Genomic Alterations in Ethanol-Exposed Trophoblast Cell Lines Induced by Chronic Ethanol Treatment

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Abstract

Background: Ethanol consumption during pregnancy results in a broad spectrum of damage, but the knowledge of its mechanism is lacking.

Objective: The aim of this study is to determine ethanol-caused genomic alterations in placental cell lines after a repeated ethanol treatment in order to describe new genomic targets of cell damage.

Methods: A model of sustained exposure to standard doses of ethanol on two in vitro human choriocarcinoma cell lines, JEG-3 and BeWo, was used. Chromosomal abnormalities and copy number alterations (CNAs) were assessed by G-Banding cytogenetics and oligonucleotide Single Nucleotide Polymorphism-Array analysis (CytoScan, Affymetrix).

Results: Chromosomal abnormalities did not change despite ethanol exposure except for the presence of a derivative chromosome 4 [add(4)(p14)] in exposed BeWo cells. Regarding SNP-Array analysis, a total of 21 CNAs were found to be caused by ethanol exposure, 16 in JEG-3 cell line and 5 in BeWo cell line, which were not found in controls. There was no coincidence between JEG-3 and BeWo regions affected by ethanol.

Conclusion: Trophoblast cell lines exposed repetitively to ethanol presented genomic instability resulting in CNAs. However, no region has been equally altered in both models to consider it an ethanol exposure target area. So, further studies involving different models and approaches that target gene regulation are required.

Keywords: Target; Ethanol; Placenta; Cytogenetics; Array; CNAs

Introduction

One of the health consequences of alcohol consumption during pregnancy is foetal alcohol syndrome (FAS) [1]. The prevalence of FAS is between 1.3 and 4.6 births per 1,000 [2], while the combined prevalence of FAS and alcohol-related neurodevelopmental disorders (ARND) is estimated to be as high as 9.1 per 1,000 [3]. This is due to the appearance of various permanent birth defects caused by the mother’s consumption of alcohol during pregnancy, called foetal alcohol spectrum disorder (FASD) [4].

For the majority of FASD cases, strategies for damage-diagnosis are lacking and there are not biomarkers that offer a reliable information about the injury in the foetus [5]. Among that, selective biological markers for intrauterine alcohol damage promise to lead to interventional strategies targeted to these spectrum of undiagnosed cases [6].

Over the past decade, studies in zygotic and dizygotic twins provided the first evidence for the involvement of genetic factors in damage risk for FASD [7]. Since that, evidence has been accumulating and models for genomic dysregulation have emerged [8]. The consequences of prenatal ethanol exposure (PEE) observed in infants could be attributable, in part, to the damage exerted to the cells and, as consequence, this cell system losses partially its function [9]. Upon delivery, placenta is the most accessible fetal-maternal tissue and carries valuable information about the pregnancy including adverse effects on maternal and/or fetal physiology [5,10]. Ethanol produces some of fetal abnormalities via actions on the placenta. These alterations are: transport of nutrients, hormone/growth factor production and its deregulation [11-13]. Altogether, ethanol shifts the trophoblast from a state of proliferation to one of cell cycle arrest or differentiation, the mechanism of these changes is not understood.

Despite being a preliminary model, cell lines are important resources in order to characterize genomic alterations in pathological conditions [14]. Although G-banding techniques enable the identification of chromosomal aberrations (structural and numerical changes), remarkable alterations could remain unidentified in complex karyotypes [15]. The combined use with Single Nucleotide Polymorphism Array (SNP-Array) allows to give an average genomic profile of copy number gains and losses for all chromosomes [16]. Some studies have been published showing advances in genomic-based alterations in FASD cases [7,17,18] but these studies have not expanded the knowledge in relation to changes in copy number...
alterations (CNAs) in placental cells. Herein we present the results of two different placental cell lines exposed chronically to ethanol and its related-genomic alterations with the aim to find out chromosomal regions that can be considered preliminary targets involved in the dysfunction observed in FASD.

Material and Methods

Cell culture

Human placental choriocarcinoma cell lines were purchased from the American Type Culture Collection (ATCC): JEG-3 (HTB-36; ATCC, Manassas, VA) and BeWo (CCL-98; ATCC). JEG-3 cells were maintained in Minimum Essential Media (MEM) supplemented with 10% (v/v) Foetal Bovine Serum (FBS), 20 mM L-glutamine, 10 mM sodium pyruvate, 100 U/mL penicillin and 100 mg/mL streptomycin and 100 U/mL penicillin. BeWo cells were maintained in Ham’s F-12 medium supplemented with 2 mM L-glutamine, 10 mM sodium pyruvate, 100 mg/mL streptomycin and 100 U/mL penicillin; all from Gibco, Montreal, CA. Cell cultures were maintained at 37°C in humidified 5% CO2 atmosphere. These lines are the best characterized [9,19,20] and also allow chronic ethanol exposure during several hours as previously described [19,20].

Experimental design

We followed the experimental procedure to expose the in vitro models chronically to ethanol designed by van Steenwyk et al. with minor modifications: 600,000 cells per 50 cm2 flask were seeded, with 5 mL of medium, until the cells were 80% confluent [21,22]. Ethanol-treated cells (50 mM) were cultured in an ethanol-saturated incubator as previously described [23]. This concentration is equivalent to the expected in the human placental tissue from moderate ethanol consumption in alcohol-dependent consumers (386 mg/dl; approximately 85 mM) [24]. Control flasks were kept in ethanol-free media and subjected to media changes at the same time as ethanol exposed cells. The ethanol treated cells were maintained in a sealed vessel in which the atmosphere was saturated with ethanol in order to maintain the ethanol concentration at the level added to the medium.

G-banding cytogenetics

G-banding study was carried out on cell lines harvested when cell growth was subconfluent and actively dividing, adding KaryoMAX Colcemid Solution 10 pg/ml (Life Technologies, Rockville, MD, USA). After that, cells were treated with hypotonic solution (postassium chloride, 0.075 M) for 30 minutes at 37°C and were fixed in Carnoy solution. Chromosomes were banded using G-banding technique with Wright solution. All products get at Sigma-Aldrich, St. Louis, MO, USA. A minimum of twenty metaphases per cell line were studied in accordance with the the International Standing Committee on Human Cytogenetic Nomenclature (ISCN) 2013 [25].

Oligonucleotide SNP-array

Genomic DNAs were extracted from cell cultures at time of harvesting the cells using the PureLink Genomic DNA Mini Kit (Life Technologies, according to manufacturer’s protocol. Genome-wide high-resolution Single Nucleotide Polymorphisms (SNPs) array CytoScan HD (Affymetrix, Santa Clara, CA, USA) was used containing both SNPs and oligonucleotide probes. Procedures for DNA digestion, ligation, PCR amplification, fractionation, labelling and hybridization with the arrays were performed according to the manufacturer’s protocols (Affymetrix). Copy Number Alterations (CNAs), mosaic/clonal status, and Loss of Heterozygosity (LOH) were analyzed using Chromosome Analyses Suite (ChAS) Software (Affymetrix).

Results

JEG-3

By karyotyping, chromosome copy number per metaphase was variable ranging to 71-73 chromosomes per cell, which hampered to characterize the karyotype compared with normal human cells. JEG-3 cell line showed a complex karyotype with many structural chromosomal aberrations (in both, control and exposed conditions) listed below: t(4;11)(p15q13), add(7)(p22), add(7)(q36), add(15)(p11), i(13)(q10), del(18)(q21), add(19)(p13) and two marker-chromosomes with material of unknown origin. No differences between exposed and non-exposed JEG-3 cells were found (Figure 1).

A total of 16 CNAs were identified in ethanol exposed cells/nuclei: 11 losses and 5 gains (Table 1).

Finally, genes contained in these CNAs JEG-3 regions are: G-protein coupled receptors (TACR1, ADRA2B and ADRA1A), transcriptional regulators (ID2, DNMT3A and EGR3) and catalytic enzymes (GAD1 and BHMT). We also group these genes as belonging to main diseases: cancer (ID2, EFEMP1, TACR1, IGFBP2, BHMT, SAMD5, EGR3 and ARF6), neurological diseases (GADI, EGR3, ADRA1A and ADNP) and developmental diseases (MAP2, SHH and OTX2).

BeWo

In this cell line, chromosome copy number per metaphase was also variable, ranging to 63-80 chromosomes per cell. We found differences due to the ethanol input regarding karyotype. In non-exposed cells, we observed a deletion in the short arm of chromosome 4 [del(4)(p11)] but not in exposed cells, where a derivative chromosome with material added to 4p [add(4)(p14)] was considered (Figure 1). The remaining chromosomal aberrations were found in both conditions: add(1)(p36), der(1)(qter→q25::p36→qter), del(18)(q21), add(19)(p13) and two marker-chromosomes (Table 1).

Only 5 altered regions were differently identified in ethanol-exposed cells by SNP-Array, one loss and four gains (Table 1).

Genes coding for receptors and transport proteins (GABRA2, GABRA4, HTT and SLC1A1) and transcriptional regulators (RBPF1, PPARGC1A and SMARCAD2) were allocated in these CNAs BeWo regions. Classifying them according to related diseases, cancer (RAC1, SMARCAD2 and JAK2) and alcohol dependence (GABRA2 and MPDZ) were the most relevant.

Discussion

Trophoblast cell lines exposed repetitively to ethanol developed several CNAs in comparison to trophoblast non-exposed to ethanol. In particular, only one structural change was found to be related with ethanol exposure in BeWo cell line: [add(4)(p14)]. Furthermore, this study can support the hypothesis that ethanol causes genomic damage but this damage occurs randomly and non-specifically.

Regarding CNAs associated with ethanol exposure, previous publications suggested that cell lines are in continuous adaptation to the environment due to their immortal capacity and that some genomic changes are likely resulting from in vitro evolution of the karyotype [26,27]. So, environmental factors such as ethanol exposure can cause clone selections. This reason could explain the no detection of common regions in both cell lines.
However, the genes located in the CNA affected areas have been involved in several pathways related to FASD pathogenesis, such as nervous system development, growth restriction, as well as metabolic pathways such as glucocorticoid signaling and retinol, insulin and nitric oxide balanced levels [28-30]. Furthermore, it is important to note that in our study we found frequent association between genes of the CNAs areas and genes involved in cancer development like TACR1, IGFBP2 and RAC1 [31-33]. Also, the genes altered in both placental cell lines (SMAD5, SHH and POMC) have been previously associated with PEE [34-36].

It worth to be mentioned that genetic factors from the mother and from the foetus could contribute to develop FASD [17]. It is known that polymorphisms in alcohol metabolizing enzymes have a significant impact on the risk for FASD [37]. For example, variations in the alcohol dehydrogenase 1B (class I) gene (ADH1B) have been reported to confer either increased or decreased likelihood of developing FASD [38,39]. So, genomic predisposition is known to be present in ethanol exposed cases but no genome-wide studies have demonstrated specific polymorphisms in alcohol metabolizing enzymes have a significant impact on the risk for FASD [38,39]. For example, variations in the alcohol dehydrogenase 1B (class I) gene (ADH1B) have been reported to confer either increased or decreased likelihood of developing FASD [38,39]. So, genomic predisposition is known to be present in ethanol exposed cases but no genome-wide studies have demonstrated specific polymorphisms in alcohol metabolizing enzymes have a significant impact on the risk for FASD [38,39].

One clear limitation in the present prospective study is that the data obtained are not homogeneous and are considered preliminary especially for describing new biomarkers. Further investigations to better understand the effects of ethanol also using other models and human cells are needed.

In summary, trophoblast cell lines chronically exposed to ethanol presented genomic instability resulting in chromosomal alterations. Despite that, as no genetic aberrations are commonly found in our in vitro models, we are not able to define any candidate damage-targets for a dysfunction of the placenta. Future work should be done with more replicates and also considering other models or even human samples. References


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