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Hydroquinone induces DNA hypomethylation-independent overexpression of retroelements in human leukemia and hematopoietic stem cells

Anastasia Conti [a], Federica Rota [b], Enrico Ragni [c], Chiara Favero [b], Valeria Motta [b], Lorenza Lazzari [c], Valentina Bollati [b, d], Silvia Fustinoni [b, d], Giorgio Dieci [a, *]

[a] Department of Life Sciences, University of Parma, Parma, Italy
[b] EPIGET – Epidemiology, Epigenetics and Toxicology Lab, Department of Clinical Sciences and Community Health, University of Milano, Milan, Italy
[c] Cell Factory, Unit of Cell Therapy and Cryobiology, Fondazione Ca' Granda, IRCCS Ospedale Maggiore Policlinico, Milan, Italy
[d] Epidemiology Unit, Fondazione Ca' Granda, IRCCS Ospedale Maggiore Policlinico, Milan, Italy

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ABSTRACT

Hydroquinone (HQ) is an important benzene-derived metabolite associated with acute myelogenous leukemia risk. Although altered DNA methylation has been reported in both benzene-exposed human subjects and HQ-exposed cultured cells, the inventory of benzene metabolite effects on the epigenome is only starting to be established. In this study, we used a monocytic leukemia cell line (THP-1) and hematopoietic stem cells (HSCs) from cord blood to investigate the effects of HQ treatment on the expression of the three most important families of retrotransposons in the human genome: LINE-1, *Alu* and Endogenous retroviruses (HERVs), that are normally subjected to tight epigenetic silencing. We found a clear tendency towards increased retrotransposon expression in response to HQ exposure, more pronounced in the case of LINE-1 and HERV. Such a partial loss of silencing, however, was generally not associated with HQ-induced DNA hypomethylation. On the other hand, retroelement derepression was also observed in the same cells in response to the hypomethylating agent decitabine. These observations suggest the existence of different types of epigenetic switches operating at human retroelements, and point to retroelement activation in response to benzene-derived metabolites as a novel factor deserving attention in benzene carcinogenesis studies.

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1. Introduction

Retrotransposons (or retroelements) account together for more than 40% of the human genome sequence. The most numerous retroelement families are those referred to as LINE-1 (long interspersed element 1, L1) and SINE (short interspersed element), which constitute together the so-called non-LTR retrotransposons, because of the absence in their sequence of long terminal repeats (LTRs). SINEs (mainly composed of *Alu* elements) and L1s contribute together more than 30% of our genome, while the so-called LTR (or virus-like) retroelements, corresponding to human endogenous retroviruses (HERVs) and related elements, cover ~8% of the genome sequence [1,2]. Most of the >500,000 L1 copies, of the >1.5 million SINE copies and of the ~500,000 HERV copies [3] annotated in the human genome tend to be either transcriptionally inactive (because of the lack of functional promoter elements) or epigenetically silenced [4–6], at least in differentiated somatic tissues. In such a general silencing context, however, varying levels of retroelement expression have been documented. A few hundreds individual Alu SINEs are detected as expressed, albeit to low levels, by RNA-seq approaches in human cell lines [7], and *Alu* expression is known to increase under various cellular stress conditions [8]. HERVs are transcriptionally active in human cells in a tissue-specific manner [9], and they are subjected to transcriptional modulation during human development [2]. Similar considerations apply to mammalian L1 [10].

Epigenetic silencing of mammalian retroelements is thought to mainly involve DNA methylation and/or histone modification, together with a number of family-specific strategies. In particular for *Alu* (and presumably SINE), H3K9 methylation is mainly

* Corresponding author.
E-mail address: giorgio.dieci@unipr.it (G. Dieci).

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responsible for silencing, while DNA methylation appears to be rather involved in suppressing recombination [5]. In the case of HERV, KRAB zinc-finger proteins with TRIM28 cofactor have been implicated in silencing via recruitment of DNA methylation machinery [3]. As to L1 silencing, studies in mammals have variously implicated DNA methylation- and piRNA dependent mechanisms in germ cells, embryo and somatic tissues [10,11].

Promoter hypomethylation and increased expression of retroelements, potentially resulting in mutagenic retrotransposition events, have been frequently reported in association with cancer [12,13]. Since changes in DNA methylation patterns frequently accompany the action of environmental carcinogens [14], the possibility exists that altered retroelement methylation and/or expression might be part of complex cellular responses leading to environmental carcinogenesis. A particularly fitting example is exposure to benzene, a widespread pollutant associated with acute myelogenous leukemia (AML) risk, which has been linked to altered DNA methylation patterns (including retroelement hypomethylation) both in cultured cell lines and in human subjects [15–17]. In spite of its possible relevance for human health, the transcriptional derepression of retroelements in response to carcinogens has not been systematically addressed, nor has the mechanistic link between retroelement methylation and expression been clarified [18].

In this study we assessed the effects of hydroquinone (HQ), a key benzene-derived metabolite, on the expression levels of L1, *Alu* and HERV retroelements in human leukemia and hematopoietic stem cells. We report evidence for HQ-induced transcriptional reactivation of retroelements uncoupled from DNA methylation changes.

2. Materials and methods

2.1. Cell culture and chemical treatment

THP-1, a human leukemia monocytic cell line, was purchased from ATCC® (TIB-202™). THP-1 cells were cultured in RPMI-1640 medium (Gibco®, Life Technologies) supplemented with 10% FBS, 1% Penicillin/Streptomycin and 0.05 mM 2-mercaptoethanol. Human hematopoietic stem cells (HSCs) were extracted from cord blood as described [19] and cultured in CellGro medium supplemented with cytokines (SCF, Flt3 ligand, TPO, IL6). Both cell types were cultured at 37 °C in a 95% humidified, 5% CO2 atmosphere. Hydroquinone (>99% purity, Sigma-Aldrich) was dissolved in culture medium to the final concentrations of 1, 5, and 15 μM and added to cell cultures at 60–70% confluence, followed by incubation for 24 or 48 h. A similar procedure was used for decitabine (5-aza-2'-deoxycytidine, Abcam) treatment of THP-1 cells. THP-1 cell vitality was evaluated by flow cytometry using propidium iodide. HSC vitality was assessed by the use of FITC Annexin V/Dead Cell Apoptosis Kit (Invitrogen). All cell treatments were conducted in triplicate.

2.2. RNA extraction and analysis

Total RNA was extracted using the mirVana™ miRNA isolation kit (Ambion, Life Technologies), and RNA quality was assessed by 2100 Agilent Bioanalyzer (Agilent Technologies, Inc.), with minimum quality defined as A260/A280 > 1.9, RIN > 8. Reverse transcription of DNase-treated total RNA (500 ng for THP-1, 100 ng for HSCs) was carried out using the iScript™ cDNA Synthesis Kit (BioRad) with oligo-dT and random hexamer priming. Quantitative PCR (qPCR) analysis of cDNA was carried out using an Applied Biosystem 7300 Real Time PCR System. Primer pairs (listed in Supplementary Table S1) were designed to predominantly target: *Alu* J subfamily (*Alu* J pair); *Alu* Y and some members of *Alu* S subfamilies (*Alu* Y pair); either the 5′UTR or the ORF1 regions of L1Hs and L1PA2 subfamilies; the human endogenous retrovirus ERVW-1(Syncytin-1) [20]. All reactions were conducted in technical triplicates and relative gene expression was calculated according to [21].

2.3. DNA methylation analysis

Genomic DNA was extracted from frozen cells using the Wizard Genomic DNA purification kit (Promega), according to the manifacturer's instructions. DNA (1 μg, at a concentration of 50 μg/ml) was treated using EZ DNA Methylation-Gold™ kit (Zymo Research), with final elution in 30 μl. Bisulphite-treated DNA was stored at −20 °C and used shortly after treatment. Analysis of DNA methylation was performed essentially as described [22]. Primer pair sequences are listed in Supplementary Table S1.

3. Results

3.1. Treatment of THP-1 and HSCs with HQ activates retroelement expression

Hydroquinone (HQ) is a benzene metabolite playing an important role in benzene-induced leukemia. THP-1 cells and HSCs were treated for either 24 or 48 h with increasing concentrations of HQ (1, 5, 15 μM). Such concentrations were chosen as roughly representative of the HQ levels expected to be produced in the human body following occupational exposure to 0.2, 0.9 and 2.7 ppm of benzene, respectively. This estimate was based on an exposure duration of 8 h (a typical work shift), an adsorption of inhaled benzene of 70%, a HQ production of 12% [23] and a distribution in 5 L blood. This exposure is in the same order of magnitude of the EU occupational limit value for benzene of 1 ppm [24]. Neither cell type displayed decreased viability upon treatment within these time and dose ranges (data not shown). Retroelement transcript analysis is complicated by their abundant presence within pre-mRNA introns or mRNA untranslated regions, making it difficult to evaluate autonomous retroelement transcription within an undefined background of 'passenger' retroelement RNA. This effect is mostly relevant in the case of SINEs, given their abundance in gene-rich regions and their small size [25]. In spite of these limitations, we chose to assess expression changes of the different retroelement families by RT-qPCR, encouraged by the success of previous studies in revealing retroelement expression modulation through this approach [2,26,27], by its relative easiness of application, and by the recent observation that even gene-hosted *Alu*s are capable of autonomous transcription [7]. In particular, we designed and used primer pairs detecting the ancient *Alu* J subfamily, the more recent *Alu* Y and *Alu* S subfamilies, the human-specific L1Hs and L1Pa2 subfamilies (targeted in both the 5′UTR and ORF1), and an individual member of the human ERV-W family, ERVW-1 [20]. Based on preliminary experiments, 48 h of treatment was chosen as a standard condition giving more consistent and reproducible results than 24 h of treatment (data not shown). As shown in Fig. 1, after 48 h of HQ treatment the levels of *Alu*, L1 and ERVW-1 transcription products were generally found to be increased in a dose-dependent manner. Such a general retroelement activation response was similarly observed in the two cell types tested, thus suggesting its likely dependence on widespread epigenetic mechanisms.

3.2. Effects of HQ treatment on retroelement methylation in THP-1 and HSCs

Hydroquinone has been reported to increase 5-

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