Aberrant gene expression associated with recurrent pregnancy loss

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Recent studies indicate that a number of factors including chromosomal abnormalities, immunological feto-maternal rejection, hormonal irregulation and anatomical factors are involved in provoking recurrent pregnancy loss (RPL). This indicates that normal cellular regulation of these factors is required for maintaining normal pregnancy. In addition, it is expected that biological processes for maintaining normal pregnancy require a series of differential gene expression. As expected, our previous investigations revealed that there are ≈30 genes showing different levels of expression between normal and RPL patients. In addition, other research groups have also identified a number of genes that are expressed aberrantly in pregnancy failure. In this review, recent study on aberrant expression levels of genes, which are grouped as immunity-related, angiogenesis-related, apoptosis-related and other groups of genes, will be discussed.

Key words: angiogenesis/apoptosis/immunosuppression/recurrent pregnancy loss

Introduction

Previous investigations have demonstrated that the cellular process of immunological, metabolic, vascular and endocrine regulation is required for maintaining normal pregnancy. Aberrant regulation of these processes may lead to pregnancy loss including recurrent pregnancy loss (RPL). Pregnancy loss is the most common complication of pregnancy, as ~10–15% of human conception terminates in a clinically detected spontaneous abortion (United States Department of Health and Human Services, 1992). It has been reported that RPL or habitual abortion, defined as three or more clinical pregnancy losses before the 20th week of gestation, occurs in ~2–5% of pregnant women (Coulam et al., 1997). A number of investigations have been carried out in order to identify factors that can cause RPL during pregnancy. However, molecular genetic mechanisms for maintaining normal pregnancy are poorly understood. Several investigations on molecular genetic mechanisms of RPL have demonstrated that immunity-, angiogenesis-, apoptosis-related and other groups of genes are involved in RPL (Bolton et al., 1987; Serle et al., 1994; Hey et al., 1995; France et al., 1996; Aarli et al., 1997; Dalton et al., 1998; Kilpatrick, 2000; Choudhury and Knapp, 2001; Zayed et al., 2001; Baek et al., 2002; Hoshimoto et al., 2002; Jokimaa et al., 2002; Choi et al., 2003; Heikkinen et al., 2003; Takakuwa et al., 2003), as listed in Tables I and II. A detailed functional analysis for these genes during normal pregnancy will help to identify pregnancies with a high risk of RPL and how to manage those pregnancies.

Aetiological factors

It has been reported that chromosome abnormalities (Lanasa et al., 2001; Bruyere et al., 2003; Rubio et al., 2003), congenital malformations of the uterus, endocrine malfunction (Costa et al., 1993; Bussen et al., 1999; Rai et al., 2000), immunological disorders (Lim et al., 1996; Eble et al., 2000; Jablonowska et al., 2002), infections (Giles, 2003), haemostatic and metabolic abnormalities (Coumans et al., 1999), and other unknown factors (Baek et al., 2002) can lead to RPL during pregnancy.

Inborn chromosomal abnormalities may be inherited or may arise by spontaneous mutations during embryonic development. The most common parental chromosomal abnormality contributing to pregnancy loss is a translocation, which involves two chromosomes in a mutual exchange of broken-off fragments. In addition, aberration of oocyte spindle formation and meiotic division can also lead to chromosomal abnormalities including aneuploidy, mosaicisms and inversions (Devine et al., 2000; Kuo, 2002). Anatomic deformation of the intrauterine cavity occurs in 10–50% of women experiencing recurrent pregnancy loss (Keltz et al., 1997). In general, congenital uterine abnormalities may be due to the limitation of space and incompetent cervix. It has been demonstrated that endocrine malfunction such as luteal phase insufficiency, hyperandrogenic disorders including the polycystic ovary syndrome (PCOS), hyperprolactinaemia, thyroid dysfunction, and diabetes mellitus is associated with recurrent pregnancy loss (Matsui et al., 1989; Roberts and Murphy, 2000).

Since the conceptus produces parental gene products and its immune differentiating antigen, it is possible that the maternal immune system recognizes these genes products as immunologically foreign, resulting in an immune response (Bolton et al., 1987; Dalton et al., 1998; Hill and Choi, 2000). Interestingly, this phenomenon is rarely seen due to the fact that uterine decidua secretes soluble proteins capable of inhibiting cell-mediated immune responses, potentially protecting the conceptus from maternal immune rejection during normal pregnancy (Olajide and Chard, 1992; Kamarainen et al., 1996). In addition, infection involved in immunological activation is one of the factors causing recurrent pregnancy loss. Haemostatic and metabolic abnormalities such as protein S deficiency, hyperhomocysteinaemia and anticardiolipin antibody are also known to be involved in causing RPL (Coumans et al., 1999). A recent investigation indicated that thrombophilic disorders play a prominent role in RPL, demonstrating that Factor V Leiden, prothrombin G20210A mutation
Table I. Immunity- and angiogenesis-related genes involved in recurrent pregnancy loss

<table>
<thead>
<tr>
<th>Classification</th>
<th>References</th>
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<tbody>
<tr>
<td>Immunity-related genes</td>
<td>Bolton et al., 1987; Dalton et al., 1998; Baek et al., 2002</td>
</tr>
<tr>
<td>PP14 (placental protein 14)</td>
<td>Choi et al., 1998; Choi et al., 2002; Choi et al., 2003</td>
</tr>
<tr>
<td>hCG</td>
<td>France et al., 1996; Zayed et al., 2001; Baek et al., 2002</td>
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<tr>
<td>MUC1</td>
<td>Serle et al., 1994; Hey et al., 1995; Baek et al., 2002</td>
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<tr>
<td>CA-125</td>
<td>Dalton et al., 1998</td>
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<tr>
<td>Annexin II</td>
<td>Aari et al., 1997</td>
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<tr>
<td>Mannan binding protein</td>
<td>Kilpatrick, 2000</td>
</tr>
<tr>
<td>Indoleamine 2,3-dioxygenase</td>
<td>Heikkinen et al., 2003</td>
</tr>
<tr>
<td>CD95</td>
<td>Hoshimoto et al., 2002</td>
</tr>
<tr>
<td>PIBF (progesterone-induced blocking factor)</td>
<td>Laskarinen et al., 2002</td>
</tr>
<tr>
<td>HLA-DRB1</td>
<td>Takakuwa et al., 2003</td>
</tr>
<tr>
<td>CD69</td>
<td>Ramhorst et al., 2003</td>
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<tr>
<td>Angiogenesis-related genes</td>
<td>Choi et al., 2003</td>
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<tr>
<td>MMP-2 (matrix metalloproteinase-2)</td>
<td>Jokimaa et al., 2002; Choi et al., 2003</td>
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<tr>
<td>MMP-9 (matrix metalloproteinase-9)</td>
<td>Choi et al., 2003</td>
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<tr>
<td>Fibronectin</td>
<td>Pijnenborg et al., 2000; Choi et al., 2003</td>
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<tr>
<td>Integrin</td>
<td>Choi et al., 2003</td>
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<tr>
<td>PAI (plasminogen activator inhibitor)</td>
<td>Choi et al., 2003</td>
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<tr>
<td>TGF-β (transforming fibroblast growth factor-β)</td>
<td>Choi et al., 2003</td>
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<tr>
<td>VEGF (vascular endothelial growth factor)</td>
<td>Choi et al., 2003</td>
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<tr>
<td>BFGF (basic fibroblast growth factor)</td>
<td>Choi et al., 2003</td>
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<tr>
<td>TIMP-1 (tissue inhibitors of metalloproteinases-1)</td>
<td>Jokimaa et al., 2002</td>
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Table II. Apoptosis- and other groups of genes involved in recurrent pregnancy loss

<table>
<thead>
<tr>
<th>Classification</th>
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<tbody>
<tr>
<td>Apoptosis-related genes</td>
<td>Choi et al., 2003</td>
</tr>
<tr>
<td>Caspase 3</td>
<td>Choi et al., 2003</td>
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<tr>
<td>Caspase 6</td>
<td>Choi et al., 2003</td>
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<td>Caspase 7</td>
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<td>Caspase 10</td>
<td>Choi et al., 2003</td>
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<tr>
<td>Caspase 12</td>
<td>Choi et al., 2003</td>
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<td>BAD</td>
<td>Choi et al., 2003</td>
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<tr>
<td>BAX</td>
<td>Choi et al., 2003</td>
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<tr>
<td>BID</td>
<td>Choi et al., 2003</td>
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<td>Fas</td>
<td>Choi et al., 2003</td>
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<tr>
<td>FasL</td>
<td>Choi et al., 2003</td>
</tr>
<tr>
<td>Other groups of genes</td>
<td>Choi et al., 2003</td>
</tr>
<tr>
<td>Cathepsin H</td>
<td>Jokimaa et al., 2002</td>
</tr>
<tr>
<td>Globin</td>
<td>Baek et al., 2002</td>
</tr>
<tr>
<td>Unknown 1</td>
<td>Baek et al., 2002</td>
</tr>
<tr>
<td>Unknown 2</td>
<td>Baek et al., 2002</td>
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and protein S deficiency are associated with RPL, whereas methyleneetahydrofolate (MTHFR) mutation, protein C and antithrombin deficiencies are not significantly associated with RPL (Rey et al., 2003).

Molecular genetic study

It has been suggested that biological processes for maintaining the stable pregnancy are mediated by a series of differential gene expression. Previous investigations demonstrated that aberrant expression of genes including matrix metalloproteinases (MMP), tissue inhibitors of metalloproteinases (TIMP)-1, cathepsin H, IL-13 and IL-15 is involved in RPL (Chegini et al., 2002; Jokimaa et al., 2002). To define the molecular regulation of these processes, cDNA subtractive hybridization analysis using human chorionic villi from normal and RPL patients has been performed (Baek et al., 2002; Choi et al., 2003). In this study, a number of genes that were identified and were classified into four groups; immunosuppression-related, angiogenesis-related, apoptosis-related, and other groups of genes including two unidentified genes. All of these genes were either more or less expressed in chorionic villi from the therapeutic abortion patients than in those of RPL patients (Baek et al., 2002; Choi et al., 2003). Even though chorionic villi samples for our study were collected from RPL patients who had at least three unexplained miscarriages and from normal patients with no history of abortion, ectopic pregnancy, preterm delivery or stillbirth as controls, cDNA subtractive hybridization analysis cannot represent all genes aberrantly expressed. The PCR-based cDNA subtractive hybridization analysis has been used for investigating a variety of subjects in living organisms (Diatchenko et al., 1996). However, it should be noted that this method may not detect all genes aberrantly expressed. In addition, it is difficult to address whether the cellular composition of the villi samples is equivalent between normal and RPL patients for unknown genes, which have not been characterized previously. Here, recent findings on aberrant expression for a group of genes in RPL patients from various groups will be discussed.

Immunity-related genes

Recently, immunological studies have been carried out more actively than before. Recent investigations have been focusing on immunological feto-maternal reaction that may cause RPL (Hill and Choi, 2000). Since half of the fetal genome derives from the father, the fetus synthesizes antigens considered to be foreign by the maternal immune system. How the conceptus evades rejection by the maternal immune system is not well-specified, but the most reliable hypothesis involves an immune barrier by the placenta (Okon et al., 1998; Hill and Choi, 2000; Lögdberg and Wester, 2000).

One immunity-related molecule is glycodelin. It has been called by various names; placental protein 14 (PP14), chorionic α2-microglobulin (CAG-2), pregesterone-associated endometrial protein (PEP) and pregnancy-associated α2-microglobulin (α2-PEG) (Rachmilewitz et al., 1999). PP14 has been isolated from human placenta and is expressed in other normal tissues including the epithelium of the Fallopian tube, ovarian surface epithelium, uterine cervix, breast tissue, sweat glands, and bone marrow aspirates (Dutta et al., 1998; Connor et al., 2000). One biological function of PP14 is to inhibit early events in T-cell receptor signalling pathway (Rachmilewitz et al., 1999, 2003). Previous studies showed that PP14 inhibits phytoceramidase-induced lymphocyte proliferation and IL-2 synthesis (Hamlin et al., 1998). Taken all together, PP14 has important roles as a possible immunosuppressive molecule capable of successive pregnancy and growth of the fetus (Ojole and Chard, 1992; Lögdberg and Wester, 2000). In addition, PP14 is known as an inducer for apoptosis in T cells (Mukhopadhay et al., 2001), and is found in carcinomas mechanisms (Tatarinov et al., 1990). It belongs to a superfamilly of lipocalin (Bratt, 2000).

hCG is a luteotrophic hormone that regulates the corpus luteum. It is detected in the maternal blood during the 2nd week of pregnancy and...
its concentration peaks during the 7th to 12th weeks of pregnancy. hCG stimulates placental steroid synthesis and the growth of the fetal adrenal gland. In addition, hCG is involved in modulating the immunological response of the maternal tissues by immunosuppressive action on maternal leukocytes in the region of the invading trophoblast (Hopper and Hart, 1985).

At the protein expression level, several proteins including PP14 and hCG were detected in higher quantities in flushings from the uterine cavity of normal patients than in that of RPL patients (Hamilton et al., 1998; Zayed et al., 2001). It has been suggested that PP14 functions as an immunosuppressor for the protection of the fetomaternal tissues from the potentially hostile maternal immune system (Bolton et al., 1987) and that the uterine PP14 concentration is an adequate indicator for diagnosing whether the pregnancy will succeed or not due to the different level of protein expression in both normal and RPL patients (Hamilton et al., 1998; Li et al., 1998). Recent investigation supports this indication, since PP14 is expressed higher in normal than in RPL patients (Baek et al., 2002). hCG shows a remarkably subnormal concentration at the transcriptional level during the first trimester in RPL patients (Baek et al., 2002), suggesting that hCG is also a useful marker for the diagnosis of early pregnancy failure. Therefore, further research on the mechanism of transcriptional and translational regulation of immunity-related genes will help in understanding their roles in maintaining normal pregnancy.

Mucin proteins generally contain a series of tandem repeat domain enriched in serine, threonine and proline residues (Lagow et al., 1999). Many human mucin genes excluding MUCSB are polymorphic. Ten mucin genes have been discovered so far and are named MUC followed by a number reflecting the order in which the particular mucin gene was isolated (MUC1, MUC2, MUC3 etc.) (Debailleul et al., 1998). Mucins have various functions including protection against bacterial infections (Cohen et al., 1984; Lamblin and Roussel, 1993), protection of proteins and cells from proteolysis, inhibition of cell attachment, promotion of cell attachment, and inhibition of immune cell function (Lagow et al., 1999). It has been found that MUC1 is a cell-surface and secretory molecule of endometrial epithelium and shows the highest expression in the mid-luteal phase (Hey et al., 1995). Previous studies demonstrated that RPL patients showed low MUC1 protein concentration relative to normal pregnancy controls analysed in uterine flushings (Serle et al., 1994; Hey et al., 1995).

In addition, immunological factors implicated in RPL include cytokines regulating the Th1/Th2 balance (Raghupathy et al., 2000), annexin II (Aarli et al., 1997), mannann binding lectin (MBL) (Kilpatrick, 2000), leukemia inhibitor factor (LIF) (Choudhury and Knapp, 2001), indoleamine 2,3-dioxygenase (IDO) (Heikkinen et al., 2003), cluster designation 95 (CD95) (Hoshimoto et al., 2002), CD69 (Ramhorst et al., 2003), and suppressor macrophage (Katano et al., 1997), CA-125 (Dalton et al., 1995), progesterone-induced blocking factor (PIBF) (Laskarin et al., 2002), in addition to PP14, hCG and MUC1 (Table I). Detailed analysis for these immunological factors involved in RPL can be found elsewhere.

**Angiogenesis-related genes**

MMP (matrix metalloproteinases) are involved in basement membrane disruption by T-lymphocytes (Goetzl et al., 1996). MMP family members have been classified according to their substrate specificity; gelatinases (MMP-2, MMP-9), stromelysins and collagenases (MMP-1) (Sato et al., 1994; Birkedal-Hansen, 1995; Bashbaum and Werb, 1996). It has been found that gelatinases degrade basement membrane components including collagen types IV and V, fibronectin, entactin and elastin (Laurie et al., 1982). Previous reports showed that MMP-2 and MMP-9 could be expressed by T-lymphocytes as well as other leukocytes (Yakubenko et al., 2000). And the relationship between MMP expression and activation by T-lymphocytes has been well-clarified (Esparza et al., 1999).

Fibronectin is a component of epithelial and endothelial cells that synthesize extracellular matrix structure (Laurie et al., 1982; Armstrong and Armstrong, 2000). Fibronectin has two forms in humans, one is a soluble dimeric plasma protein and the other is an insoluble multimeric matrix protein (Armstrong and Armstrong, 2000). Both forms are similar in many biological functions, including platelet adhesion, endothelial cell integrity, and cell migration during blood vessel repair (Clark, 1995; Labat-Robert, 2002). In addition, the insoluble fibronectin in the matrix plays a role in the mediation of attachment and migration during embryonic development and tissue rearrangement (Langenbach and Sottile, 1999). It has been demonstrated that fibronectin up-regulates MMP-2 and MMP-9 in human T-lymphocyte-mediated adhesion and migration, which are associated with wound healing in lymphocytes (Esparza et al., 1999; Pijnenborg et al., 2000). However, it has been shown that there is increased frequency of T cell adhesion to extracellular matrix fibronectin in women suffering RPL (Jerzak et al., 2000). Fibronectin also protects against TNF-α-induced toxicity in human trophoblast (Pijnenborg et al., 2000). Thus, this molecule seems to have various functions in immune response and further investigations need to be performed to better understand its cellular functions. In addition to the functional roles of MMP and fibronectin, detailed molecular mechanisms of other angiogenesis-related genes including plasminogen activator inhibitor (PAI), integrin, transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF), which were shown to have lower levels of gene expression in chorionic villi from RPL patients than in those of normal patients (Table I), during normal pregnancy should be elucidated to clarify their functional roles.

**Apoptosis-related genes**

Apoptosis, or programmed cell death, is responsible for a number of normal developmental processes (William et al., 1999; Kumagai et al., 2001). Since the regulation of cell death and proliferation is required for the successful human pregnancy, apoptosis in normal pregnancy is critical (Chatzaki et al., 2001; Jerzak and Brschof, 2002). However, it has not been demonstrated how apoptosis is involved in normal development of the fetal chorionic villi during pregnancy at the molecular level. Our preliminary observation revealed that telomerase activity was suppressed in chorionic villi tissues obtained from RPL patients, supporting the hypothesis that aberrant expression of apoptosis-related genes during development is also involved in RPL. Signalling pathways leading to apoptosis converge on a common machinery of cell destruction activated by a family of cysteine proteases that cleave proteins at aspartate residues (caspases), members of the tumour necrosis factor (TNF) receptor (TNF-R) superfamily, and members of the Bcl-2 family proteins. Interestingly, high expression levels of apoptosis-related genes such as caspase 3, 6, 7, 8, 9, 10, 12, BAD, BAX, BID, Fas and FasL were shown in chorionic villi from RPL patients than those from normal patients (Choi et al., 2003), as listed in Table II. However, anti-apoptosis-related genes (Bcl-2 and Bcl-xL) revealed various expression levels among chorionic villi derived from normal and RPL patients. This indicates that gene products of apoptosis-related genes directly regulate the embryonic development during pregnancy.

**Other groups of genes**

The human globin genes are composed of multigene locus (ε, γ, δ and β). Each gene is expressed in different developmental stages.
There is a switch from fetal (Hb F) to adult (Hb A) haemoglobin throughout development (Jane and Cunningham, 1998). For instance, embryonic (ε) gene is expressed until the 5th week of gestation in the yolk sac. After ε gene expression, the first change is the conversion of gene expression to fetal globin (γγ and αγ) genes, until the birth. After the expression of fetal globin genes, the subsequent change occurs with the expression of adult globin (β) gene (Orkin, 1995; Jane and Cunningham, 1998). However, the expression of fetal globin gene continues throughout life, but to a much lesser extent (1%) than that of adult globin gene (98%) (Hill et al., 1992). Therefore, detailed analysis of globin expression during embryonic and fetal development is required for understanding its biological functions.

In addition to globin gene, two unidentified genes were also expressed less in chorionic villi from RPL patients than those from normal patients. They are located on chromosomes 9 and 21, based on human genome sequence database (Baek et al., 2002). Since both genes are down-regulated in chorionic villi (98%) (Hill et al., 1992). Therefore, detailed analysis of globin expression during embryonic and fetal development is required for understanding its biological functions.

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Combinatorial regulations

It is possible that cellular functions for gene products mentioned above may be related to each other (Figure 1). Immunofluorescence staining of chemo-attractant receptor-homologous molecule (CRTH2) revealed that accumulation of T cells (both Th2 and Tc2 cells) decreased in the decidua bassalis in RPL patients, indicating that their reduction in numbers at the implantation site may contribute to RPL (Michimata et al., 2003). Th1 cytokines, such as gamma-interferon (IFN-γ) and tumour necrosis factor (TNF-α), may give embryotoxic effects in RPL patients (Hill et al., 1992). Approximately 25% of women with a history of RPL show an elevated immune response to trophoblast, increased proliferation of inflammatory cells, and preferential secretion of embryotoxic Th1 cytokines (Hill et al., 1992, 1995). TNF-α is produced from activated macrophages and its cytotoxic effects appear in collaboration with IFN-γ (Yui et al., 1994). In addition, TNF-α-mediated signal transduction pathway results in various effects including apoptosis (Liu et al., 1996), cell proliferation (Kasahara et al., 2003) and cell differentiation (Kumakura et al., 2003). On the contrary, it has been demonstrated that fibronectin plays a role in blocking cytotoxic effects of TNF-α in cultured human trophoblast cells (Pijnenborg et al., 2000).

It has been shown that MMP-2 and MMP-9 are induced in T-lymphocytes upon engagement of integrin α4β1 by its two major ligands, VCAM-1 and the CS-1 sequence of fibronectin in a ligand-dependent manner (Yakubenko et al., 2000). IL-2 or phobol ester upregulates the expression of MMP-9, whereas the expression of MMP-2 depends on the duration of IL-2 exposure (Yakubenko et al., 2000). Fibronectin not only induces MMP-2 and MMP-9 expression by T-lymphocytes, but promotes MMP-2 activation (Esparza et al., 1999). In addition, it has been reported that MMP-2 and MMP-9 are expressed in trophoblast cells during the first trimester (Xu et al., 2001). Thus, these genes play positive and negative roles in maintaining a normal pregnancy. Immunity-related genes PP14 and

**Figure 1.** A speculative model linking some gene products identified as differentially expressed in recurrent pregnancy loss (RPL). Fibronectin stimulates the production of matrix metallocprotease (MMP)-2 and MMP-9 from T-lymphocyte and trophoblast cells and is disrupted by MMP-2 via feedback inhibition. In addition, fibronectin functions to block the cytotoxic effect of Th1 cytokine tumour necrosis factor (TNF-α) produced by activated macrophages. MMP-2, an angiogenesis-related gene, is produced in T-lymphocyte not only by fibronectin, but also Th1 cytokine interleukin (IL)-2 stimulation. Accumulation of T cells (both Th2 and Tc2 cells) becomes reduced in the decidua basalis in RPL patients, leading to less expression of MMP-2 and MMP-9. Placental protein 14 (PP14), an immunity-related gene, is known to inhibit the proliferation of T-lymphocyte in immune reaction.
MUC1 are involved in immunosuppressive reaction (Bolton et al., 1987; Lamblin and Roussel, 1993; Kamarainen et al., 1996; Lagow et al., 1999; Rachmiliwitz et al., 1999; Lögberg and Wester, 2000; Mukhopadhyay et al., 2001) and fibronectin, one of the angiogenesis group of genes, induces MMP-2 expression, and in turn MMP-2 disrupts basement membrane, resulting in an immune response (Laurie et al., 1982; Pijnenborg et al., 2000). However, the molecular mechanisms are not elucidated yet. Besides, the fetal globin gene, a major component in fetal blood, cathepsin H and two unidentified genes may play essential roles in these processes during pregnancy. Therefore, the genetic and molecular analysis of these genes will clarify their functions and relationships during human development.

**Future directions**

Our previous PCR-based subtractive hybridization analysis showed a limited number of genes that are expressed either more or less in chorionic villi from RPL patients than those from normal patients. Since the PCR-based cDNA subtractive hybridization analysis may not represent all genes differentially expressed, it is expected that there should be more genes that are involved in the process of establishing and maintaining pregnancy. On the basis of studies to date, it can be concluded that ≥40 gene products are differentially expressed in RPL and thus may have regulatory roles in establishing or maintaining normal pregnancy. However, the question of cause versus effect resulting in RPL should be resolved. In order to address causal linkages, genetically modified animal models will help to establish their cellular functions during development. In addition, in vitro studies with overexpression and knock-down of these genes will help to clarify the question of cause versus effect resulting in RPL. The purpose of these studies mentioned above is to find factors that may be involved in RPL at the molecular level and to classify angiogenesis- and apoptosis-related genes as well as immunity-related. Interestingly, the partial sequences of unidentified genes were localized in two different chromosomes (9, 21) based upon the human genome sequence database. Using PCR primers derived from a putative open reading frame for these genes, we observed that the expression was less in RPL samples than in normal samples. The cloning of these genes is under way and the characterization of these gene products derived from RPL patients is being investigated. Thus, further research is necessary to confirm the clinical relevance of these genes and to classify angiogenesis- and apoptosis-related genes as well as immunity-related. Interestingly, the partial sequences of unidentified genes were localized in two different chromosomes (9, 21) based upon the human genome sequence database. Using PCR primers derived from a putative open reading frame for these genes, we observed that the expression was less in RPL samples than in normal samples. The cloning of these genes is under way and the characterization of these genes must be investigated to better understand their cellular functions for maintaining normal pregnancy. In addition, the identification of differentially expressed genes in maternal endometrium and decidua derived from RPL patients is being investigated. Thus, further research is necessary to confirm the clinical relevance of these genes identified for abnormal expression in RPL patients. Therefore, finding any aberrant expression of these genes may delineate general health during pregnancy and a better understanding of the physiological significance of these genes may help in controlling the management of subsequent pregnancies.

**Acknowledgements**

I would like to thank members of the Infertility Medical Center and Cell and Gene Therapy Research Institute at Pochon CHA University and CHA General Hospital. I also thank reviewers for their critical comments. This study was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (01-PJ10-PG6-01GN13-0002).

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Submitted on January 25, 2004; resubmitted on February 9, 2004; accepted on February 16, 2004