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Localization and possible role of membrane type metalloproteinase and tissue inhibitors of metalloproteinase-1 in early stages of placentation

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Abstract   Human placental tissues from the first and second trimesters of gestation have been investigated using riboprobe *in situ* hybridisation of mRNA sequences coding for membrane type metalloproteinase (MT-1-MMP) and tissue inhibitors of metalloproteinase-1 (TIMP-1). Results show

that (i) both mRNAs express at a relatively high level in the chorion laeve trophoblast cells and the adjacent decidual cells of fetal membrane; (ii) the most abundant expression of the two mRNAs was found in the extravillous trophoblast between Rohrs and Nitabuch striae of basal plate, trophoblast shell and gland cells of the decidua; (iii) isolated or small groups of cytotrophoblast cells in the chorionic villi and in the cells lining arterioles in decidua and stem villi also expressed both MT-1-MMP and TIMP-1 at defferent extents. The data suggest that the coordinated expression of the MT-MMP and its inhibitor TIMP in defferent cells of the placental tissue may play an essential role in trophoblast invasion and angiogenesis related to placentation in the first two trimesters of gestation. They may also have an ability to effect separation of fetal from material tissue at a favorable junctional site during parturition.

Keywords: placenta, trophoblast, basal plate, fetal membrane, MT-1-MMP, TIMP-1, *in situ* hybridization.

Changes in composition of extracellular matrix (ECM) are important in modulating cell motility, migration, differentiation, proliferation, cell to cell signal transduction and metabolic function[1—3]. Proteolytic activity generated by the two different systems, plasminogen activator (PA) and matrix metallo-proteinase (MMP), is a versatily and temporally controlled enzymatic system[2, 3]. The MMPs are a familily of zinc- and calcium-dependent endopeptidases that consist of an increasing number of family members[4, 5]. The recent cloned membrance type MMP (MT-MMP) is uniquely different from other MMPs, since it contains a membrane-spanning sequence in its carboxy-terminal domain and can induce specific activation of pro-gelatinases A *in vitro*[5—7]. Three types of tissue inhibitors of metalloproteinases (TIMPs) have been identified as TIMP-1, TIMP-2 and TIMP-3 and are effective to inhibit all the MMP activities so far identified. TIMP-1 is the most abundant expressed TIMP, and can bind and irreversibly inactivate the active forms of all MMPs as well as the latent form of 92-ku gelatinase[8, 9]. The PA and MMP systems are supposed to be interactive and appear to form a lytic cascade which can completely denature interstitial molecules including collagens, fibrin, fibronectin, vitronectin, laminin and proteoglycans which have been widely identified in uterus and placental tissues[9].

Proteolytic activity generated by the two systems has been reported to be associated with various reproductive processes, such as ovulation[10, 11] and embryo implantation[8]. However no data are available in the literature to show specific cell localization and associated functions of MMP and TIMP in the early stage of placentation. This study was therefore undertaken in an attempt to look at cell-specific localization and the function of MT-1-MMP and TIMP1 in the first and second trimesters of gestation.

1 Materials and methods

(i) Reagents.   The riboprobe system for Northern blot analysis was purchased from Promega Co. (Madison, WI); restriction enzymes, Tag polymerase, 4-nitro blue tetrazolium chtoride, 5-bromo-4-chloro-3-indolyl-phosphate, antidigoxygenin-APF(ab) fragments, the Dig RNA Labelling Kit and blocking reagent for riboprobe of *in situ* hybridization were purchased from Boehringer Mannheim (Mannheim, Germany).

(ii) Tissue preparation.   Seven trophoblast and decidua tissue samples in the first trimester (6—9 weeks), 3 placental materials of the second trimester of gestation (4—6 months), 10 normal term placentae and 1 one-month-overtime gestation placenta by surgery were obtained from Beijing Zhongguancun Hospital and immediately returned to the laboratory. The 2nd trimester termination masterials were entirely volumtary operations undertaken for therapeutic purposes and that full informed consent was given for the use of the tissue by the patients and approved by the Ethic Review Committee in the Hospital. The fetal membrane, the basal and chorionic plates as well as the trophoblast and decidual tissues were gently cut into 20 mm × 10 mm strips and frozen either in liquid nitrogen or in hexane/CO_2 slush in disposable paper cups which contained Tissue-O.T.C. embedding compound (Miles Inc.Diagnostic Division Elkhart, IN 46515, USA). Cryosections (8—12 μm thick) were cut using the method of Liu[12] and melted onto 10-well Multitiest slides (Flow Laboratoties).

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(iii) Synthesis of MT-MMP and TIMP-1 RNA probes. The mouse MT-1-MMP(nucleotides 947-1464) and TIMP-1(nucleotides 22-663) cDNA fragments, provided by Prof. Ny of Umea University (Sweden), were used for detection of the mRNAs by *in situ* hybridization as described previously[13]. Before transcription, plasmids were linearized, and antisense or sence RNA probes could be produced. Transcription was performed using an *in vitro* transcription system (Promega) and appropriate RNA polymerase. For *in situ* hybridization, plasmids were linearized in the same way, and riboprobes were made using a Dig RNA Labeling Kit from Boehring Mannheim.

(iv) *In situ* hybridization.　*In situ* hybridization was performed basically according to the method described by Schaeren-Wiemers et al.[14] with digoxigenin-labeled MT-MMP and TIMP-1 riboprobes. To monitor background levels and the specificity of hybridization, the sense strands of the above mentioned probes were included in each experiment. Photographs were taken with E6 process color films (Kodak and Fujichrome).

(v) Data presentation.　The experiments for *in situ* hybridization were repeated more than three times from 3—5 different samples each time. Each of the three similar representative pictures is shown in this note.

2 Results and discussion

(i) Expression of MT-1-MMP and TIMP-1 in villi.　As shown in fig. 1, both MT-1-MMP and TIMP-1 mRNAs were strongly expressed in the blood vessel wall of the stem villi and in decidua. This observation is important because placentation and establishment of an adequate chorino-decidual blood flow in normal pregnancy depend on endovascular invasion by trophoblast in the first two trimesters of pregnancy[15, 16]. The invasive trophoblast has been reported to be characteristically buried in the vessel walls[16], implying that the observed expression of MT-1-MMP and TIMP-1 mRNAs in the blood vessel of both stem villi and decidua may be important in the process of conversion of spiral arteries into uteroplacental arteries.

Fig. 1.　Localization of MT-1-MMP and TIMP-1 mRNA in the villi of the second trimester placenta. (a)　Strong MT-1-MMP mRNA expression (arrows) was noted in the blood vessel wall of the stem villi; (b) TIMP-1 mRNA was mainly expressed in the cytotrophoblast cells and the blood vessels wall. No expression of the mRNA was observed in the syncytiotroblast cells (arrows) of the villi where only the stained nuclei were noted; (c) strong MT-1-MMP mRNA was noted in the trophoblast cell islands; (d) sense MT-1-MMP probe as negative control.

(ii) Expression of MT-MMP and TIMP in decidua and basal plate.　As shown in fig. 2, both molecules were highly expressed in the trophoblast shell (materno-fetal junction), gland cells and

Fig. 2. Localization of MT-1-MMP and TIMP-1 mRNA in the decidua and basal plate of the first two trimesters of gestation. (a) Strong MT-MM-1 mRNA was noted in the trophoblast shell of the first trimester decidual tissues; (b) strong MT-1-MMP mRNA was noted in the gland cells of the first trimester decidual tissues; (c) strong MT-1-MMP mRNA was noted in the extravillous cytotrophoblast cells (*) of the basal plate of the second trimester; (d) the extravillous cytotrophoblast cells expressed abundant TIMP-1 mRNA; (e) the trophoblast shell expressed abundant TIMP-1 mRNA; (f) sense TIMP-1 probe as negative control.

extravillous cytotroblast cells of the first two trimesters of pregnancy, indicating that the coordinated expression of MT-1-MMP and TIMP-1 in the particular areas may be essential in controlling decidualization, the growth of placenta and placentation through local extracellular matrix degradation generated by the two molecules.

    (iii) Expression of MT-1-MMP, TIMP-1 and tPA mRNAs in fetal membrane. Fig. 3 shows strong expression of both MT-1-MMP and TIMP-1 mRNAs in the chorionic and decidual cells of fetal membranes in the second trimester. Term fetal membrane also expressed the two molecules and tPA, which has been demonstrated to play a role in materno-fetal seperation at labour[17,18], indicating that the PA and MMP systems may coordinately play a role in the control over abscission during the third stage of labour. This suggestion is further confirmed by the fact that only the inhibitor TIMP-1 mRNA, but not the activator MT-1-MMP and tPA mRNAs was demonstrated in the surgical one month over-gestation fetal membrane.

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Fig. 3. Expression and localization of MT-1-MMP, tPA and TIMP-1 mRNA in the fetal membranes of the second trimester, term and one-month overtime gestation pregnancy. (a) Strong expression of MT-1-MMP mRNA in the trophoblast and decidual cells of the second trimester fetal membrane; (b) strong expression of TIMP-1 mRNA in the trophoblast and decidual cells of the second trimester fetal membrane; (c) sense TIMP-1 probe as negative control; (d) trophoblast and decidual cells of the fetal membrane obtained from formal term pregnancy expressed MT-1-MMP mRNA; (e) trophoblast and decidual cells of the fetal membrane obtained from formal term pregnancy expressed TIMP-1 mRNA; (f) trophoblast and decidual cells of the fetal membrane obtained from formal term pregnancy expressed high tPA mRNA; (g) no expression of MT-1-MMP mRNA was observed in the fetal membrane of one month over-gestation placenta obtained by surgery; (h) high TIMP-1 mRNA was observed in the fetal membrane of one month over-gestation placenta obtained by surgery; (i) no expression of tPA mRNA was observed in the fetal membrane on one month over-gestation placenta obtained by surgery. A, Amnion; C, chorion; D, decidua.

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