Identification of secretion sites of tissue plasminogen activator and plasminogen activator inhibitor type-1 in basal plates of human and rhesus monkey placentae

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INCREASING evidence has demonstrated that the locally controlled proteolytic activity generated by coordinated expression of tPA and PAI-1 in different tissues may play an important role in many reproductive events. These are largely related to fibrinolytic activity. They include follicle rupture, luteolysis, spermatogenesis and trophoblast implantation[1—4]. Parturition, which is a complex process, may also be associated with tissue destruction. Detachment of placental decidua and partial breakdown of fetal membranes are possible examples. It has been reported that tPA and PAI-1 increase in the peripheral circulation after the third trimester of pregnancy, and reach maximum levels during the first stage of labour[5, 6]. Further studies indicate that tPA is localized in amniotic epithelium and chorion laeve trophoblast epithelium, whereas PAI-1 mainly distributes in the decidua associated with chorion laeve (to be published in separate paper). To further study whether the coordinated expression of tPA and PAI-1 in the tissue also plays a role in the process of parturition, we have examined the secretion sites of tPA and PAI-1 in basal plates of both human and rhesus monkey placentae by confocal and conventional indirect immunofluorescence techniques[7]. The experimental results show that the immunofluorescence of either tPA or PAI-1 is localized in the decidual cells along the border of the detachment sites both in human and rhesus monkey basal plates. PAI-1 is also expressed in the decidual cells surrounding blood vessel walls, but in other decidual tissue or in the uterine myometrium cells, no evident immunofluorescence specific for tPA and PAI-1 was observed.

1 Materials and methods

Ten human term placentae were obtained from Beijing Zhongguancun Hospital and immediately returned to the laboratory. The basal plates were gently cut from the maternal sides of the placentae into 20 mm by 10 mm strips and snap-frozen in disposable paper cups which contained Tissue-Tek O.T.C. embedding compound (Miles Inc. Diagnostic Division Elkhart, IN 46515, USA). Five rhesus monkey placentae after pregnancy for 130—150 d were collected following the terminations performed for other purposes at Kunming Institute of Medical

Biology. The basal plates were dissected from the placentae and snap-frozen in the same way as the samples from human placentae. Cryosections (10—12 μm in thickness) were cut using a Leitz cryostat and melted onto 10-well multistat slides.

The sections were fixed in 3% formaldehyde in 0.25 mol/L PBS (pH 7.4), washed twice with PBS and permeabilised in 0.05% Triton X100. The sections were washed again and immersed in 0.25 mol/L L-lysine in PBS for 1 h at 4℃. After thorough removing of the excess PBS/lysine from the slides using a vacuum driven suction pump the first stage antibody was added to each experimental well and incubated overnight at 4℃. After washing off the sections in PBS for 1 h at room temperature, the corresponding second stage antibodies which were conjugated to fluorescein isothiocyanate (FITC) were added to all wells and the tissue was incubated for 1 h at 37℃. The sections were thoroughly washed in PBS and then mounted in antiphotobleaching mountant (Citifluor) and sealed with nail varnish. Immunofluorescence specimens were examined under a Zeiss standard epifluorescence microscope. Photographic recording was achieved using a Zeiss MC63 camera attachment and the 400 ASA Fujichrome colour slide film.

Sections were examined more critically using a Zeiss Axio Vert 10 epifluorescence microscope equipped with a Biorad Lasersharp MRC600 confocal laser scanning attachment. Photographic recording from the confocal system was undertaken using, for black and white photography, a Shackman flat screen monitor with camera attachment loaded with Ilford FP4 or, for false colour, a Polaroid Quickprint loaded with E6 colour transparency film such as Polaroid presentation chrome. The instrument was calibrated for measurement purposes using a reflective slide-mounted grating (Biorad, UK)[7].

2 Results and discussion

By immunofluorescence microscopy extremely high tPA immunofluorescence (fig. 1(a)), as compared with the control (fig. 1(b)), was localized in the decidual cells along the border of the detachment sites (m) in the placentae of rhesus monkey. No immunofluorescence for tPA was evidently observed in other areas of decidual tissue (not shown) or in the uterine myometrial cells of the basal plate. Further examination using confocal epifluorescence microscopy showed that the tPA immunofluorescence was cytoplasmic in origin (not shown).

Figure 1(c) is from a representative experiment showing the localization of PAI-1 in the decidual cells of the basal plate of human placentae by confocal epifluorescence microscopy. Similar to the PAI-1 localization in the human basal plate (fig. 1(a)), the secreting sites of PAI-1 were also localized in the decidual cells of the monkey close to the uterine detached tissue (m). Fig. 1(d), (e) and (f) represent respectively the distribution of PAI-1 in the monkey basal plate at different gestational ages. Similar to the distribution of PAI-1 immunofluorescence in human basal plates, the inhibitor was also defined in the decidual cells (fig. 1(e) and (f)) along the uterine detached sites (m) in the monkey basal plates. Some decidual cells surrounding the blood vessel walls (fig. 1(d)) were also sites of secretion of PAI-1, implying that PAI-1 may also be important in protecting the vessels from possible damage by tPA. Other decidual (fig. 1(e)-A) or uterine myometrial tissues (m) appear the same as the control (fig. 1(e)-B). They contain no immunofluorescence more intense than background. The Nomarski differential interference contrast micrograph shows the morphology of the monkey basal plate in the experimental (fig. 1(e)-C and (f)-B) and the control (fig. 1(e)-D) groups.

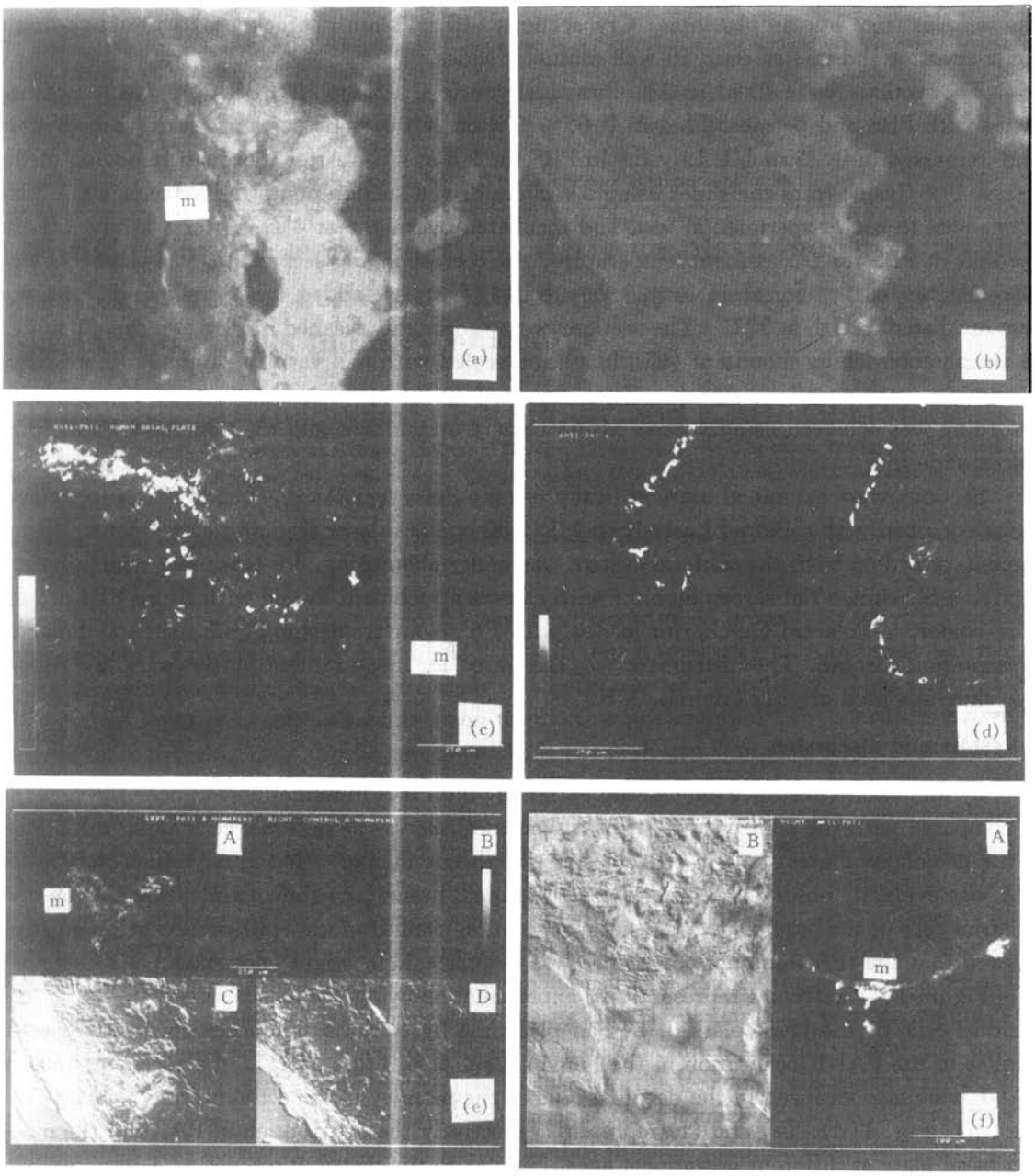

Fig. 1. Distribution of immunofluorescence of tPA ((a) and (b)) and PAI-1 ((c), (d), (e) and (f)) in placental basal plates of human ((c) and (e)) and rhesus monkey ((a), (b), (d) and (f)) placentae. (a) tPA immunoreactivity in basal plate of rhesus monkey placenta. Fluorescence is associated with the decidual cells along the detachment sites (m). (b) Control for (a). (c) Distribution of immunofluorescence of PAI-1 in basal plate of human placenta. (d) PAI-1 immunofluorescence in the decidual cells of the basal plate of monkey placenta surrounding the blood vessesl. (e) A, Anti-PAI-1 on the 150th day of gestation rhesus monkey basal plate; B, control with the first step anti-PAI-1 omitted; C, Nomarski DIC of the same area as shown in (a); D, Nomarski DIC of the same area as shown in the control. (f) A, PAI-1 in rhesus monkey basal plate after 140 d of gestation; B, Nomarski DIC of the same area.

This is the first report on the localization of tPA and PAI-1 in the basal plate of human and monkey term placentae. We have also provided the direct evidence which shows that the coordinated expression of tPA and PAI-1 in placenta may be important for the detachment of decidua from the uterus during the process of parturition.

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