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Research Article

CpG methylation suppresses transcriptional activity of human *syncytin-1* in non-placental tissues

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ABSTRACT

Syncytin-1 is a captive envelope glycoprotein encoded by one of human endogenous retroviruses W. It is expressed exclusively in the placental trophoblast where it participates in cell-to-cell fusion during differentiation of syncytiotrophobast. In other tissues, however, *syncytin-1* expression must be kept in check because inadvertent cell fusion might be dangerous for tissue organization and integrity. We describe here an inverse correlation between CpG methylation of *syncytin-1* 5′ long terminal repeat and its expression. Hypomethylation of the *syncytin-1* 5′ long terminal repeat in the placenta and in the choriocarcinoma-derived cell line BeWo was detected. However, other analyzed primary cells and cell lines non-expressing *syncytin-1* contain proviruses heavily methylated in this sequence. CpG methylation of *syncytin-1* is resistant to the effect of the demethylating agent 5-azacytidine. The inhibitory role of CpG methylation is further confirmed by transient transfection of in-vitro-methylated *syncytin-1* promoter-driven reporter construct. Altogether, we conclude that CpG methylation plays a principal role in the transcriptional suppression of *syncytin-1* in non-placental tissues, and, in contrast, demethylation of the *syncytin-1* promoter in trophoblast is a prerequisite for its expression and differentiation of multinucleated syncytiotrophoblast.

Introduction

Human endogenous retroviruses (HERVs) originate from ancient retroviral infections that entered the germ line and were fixed in populations of our ancestors. The human genome contains around 200 sequentially distinct HERV families [1,2]. The HERV-W family, one of the most extensively studied, comprises some 250 retrovirus sequences with striking prevalence of processed pseudogenes [3,4]. This family invaded the human and Old World monkeys lineage approximately 25–40 million years ago and has been active for a short period of some five million years [5]. Most of HERV-Ws, like members of other HERV families, are compromised by large deletions and mutations so that only three of them retain ORFs longer than 1000 bp [3]. HERV-W envelope (*env*) sequences form a homogenous group and cluster together with HERV-F, HERV-H and ERV9 *envs* in phylogenetic alignment of their transmembrane (TM) subunits [6].

Despite the scarcity of intact ORFs within the HERV-W family, the defective ERVWE1 element localized at chromo-

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somal position 7q21–22 contains an intact and full-length *env* gene [7], so called *syncytin-1*. The mature glycoprotein encoded by this gene displays cell-to-cell fusion capacity and induces differentiation of the placental syncytiotrophoblast in vitro and in vivo [8,9]. *Gag* and *pol* ORFs of ERVWE1 are interrupted by frame-shifts and stop codons [10]. Similarly, as in the envelope protein in exogenous viral particles, the TM subunit of syncytin-1 protein induces membrane fusion after binding of the surface subunit (SU) to its specific receptor. As to the receptor usage, HERV-Ws interfere with RD114 feline endogenous virus, baboon endogenous virus, simian retroviruses type D and avian reticuloendotheliosis virus and compete for the human sodium-dependent neutral amino acid transporter 2 (hASCT2) [11,12]. Furthermore, HERV-W env glycoproteins also efficiently use hASCT1 as a receptor [13]. Inhibition of *syncytin-1* expression by specific anti-sense oligonucleotides leads to a decrease in the trophoblast fusion and differentiation in vitro [14].

Recently, other fusogenic retroviral envelope glycoproteins have been found to play a role in placentation. Out of 16 identified full-length HERV *env* genes, one belonging to the HERV-FRD family and dubbed *syncytin-2* was shown to be specifically expressed in the placenta, to induce cell-to-cell fusion and to confer infectiveness on lentiviral pseudotypes [15,16] in addition to *syncytin-1*. Env protein of the single-copy HERV ERV3 is also specifically expressed in syncytiotrophoblast. Since it is truncated at the 3′ end by a stop codon in the transmembrane domain, it is a soluble protein that might be involved in the development of the placenta acting as a regulatory or immunosuppressive factor instead of inducing cell-to-cell fusion. This protein, however, is not essential because at least 1% of the fertile population contain another stop codon within its coding sequence [17]. Two other full-length *env* genes, *env*V and *env*P(b), belonging to newly characterized HERV families HERV-V and HERV-P(b), respectively, do not fulfil the criteria for sensu stricto syncytins because *env*V does not display any fusogenic activity and *env*P (b) is not specifically expressed in placenta [18]. Two retroviral *env* genes, *syncytin-A* and *-B*, were also identified in the mouse genome. They are specifically expressed in placenta, display fusogenic activity and their orthologous loci are conserved in the family Muridae [19].

The transcriptional activity of HERV-W elements was found to be high in most human tissues analyzed by a retrovirus-specific microarray and real-time RT-PCR [20]. Expression of *syncytin-1*, however, is tightly restricted to the trophoblast, particularly to the multinucleated syncytiotrophoblast layer of the placenta [8,9,14], although weak expression was observed in testes [9]. In the process of placenta morphogenesis, cytotrophoblastic cells serve as precursors for the syncytiotrophoblast differentiation. The syncytiotrophoblast layer is necessary for normal physiological functions of the placenta, i.e. hormone production and gas/substrate exchange between the maternal and fetal blood circulations. Altered cytotrophoblast fusion during placentogenesis and hypertensive disorders in pregnancy such as preeclampsia and HELLP syndrome is often accompanied by deregulation of *syncytin-1* expression or its incorrect localization [21,22]. Reduced *syncytin-1* expression correlated with insufficient or late syncytiotrophoblast differentiation in Down's syndrome pregnancies [23]. The human choriocarcinoma cell line BeWo has been widely used as a relevant model of trophoblast differentiation. These cells can be induced to cell fusion with forskolin, whereas a rabbit polyclonal antibody raised against syncytin-1 inhibits heterologous fusion between BeWo and COS cells [9].

The promoter region and transcription initiation site of *syncytin-1* were localized within the 5′ long terminal repeat (LTR) of ERVWE1 provirus by deletion analysis and reporter assay for the basal promoter activity [24]. Mutagenesis of this promoter region together with DnaseI footprint analysis revealed that the CCAAT motif and the octamer protein binding site are critical for transcriptional regulation of *syncytin-1*. Another study [25] showed that a transcription factor called Glial Cell Missing a (GCMa) binds to two GCMa-binding sites upstream of the 5′ LTR and enhances *syncytin-1* expression in BeWo and JEG3 choriocarcinoma cells. Conversely, mutation of the ecdysone receptor response element slightly increases basal promoter activity, suggesting that this nuclear hormone receptor is a negative regulator of the *syncytin-1* gene [24]. Similarly, a correlation between the decreased level of oxygen in placenta and insufficient expression of *syncytin-1* was also detected in BeWo cells [26]. However, the precise mechanisms of *syncytin-1* gene regulation remain unclear. Of particular importance is the question of transcriptional suppression of *syncytin-1* in non-placental tissues, where its inadvertent expression could be dangerous given the almost ubiquitous expression of hASCT receptors.

Usually, methylation of cytosines in CpG dinucleotides has profound impacts on promoter activity. As well as with genes, the transposable elements integrated in the genomic DNA are subverted to this host defense mechanism, which protects the cell against intragenomic parasites [27]. The methylation status of both exogenous and endogenous retroviruses correlates with the level of their expression [28–30], and the promoter activities of in-vitro-methylated LTRs are dramatically reduced when measured in transient reporter assays [30–32]. Transcription of hypomethylated HERVs, particularly HERV-Ks, has been implicated in tumors [33] and systemic lupus erythematosus [34]. Elevated HERV transcription in placenta and other reproductive tissues [17,20,35], associated even with budding of retroviral particles, corresponds well with a decreased level of methylation in the trophoblast ([36], for the review). There are, however, individual HERV loci or HERV subfamilies that reveal exclusive expression in non-placental tissues and organs [20,35]. In addition to the *syncytin-1*-mediated cell-to-cell fusion, HERV-encoded envelope glycoproteins may play other beneficial roles, such as suppression of maternal immunoreactivity against the fetus [37] and competition with infectious exogenous retroviruses transferred from maternal blood circulation [38]. On the other hand, syncytin-1 can pseudotype human immunodeficiency virus type 1 (HIV-1) [39] and, due to the particular expression of hASCT1 in liver and brain [40], could broaden the range of HIV-1 infectiveness. A high transcriptional activity of hypomethylated HERVs in reproductive tissues is necessary for their efficient amplification, retrotransposition, penetration into the germ line and subsequent genetic fixation.

The aforementioned data suggest that high expression of HERV-Ws in placenta is enabled by the hypomethylation of