOH 0.1	18.03482	429.2533	4 D	−1.80751	0.006213	Unknown	
ETOH 0.1	30.24237	429.2535	4 D	1.804876	0.044332	Unknown	
ETOH 0.1	35.58196	431.2501	4 D	1.532408	0.037928	Unknown	
ETOH 0.1	32.18393	437.2042	24 H	24.53212	0.001124	Unknown	
ETOH 0.1	4.808947	440.0223	4 D	−1.52785	0.03034	Unknown	
ETOH 0.1	24.13915	443.2381	4 D	2.985557	0.023682	Unknown	

TABLE 7-continued

Experiment	Retention time	Mass	Time	Fold	p-value	Compound 1	Compound 2
	Cellular metabolites measured in hES cells treated with alcohol						
ETOH 0.1	67.0705	443.3216	4 D	1.751997	0.037074	Unknown	
ETOH 0.1	51.58546	444.2237	4 D	2.130512	0.031074	Unknown	
ETOH 0.1	33.51823	460.9391	4 D	−4.51805	0.005949	Unknown	
ETOH 0.1	22.95657	462.2217	24 H	−1.98563	0.0437	Unknown	
ETOH 0.1	25.3287	464.225	24 H	−1.63071	0.025852	Unknown	
ETOH 0.1	46.51446	467.3804	4 D	2.068091	0.042613	Unknown	
ETOH 0.1	51.6158	468.2002	4 D	1.875012	0.015762	Unknown	
ETOH 0.1	30.37291	471.1928	4 D	2.001387	0.022662	glucuronide	
ETOH 0.1	30.72707	471.7804	4 D	−2.83569	0.037476	Unknown	
ETOH 0.1	30.28867	478.2761	4 D	1.698899	0.000475	Unknown	
ETOH 0.1	72.7735	482.3062	24 H	−1.95925	0.042853	Unknown	
ETOH 0.1	6.676207	485.2069	24 H	−2.01419	0.013732	Unknown	
ETOH 0.1	66.73744	487.3472	4 D	2.931014	0.007475	Unknown	
ETOH 0.1	21.72729	489.2127	4 D	−1.51037	0.0314	Unknown	
ETOH 0.1	31.22083	510.8202	4 D	2.488196	0.011267	Unknown	
ETOH 0.1	34.35986	521.9924	24 H	−1.44593	0.032994	Unknown	
ETOH 0.1	34.57864	525.3161	4 D	1.551324	0.037545	Unknown	
ETOH 0.1	51.73057	526.2773	4 D	3.445707	0.006877	Unknown	
ETOH 0.1	23.87065	530.314	4 D	1.964552	0.006366	L-Oleandrosyl-oleandolide	
ETOH 0.1	32.50661	531.2876	4 D	2.106138	0.024689	Unknown	
ETOH 0.1	35.58454	531.3191	4 D	−1.25162	0.027019	Unknown	
ETOH 0.1	66.31491	531.3736	4 D	3.862674	0.01116	Unknown	
ETOH 0.1	32.24719	541.3274	4 D	2.161601	0.038319	Unknown	
ETOH 0.1	17.6573	545.3029	4 D	−1.39484	0.043629	Unknown	
ETOH 0.1	31.891	554.8471	4 D	2.038489	0.037688	Unknown	
ETOH 0.1	15.78741	555.2406	4 D	1.835025	0.014923	Unknown	
ETOH 0.1	5.742094	555.8505	4 D	−1.28922	0.017625	Unknown	
ETOH 0.1	88.02533	556.3971	4 D	−3.11839	0.016192	Unknown	
ETOH 0.1	31.97996	559.8329	4 D	−1.7213	0.049678	Unknown	
ETOH 0.1	24.7039	574.3397	4 D	1.58436	0.02116	Unknown	
ETOH 0.1	31.71105	574.3427	4 D	1.750055	0.026643	Unknown	
ETOH 0.1	47.90467	576.096	4 D	1.361314	0.021201	Unknown	
ETOH 0.1	16.90923	577.2825	24 H	1.727637	0.047154	Unknown	
ETOH 0.1	35.26918	589.6938	4 D	1.819573	0.027966	Unknown	
ETOH 0.1	31.54325	591.3789	4 D	1.578222	0.000714	Unknown	
ETOH 0.1	25.14927	596.3543	4 D	1.395808	0.049214	L-Urobilinogen;	
ETOH 0.1	32.67372	603.3535	4 D	−2.62952	0.015253	Unknown	
ETOH 0.1	8.056862	612.1509	24 H	−1.47366	0.02889	Oxidized glutathione	Oxidized glutathione; Glutathione disulfide; GSSG; Oxiglutatione
ETOH 0.1	33.68866	619.3409	4 D	1.856648	0.030722	Unknown	
ETOH 0.1	32.73907	620.8861	4 D	2.248558	0.00893	Unknown	
ETOH 0.1	32.08734	635.4065	4 D	1.392618	0.030885	Unknown	
ETOH 0.1	5.903429	646.7084	4 D	−1.27147	0.020652	Unknown	
ETOH 0.1	26.7452	661.3846	4 D	1.295402	0.046573	Unknown	
ETOH 0.1	33.48766	677.9101	24 H	−2.22083	0.028347	Unknown	
ETOH 0.1	31.11764	693.4124	4 D	2.318353	0.028562	Unknown	
ETOH 0.1	28.2045	695.4286	24 H	−32.9384	0.027453	Unknown	
ETOH 0.1	33.70266	699.9225	4 D	1.719036	0.039493	Unknown	
ETOH 0.1	40.84822	702.2497	4 D	−2.76945	0.001031	Neu5Acalpha2-3Galbeta1-4Glcbeta-Sp	Neu5Acalpha2-6Galbeta1-4Glcbeta-Sp
ETOH 0.1	34.62439	707.3928	4 D	2.136871	0.0353	Unknown	
ETOH 0.1	56.18269	707.4296	24 H	1.489574	0.046762	Unknown	
ETOH 0.1	33.73428	708.9387	4 D	2.159654	0.006959	Unknown	
ETOH 0.1	4.826824	711.8344	24 H	−3.02053	0.013448	Unknown	
ETOH 0.1	33.89008	730.4494	4 D	2.613893	0.009133	Unknown	
ETOH 0.1	47.76987	731.0954	4 D	−2.77579	0.004811	Unknown	
ETOH 0.1	5.918027	732.007	4 D	1.795393	0.021782	Unknown	
ETOH 0.1	35.028	751.4193	4 D	1.87008	0.032616	Unknown	
ETOH 0.1	34.12668	752.4629	4 D	2.066515	0.031345	Unknown	
ETOH 0.1	69.32865	765.5211	24 H	1.873064	0.033127	Unknown	
ETOH 0.1	34.33545	774.4767	4 D	2.103658	0.020363	Unknown	
ETOH 0.1	89.29926	774.5055	4 D	−2.40077	0.006755	Unknown	
ETOH 0.1	5.886782	780.241	4 D	−1.31403	0.025516	Unknown	
ETOH 0.1	34.52749	796.4891	4 D	1.926524	0.049615	Unknown	
ETOH 0.1	34.60124	796.9917	4 D	1.818186	0.015806	Unknown	
ETOH 0.1	4.613879	820.8181	4 D	−1.47009	0.047535	Unknown	
ETOH 0.1	5.259716	888.8041	4 D	−1.45771	0.021932	Unknown	
ETOH 0.1	8.502051	909.5934	24 H	2.274264	0.037836	Unknown	
ETOH 0.1	5.217833	913.8074	24 H	−1.86064	0.028059	Unknown	
ETOH 0.1	5.399211	921.0025	4 D	1.677834	0.001526	Unknown	
ETOH 0.1	3.646902	994.0917	24 H	1.441829	0.019979	Unknown	
ETOH 0.1	3.705141	1008.072	4 D	1.30378	0.048393	Unknown	
ETOH 0.1	5.177162	1038.786	4 D	1.677834	0.030851	Unknown	

TABLE 7-continued

Cellular metabolites measured in hES cells treated with alcohol

Experiment	Retention time	Mass	Time	Fold	p-value	Compound 1	Compound 2
ETOH 0.3	85.57399	83.0372	24 H	2.472036	0.010882	Unknown	
ETOH 0.3	15.48433	99.0689	4 D	1.337742	0.043286	N-Methyl-2-pyrrolidinone	
ETOH 0.3	15.48433	99.0689	24 H	1.467845	0.043638	N-Methyl-2-pyrrolidinone	
ETOH 0.3	52.01225	99.1043	24 H	3.331103	0.000378	Unknown	
ETOH 0.3	10.21225	101.1201	24 H	2.191927	0.043209	Hexylamine	
ETOH 0.3	4.032816	111.9839	4 D	−8.67642	0.018374	Thiosulfate	
ETOH 0.3	3.767232	120.0436	4 D	1.936297	0.034585	3,4-Dihydroxybutyric acid	
ETOH 0.3	13.40565	120.2112	4 D	3.90007	0.046375	Unknown	
ETOH 0.3	16.73904	129.0452	24 H	1.795891	3.32E−05	3,4-Dihydroxybutyric acid	
ETOH 0.3	88.64043	130.9541	24 H	2.024969	0.006982	Unknown	
ETOH 0.3	22.22892	131.0746	4 D	2.502205	0.049833	3-Methylindole	
ETOH 0.3	14.35336	131.076	4 D	4.050219	0.020549	Unknown	
ETOH 0.3	3.958833	148.0052	24 H	−1.72967	0.043053	Unknown	
ETOH 0.3	7.479235	149.0511	4 D	−1.30477	0.014313	Amino-4methylthiobutyric acid	
ETOH 0.3	27.80141	153.0811	24 H	−1.6976	0.025771	Unknown	
ETOH 0.3	5.559732	155.0681	4 D	1.637846	0.022817	L-Histidine	4-propionic acid
ETOH 0.3	14.22357	161.0805	24 H	−6.43527	0.032474	Unknown	
ETOH 0.3	44.88033	162.0662	4 D	1.799131	0.037703	Unknown	
ETOH 0.3	23.6763	167.0941	24 H	−2.83	0.012984	3-Methoxytyramine	Phenylephrine
ETOH 0.3	19.18355	168.0434	4 D	1.281914	0.028006	Homogentisic acid	Vanillic acid
ETOH 0.3	26.70635	171.1244	24 H	3.755227	0.001253	GABA analogue	
ETOH 0.3	20.23014	173.084	24 H	−1.43983	0.019446	1,3-Dimethyl-8-isoquinolinol	
ETOH 0.3	28.52393	178.5546	24 H	−2.76389	0.001401	Unknown	
ETOH 0.3	5.935143	194.073	4 D	−1.39988	0.002956	Phenanthrene-9,10-oxide	9-Hydroxyphenanthrene; 9-Phenanthrol
ETOH 0.3	22.61355	194.0836	24 H	−1.70303	0.030955	Unknown	
ETOH 0.3	31.19917	195.124	4 D	−1.86013	0.015911	Benzenemethanol, 2-(2-aminopropoxy)-3-methyl-	a-[1-(ethylamino)ethyl]-p-hydroxy-Benzyl alcohol
ETOH 0.3	19.48063	201.1709	4 D	−2.97874	0.009458	Unknown	
ETOH 0.3	6.747279	205.1304	4 D	−1.33858	0.014168	Pantothenol	dimethylbutanamide;
ETOH 0.3	36.18938	210.0922	4 D	1.849968	0.042032	3-(2,5-Dimethoxy phenylpropionic acid	
ETOH 0.3	6.62669	218.0762	4 D	−1.77129	0.047105	Unknown	
ETOH 0.3	27.57188	222.0401	24 H	−2.11155	0.040386	Unknown	
ETOH 0.3	13.6845	223.119	24 H	−2.73967	0.024694	Unknown	Unknown
ETOH 0.3	24.52067	229.0949	4 D	−1.74038	0.010344	Malonylcarnitine	Malonylcarnitine
ETOH 0.3	55.50731	229.1457	4 D	−1.53336	0.028395	Unknown	
ETOH 0.3	32.9941	229.2025	24 H	−1.37697	0.03113	Unknown	
ETOH 0.3	47.0879	234.125	24 H	−1.63184	0.029169	5-Methoxytryptophan	
ETOH 0.3	53.26863	234.1253	4 D	−1.62383	0.026291	5-Methoxytryptophan	
ETOH 0.3	3.673694	237.0041	24 H	2.989077	0.011016	Unknown	
ETOH 0.3	5.176232	239.9592	24 H	1.640005	0.018097	Unknown	
ETOH 0.3	27.39631	243.11	4 D	−1.49547	0.024259	Unknown	
ETOH 0.3	6.626769	247.1049	4 D	−4.47566	0.039425	Unknown	
ETOH 0.3	9.0276	247.1408	4 D	−2.75089	0.000424	Unknown	
ETOH 0.3	18.84552	256.1066	4 D	−2.14073	0.02288	5-Ethyl-5-(1-methyl-3-carboxypropyl)barbituric acid	
ETOH 0.3	40.75868	267.2543	4 D	−1.6481	0.029485	Unknown	
ETOH 0.3	40.75868	267.2543	24 H	−1.49599	0.021245	Unknown	
ETOH 0.3	42.3656	268.2487	4 D	−1.51708	0.030774	Unknown	
ETOH 0.3	4.86619	271.9364	24 H	4.069637	0.02742	Unknown	
ETOH 0.3	22.86183	275.1193	24 H	−2.23674	0.044504	Unknown	
ETOH 0.3	5.69776	284.9798	4 D	−1.2223	0.026683	Unknown	
ETOH 0.3	14.88092	286.1519	4 D	−1.86167	0.033393	Unknown	
ETOH 0.3	75.76147	288.2632	24 H	1.96905	0.001271	Unknown	
ETOH 0.3	66.86661	293.1952	4 D	−2.15382	0.004487	Unknown	
ETOH 0.3	4.014175	293.9773	4 D	−2.26718	0.013307	Unknown	
ETOH 0.3	20.67831	294.1535	4 D	−1.42237	0.008416	Unknown	
ETOH 0.3	24.21651	294.1531	24 H	−1.89159	0.02267	Unknown	
ETOH 0.3	8.967061	295.1521	4 D	1.467032	0.021323	Unknown	
ETOH 0.3	66.35884	298.1537	24 H	3.758351	0.016783	Unknown	
ETOH 0.3	19.66398	299.1929	24 H	−1.57058	0.041283	Unknown	
ETOH 0.3	7.719471	301.1345	4 D	2.678267	0.046654	Unknown	
ETOH 0.3	4.954547	303.8875	24 H	2.069669	0.049847	Unknown	
ETOH 0.3	44.09424	313.199	24 H	2.291989	0.005146	Unknown	
ETOH 0.3	26.51229	315.6732	4 D	−2.86533	0.000573	Unknown	
ETOH 0.3	23.90107	322.1166	4 D	−2.4215	0.010241	Unknown	
ETOH 0.3	30.19245	324.1666	24 H	−1.59251	0.037632	Unknown	
ETOH 0.3	20.72194	332.1367	4 D	−2.077	0.009795	Unknown	

TABLE 7-continued

Cellular metabolites measured in hES cells treated with alcohol							
Experiment	Retention time	Mass	Time	Fold	p-value	Compound 1	Compound 2
ETOH 0.3	8.542672	337.2012	24 H	1.287435	0.004465	Unknown	
ETOH 0.3	5.033976	340.9252	24 H	−1.83604	0.037709	Unknown	
ETOH 0.3	5.033976	340.9252	4 D	2.624968	0.049915	Unknown	
ETOH 0.3	68.02448	342.1482	24 H	−1.85279	0.043066	Unknown	
ETOH 0.3	3.85935	353.2765	4 D	4.904139	0.000229	Unknown	
ETOH 0.3	18.50245	360.1321	24 H	−2.28881	0.005116	Unknown	
ETOH 0.3	83.72506	362.2787	24 H	−2.54153	0.005266	Unknown	
ETOH 0.3	20.16372	365.1606	4 D	−1.92519	0.027055	Unknown	
ETOH 0.3	11.47109	375.1898	4 D	2.131693	0.028486	Unknown	
ETOH 0.3	26.07722	375.1886	4 D	−4.75123	0.000746	Unknown	
ETOH 0.3	41.28494	378.2956	24 H	1.636485	0.027631	Unknown	
ETOH 0.3	15.20829	383.1721	4 D	3.377369	0.002214	Unknown	
ETOH 0.3	51.65871	386.1724	4 D	4.85106	0.031786	(+)-Eudesmin	(+)-Eudesmin
ETOH 0.3	19.97914	387.0812	4 D	−1.69949	0.015257	Unknown	
ETOH 0.3	46.346	388.2349	4 D	−1.53209	0.040255	Unknown	
ETOH 0.3	15.90129	393.1889	24 H	−1.51089	0.011717	Unknown	
ETOH 0.3	19.69092	393.1886	4 D	−2.05894	0.011258	Unknown	
ETOH 0.3	6.259963	396.1687	4 D	2.422002	0.013544	Unknown	
ETOH 0.3	17.27421	403.1984	4 D	−2.30266	0.00281	Unknown	
ETOH 0.3	21.14975	417.2386	4 D	−1.57309	0.03368	Unknown	
ETOH 0.3	33.09057	417.2338	4 D	−1.77978	0.026439	Unknown	
ETOH 0.3	13.35295	420.0513	4 D	−1.90198	0.003417	Unknown	
ETOH 0.3	27.46219	421.2201	4 D	−2.27332	0.012803	Unknown	
ETOH 0.3	18.03482	429.2533	4 D	−2.20091	0.002807	Unknown	
ETOH 0.3	54.46495	440.0284	24 H	2.51037	0.011972	Unknown	
ETOH 0.3	30.87201	443.2339	4 D	2.171362	0.0186	Unknown	
ETOH 0.3	67.0705	443.3216	4 D	1.688451	0.048544	Unknown	
ETOH 0.3	51.58546	444.2237	24 H	2.241866	0.044444	Unknown	
ETOH 0.3	51.58546	444.2237	4 D	2.030169	0.032667	Unknown	
ETOH 0.3	30.05687	444.2789	24 H	−1.83503	0.021764	Unknown	
ETOH 0.3	54.8719	446.0431	24 H	−2.22145	0.049006	Unknown	
ETOH 0.3	54.8719	446.0431	4 D	1.933882	0.047398	Unknown	
ETOH 0.3	29.86415	447.2509	4 D	−1.85819	0.025964	Unknown	
ETOH 0.3	29.86415	447.2509	24 H	2.10322	0.036254	Unknown	
ETOH 0.3	30.45343	449.2653	4 D	−2.41429	0.016795	Unknown	
ETOH 0.3	44.98056	449.2611	24 H	−3.47136	0.038457	Unknown	
ETOH 0.3	28.27806	455.2052	24 H	−2.50898	0.005338	Unknown	
ETOH 0.3	33.51823	460.9391	4 D	−3.60151	0.010558	Unknown	
ETOH 0.3	22.95657	462.2217	4 D	1.935626	0.03474	Unknown	
ETOH 0.3	33.5733	463.2914	24 H	−1.66521	0.034862	Unknown	
ETOH 0.3	25.3287	464.225	24 H	−1.71665	0.033532	Unknown	
ETOH 0.3	30.46172	466.2921	24 H	−1.95938	0.012601	Unknown	
ETOH 0.3	33.68894	466.615	24 H	−3.27865	0.008212	Unknown	
ETOH 0.3	46.51446	467.3804	24 H	−2.13465	0.013009	Unknown	
ETOH 0.3	51.6158	468.2002	4 D	1.919859	0.003122	Unknown	
ETOH 0.3	30.37291	471.1928	4 D	2.725649	0.008164	glucuronide	
ETOH 0.3	30.72707	471.7804	4 D	−3.44069	0.010281	Unknown	
ETOH 0.3	30.28867	478.2761	4 D	1.509949	0.018484	Unknown	
ETOH 0.3	10.82859	482.1942	24 H	−1.52795	0.033711	Unknown	
ETOH 0.3	72.7735	482.3062	24 H	−2.61806	0.0161	Unknown	
ETOH 0.3	10.8217	485.204	24 H	2.038065	0.038466	Unknown	
ETOH 0.3	66.73744	487.3472	4 D	2.877867	0.020235	Unknown	
ETOH 0.3	30.83472	488.305	24 H	−2.18525	0.009407	Unknown	
ETOH 0.3	30.88032	488.8071	24 H	−1.91959	0.040672	Unknown	
ETOH 0.3	21.72729	489.2127	4 D	2.372158	0.000369	Unknown	
ETOH 0.3	13.78553	502.2258	4 D	1.866325	0.010364	Unknown	
ETOH 0.3	18.36887	505.2616	4 D	2.008892	0.036368	Unknown	
ETOH 0.3	5.891069	509.6704	4 D	1.467845	0.0228	Unknown	
ETOH 0.3	31.20706	510.3182	24 H	−2.26373	0.006715	Unknown	
ETOH 0.3	31.22083	510.8202	24 H	−2.04514	0.041219	Unknown	
ETOH 0.3	31.22083	510.8202	4 D	2.502378	0.010461	Unknown	
ETOH 0.3	46.57666	518.3914	4 D	2.288814	0.002533	Unknown	
ETOH 0.3	46.57666	518.3914	24 H	−2.70682	0.017765	Unknown	
ETOH 0.3	34.35986	521.9924	24 H	−1.58403	0.024454	Unknown	
ETOH 0.3	31.53434	523.8187	24 H	−2.17272	0.02888	Unknown	
ETOH 0.3	51.73057	526.2773	4 D	2.714525	0.010415	Unknown	
ETOH 0.3	71.36012	528.3631	4 D	2.361003	0.026297	Unknown	
ETOH 0.3	23.87065	530.314	4 D	2.211921	0.001298	L-Oleandrosyl-oleandolide	
ETOH 0.3	32.50661	531.2876	4 D	2.36084	0.014947	Unknown	
ETOH 0.3	35.58454	531.3191	4 D	−3.0409	0.000281	Unknown	
ETOH 0.3	66.31491	531.3736	4 D	2.87388	0.031879	Unknown	
ETOH 0.3	31.58889	532.8335	24 H	−2.16926	0.015971	Unknown	
ETOH 0.3	51.52268	539.4374	24 H	−1.92987	0.031525	Unknown	
ETOH 0.3	32.24719	541.3274	24 H	−2.12255	0.01445	Unknown	
ETOH 0.3	31.8519	554.3444	24 H	−2.36953	0.00655	Unknown	

TABLE 7-continued

Cellular metabolites measured in hES cells treated with alcohol							
Experiment	Retention time	Mass	Time	Fold	p-value	Compound 1	Compound 2
ETOH 0.3	31.891	554.8471	24 H	−2.29708	0.010734	Unknown	
ETOH 0.3	15.78741	555.2406	4 D	2.470837	0.001092	Unknown	
ETOH 0.3	5.742094	555.8505	4 D	−1.3396	0.023948	Unknown	
ETOH 0.3	5.742094	555.8505	24 H	−1.51803	0.031838	Unknown	
ETOH 0.3	88.02533	556.3971	4 D	−2.38386	0.035829	Unknown	
ETOH 0.3	31.97996	559.8329	4 D	−2.56596	0.002155	Unknown	
ETOH 0.3	14.9927	566.2265	4 D	1.624054	0.006806	Unknown	
ETOH 0.3	24.7039	574.3397	4 D	1.523934	0.011558	Unknown	
ETOH 0.3	47.90467	576.096	4 D	1.557573	0.020904	Unknown	
ETOH 0.3	32.15777	576.3582	24 H	−2.20886	0.012421	Unknown	
ETOH 0.3	16.90923	577.2825	4 D	−8.91726	0.02781	Unknown	
ETOH 0.3	5.903169	579.2519	4 D	−2.11184	0.001075	Ethanesulfonic acid	
ETOH 0.3	5.903169	579.2519	24 H	−1.48021	0.01083	Ethanesulfonic acid	
ETOH 0.3	31.54325	591.3789	24 H	−1.98165	0.010883	Unknown	
ETOH 0.3	25.14927	596.3543	4 D	1.545421	0.015277	L-Urobilinogen;	
ETOH 0.3	25.14927	596.3543	24 H	1.834898	0.021555	L-Urobilinogen;	
ETOH 0.3	32.67372	603.3535	4 D	−2.93997	0.007904	Unknown	
ETOH 0.3	56.3513	611.4952	24 H	−1.9811	0.007557	Unknown	
ETOH 0.3	4.936704	611.8727	4 D	2.009588	0.018619	Unknown	
ETOH 0.3	32.70236	611.871	24 H	−2.19208	0.044039	Unknown	
ETOH 0.3	8.056862	612.1509	24 H	−1.59538	0.010611	Oxidized glutathione	
ETOH 0.3	33.68866	619.3409	4 D	2.125939	0.015211	Unknown	
ETOH 0.3	32.73907	620.8861	24 H	−2.32076	0.039661	Unknown	
ETOH 0.3	32.08734	635.4065	4 D	1.394744	0.031645	Unknown	
ETOH 0.3	32.95522	642.397	24 H	−2.21146	0.035834	Unknown	
ETOH 0.3	5.903429	646.7084	4 D	2.946495	2.66E−05	Unknown	
ETOH 0.3	8.787827	658.2544	4 D	−3.20961	0.030138	Unknown	
ETOH 0.3	26.7452	661.3846	4 D	1.546707	0.010714	Unknown	
ETOH 0.3	16.3338	666.3836	24 H	−1.37135	0.037278	Unknown	
ETOH 0.3	33.48766	677.9101	24 H	−2.64653	0.009907	Unknown	
ETOH 0.3	40.84822	702.2497	24 H	1.283337	0.019733	Neu5Acalpha2-3Galbeta1-4Glcbeta-Sp	
ETOH 0.3	40.84822	702.2497	4 D	−2.21591	0.004389	Neu5Acalpha2-3Galbeta1-4Glcbeta-Sp	
ETOH 0.3	30.43889	707.3933	24 H	−1.79166	0.048232	Unknown	
ETOH 0.3	56.18269	707.4296	24 H	−2.43361	0.009442	Unknown	
ETOH 0.3	4.826824	711.8344	24 H	−2.25605	0.017007	Unknown	
ETOH 0.3	47.76987	731.0954	4 D	−3.25036	0.027339	Unknown	
ETOH 0.3	5.918027	732.007	4 D	1.76125	0.038454	Unknown	
ETOH 0.3	35.028	751.4193	24 H	−1.70279	0.045682	Unknown	
ETOH 0.3	35.028	751.4193	4 D	1.845101	0.0375	Unknown	
ETOH 0.3	69.32865	765.5211	24 H	1.884393	0.027105	Unknown	
ETOH 0.3	34.33545	774.4767	24 H	−2.34161	0.036388	Unknown	
ETOH 0.3	34.60124	796.9917	4 D	1.688919	0.047852	Unknown	
ETOH 0.3	4.613879	820.8181	24 H	−1.85112	0.038895	Unknown	
ETOH 0.3	4.613879	820.8181	4 D	−1.57113	0.014605	Unknown	
ETOH 0.3	5.259716	888.8041	24 H	1.221624	0.043348	Unknown	
ETOH 0.3	5.217833	913.8074	24 H	−1.88465	0.026148	Unknown	
ETOH 0.3	5.399211	921.0025	4 D	1.944905	2.78E−05	Unknown	
ETOH 0.3	5.387775	922.0048	24 H	−2.75471	0.015691	Unknown	
ETOH 0.3	3.680188	980.075	4 D	1.480926	0.012893	Unknown	
ETOH 0.3	3.646902	994.0917	4 D	1.604696	0.000395	Unknown	
ETOH 0.3	3.705141	1008.072	4 D	1.415783	0.021503	Unknown	
ETOH 0.3	5.177162	1038.786	4 D	1.588208	0.030971	Unknown	
ETOH 0.3	5.8905	1040.323	4 D	−1.47887	0.009656	Unknown	

All references cited herein are incorporated by reference. In addition, the invention is not intended to be limited to the disclosed embodiments of the invention. It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

What is claimed is:

1. A method for identifying one or a plurality of cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons that is differentially produced in human embryonic stem cells (hESCs) or human pluripotent stem cells contacted with a test compound from a population of secreted cellular metabolites, the method comprising the steps of:

a) culturing hESCs or human pluripotent stem cells in the presence or absence of a test compound;

b) separating members of the population of cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons that are secreted from hESCs or human pluripotent stem cells;

c) detecting one or a plurality of differentially produced cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons from hESCs or human pluripotent stem cells; and

d) identifying at least one cellular metabolite having a molecular weight of from about 10 to about 1500 Daltons that is differentially produced in cells cultured in the presence of the test compound.

2. A method according to claim **1**, wherein at least one of the cellular metabolites is produced in greater amounts in the presence of the test compound than in the absence of the test compound.

3. A method according to claim **1**, wherein at least one of the cellular metabolites is produced in greater amounts in the absence of the test compound than in the presence of the test compound.

4. A method according to claim **1**, wherein the cellular metabolite has a molecular weight of from about 100 to about 1000 Daltons.

5. A method according to claim **1**, wherein the test compound is a toxic or teratogenic compound.

6. A method according to claim **1**, wherein one or a plurality of cellular metabolites is separated using a physical separation method.

7. A method according to claim **6**, wherein the physical separation method is liquid chromatography/electrospray ionization time of flight mass spectrometry.

8. A method according to claim **1**, wherein the cellular metabolites are tetrahydrofolate, dihydrofolate or other metabolites in the folate metabolic pathway, glutathione, or oxidized glutathione.

9. A method according to claim **1**, wherein the cellular metabolites are kynurenine, 8-methoxykynurenate, N'-formylkynurenine 7,8-dihydro-7,8-dihydroxykynurenate, 5-Hydroxytryptophan, N-acetyl-D-tryptophan, glutamate, pyroglutamic acid or other metabolites in the tryptophan or glutamate metabolic pathways, histamine, dopamine, 3,4-dihydroxybutyric acid, serotonin, or gamma-aminobutyric acid (GABA).

10. A method according to claim **1**, wherein a plurality of cellular metabolites are identified.

11. A method according to claim **10** wherein the plurality of identified cellular metabolites comprise a biomarker profile.

12. A method according to claim **11**, wherein one of the cellular metabolites comprising a biomarker profile is kynurenine.

13. A method according to claim **10**, wherein the test compound is a toxic or teratogenic compound.

14. A method according to claim **13**, wherein the plurality of identified cellular metabolites comprise a biomarker profile characteristic of hESC or human pluripotent stem cell response to a toxic or teratogenic compound.

15. A method of claim **1**, further comprising the step of identifying at least one cellular metabolite having a molecular weight of from about 10 to about 1500 Daltons that is differentially produced in the cells in the presence or absence of the test compound in a biomarker profile comprising one or a plurality of cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons that are differentially produced in human embryonic stem cells (hESCs) or human pluripotent stem cells contacted with a toxic compound or compounds.

16. The method of claim **1**, wherein at least two cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons that are differentially produced in the cells in the presence or absence of the test compound are detected.

17. A method for screening a test compound to identify an effect of the compound on human embryonic stem cells (hESCs) or human pluripotent stem cells contacted with the test compound, the method comprising the steps of:

a) culturing hESCs or human pluripotent stem cells in the presence or absence of a test compound;

b) separating members of a population of cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons that are secreted from hESCs or human pluripotent stem cells

c) detecting one or a plurality of differentially produced cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons; and

d) identifying the effect of a compound on human embryonic stem cells (hESCs) or human pluripotent stem cells by identifying at least one cellular metabolite having a molecular weight of from about 10 to about 1500 Daltons that is differentially secreted between cells cultured in the presence versus the absence of the test compound.

18. A method according to claim **17**, wherein at least one of the cellular metabolites is produced in greater amounts in the presence of the test compound than in the absence of the test compound.

19. A method according to claim **17**, wherein at least one of the cellular metabolites is produced in greater amounts in the absence of the test compound than in the presence of the test compound.

20. A method according to claim **17**, wherein at least one of the cellular metabolites has a molecular weight of from about 100 to about 1000 Daltons.

21. A method according to claim **17**, wherein the test compound is a toxic or teratogenic compound.

22. A method according to claim **17**, wherein one or a plurality of cellular metabolites is separated using a physical separation method.

23. A method according to claim **22**, wherein the physical separation method is liquid chromatography/electrospray ionization time of flight mass spectrometry.

24. A method according to claim **17**, wherein the cellular metabolites are tetrahydrofolate, dihydrofolate or other metabolites in the folate metabolic pathway, glutathione, or oxidized glutathione.

25. A method according to claim **17**, wherein the cellular metabolites are kynurenine, 8-methoxykynurenate, N'-formylkynurenine 7,8-dihydro-7,8-dihydroxykynurenate, 5-Hydroxytryptophan, N-acetyl-D-tryptophan, glutamate, pyroglutamic acid or other metabolites in the tryptophan or glutamate metabolic pathways, histamine, dopamine, serotonin, gamma-aminobutyric acid (GABA) or other butyric acid species.

26. A method according to claim **17** wherein a plurality of cellular metabolites are identified.

27. A method according to claim **26**, wherein the plurality of identified cellular metabolites comprise a biomarker profile.

28. A method according to claim **27**, wherein one of the cellular metabolites comprising a biomarker profile is kynurenine.

29. A method according to claim **27**, wherein the test compound is a toxic or teratogenic compound.

30. A method according to claim **29**, wherein the plurality of identified cellular metabolites comprise a biomarker profile characteristic of hESC or human pluripotent stem cells response to a toxic or teratogenic compound.

31. A method of claim **17**, further comprising the step of identifying at least one cellular metabolite having a molecular weight of from about 10 to about 1500 Daltons that is differentially produced in the cells in the presence or absence of the test compound in biomarker profile comprising one or a plurality of cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons that are differentially produced in human embryonic stem cells (hESCs) or human pluripotent stem cells contacted with a toxic compound or compounds.

32. The method of claim **17**, wherein at least two cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons that are differentially produced in the cells in the presence or absence of the test compound are detected.

33. A method for assaying a test compound for toxicity or teratogenicity to hESC-derived lineage-specific cells or human pluripotent stem cell-derived lineage-specific cells contacted with the test compound, the method comprising the steps of:

a) culturing hESC-derived lineage-specific cells or human pluripotent stem cell-derived lineage-specific cells in the presence or absence of a test compound;

b) separating members of a population of cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons secreted from or hESC-derived lineage-specific cells or human pluripotent stem cell-derived lineage-specific cells;

c) detecting one or a plurality of cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons produced by hESC-derived lineage-specific cells or human pluripotent stem cell-derived lineage-specific cells contacted with a compound; and

d) identifying the toxicity or teratogenicity of the test compounds wherein hESC-derived lineage-specific cells or human pluripotent stem cell-derived lineage-specific cells contacted with a test compound differentially produce one or a plurality of cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons.

34. A method according to claim **33**, wherein at least one of the cellular metabolites is produced in greater amounts in the presence of the test compound than in the absence of the test compound.

35. A method according to claim **33**, wherein at least one of the cellular metabolites is produced in greater amounts in the absence of the test compound than in the presence of the test compound.

36. A method according to claim **33**, wherein the cellular metabolite has a molecular weight of from about 100 to about 1000 Daltons.

37. A method according to claim **33**, wherein the test compound is a toxic or teratogenic compound.

38. A method according to claim **33**, wherein one or a plurality of cellular metabolites is separated using a physical separation method.

39. A method according to claim **38**, wherein the physical separation method is liquid chromatography/electrospray ionization time of flight mass spectrometry.

40. A method according to claim **33**, wherein the cellular metabolites are tetrahydrofolate, dihydrofolate or other metabolites in the folate metabolic pathway, glutathione, or oxidized glutathione.

41. A method according to claim **33**, wherein the cellular metabolites are kynurenine, 8-methoxykynurenate, N'-formylkynurenine 7,8-dihydro-7,8-dihydroxykynurenate 5-Hydroxytryptophan, N-acetyl-D-tryptophan, glutamate, pyroglutamic acid or other metabolites in the tryptophan or glutamate metabolic pathways, histamine, dopamine, 3,4-dihydroxybutyric acid, serotonin, gamma-aminobutyric acid (GABA) or other butyric acid species.

42. A method according to claim **33**, wherein a plurality of cellular metabolites are identified.

43. A method according to claim **42**, wherein the plurality of identified cellular metabolites comprise a biomarker profile.

44. A method according to claim **43**, wherein one of the cellular metabolites comprising a biomarker profile is kynurenine.

45. A method according to claim **44**, wherein the test compound is a toxic or teratogenic compound.

46. A method according to claim **33**, wherein the plurality of identified cellular metabolites comprise a biomarker profile characteristic of hESC response to a toxic or teratogenic compound.

47. A method of claim **33**, further comprising the step of identifying at least one cellular metabolite having a molecular weight of from about 10 to about 1500 Daltons that is differentially produced in the cells in the presence or absence of the test compound in a biomarker profile comprising one or a plurality of cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons that are differentially produced in hESC-derived lineage-specific cells or human pluripotent stem cell-derived lineage-specific cells contacted with a toxic compound or compounds.

48. The method of claim **33**, wherein at least two cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons that are differentially produced in the cells in the presence or absence of the test compound are detected.

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