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Increased kisspeptin mRNA expression derived from abnormally adhered placenta: compensatory inhibition of abnormal placental invasion?

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Summary

Purpose of investigation: In normal conditions, circulating kisspeptin levels dramatically increase during the first trimester of pregnancy and fall approximately five days post-delivery to concentrations comparable to those detected prior to pregnancy. Dysregulation of trophoblast invasion results in a wide spectrum of abnormal pregnancies such as preeclampsia, premature delivery, and choriocarcinoma. The aim of the present study was to investigate the expression profile of KiSS-1 in abnormally adherent placenta specimens (placenta accreta and placenta praevia). *Materials and Methods:* Ten term trophoblast preparations (five normal adhesions, four placenta accretae and one placenta praevia) were obtained for RNA extraction and immunohistochemistry in order to determine KiSS-1 mRNA and protein levels. *Results:* KiSS-1 mRNA expression was found to be increased in conditions of pathological placental adhesion compared to normal tissues. *Conclusion:* KiSS-1 is implicated in the regulation of placental invasion as part of a compensatory inhibition to abnormal placental adhesion.

Key words: Kisspeptin; Placenta; Placenta accreta.

Introduction

Kisspeptins comprise a family of peptides (kisspeptin-54, kisspeptin-14, kisspeptin-13, and kisspeptin-10) deriving from the proteolytic cleavage of kisspeptin, a 145-amino-acid residue polypeptide, which is the primary translation product of the KiSS-1 gene [1, 2]. Kisspeptins have been shown to be implicated in multiple biological procedures and due to this biological multi-functionality they are in the center of research interest.

Initially, the largest cleavage product, kisspeptin-54 (Kp-54) was identified for its ability to suppress the metastatic potential of malignant melanoma cells and it was therefore termed 'metastin' [3]. Since then, kisspeptins and their endogenous ligand, the G-protein coupled receptor, KiSS-1R, have been studied in various cancer types and have been suggested to play multiple roles in cancer development and metastasis [4]. Particularly, kisspeptins have well established antimetastatic properties. Most studies so far, support a strong association between down-regulation of KiSS-1 expression in malignant tissues and poor clinical outcome due to tumor metastasis [5], whereas in the case of ovarian cancer, KiSS-1 suppressed ovarian cancer cell invasion [6]. Additionally, it has been shown that KiSS-1 is capable of suppressing the migration of transfected cancer cell lines in vitro [7].

In addition to kisspeptins' role in cancer, several data indicate that KiSS-1 is implicated in placental adhesion during pregnancy. The invasion of extravillous trophoblasts in the uterine decidua displays a phenotypic similarity to cancer cells [8], resembling tumour metastasis [9-11]. Therefore, the normal trophoblast has been termed as 'pseudo-malignant'. However, in sharp contrast to cancer, this physiological invasion process is tightly regulated in temporal and spatial manner [12]. Thus, molecules that interfere with cancer cell invasion and metastasis have been studied in order to investigate their possible role in placental biology.

It has been shown that in the human placenta, KiSS-1 mRNA, and Kp-54 (metastin) are expressed in the syncytiotrophoblast, while KiSS-1R is expressed in the syncytiotrophoblast but also in the villous and the invasive extravillous trophoblasts [13], indicating autocrine/paracrine signaling. The anti-metastatic properties of kisspeptins indicate high kisspeptin expression in normal placental tissue. Plasma KiSS-1 levels are raised during the first trimester of pregnancy in women, whereas KiSS-1 mRNA and protein are highly expressed in fetal placenta

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[14]. The placental expression of KiSS-1 and KiSS-1R is particularly striking in the first trimester of gestation and decreases throughout pregnancy [15]. Therefore, KiSS-1 and KiSS-1R expression peaks at the same time period of pregnancy when regulation and limitation of trophoblast invasion is of particular importance.

Many placental pathologies associated with abnormal invasion originate in the first trimester of pregnancy, highlighting the importance of tight control mechanisms during this crucial period. In normal conditions, circulating kisspeptin levels dramatically increase during the first trimester of pregnancy and fall approximately five days post-delivery to concentrations comparable to those detected prior to pregnancy, implicating a placental source of the peptide [14]. Dysregulation of trophoblast invasion results in a wide spectrum of abnormal pregnancies such as preeclampsia, premature delivery, and choriocarcinoma [16] in which the possible roles of KiSS-1 and KiSS-1R abnormalities have been studied.

The aim of the present study was to investigate the expression profile of KiSS-1 in abnormally adherent placenta specimens (placenta accreta and placenta praevia) derived from term trophoblast preparations.

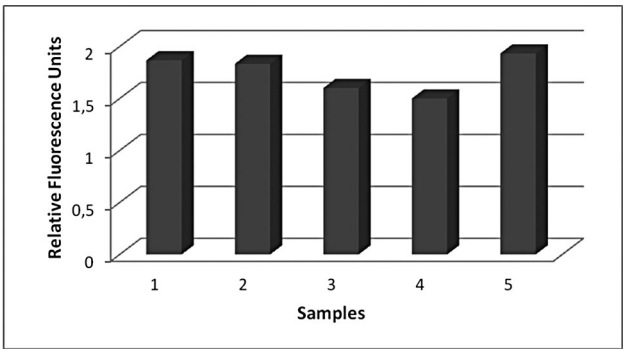

Figure 1. — Normalised data for KiSS-1 expression in abnormal placenta samples. Data represents the fold-difference of expression in comparison to control. Normal placenta specimens were used as control. Samples 1-4 represent KiSS-1 relative expression in placeta accreta samples, while sample 5 represents KiSS-1 relative expression in placeta praevia.

Materials and Methods

The study population comprised a total of ten pregnant women. Five placental specimens were characterized as normal adhesion, four placental specimens were characterized as placenta accreta, and one placental specimen was characterized as placenta praevia. Biological specimens were obtained immediately post-delivery and preserved in RNAlater buffer until RNA extraction.

RNA was extracted from placental specimens using the commercially available RNeasy lipid tissue mini kit. RNA integrity was checked by formamide agarose gel electrophoresis, while RNA concentration and purity were estimated by measuring optical absorption at 260 nm and calculating the ratio 260/280 nm, respectively. One μg of total RNA was used as template in cDNA synthesis with PrimeScript first strand cDNA synthesis kit.

KiSS-1 mRNA relative expression was estimated by Real-time PCR in the LightCycler 1 instrument. A 294 bp fragment of KISS-1 gene was amplified using the following primers forward: 5'-cactttggggagccattaga-3', reverse: 5'- ccagttgtagttcggcaggt-3', in a final concentration of 10 uM. A total of 100 ng of cDNA was used as template. Cycling conditions were as follows: initial denaturation was performed at 95°C for three minutes, followed by 35 cycles of denaturation at 95°C for 10 seconds, annealing at 62°C for 20 seconds, and extension at 72°C for 20 seconds. PCR products were analyzed by the melting curve method and relative expression was estimated by the $\Delta\Delta C_T$ method. GAPDH and β-actin were used as housekeeping genes.

Placental specimens were placed immediately after delivery in 10% v/v formalin solution. All tissues were paraffin-embedded and four-micrometer thick sections were used for immunohistochemistry, as previously described. KiSS-1 detection was performed using the anti-Kisspeptin antibody (ab5538), in a dilution of 1:100.

Results

The investigation of KiSS-1 relative expression in placental samples revealed that samples from placenta accreta and placenta praevia showed 1.5 - 1.8-fold higher expression in comparison with normal placenta (Figure 1). On the contrary no significant differences were detected in the expression of KISS-1 at the protein level by immunohistochemistry (Figure 2).

Discussion

The results of the present study demonstrate an increased full term placental expression of kisspeptins' mRNA in conditions of abnormal placental adhesion such as placenta accreta and placenta praevia compared to normally adhered placental specimens.

Although the precise role of kisspeptin during pregnancy is not well understood yet, the implication of kisspeptins in placental implantation is well documented by several studies [17, 18]. So far, the available data indicate that placenta produces kisspeptin, which is implicated in the regulation of trophoblast invasion into the maternal uterus decidua during the first trimester of pregnancy [14]. Therefore, increased kisspeptin expression during the first trimester of normal pregnancies acts as part of a regulatory mechanism

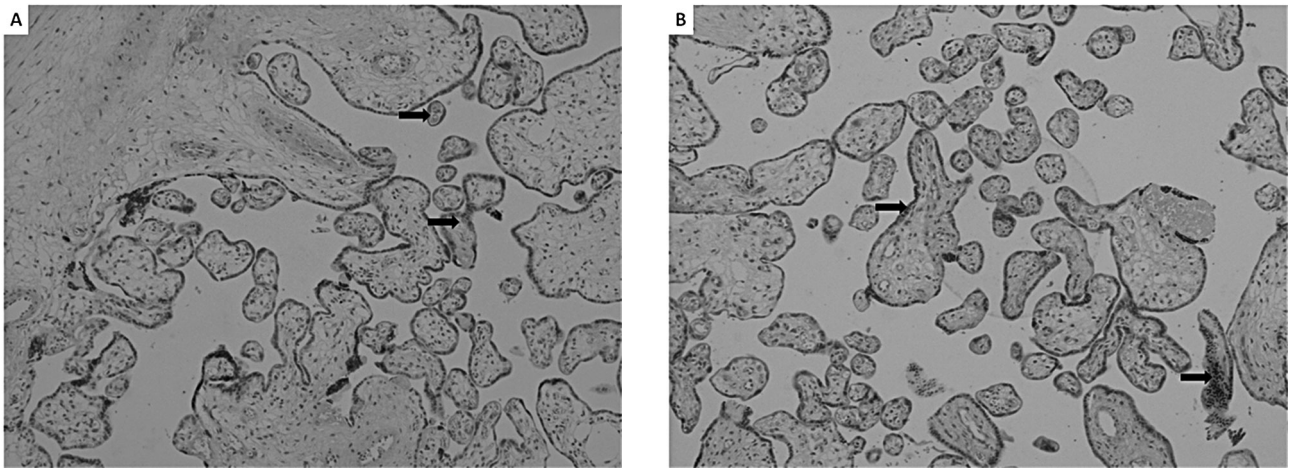

Figure 2. — Immunohistochemical staining of placental specimens. A) Normal placenta section stained with hematoxylin, eosin, and anti-KiSS-1 antibody. Mild KiSS-1 expression (arrow). B) Abnormal placenta section stained with hematoxylin, eosin, and anti-KiSS-1 antibody. Mild Kiss-1 expression (arrow).

preventing abnormal placental invasion [17].

Interestingly, increased kisspeptin mRNA expression has been noticed in different cancer cell types, where KiSS-1 exerts its action on tumors' invasiveness and metastatic potential. Tumor metastasis and placental ontogenesis are thought to have similar biological behavior. Regarding cancer tissues, most studies so far support a strong association between down-regulation of KiSS-1 expression in malignant tissues and poor clinical outcome due to tumor metastasis and invasion [5]. However, expression data from breast cancer and hepatocellular carcinoma (HCC) tissues are contradictive indicating up-regulation of KiSS-1 in highly metastatic cancer tissues [19-21]. Specifically, Martin *et al.* analyzed by immunohistochemistry and quantitative PCR 124 breast tumor samples and 33 matched controls revealing that KiSS-1 was significantly elevated in tumor and lymph node positive tumors., as compared to normal tissues [19]. Moreover, Ikegushi *et al.* reported over-expression of KiSS-1 at all advanced stage HCCs, whereas Schmid *et al.* showed a strong correlation between KiSS-1 expression levels and disease early local recurrence and metastasis [20, 21].

These findings in accordance with the results of the present study suggest a multi-functionality of KiSS-1 in cellular invasion and migration. It has been shown that KiSS-1 is among the strongest differentially expressed genes in invasive first trimester versus non-invasive term trophoblast preparation. The absence or low levels of invasion in situ at term despite low KiSS-1 levels suggest that other anti-invasive factors prevail at the end of gestation. Hence, the anti-invasive effect of KiSS-1/ KiSS-1R system may only be important in the first trimester [17]. Therefore, in conditions of pathological placental adhesion, kisspeptin mRNA expression might be increased as a protective mechanism against inappropriate trophoblast invasion into the maternal uterine decidua. This increased kisspeptin expression in the mature placenta accreta and praevia suggests that kisspeptin might be part of a compensatory inhibitory mechanism of abnormal placental invasion. An interesting hypothesis about the biological network implicated in this phenomenon has been reviewed by Makri *et al.* [22]. In brief, they hypothesized that the final trophoblast invasion outcome, leading to the formation of either normal placentae or abnormally adhered ones, depends on the expression prevalence of either KiSS-1 gene, which displays anti-metastatic properties, or the competitive invasion-related uPA/plasmin/MMP/TIMPs genes, as well as MMP-regulating factors [22]. Taken together, it seems that different molecules are the major regulators of trophoblast invasion depending on the age of pregnancy. Actually, up-regulation of MMP-9, MMP-2 in placenta accompanied by down-regulation of metalloproteinase tissue inhibitor TIMP-1 in uterine decidua have been shown to be involved in occurrence of the placental accreta [23]. Additionally, MMPs play an important role in cancer cell invasion, and metastasis by degrading the extracellular matrix (ECM). MMP-9, MMP-2, and MMP-7, have been shown to be over-expressed in ovarian malignant tissues [24], colorectal carcinoma [25] and breast cancer cells [26], as well as renal tumors [27]. Finally, it should be mentioned that the degradative activity of various MMPs is present throughout human gestation, unlike KiSS-1 gene that is highly expressed only in the first trimester [28]. Nevertheless, in order to elucidate this paradox, additional studies regarding KiSS-1 protein expression and its receptor, KiSS-1R, are required. In the present study, no significant differences were detected between abnormal adhesion placental samples and matched normal placental samples at the protein

level by immunohistochemistry. However, it should be underlined that KiSS-1 is a multifunctional biological molecule with complex regulation both at mRNA and protein expression level. Thus, the results of the present study at the protein level could be interpreted as down-regulation of KiSS-1 at the protein level. That is, the KiSS-1 protein is down-regulated by spontaneous proteolytic cleavage and therefore it has a limited half life. Pharmacokinetic study in rats showed that low ng/mL kisspeptin-10 was detected in the first few minutes, and eliminated rapidly and became undetectable 30 minutes after intravenous (i.v.) bolus administration of 1.0 mg/kg kisspeptin-10 [29]. The short half life of the protein inserts technical biases in protein detection obscuring KiSS-1 measurements at the protein level.

In conclusion, KiSS-1 mRNA expression was found to be increased in conditions of pathological placental adhesion i.e. mature placenta praevia and placenta accreta as compared to normal tissues, indicating the implication of KiSS-1 in the regulation of placental invasion as part of a compensatory inhibition to abnormal placental adhesion. The small number of specimens studied denote the need for further investigation regarding the expression of KiSS-1 at the protein level and its receptor.

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