行政院國家科學委員會專題研究計畫　成果報告

侵襲性滋養層細胞於著床後對子宮螺旋動脈血管重塑扮演角色之探討(3/3)

計畫類別： 個別型計畫
計畫編號： NSC92-2314-B-002-155-
執行期間： 92 年 08 月 01 日至 93 年 07 月 31 日
執行單位： 國立臺灣大學醫學院婦產科

計畫主持人： 施景中

計畫參與人員： 謝豐舟　錢宗良 徐明洸 李建南

報告類型： 完整報告

處理方式： 本計畫可公開查詢

中　華　民　國 93 年 12 月 7 日

行政院國家科學委員會補助專題研究計畫成果報告

※※※※※※※※※※※※※※※※※※※※※※※※

侵襲性滋養層細胞於著床後對子宮螺旋動脈血管重塑扮演角色之探討

The role of invasive extravillous trophoblast in the remodeling of spiral artery during normal and abnormal implantation

※※※※※※※※※※※※※※※※※※※※※※※※

計畫類別：☑個別型計畫　　□整合型計畫
計畫編號：NSC－90－2314－B－002－452
執行期間：92　年　8 月　1 日 至　93　年　7 月　31 日

計畫主持人：施景中
共同主持人：謝豐舟　錢宗良

本成果報告包括以下應繳交之附件：
□赴國外出差或研習心得報告一份
□赴大陸地區出差或研習心得報告一份
□出席國際學術會議心得報告及發表之論文各一份
□國際合作研究計畫國外研究報告書一份

執行單位：台大醫院婦產部

中　華　民　國　93 年　10　月　15　日

行政院國家科學委員會專題研究計畫成果報告

侵襲性滋養層細胞於著床後對子宮螺旋動脈血管重塑扮演角色之探討

The role of invasive extravillous trophoblast in the remodeling of spiral artery during normal and abnormal implantation

計畫編號：NSC－90－2314－B－002－452
執行期限：92 年 8 月 1 日 至 93 年 7 月 31 日
主 持 人： 施景中 台大醫院婦產部
共同主持人： 謝豐舟 台大醫院婦產部
錢宗良 台大醫學院解剖暨細胞生物學研究所
計畫參與人員：徐明洸 李建南 台大醫院婦產部

一、中文摘要

成功的人類著床倚賴絨毛外滋養層細胞透過增殖、轉移、侵入母體的蛻膜，並適度的轉換血管內皮細胞，以達成有效建立子宮胎盤循環。可是至今，這些滋養層細胞如何引發並執行母體子宮螺旋動脈轉換及血管重塑，真正機轉仍不明朗。一個重要的因子為這些滋養層細胞侵入血管管腔內，並跨站於原來的內皮細胞上將其取代轉換，目前的主流觀念為 Hamilton 於 1965 年於 Nature 中所提出，這些侵襲性滋養層細胞來自基本板，由子宮螺旋動脈的末端逆流而上，並非由蛻膜的間質區裡的侵襲性滋養層細胞所侵入。這個主流觀念衍生一些重要問題尚未能解答，第一，若是如此，蛻膜的間質區裡的侵襲性滋養層細胞於人類著床中所扮演的角色為何，為什麼這些滋養層細胞要千里迢迢至母體胎兒的交界面—細胞板，穿越母體的蛻膜，最後有些遠遠地落腳在子宮肌肉層裡？第二、何者觸發基本板的滋養層細胞去對抗動脈高壓、逆流而上？

蛻膜的間質區裡的侵襲性滋養層細胞，早先被認為與人類著床的免疫調節有關，其實它的真正角色從未被清楚確定。晚近，有學者發現到這些細胞自脫離了細胞板後，大量集結在蛻膜血管的周遭，這些學者因此假定：這些細胞極有可能在子宮螺旋動脈的血管重塑扮演重要角色，可能是集結在蛻膜血管的周遭後，與血管產生互動，進而放出訊號使基本板的滋養層細胞去對抗動脈高壓、逆流而上侵入血管。事實上侵襲性滋養層細胞侵入母體組織也血管內滋養層細胞空間較深遠而且時序上也較早，這些重要的著床發育上的問題正是本計劃欲探討的主軸。

在本計劃的第一年裡，我們收取早期妊娠流產手術的蛻膜組織，以及足月正常妊娠及子癲前症的胎盤底部採樣(剖腹產時取)，我們進一步將以免疫螢光反應、共軛焦距螢光顯微、掃瞄式電子顯微鏡、穿透式電子顯微鏡、免疫電子顯微鏡，去觀察原位標本下，侵襲性滋養層細胞(含侵襲性滋養層細胞、血管內滋養層細胞、多核滋養層巨細胞)本身、及與著床微環境互動下的超微結構變化(因細胞執行既定命令時仍須透超微結構改變) ，這些觀察將對滋養層細胞著床提供重要資訊。本年計劃的亦將體外培養及分離滋養層細胞以供第二年實驗之用。

關鍵詞：侵襲性滋養層細胞，著床，血管重塑，人類，細胞生長因子。

Abstract

Successful human placentation depends on adequate transformation of the uteroplacental circulation by extravillous trophoblast proliferation, migration, and invasion into the maternal decidua. Thus far, how these trophoblasts execute the process of spiral arteries transformation is still not clear. One key factor is that the trophoblasts which invade the lumen and straddle on the endothelial cells. The prevailing view of this endovascular trophoblast was first addressed by Hamilton and Boyd in 1965 in *Nature*. After reviewing numerous sections of placental bed biopsy, he concluded that cells in the lumen of spiral arteries which responsible for

vascular remodeling is the progeny of fetal cytotrophoblast. Moreover, this specialized cytotrophoblast is not from directly invading of the interstitial trophoblast, rather, should be from the trophoblast in the basal plate and migrating up to transform the spiral arteries. However, it arises some important queries to be answered. First, if the cytotrophoblast in the interstitial area does not invade the spiral artery directly, what is the purpose for its journey across the long distance area, from tips of cell column, pass by the decidual cells, and finally to the muscular area of the uterus? Second, what factor(s) is (are) the key driving force for these EVTs within the vessel lumen to against the arterial pressure to migrate up the spiral artery?

The interstitial cytotrophoblast once was thought to play a role in the immuno- regulation of embryo implantation. Nevertheless, the actual function of interstitial trophoblast is never elucidated. Recently, authors found these interstitial trophoblasts, after detaching the cell columns, usually colonize around the spiral artery. Therefore the authors presume that these interstitial trophoblasts may probably play a role in the transformation of spiral arteries, possibly priming some events, then the endovascular trophoblasts receive the signals and migrate up to the maternal uterine circulation. In fact, the invasion of interstitial trophoblast is also deeper than endovascular trophoblast: reaching a peak by the end of the first trimester and declined rapidly thereafter, the second wave in around 16 weeks and may deep into the myometrium.

In the first year of this project, we collect the sample from the decidua from the first-trimester D&C specimen, and also collect the placental bed biopsy form normal and preeclampsia third-trimester pregnancies during Cesarean section. We examine the interstitial trophoblasts in situ by immunohistocytochemistry, confocal microscopy, scanning electromicroscopy, transmission electromicroscopy. The aims of these examination are to understand the interrelationship of the trophoblast (interstitial, endovascular, multinucleated giant cells) and the implantation microenvirionment. The electromicroscopy may provide valuable information for the ultrastructure changes of trophoblast during implantation. Besides, we start the in vitro vulture and isolation of the trophoblast for the use of second year project.

Keywords: invasive trophoblast, implantation, vascular remodeling, human, cytokines.

二、緣由與目的

Successful human placentation depends on adequate transformation of the uteroplacental circulation by extravillous trophoblast (EVT) proliferation, migration, and invasion into the maternal decidua. This process rises to a peak by the end of the first trimester and declined rapidly thereafter. Human placental development depends critically on the differentiation of the placenta's specialized epithelial cells, termed cytotrophoblast. Two differentiation pathways exist. In one, cytotrophoblast remain in the fetal compartment and fuse to form multinucleate syncytiotrophoblasts that cover the floating chorionic villi, whihch were direct contact with maternal blood in the intervillous space and, perform nutrient and gas exchange for the fetus. In the second pathway, a subset of cytotrophoblasts in anchoring villi aggregate into cell columns that attach to the uterine wall. From there, cytotrophoblast (CTB) invade the uterine wall and (interstitial invasion) and the decidual vessels (endovascular invasion) as far as the first third of the myometrium. As a net result, oxygenated maternal blood can thus largely flow into the intervillous space and provide nutrient for the fetus.

In normal pregnancy, CTB invade arteries more deeply than veins. Cells that are participating in endovascular invasion have two types of interactions with maternal arterioles. In the first type of interaction, large aggregates of these fetal cells are found primarily inside the vessel lumen. These aggregate can either lie adjacent to the apical surface of the resident endothelium or replace it such that they appear directly attached to the vessel wall. In the second type interaction, CTB are found within the vessel wall rather in the lumen. In this situation, they colonize the smooth muscle layer of vessel wall and lie adjacent to the endothelium. These two types of interactions may be representatives of the progressive stages in a single process, or indicative of different strategies by which CTB accomplish endovascular invasion. In either case, the stage in which fetal CTB cohabit with maternal endothelium in the spiral arterioles

is transient. By late second trimester, these vessels are lined exclusively by endometrial or the superficial portions of their myometrial segments. The specific purpose of this project is designed for the identifying the driving force of these EVTs and the whole process of the spiral transformation.

三、研究方法

I. SAMPLE COLLECTION: Chorionic villi and deciduas of the first-trimester pregnancy will be obtained from women undergoing elective termination due to blighted ovum or other psycosocial problems. Third-trimester specimen will be obtained during cesarean section at term.

II. IMMUNOHISTOCYTOCHEMISTRY

Tissue fixation, and immunocytochemistry

The tissues are fixed/rinsed/blocking according to the standard condition described *(Cell, Volume II: Subcellular localization of genes and their products, Spector DL et al., 1998 Cold Spring Harbor Laboratory Express).*

1. Tissues sections (5μm) are dewaxed and rehydrated conventionally.
2. Quenching of endogenous peroxidase is achieved by incubation with 0.3% hydrogen peroxide in methanol for 30 mins at room temperature.
3. All tissue sections are exposed to a non-immuned block with normal rabbit serum for 30 minutes at room temperature.
4. Incubation with the primary antibodies is carried out at 4°C overnight with various dilutions for the specific antibodies.
5. Thereafter tissues sections are labeled with an avidin-biotin-peroxidase detection system Vectastain (Vector Lab, Burlington VT, USA).
6. Each step is followed by a meticulous washing with PBS.
7. Finally, 3,3'-diaminobenzidine is used as chromogen.
8. Counterstaining was performed with hematoxylin.

For cryostat section (8-10μm), the steps are the same except skip the procedures of dewaxing and dehydration.

Antibodies

Markers for cytotrophoblasts

In chorionic villi and column portion of anchoring villi, cytokeratins are the most useful markers. Anti-pancytokeratins can stain syncytiotrophoblast, cytptrophoblast (stem cell), and also the invasive interstitial trophoblast. Nonetheless, the glandular epithelium, which thereotically regresses after 10 weeks' gestation, will be stained positive for anti-pancytokeratin. The architecture of glandular epithelium loos like transformed spiral artery with endovascular trophoblast in the lumen. We use CD56 (NCAM) as an immunomarker for the differentiation between endovascular trophoblast ($CD56^+$) and glandular epithelium ($CD56^-$).

Markers for smooth muscles component of the decidual vessels

We use phalloidin to recognize all the F-actin components in the placental bed biopsy. Phalloidin is a non-specific actin-binding toxin derived from mushroom. Either α, β, and γ forms of actin will be recognized by phalloidin. This will help to localize the decidual vessels (either vein or artery). We also use CD31 (PECAM) as a marker to localize the endothelial cells in the lumen of spiral artery.

Markers for cytokines that are putatively essential for implantation

We use a panel of antibodies to cytokines and their receptors that we intend to test the roles in the embryonic implantation.

Immunostaining

We use fluorescein-conjugated anti-mouse IgG as secondary antibodies to label the monoclonal antibodies, and rhodamine-antimouse IgG as seconday antibodies to label the polyclonal antibodies. Other fixation, washing, incubation techiniques are followed the standard protocols (see *Cells: a laboratory manual, by Spector DL, et al., Volume 3 Subcellular location of genes and their products*).

III. Transmission electromicroscopy (TEM)

IV. Scanning electromicroscopy (SEM)

V. CONFOCAL MICROSCOPY

Tissues sections (30μm) are fixed and stained in the standard processes of immunohistocytochemistry. The

post-stained slides will be examined using Zeiss confocal microscope (LSM 510). Optical sections will be collected at 0.5 micrometer. Steps through individual cell nuclei for analysis. Distribution of EGFP will be localized on the different optical sections.

四、結果與討論

The followings are illustrations supporting our concept in the 3 years projects.

.

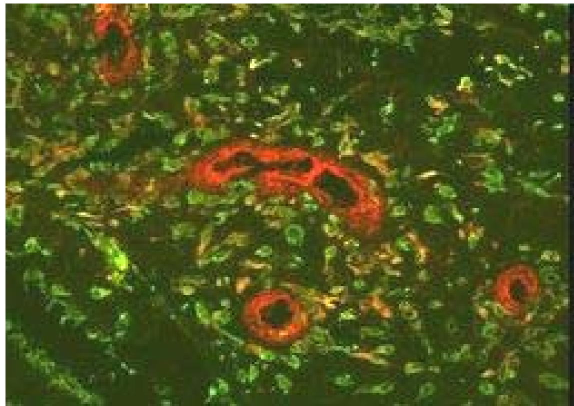


Fig.1 Confocal laser microscopy of placental bed biopsy of 8-week pregnancy demonstrated the lost epithelial polarity of invasive trophoblast in the decidua. The result of loosing polarity enhances the architecture change of trophoblast into tadpole shape and enables the motility of the cell. (CK7-FIGAM + Phalloidin-Rhodamin)

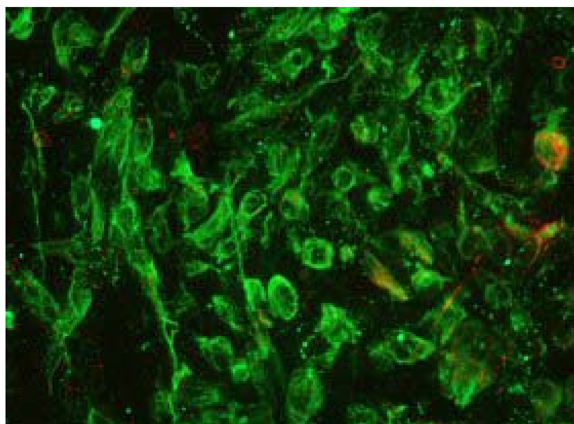
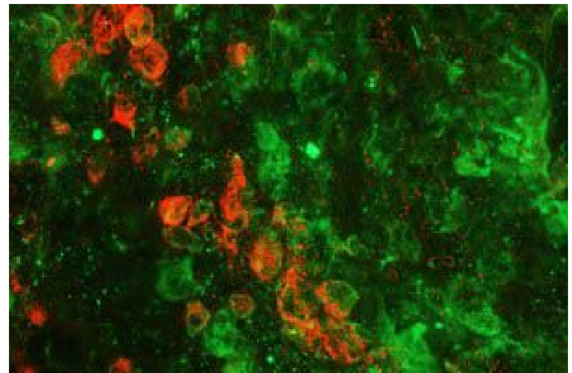


Fig.2 Confocal laser microscopy of a myometrium specimen from a 7-week gestation uterus. In the left side, the invasive trophoblasts still keep the tadpole shape, indicating their ability to move. In the right side of figure, the trophoblast retracts their pseudopad, indicating their motility being decreasing. These cells also shows the tendency of increasing expression of TGF β type I receptor..
(CK7-FIGAM + TGF β RI-ROGAR)



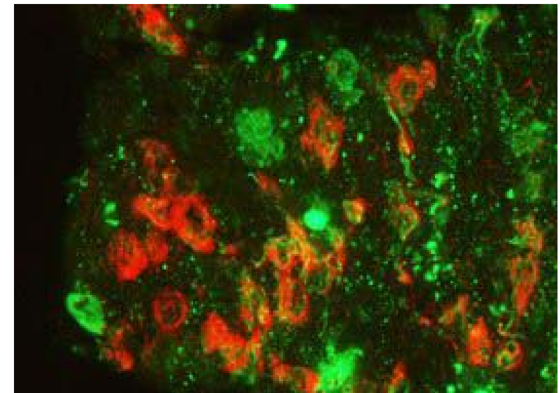


Fig.3 Double immuofluorescence microscopy demonstrating these interstital trophoblast upregulate their TGF β RI. These upregulation also coincide the event of retract pseudopad of trophoblast and loss motility. (CK7-FIGAM + TGF β RI-ROGAR)

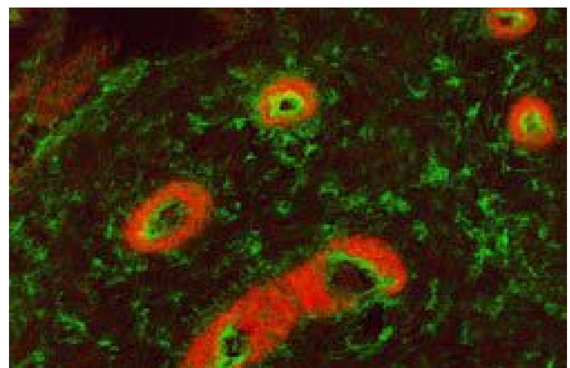


Fig. 4 Double immuofluorescence microscopy demonstrating the endometrium of spiral artery are strongly expressing TGF β RI , indicating a cue for endovascular trophoblast invasion and harboring.
(TGF β RI-FIGAM+ Phalloidin-Rhodamin)

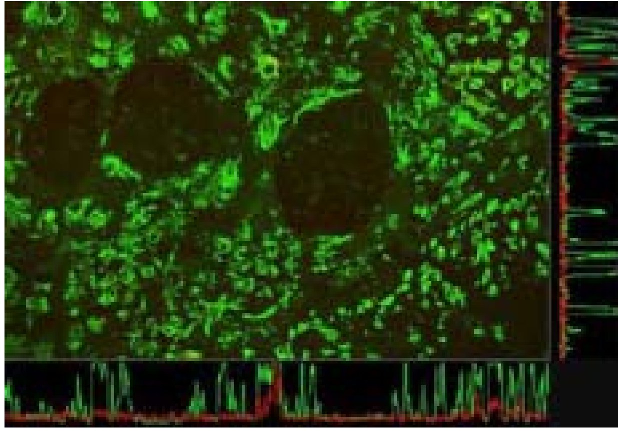


Fig. 5 Confocal laser microscopy of placental bed biopsy of 10-week pregnancy demonstrated the stellate transformation of perivascular invasion cytotrophoblast. Particularly, these cell also have remarkably high expression of TGF β RI, indicating the change of cell architecture being associated with this gene upregulation. And also, the stellate transformation also leads to the subsequent physiological transformation of spiral artery.
(CK7-FIGAM + TGF β R1-ROGAR)

Following is the text explains and summarizes our findings.

Placental bed biopsy specimens from 112 patients met the inclusion criteria in the following categories: (1) first trimester pregnancy (n =97) with positive fetal pole and cardiac action, (2) third trimester pregnancy (n = 15).

Characterization of epithelial polarity of extravillous trophoblast along the invasive pathway in the first trimester

Epithelial cells exhibit a structural asymmetry of the cytoplasm and the plasma membrane is compartmentalized into distinct apical and basolateral domains with characteristic lipid and protein compositions. Differentiated epithelial cells are characterized by an apical-basolateral polarity and specialized cell-cell contacts. Depolarization, loss of adhesiveness and invasion of epithelial cells occur in normal processes (such as organ development and remodeling, wound healing) and in a deregulated manner during carcinogenesis.

The highly differentiated trophoblast is a derivative of epithelial cell. To examine the epithelial polarity of trophoblast in their different locations, we observed the trophoblast under confocal laser microscopy and immunofleuororescence microscopy. Under microscopy examination, clusters of cells in selected regions leave this basement membrane to form columns of polarized cells that attach to and then penetrate the uterine wall. The ends of the columns terminate within the superficial endometrium, where they give rise to invasive cytotrophoblasts.

Cell column is the compact cellular layer at the border to the anchoring villus. The trophoblastic cells in cell columns consist several layers of proliferating extravillous cytotrophoblasts. Morphologically, they appeared as round to polygonal cells that are mostly grouped in strings (Fig 1a). In this area, the epithelial polarity is consisted preserved. The more distal cells that invade the basal plate begin to change their cell structure.

As soon as the trophoblastic cells that leave cell column, they invade the interstitial area and change their cell shapes from a round or polygonal shape to a unipolar shape. Their cellular architecture mimics the extending neurite or the motile sperm. The prominent cell body with cellular processes (most only have one), are traversed in the interstitial area. Particularly, they are drawn up in orderly ranks in compare to the third-trimester interstitial trophoblast (Fig 1b). The cell polarity are notably absent in this area.

The distribution of interstitial trophoblast is more crowded in the superficial deciduas. In the deep decidua, only some scattered interstitial trophoblasts were found. The morphology of them was similar with those in superficial decidua – unipolar cell body with an extending process (data not shown).

Surprisingly, while the myometrial area was detailed examined, the existence of extravillous trophoblast was even more than the deep deciduas. Clusters of trophoblast lied between the myometrial fibers. The cell architecture changed into a round shape, without cell processes in most of them (Fig 1c). In compare to the trophoblasts in cell column, almost no cell contact occurred in this area. Thus the epithelial polarity was also absent in myometrial area.

The monoclonal antibody, anti-cytokeatin 7, also crossly reacts with the glandular cells of the endometrial glands. However, these glands usually reside deep in the deciduas. The glandular cells are arranged in a rosette or fence shape, and thus can be easily differentiated with the interstitial trophoblast.

Hematoxylin eosin counterstaining of paraffin sections shows that EVT columns form at the tips of the placental villi and adhere and penetrate the decidual surface. The decidual blood vessels in the path of the EVT show morphologic disruption.

Stellate transformation of interstitial trophoblast in the third trimester

We examined the placental bed biopsies and also the tissue from cesarean hysterectomy due to other gynecology indications. In the border between placenta villi and maternal decidua, cell column can be still observed. However, the number and thickness was apparently less than those of the first-trimester cell column. Notably, many trophoblasts discretely distributed in the loose connective tissue, and changed into a stellate architecture with five to ten cell processes (Fig 1d).

In the superficial decidua, numerous interstitial trophoblasts can still be seen. The cell architecture was similar to the invading trophoblast of the same area in the first trimester. Nevertheless, the arrangement of them was quite chaotic, which was apparently different with the trophoblast of the same area in the first trimester (Figure 1e).

Only scattered trophoblasts can be observed in the third-trimester myometrium. These cells exclusively exhibited a stellate architecture, with 10 or more cellular processes (Fig 1f). The epithelial polarity were absent in all layers of interstitial trophoblasts at the third trimester specimens.

Interactions between invasive cytotrophoblasts and uterine blood vessels before 7 weeks

Important steps in cytotrophoblast invasion of uterine arterioles are observed from visualization of gestational sac (around 6 weeks of gestation) to the end of the first trimester (11 weeks of gestation). Consecutive changes of interstitial trophoblasts were inspected with the focus on the interaction between these invasive trophoblast and maternal uterine arterioles.

In the first step, cytotrophoblasts that leaving cell columns manifested with an invasive form with a unipolar cell body and a cell process. Along the invasive pathway, some of the cytotrophoblasts target uterine vessels. These cells approach uterine arteries, but not veins (Fig. 2a). Many of them seemed to be stuck against the vessel wall. Nonetheless, neither cytotrophoblasts nor vessel itself have been changed their architecture at this stage. Notably, not all the cytotrophoblasts nearby the vessel were trapped by the vessel wall. There were still quite a few cytotrophoblasts moving away the vessel wall.

Interactions between invasive (fetal) cytotrophoblasts and uterine (maternal) blood vessels between 7-8 weeks

The interaction between invasive trophoblast and uterine arterioles is particularly evident and splendid in this gestational age. In the specimens of the earlier pregnancy before 7 weeks of gestation, the cytotrophoblasts approach and attach against the vessel walls. In this stage, large aggregates of cytotrophoblasts initially colonize around the uterine arteriole. After that, some of these these invasive cytotrophoblasts in the perivascular area extended their multiple cellular processes, and parts of these processes penetrate into the vascular wall (Fig 2b). Afterward, more and more cytotrophoblast underwent stellate transformation, and started to invade and harbor inside the vessel wall. The stellate transformation of cytotrophoblast was also observed in the third trimester myometrial biopsies (Fig 1f). At this stage, the vessel wall of uterine arteriole did not have major changes, such as thinning or vacuolization of smooth muscle layers.

As more and more invasive cytotrophoblasts invade into the vascular wall, the vessel wall was infiltrated by these stellate cytotrophoblast. Many crowded cellular processes of these stellate cytotrophoblast can be observed to interlace inside the vascular wall. These vessels were also examined with Rhodamine-Phalloidin antibody only. The muscular layers of arteriole with stellate cytotrophoblast infiltration were significantly less than those without such cells infiltration (Fig 2). The PAS reaction also showed positive necrosis reaction in these vessels with stellate cytotrophoblast infiltration (Fig 2). Notably, a large, clear lacuna can be also identified around the stellate cytotrophoblast inside the vascular wall (Fig 2). In brief, the interaction of invasive cytotrophoblast and maternal decidual arteriole can be summarized as stellate transformation of cytotrophoblasts, penetration and infiltration of vascular wall, destruction of vascular wall. All these interaction can be sometimes observed in the same slice of specimen, reflecting it is a gradual transition rather than an abrupt process.

Interactions between invasive (fetal) cytotrophoblasts

and uterine (maternal) blood vessels after 9 weeks

In compare to the aforementioned physiological changes, the most pronounced event in this stage is the endovascular retrograde migration into the lumen of decidual arteriole. After extensively examination, this retrograde migration of endovascular trophoblast only occurs in those vessels with stellate cytotrophoblasts infiltration. We never identified such retrograde migration of endovascular trophoblasts occurs in the vessels without stellate cytotrophoblast transformation. Interestingly, we also the tendency of decreasing numbers of perivascular stellate trophoblast in the full transformed decidual arteriole. In these full transformed vessels, the vascular lumen was filled with plug endovascular trophoblast. Only scattered stellate cytotrophoblasts with weak reactivity of anti-cytokeratin 7 were observed in the perivascular area, leaving a large acellular area. This acellular area, similar with appearance of Nitabuch layer, can be confirmed by hematoxylin-stained to be nucleus-negative. We have noted that the endovascular cytotrophoblast never existed in the arteriole of myometrium

Discussion

Until recently, much debate still exists in the process of physiological changes of spiral arteries during pregnancy. Some authorities regarded the large aggregates of endovascular trophoblast induce the fibrinoid change of the turnica media spiral artery, with subsequent development of sac-like vessels which allow continuous low-resistant maternal blood streaming in the intervillous space. In contrast, some pioneer view indicated that cytotrophoblast invade the interstitial area elicit this physiological process, with subsequent priming of the endovascular invasion. In this point of view, interstitial cytotrophoblast invading spiral artery causes the fibrinoid change of smooth muscle layer of spiral artery, and endovascular trophoblast replaced the resident endothelial cell of spiral artery.

Our report first characterized the stellate transformation of interstitial trophoblast. This particular transformation of cytotrophoblast is especially evident in the Nitabuch layer (fibrinoid layer beneath the junction of free-floating villi and superficial decidua) and also the myometrium in the third trimester. A recent *in vitro* model model of dendritic cell dynamics provided by … may explain the *in vivo* counterpart of cytotrophoblast migration (Ref). The extravillous cytotrophoblasts first exit cell column, and then travel into the interstitial area. The journey of these cytotrophoblast ends at the superficial myometrium. This CTB invasion pathway would be expected to involve translocation through cellular barriers, basement membranes, interstitial area, connective tissues and myofibrils. It is, therefore, highly unlikely that the process of CTB migration can be achieved passively. These CTBs must be able to crawl through all the barriers, and eventually put a brake in their end of journey. Although the invasion pathway of CTB has already well described in previous literature, little is known about the motile behavior of CTBs in either location.

Our current report described the epithelial polarity of interstitial trophoblast along the invasive pathway in different gestational ages. The epithelial polarity of extravillous cytotrophoblast apparently related with motility and phenotypes of these trophoblast. Those cytotrophoblasts in the cell column manifest distinct epithelial polarity. Therefore these cells closely contact with each other, resembling those cancer cells without metastasis. Those cytotrophoblasts in the cell column are also the only site that extravillous cytotrophoblast manifest distinct epithelial polarity. Once the polarity is perturbed in the distal column, the cytotrophoblasts begin to loosely attach with each other. Moreover, those cytotrophoblasts losing polarity commence to change their architecture into an invasive form (tadpole-shape cytotrophoblast) and start to migrate in the interstitial area. From now on, those cytotrophoblast has never regained their epithelial polarity. As long as the interstitial cytotrophoblast entering the myometrium, the architecture changes into round shape without a motile cellular process. That may enlighten why extravillous cytotrophoblast never penetrate myometrium in physiological condition. This finding also explain why cytotrophoblasts resident in myometrium are sometimes crowded than distal portion of decidua. That is, those cytotrophoblasts

in deep decidua are still moving, but put a brake when they reach their goal- myometrium. The net result is that, more and more immotile cytotrophoblasts stuff at myometrium.

The motile behavior of differentiated CTB can be comparable to that of a number of motile dendritic cells (DC) analyzed by the computer-assisted methods. Undifferentiated DCs translocated in persistent fashion through extension of pseudopods. In stellate differentiated DCs, F-actin staining was localized in the cortex of the entire cell body, in bursts of numerous fine fibers emanating from the surface of the cell body and dendritic processes, and in punctate clusters. In most cases, differentiated cells did not exhibit discernible polarity or a discernible dominant anterior pseudopod. Therefore, differentiated DCs did not translocate under the same conditions that supported persistent translocation of undifferentiated DCs. The absence of significant translocation, however, did not equate with the absence of all motile behavior. As noted, the ends of dendritic processes continually changed shape, and the The general morphology of differentiated DCs suggested a major reduction or loss of polarity. Most differentiated DCs extended major dendritic processes in a nonmmotile, stellate signature phenotype.

The sequential phenotypes changes and fluorescent staining of F-actin reveal unique behavioral states and unique cellular architecture consistent with inferred in vivo function indicated in our results.

The stellate transformation of CTBs poses important implication in invasion process and decidual vascular remodeling. As illustrated in the in vitro DCs translocation model, the differentiated DCs expressed stellate phenotypes manifested immotile behavior. According to our observations, stellate CTBs exist in the situations of the third trimester Nitabuch layer and myometrium, and also the habit inside and around those decidual vessels underwent remodeling. We suggest that there are two important physiological consequences of this stellate CTBs transformation. First, those CTBs with stellate transformation lost their motile behavior and invasion capability, such as the CTBs in the myometrium. Second, we also observed the CTBs reside in clear lacuna and loose stroma. We hypothesize that those stellate CTBs may have the potential to secret some enzymes to break down the extracellular matrix, with subsequent formation of Nitabuch layer. The formation of Nitabuch layer can enhance the placenta detachment of full term delivery of the baby, and lochia sluggish with involution of uterus into prepartum state. Of particular interest is the stellate CTBs colonize inside and around the decidual vessel underwent remodeling. It is reasonably to assume that these stellate CTBs are actively immotile rather than passively stick by the vessel. In our opinion, decidual vessels serve a cue for stellate CTB transformation. Those tadpole-like invasive form CTB underwent stellate transformation and stop at the perivascular area. Further, the stellate CTB produces some enzymes to break down the tunica media of the decidua vessel, as the clear lacuna seen under PAS staining. It is likely that this step of interaction further switch on the cue for endovascular invasion. Besides, those stellate CTBs also produces large territory of necrotic area in the perivascular space. Accompanied with the disrupted vessel wall, they can pass out as well as expulsed placenta to reduce the blood loss from the implantation site.

This stellate transformation should serve as a starting point for elucidating the cues and molecular mechanisms involved in the regulation of cytotrophoblast differentiation and motility. Third trimester also has invasive form, but the direction is quite irregular. Probably is cue is perturbed in the late gestation.

五、計畫成果自評

The results obtained from current project were exciting. Not only we found the role of TGFβ in implantation and associated with integrins repertoire switching, but also identified the important event in the process of spiral remodeling.

六、參考文獻

1. Murray MJ. Lessey BA. Embryo implantation and tumor metastasis: common pathways of

invasion and angiogenesis. [Review] Seminars In Reproductive Endocrinology. 17(3):275-90, 1999.

2. Zhou Y. Genbacev O. Damsky CH. Fisher SJ. Oxygen regulates human cytotrophoblast differentiation and invasion: implications for endovascular invasion in normal pregnancy and in pre-eclampsia. [Review] Journal of Reproductive Immunology. 39(1-2):197-213, 1998.

3. Lim KH. Zhou Y. Janatpour M. McMaster M. Bass K. Chun SH. Fisher SJ. Human cytotrophoblast differentiation/invasion is abnormal in pre-eclampsia. American Journal of Pathology. 151(6):1809-18, 1997.

4. Caniggia I. Taylor CV. Ritchie JW. Lye SJ. Letarte M. Endoglin regulates trophoblast differentiation along the invasive pathway in human placental villous explants. Endocrinology. 138(11):4977-88, 1997.

5. Zhou Y. Fisher SJ. Janatpour M. Genbacev O. Dejana E. Wheelock M. Damsky CH. Human cytotrophoblasts adopt a vascular phenotype as they differentiate. A strategy for successful endovascular invasion? Journal of Clinical Investigation. 99(9):2139-51, 1997.

6. Burrows TD. King A. Smith SK. Loke YW. Human trophoblast adhesion to matrix proteins: inhibition and signal transduction. Human Reproduction. 10(9):2489-500, 1995.

7. Bischof P. Haenggeli L. Campana A. Gelatinase and oncofetal fibronectin secretion is dependent on integrin expression on human cytotrophoblasts. Human Reproduction. 10(3):734-42, 1995.

8. Damsky CH. Librach C. Lim KH. Fitzgerald ML. McMaster MT. Janatpour M. Zhou Y. Logan SK. Fisher SJ. Integrin switching regulates normal trophoblast invasion. Development. 120(12):3657-66, 1994.

9. Fisher SJ. Damsky CH. Human cytotrophoblast invasion. [Review] Seminars in Cell Biology. 4(3):183-8, 1993.

10. Schilling B. Yeh J. Transforming growth factor-β1, -β2, -β3 and their type I and II receptors in human term placenta. *Gynecologic & Obstetric Investigation. 50(1):19-23, 2000.*

11. Bischof P. Meisser A. Campana A. Mechanisms of endometrial control of trophoblast invasion. [Review] *Journal of Reproduction & Fertility - Supplement. 55:65-71, 2000.*

12. Caniggia I. Mostachfi H. Winter J. Gassmann M. Lye SJ. Kuliszewski M. Post M. Hypoxia-inducible factor-1 mediates the biological effects of oxygen on human trophoblast differentiation through TGFβ3. *Journal of Clinical Investigation. 105(5):577-87, 2000.*

13. Caniggia I. Grisaru-Gravnosky S. Kuliszewsky M. Post M. Lye SJ. Inhibition of TGF-β3 restores the invasive capability of extravillous trophoblasts in preeclamptic pregnancies. *Journal of Clinical Investigation. 103(12):1641-50, 1999.*

14. Meisser A. Chardonnens D. Campana A. Bischof P. Effects of tumour necrosis factor-alpha, interleukin-1 alpha, macrophage colony stimulating factor and transforming growth factor beta on trophoblastic matrix metalloproteinases. *Molecular Human Reproduction. 5(3):252-60, 1999.*

15. Getsios S. Chen GT. Huang DT. MacCalman CD. Regulated expression of cadherin-11 in human extravillous cytotrophoblasts undergoing aggregation and fusion in response to transforming growth factor beta 1. *Journal of Reproduction & Fertility. 114(2):357-63, 1998.*

16. Morrish DW. Dakour J. Li H. Functional regulation of human trophoblast differentiation. [Review] *Journal of Reproductive Immunology. 39(1-2):179-95, 1998.*

17. Irving JA. Lala PK. Functional role of cell surface integrins on human trophoblast cell migration: regulation by TGF-β, IGF-II, and IGFBP-1. *Experimental Cell Research. 217(2):419-27, 1995.*

18. Levy R. Smith SD. Chandler K. Sadovsky Y. Nelson DM. Apoptosis in human cultured trophoblasts is enhanced by hypoxia and diminished by epidermal growth factor. *American*

Journal of Physiology - Cell Physiology. 278(5):C982-8, 2000.

19. Payne SG. Brindley DN. Guilbert LJ. Epidermal growth factor inhibits ceramide-induced apoptosis and lowers ceramide levels in primary placental trophoblasts. *Journal of Cellular Physiology. 180(2):263-70, 1999.*
20. Dakour J. Li H. Chen H. Morrish DW. EGF promotes development of a differentiated trophoblast phenotype having c-myc and junB proto-oncogene activation. *Placenta. 20(1):119-26, 1999 .*
21. Mochizuki M. Maruo T. Matsuo H. Samoto T. Ishihara N. Biology of human trophoblast. *International Journal of Gynaecology & Obstetrics. 60 Suppl 1:S21-8, 1998.*
22. Baker VL. Murai JT. Taylor RN. Downregulation of protein kinase C by phorbol ester increases expression of epidermal growth factor receptors in transformed trophoblasts and amplifies human chorionic gonadotropin production. *Placenta. 19(7):475-82, 1998.*
23. Matsumoto K. Yamamoto T. Kurachi H. Nishio Y. Takeda T. Homma H. Morishige K. Miyake A. Murata Y. Human chorionic gonadotropin-alpha gene is transcriptionally activated by epidermal growth factor through cAMP response element in trophoblast cells. *Journal of Biological Chemistry. 273(14):7800-6, 1998.*
24. Li RH. Zhuang LZ. The effects of growth factors on human normal placental cytotrophoblast cell proliferation. *Human Reproduction. 12(4):830-4, 1997.*
25. Garcia-Lloret MI. Yui J. Winkler-Lowen B. Guilbert LJ. Epidermal growth factor inhibits cytokine-induced apoptosis of primary human trophoblast. *Journal of Cellular Physiology. 167(2):324-32, 1996.*
26. Maruo T. Matsuo H. Otani T. Mochizuki M. Role of epidermal growth factor (EGF) and its receptor in the development of the human placenta. [Review] *Reproduction, Fertility, & Development. 7(6):1465-70, 1995.*
27. Machida T. Taga M. Minaguchi H. Effects of epidermal growth factor and transforming growth factor alpha on the mouse trophoblast outgrowth in vitro. *European Journal of Endocrinology. 133(6):741-6, 1995.*
28. Amemiya K. Kurachi H. Adachi H. Morishige KI. Adachi K. Imai T. Miyake A. Involvement of epidermal growth factor (EGF)/EGF receptor autocrine and paracrine mechanism in human trophoblast cells: functional differentiation in vitro. *Journal of Endocrinology. 143(2):291-301, 1994.*
29. Bass KE. Morrish D. Roth I. Bhardwaj D. Taylor R. Zhou Y. Fisher SJ. Human cytotrophoblast invasion is up-regulated by epidermal growth factor: evidence that paracrine factors modify this process. *Developmental Biology. 164(2):550-61, 1994.*