Placenta 44 (2016) 46–53

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Epigenetic regulation of *STAT5A* and its role as fetal DNA epigenetic marker during placental development and dysfunction

Beenish Rahat [a], Shilpa Thakur [a], Rashmi Bagga [b], Jyotdeep Kaur [a, *]

[a] Department of Biochemistry, Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India
[b] Department of Obstetricsand Gynaecology, Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India

ARTICLE INFO

Article history:
Received 12 March 2016
Received in revised form
19 May 2016
Accepted 7 June 2016

Keywords:
STAT5A
Placenta
Epigenetic regulation
Hydatidiform mole
Pre-eclampsia

ABSTRACT

Introduction: Development of normal placenta requires regulated apoptosis of trophoblasts. However, uncontrolled apoptosis has been seen in the pregnancy related complications like hydatidiform mole and pre-eclampsia. *STAT5A* is a transcription factor with well-known anti-apoptotic role. Thus, we sought to study the role of *STAT5A* and its epigenetic regulation in placental development and pathologies and its use as fetal DNA epigenetic marker.

Methods: The present study was conducted on pregnant women who were enrolled in five groups, based on the three trimesters in normal pregnancy and two pregnancy related disorder groups: pre-eclampsia and hydatidiform mole. Placental villi samples and maternal blood were obtained from each pregnant woman and were analyzed for promoter region methylation (via methylation sensitive high resolution melting) and histone trimethylations (via chromatin immunoprecipitation) of *STAT5A*.

Results: Our data revealed higher expression of *STAT5A* in first trimester villi, which decreased with advancing gestation with corresponding increased DNA methylation and H3 trimethylations. Development of choriocarcinoma was associated with DNA methylation associated lower expression of *STAT5A*. The pattern of promoter methylation of *STAT5A* in cell free DNA within maternal plasma was observed to be similar to its promoter methylation in placental villi during normal pregnancy, pre-eclampsia and molar complications, which suggested its use as a novel fetal DNA epigenetic marker.

Discussion: Our results suggest the regulation of *STAT5A* via epigenetic mechanisms during normal pregnancy and the association of *STAT5A* epigenetic dysregulation in pregnancy related complications. Further, hypermethylated *STAT5A* can be utilized as novel fetal DNA epigenetic marker.

1. Introduction

Development of placenta during pregnancy is a complex event that establishes the supply of nutrients to the growing fetus [1,2]. Placenta is a highly organized and vascularized organ that connects the fetal tissues to the uterine wall. Although, placenta is a normal tissue but its constituent trophoblastic cells share various features with tumor cells [3]. These cells are highly proliferating in nature with migratory and invasive properties which make placenta a pseudomalignant tissue [1,3]. Placental development is under strict regulatory controls which results in differential expression of genes that vary with gestation age [4].

There are various cytokines and growth factors that influence trophoblastic proliferation, migration and invasion. These factors utilize at least one pathway i.e. either Jak-Stat or receptor-associated tyrosine kinase-mitogen activated protein kinase (RTK-MAPK) or both for intracellular signaling in the trophoblast pathways [5]. This function of JAK-STAT pathway highlights the possible role of *STAT5A* (signal transducer and activator of transcription 5A) in placentation. *STAT5A* is a member of a well-known group of STAT transcription factors. It is a transcription factor that plays its role via canonical JAK-STAT signaling pathway. Activation of JAK-STAT pathway results in its phosphorylation and dimerization and finally its movement to the nucleus, where it activates transcription of particular genes [6]. *STAT5A* is known to play an important role during normal mammary gland development. In mammary epithelial cells, the STAT5 pathway modulates three different cellular outcomes: differentiation, survival, and proliferation [7]. As revealed by several knockout experiments, JAK-STAT and receptor associated tyrosine kinase-mitogen activated protein kinase (RTK-

* Corresponding author.
E-mail address: jyotdeep2001@yahoo.co.in (J. Kaur).

http://dx.doi.org/10.1016/j.placenta.2016.06.003
0143-4004/

MAPK) signaling pathways are detrimental for development of placenta and reproduction. However till date, there is no direct study to show the role of *STAT5A* in placentation and during advancing gestation.

Identification of fetal epigenetic markers in the maternal blood as a method of prenatal screening and diagnosis of pregnancy related disorders holds a great scope in the field of diagnosis [8–10]. There are numerous studies that depicted the presence of fetal cells and circulating cell free DNA in maternal plasma [11–13]. However, reliability of fetal cells as a source of fetal genetic material is limited due to their extremely rare occurrence [14,15] and their continued presence even after years of earlier pregnancies in maternal plasma [16]. In comparison, cell free fetal DNA can be a much better source of fetal DNA, since it is comparatively in higher amount in the maternal circulation and unique to the current pregnancy. This cell free DNA quickly gets cleared from the maternal plasma within 24 h after delivery. Thus, analysis of cell free fetal DNA in maternal plasma opens a new field for the development of non invasive prenatal diagnostics [17].

Various pregnancy related disorders like pre-eclampsia [18,19], trisomy 21 [20,21], preterm labor, hyperemesis gravidarum [22] and invasive placentation [23] are known to be associated with quantitative aberrations of circulating cell free fetal DNA. However, till date there are only a few epigenetic markers that can be used as a positive control for fetal DNA in maternal plasma. Chim et al. (2005) reported the detection of a comparative hypomethylated pattern of maspin gene in maternal plasma relative to maternal blood cells, which mimics the pattern of methylation observed in placenta by these authors, further reporting its clearance from maternal plasma after delivery confirming its fetal origin [24]. Chan et al. (2006) have discovered an exactly opposite pattern for the promoter of the RASSF1A tumor suppressor gene with hypermethylation in placental villi as compared to maternal blood cells [25]. Based on these studies it can be suggested that such epigenetic fetal DNA markers can show different levels of DNA methylation. Hence, it becomes important to look for more epigenetic markers which may also exhibit some disease related methylation pattern, like DNA methylation changes specific to pre-eclampsia or gestational trophoblastic diseases (GTDs).

Keeping this in view, we have analyzed the promoter region methylation of *STAT5A* in placenta in reference to its status in maternal blood. In addition, epigenetic regulation of *STAT5A* has also not been yet studied in placental villi during gestation and in placental disorders. In the present study, we analyzed the methylation status of *STAT5A* in placental villi and corresponding maternal leukocytes in different groups (normal, pre-eclamptic and molar) of pregnant women, followed by its methylation profiling in cell free DNA in maternal plasma in order to evaluate the potential of *STAT5A* promoter to act as a fetal DNA epigenetic marker in maternal plasma.

2. Materials and methods

2.1. Study design and ethical approval

In this study we have included pregnant women with normal pregnancy in three different trimesters namely first, second, third and pregnant women with complicated pregnancies like pre-eclampsia and hydatidiform mole. Pregnant women with normal pregnancy of 6–11 weeks were included in the first trimester group, 16–20 weeks were included in the second trimester group and 37–40 weeks pregnancy were included in the third trimester group. Pregnant women diagnosed with clinical symptoms of systolic pressure of 140 mm Hg and diastolic pressure of 90 mm Hg, proteinuria >300 mg in 24 h were included in pre-eclamptic group while hydatidiform molar pregnancy was diagnosed by ultrasonography and confirmed by histopathology in the first or early second trimester pregnant women. Thirty pregnant women were included in each group except molar group which included 15 patients. Clinical characteristics of the pregnancies included in this study are presented in Table 1. All the participating subjects were informed about the study and a written consent was obtained from all of them. The study had also been approved by Institute Ethics Committee (IEC) of Postgraduate Institute of Medical Education and Research, Chandigarh, India.

2.2. Sample collection

From each pregnant woman we obtained blood and placental villi samples. Blood was collected from pregnant women before any obstetric procedures. Placental villi samples were taken after vaginal deliveries or caesarean sections in case of normal third trimester and pre-eclamptic pregnancies while after elective termination of pregnancies in case of normal first and second trimester and molar pregnancies. Maternal blood samples were used for the isolation of plasma and maternal blood leukocytes [26]. In addition maternal blood samples were also collected after 24 h of delivery in case of normal third trimester group, which were used for isolation of plasma.

2.3. In-vitro cell culture based studies

In-vitro studies were done using JEG3 cell line. It is a human placental choriocarcinomic cell line. JEG3 cells were cultured in DMEM-high glucose medium supplemented with 10% FBS. JEG3 cells were used to study the expression of *STAT5A* and its regulation via DNA methylation.

2.4. Quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR)

Isolation of total RNA from placental villi samples and JEG3 cell line was done using TRIzol (Ambion). Thereafter, reverse transcription of 1 μg of total RNA was carried out using RevertAidTM M-MuLV-RT kit (Fermentas) as per manufacturer's instructions. qPCR analysis was carried out on Applied Biosystems real time PCR system using SYBR Green master mix. Gene specific primers were used for qPCR analysis as indicated in Table 2. The stability of glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was initially verified in all study groups prior to its selection as the endogenous control for this study. The comparative threshold cycle or C_t method ($\Delta\Delta C_T$) [27] was used to quantify the amplified transcripts.

2.5. Methylation sensitive high resolution melting PCR (MS-HRM)

The promoter region methylation analysis was done by MS-HRM as described previously [4]. For MS-HRM, bisulphite conversion of circulating DNA and DNA extracted from placental villi samples, maternal blood leukocytes and JEG3 cells was carried out. Thereafter, DNA was analyzed promoter region CpG methylation using gene specific primers (Table 2). MS-HRM analysis was performed by real time based PCR amplification in Applied Biosystems® StepOnePlus™ Real-Time PCR, followed by melt curve analysis. For each assay a series of different methylation standards ranging from 0% to 100% CpG methylation were obtained by mixing 100% and 0% DNA methylation standards in proper proportion. After analyzing the raw melt curves in MS-HRM software, the exact percentage methylation of unknown samples was obtained by polyfit interpolating function within program MatLab (The MathWorks, Inc., USA).