Involvement of MAPK Signalling in Human Villous Trophoblast Differentiation

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Abstract: Human villous trophoblast differentiation is a complex and highly regulated process essential for the well-being of the pregnancy and fetal development. In this review, we present an overview of the role of MAPKs signalling in morphological and functional differentiation of villous trophoblast.

Key Words: Human placenta, villous cytotrophoblast, differentiation, mitogen-activated protein kinases, cell signalling, syncytiotrophoblast, peroxisome proliferator-activated receptors, Src family kinase, drug.

I. HUMAN PLACENTAL DEVELOPMENT

In humans, the placenta plays a key role in pregnancy and the development of the embryo and the fetus: 1) by exchanging ions, metabolites and waste; 2) by producing specific hormones (steroids and peptides); and 3) through its important immunological function. The human placenta is characterized by an extensive invasion of trophoblastic cells into the maternal uterus leading to a hemomonochorial placentation where the chorionic villus (or placental villus) is the structural and functional unit [1]. The chorionic villi are composed of fetal connective tissue covered by an epithelial layer, the trophoblast. The villi can be classified as anchoring villi (or stem villi), promoting implantation and maintenance of early pregnancy, or floating villi, mediating placental growth and transplacental exchanges (Fig. (**1**)).

The process of placental development involves two pathways of differentiation that lead to the formation of two distinct phenotypes: villous trophoblast (differentiative/fusion phenotype) and extravillous trophoblast (proliferative/invasive phenotype) (Fig. (**2**)). The extravillous trophoblastic tissue is composed of extravillous cytotrophoblasts (evCTB), a heterogeneous cell population including proliferative evCTB, invasive evCTB, endovascular evCTB and trophoblast giant cells (GC). Proliferative evCTB are located in the cell column, at the tip of the anchoring villi. They proliferate and migrate towards to the uterine wall, acquiring an invasive phenotype [2]. Those invasive evCTB will then, either invade the decidua and terminally differentiate in GC, or invade the uterine spiral arteries (SA), acquire an endothelial-like phenotype and terminally differentiate in endovascular evCTB [3,4]. The invasion of decidua and SA is essential to allow an increase of maternal blood flow in the intervillous space (IVS), which is necessary to a proper fetal development [5].

The villous phenotype is formed from villous cytotrophoblasts (vCTB; mononucleated) that have fused and differentiated into syncytiotrophoblast (STB; multinucleated) (Figs. (**1**, **2**)). This phenomenon called syncytialization is rare biological event. In humans, three tissues originate from syncytial fusion: STB, skeletal muscles, and osteoclasts [6,7].

The STB contacts maternal blood in the IVS and forms a continuously regenerated, polarized and non proliferative layer that covers adjacent vCTB on its basal plasma membrane, and faces the maternal blood on its brush-border membrane [8]. Thus, the STB is regenerated by continuous turnover process including proliferation of CTB stem cells, fusion of differentiating vCTB into STB and progression toward apoptosis. The syncytium is responsible for most of the endocrine functions supported by the placenta. Indeed, the STB has an intense steroidogenic activity, secreting high levels of estrogen and progesterone [9,10]. Several peptide hormones are also produced by the STB, such as human chorionic gonadotropin (hCG), human placental lactogen (hPL), placental growth hormones (pGH), neuropeptide Y and leptin [11-15]. Furthermore, the STB plays a fundamental role by allowing adequate exchange of nutrients and hormones as well as other components between the mother and her fetus.

Villous Trophoblast Differentiation

Villous trophoblast differentiation is a complex biological process occurring both *in vivo* and *in vitro*. Despites its essential function in pregnancy and fetal development, the mechanisms that control villous trophoblast differentiation remains poorly-understood. The first convincing evidences of *in vivo* cytotrophoblastic fusion to form a syncytium were given by Richart *et al*. (1961), using [^{3}H]-thymidine labeling studies [16]. The characterization of villous trophoblast differentiation gained from the capacity to isolate and

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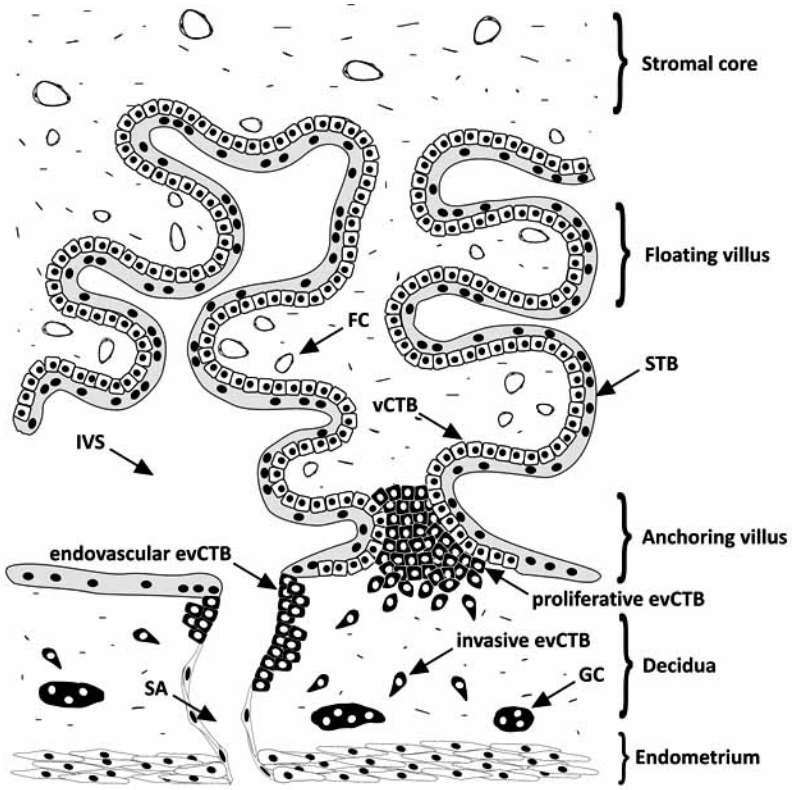

Fig. (1). Schematic representation of chorionic villi at fetomaternal interface. The floating villus is in direct contact with the maternal blood in the intervillous space (IVS). The floating villus is composed of the villous cytotrophoblasts (vCTB) and the syncytiotrophoblast (STB). The anchoring villus is composed of a heterogeneous population of extravillous cytotrophoblasts (evCTB) and the trophoblast giant cells (GC). SA, uterine spiral arteries; FC, fetal capillaries.

maintained vCTB in primary culture [2]. Kliman *et al*. (1986) developed a method to isolate and purify vCTB from human placenta and demonstrated that freshly isolated vCTB fuse and differentiate into functional STB *in vitro* [17] (Fig. (**3**)). Animal models are not suitable for the study of human villous trophoblast differentiation [18]. Thus, primary cultures of isolated vCTB are the model of choice to investigate human villous trophoblast differentiation. In this *in vitro* model, vCTB differentiated in STB in four days of culture [17] (Fig. (**3**)). This process is characterized by a morphological (fusion) and biochemical (or functionnal) differentiation. The morphological differentiation is defined by the fusion of mononucleated cytotrophoblasts with adjacent syncytium [19], while the biochemical differentiation is characterized by the production of hormones such as hCG and hPL [17,20,21].

The assessment of villous trophoblast morphological differentiation is generally performed by immunocytochemical staining of desmoplakin and nuclei [22,23]. During vCTB fusion, a redistribution in desmoplakin localization, a protein associated with desmosome junctional complex, occurs [23]. Hence, while cells boundaries are abolished with syncytium formation, the staining of desmoplakin disappears. Typically, *in vitro*, a syncytium is defined as three or more nuclei in the same cytoplasm without intervening desmoplakin membrane staining [22]. Biochemical differentiation is mainly evaluated by measuring the mRNA expression (real-time PCR) and secretion in the cell culture media (enzyme immunoassay (ELISA)) of hCG, hPL or pGH [17,24,25]. Both the assessment of morphological and biochemical differentiation, which can be independently controlled, are required to analyse the differentiation of vCTB into STB.

In vitro, the presence of serum is required for the complete morphological and biochemical differentiation of the villous trophoblast. Indeed, isolated vCTB maintained in serum free conditions either cannot aggregate or fuse [26] or display a small degree of spontaneous differentiation [27]. *In vitro* studies have shown that the differentiation of vCTB into STB can be induced or inhibited by different factors. For example, growth factors like epidermal growth factor (EGF) and granulocyte-macrophage colony-stimulating factor (GM-CSF), cyclic AMP (cAMP), polypeptide or steroid hormones such as estrogens, hCG [27-34], and glucocorticoids induce differentiation. Other factors described to promote syncytialization are colony-stimulating factor (CSF), TGF-α, leukemia-inhibitory factor (LIF), and vascular endothelial growth factor (VEGF) [28,33,35]. In contrast, hypoxia, transforming growth factor (TGF)-β, tumor necrosis factor (TNF)-α, and endothelins impaired syncytium formation of villous trophoblasts *in vitro* and inhibited secretion of hCG and hPL [36-39].

The vCTB cells fusion is a key event of the formation of the STB. Numerous studies have looked at the mechanism

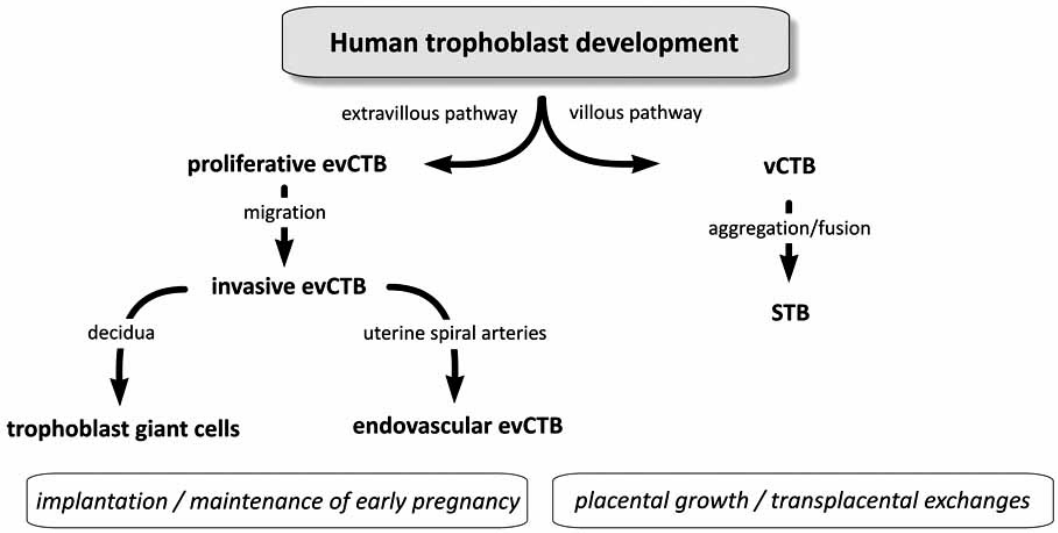

Fig. (2). Human trophoblast development pathways. In humans, trophoblast development follows two pathways. The extravillous trophoblast is responsible for uterine implantation and maintenance of early pregnancy while the villous trophoblast mediated placental growth and transplacental exchanges. In the extravillous pathway, proliferative extravillous cytotrophoblast (evCTB) will migrate from the top of the anchoring villi towards the uterus, acquiring an invasive phenotype. The invasive evCTB either invade the decidua and differentiate in trophoblast giant cells (GC) or invade the uterine spiral arteries (SA) and differentiate in endovascular evCTB. In the villous pathway, mononucleated villous cytotrophoblasts (vCTB) will aggregate and fused to differentiate in an uninterrupted multinucleated syncytium, the syncytiotrophoblast (STB).

behind villous trophoblast cells fusion. Indeed, proteins implicated in cell-cell interactions are likely to be involved in vCTB cells fusion. For example, the expression of connexin-43, a gap junction protein, has been demonstrated to be essential for syncytial formation [40,41]. Recent studies have also shown a role for the human endogenous retrovirus (HERV) envelope proteins in vCTB fusion. Additional molecules involved in cell fusion have been outlined but further studies are needed to correctly address their involvement in trophoblasts syncytialization [41-47].

Other candidate proteins that were recently considered to be involved in vCTB fusion were members of the ADAM (A Disintegrin And Metalloproteinase) family [48,49]. Caspase

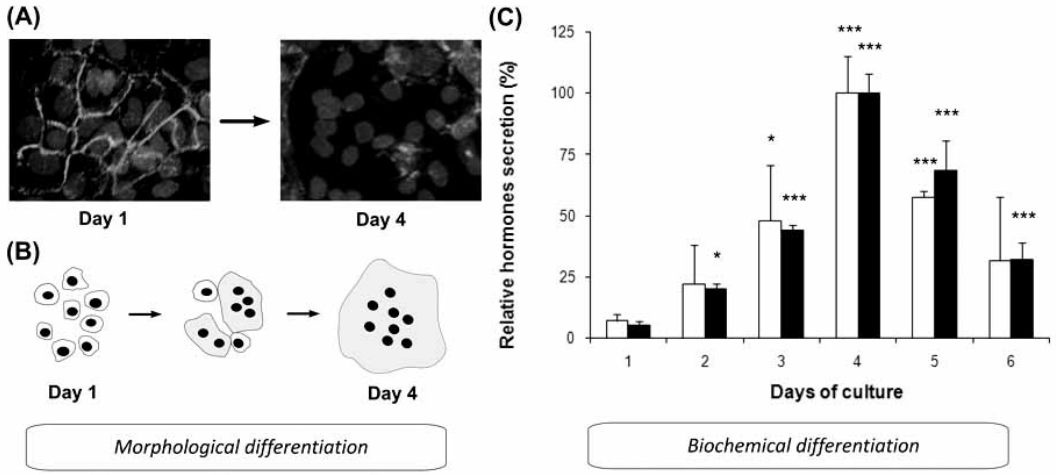

Fig. (3). Morphological and biochemical components of human villous trophoblast differentiation. (**A**) Morphological differentiation in villous trophoblast cells from primary cultures of human term placenta. Confocal microscopy staining of desmoplakin and nuclei after 1 and 4 culture (400X magnification). (**B**) Schematic representation of *in vitro* villous trophoblast morphological differentiation. (**C**) Biochemical differentiation in villous trophoblast cells from primary cultures of human term placenta. Relative secretion of human chorionic gonadotropin (hCG) (□) and human placental lactogen (hPL) (■) at 1 to 6 days of culture. Values are expressed as relative hCG or hPL secretion ± SEM compared to secretion at 4 days of culture (maximal secretion). **P*<0.05; ****P*<0.001 compared to day 1 of culture.

(cysteine aspartase) are specific proteases involved in degradation of cytoskeletal and cell adhesion proteins [50]. In human placenta, caspase 8 was considered to play a role in differentiation of vCTB into STB [8,51]. Moreover, caspase 14 was also suggested to participate in vCTB fusion [52]. Pregnancy-associated placental protein-A (PAPP-A), a metalloprotease which increases in maternal serum during pregnancy and decline after delivery, was demonstrated to increase during STB formation [53]. However, whether PAPP-A promotes vCTB differentiation or its increased expression is just a consequence of the syncytialization remains to be studied.

Alteration of Villous Trophoblast Differentiation and Obstetric Complications

In human placenta, alteration of villous trophoblast differentiation adversely affects the maintenance and integrity of placental functions and barrier. Abnormal syncytial fusion seems to be implicated in the development of trisomy 21, anti-phospholipid syndrome, preeclampsia, and hemolysis elevated liver enzymes and low platelets (HELLP) syndrome [54-61]. Therefore, the elucidation of the signalling mechanisms regulating villous trophoblast differentiation is primordial to better understand the pathogenesis of pregnancy complications related to abnormal villous trophoblast differentiation.

II. MAPK SIGNALLING IN VILLOUS TROPHOBLAST DIFFERENTIATION

Mitogen-Activated Protein Kinases

Mitogen-activated protein kinases (MAPK) are a family of protein kinases involved in many physiological functions and regulatory mechanisms [62]. There are four subfamilies of MAPK (Fig. (**4**)):i) extracellular signal-regulated kinases (ERK1 and ERK2, namely ERK1/2 subfamily), ii) p38 kinases (p38α, p38β, p38γ, and p38δ), iii) c-Jun NH2-terminal kinases (JNK 1, JNK 2, and JNK 3), and iv) ERK5. This family of protein kinases controls many cellular events such as cell proliferation, differentiation, apoptosis, embryogenesis, inflammation, and stress responses [63,64]. The ERK1/2 and p38 pathways are implicated in the differentiation of several cell types inclu-ding adipocytes [65], cardiomyocytes [66], chondroblasts [67], myoblasts [68], and neurons [69,70].

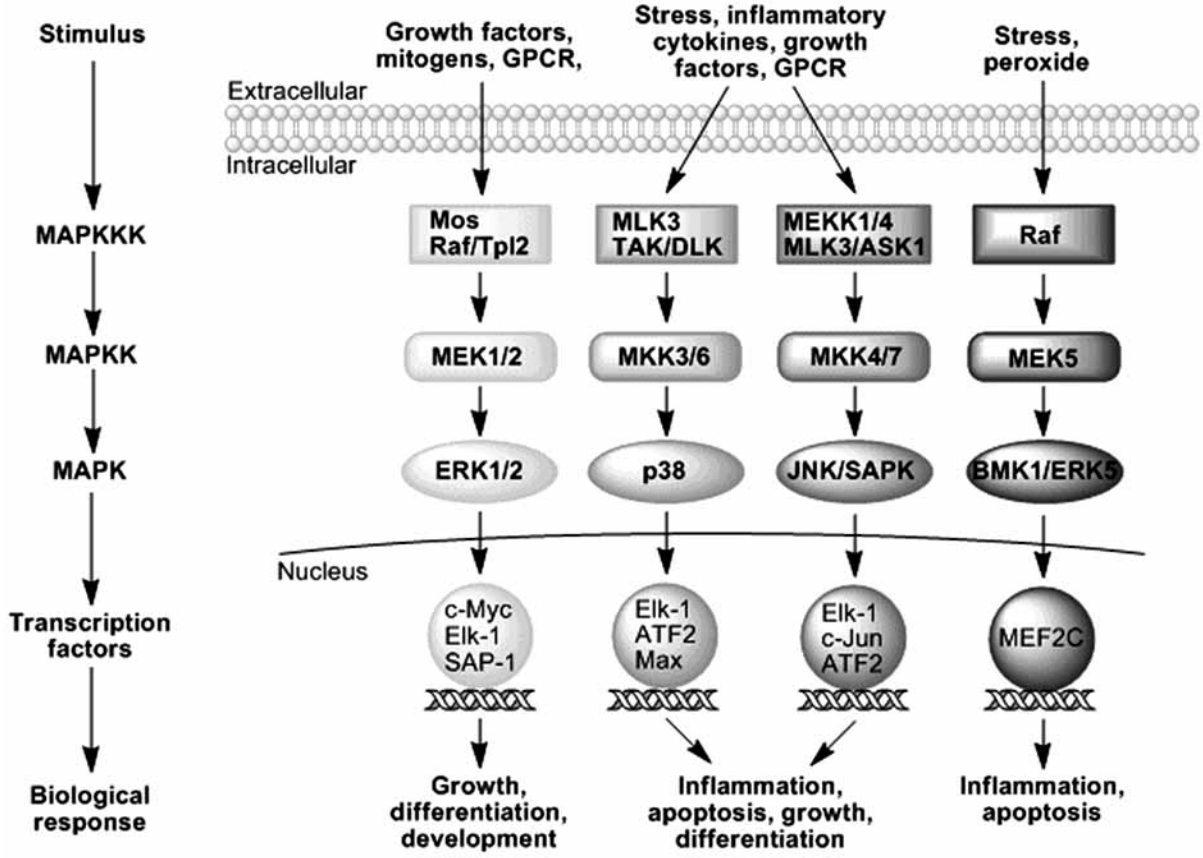

Fig. (4). MAPK signal transduction pathways. The MAPK are activated by MAPKK, which are activated by MAPKKK. Ras and Rho-family GTPases transduce extracellular signals to the MAPK cascades. Abbreviations: MAPK: mitogen-activated protein kinases; MAPKK: MAPK kinase; MAPKKK: MAPKK kinase; GPCR: G-protein-coupled receptors; SAP-1: serum response element accessory protein; ERK, extracellular signal-regulated kinase; MEK: MAP/ERK kinase; ATF: activating transcription factor; MKK: MAPK kinase; MLK3: Mixed lineage kinase-3; TAK: TGF (transforming growth factor)-β activated kinase 1; DLK: dual leucine zipper kinase; JNK: c-Jun amino-terminal kinase; SAPK: stress-activated protein kinase; MEKK, MEK kinase; ASK: Apoptosis Signal-regulating Kinase 1; MEF2C: myocyte enhancer factor 2C; BMK: BiMC related Kinase.

Whereas, JNK is involved in the regulation of apoptosis and in immune cell biology [62]. This review focuses on ERK1/2 and p38 signalling, which were suggested to play significant roles in initiating villous trophoblasts differentiation and fusion.

Specific Inhibitors for ERK1/2 and p38

The role of ERK1/2 and p38 has been evaluated using specific inhibitors (Fig (**5**)). The PD98059 compound or [2-(2-Amino-3-methoxyphenyl)-4H-1-benzopyran-4-one] is a selective and cell-permeable inhibitor of MEK that results in the inhibition of the activation (phos-phorylation) of ERK1/2. Its IC_{50} is between 2 and 7 μM [71]. The U0126 or [1,4-Diamino-2,3-dicyano-1,4-bis(2-amino-phenylthio) buta-diene] is a specific inhibitor of MEK1 (IC_{50} = 72 nM) and MEK2 (IC_{50} = 58 nM). It has very little effect on other kinases such as Abl, Cdk2, Cdk4, ERK, JNK, MEKK, MKK-3, MKK-4/SEK, MKK-6, PKC, and Raf [72]. The SB203580 compound or [4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1H-imidazole] is a highly selective, and cell-permeable p38 inhibitor (IC_{50} = 50 nM for p38α and 500 nM for p38β) [73]. The SB202190 or [4-(4-Fluoro-phenyl)-2-(4-hydroxyphenyl)-5-(4-pyridyl)1H-imida-zole] is a cell-permeable inhibitor of p38. It inhibits p38 phos-phorylation of myelin basic protein (MBP) with no effect on the activity of ERK nor JNK MAPK subfamilies. This compound inhibits the kinase activity of p38β (IC_{50} = 50 nM) and p38 phosphorylation of the activating transcrip-tion factor 2 (ATF-2; IC_{50} = 280 nM) [74,75]. These specific inhibitors for ERK1/2 and p38 impaired differentiation and syncytialization in primary villous trophoblast cultures [22].

Role of ERK1/2 and p38 in Differentiation of Villous Trophoblast Cells

Few studies have examined the implication of MAPK pathways in human trophoblasts differentiation. Null mutation in p38α gene, in mouse, results in embryonic lethality at mid-gestation, characterized by a severe reduction in the

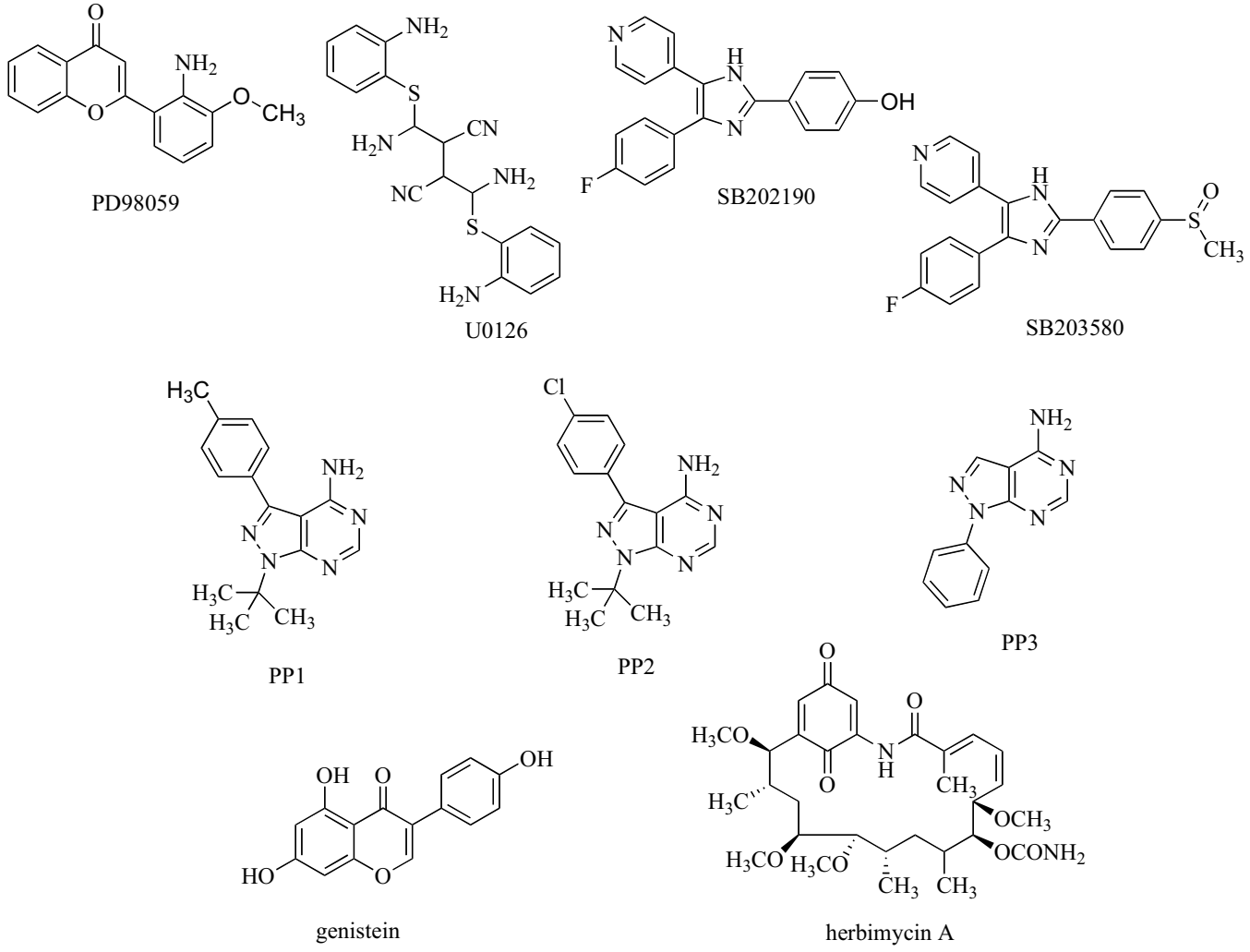

Fig. (5). Structure formulas of extracellular signal-regulated kinase (ERK), p38 MAPK and Src family kinase (SFK) inhibitors used in studies. PP1: 4-Amino-1-tert-butyl-3-(1′-naphthyl) pyrazolo[3,4-d] pyrimidine; PP2: 4-Amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo [3,4-d] pyrimidine; PP3: 4-Amino-7-phenylpyrazol[3,4-d]pyrimidine; U0126: 1,4-Diamino-2,3-dicyano-1,4-bis(2-aminophenylthio) butadiene; PD98059: 2-(2-Amino-3-methoxyphenyl)-4H-1-benzopyran-4-one; SB202190: 4-(4-Fluorophenyl)-2-(4-hydroxyphenyl)-5-(4-pyridyl)1H-imidazole; SB203580: [4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1H-imidazole].

spongiotrophoblast layer and a lack of vascularization of the labyrinth layer [76]. This suggests an important role for p38 MAPK signalling in placentation and trophoblast cells differentiation in mice. ERK2 knockout mouse leads to embryonic lethality in early gestation. Specifically, there is no formation of ectoplacental cone and extra-embryonic ectoderm, both are important for the formation of the trophoblasts. ERK1 do not compensate for the ERK2 function suggesting that ERK2 is essential for normal trophoblasts development in mouse [77].

In human placenta, ERK1/2 are present in the vCTB cells throughout pregnancy but not in the STB and phosphorylated ERK1/2 are immunolocalized in the vCTB up to 12 weeks of gestation [78]. In human primary villous trophoblast cells culture, from term placenta, the expression of ERK1/2 and p38 dramatically decrease in a time dependent manner, to reach an undetectable level after 5 days of culture. Furthermore, ERK1/2 and p38 are rapidly activated upon addition of fetal bovin serum (FBS), an essential factor for villous trophoblasts differentiation *in vitro* [22].

Treatment of villous trophoblast cells culture with ERK1/2 and p38 inhibitors reduces hCG and hPL secretion, with not effect on their synthesis, in a dose dependent manner. SB203580 is more effective than PD98059 to inhibit hCG and hPL secretion. Impaired fusion of the vCTB into syncytium is also observed [22]. These results also demonstrate a stronger effect for the p38 signalling pathway compared to ERK1/2, in the initiation of the villous trophoblasts differentiation process.

Implication of other Kinases Acting Upstream and/or Downstream of ERK1/2 and p38 in the Signalling Cascade Controlling Villous Trophoblasts Differentiation

Upstream events

SFK Pathway

The ERK1/2 and p38 signalling pathways can be activated by numerous stimuli. One of them is the tyrosine phosphorylation, known as major mechanism controlling many biological process including cells differentiation, morphology, proliferation, migration, and survival [79]. Protein tyrosine kinases (PTK) include receptor and non-receptor PTK. The Src family kinases (SFK) is one member of the non-receptor PTK.

The SFK family consists of nine proteins that can be divided in two groups: (1) Src, Fyn and Yes are ubiquitously expressed and (2) SFK, Blk, Fgr, Hck, Lck and Lyn are mainly found in hematopoietic cells and neurons [80-82]. These proteins have a common structure composed of six domains (1) SH4 domain, or N-terminal membrane-anchoring domain, responsible for recruiting SFKs to the membrane; (2) unique domain that is distinct and specific for each member; (3) SH3 domain which binds proline-rich sequences; (4) SH2 domain which binds to short amino acid sequences containing phosphotyrosine; (5) catalytic domain containing an autophosphorylation site at tyrosine (Tyr)-416, important for the regulation of kinase activity; and (6) short C-terminal domain containing a negative regulatory tyrosine residue, Tyr-527 [80]. SFKs mediate a variety of signalling pathways, including cell differentiation process of several cell types [83].

Specific Inhibitors for the SFK Family

Different types of SFK inhibitors are commercially available (Fig. (**5**)). Herbimycin A is a potent and cell-permeable PTK inhibitor with a Src selectivity (IC_{50} = 12 μM). Herbimycin A covalently interacts with thiol groups on protein tyrosine kinases [84]. PP1 or [4-Amino-1-tert-butyl-3-(1′-naphthyl) pyrazolo[3,4-d] pyrimidine] and PP2 or [4-Amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo[3,4-d] pyrimidine] are cell-permeable inhibitors of SFK. PP1 and PP2 act as competitive inhibitors of ATP for SFK [85]. PP1 inhibits v-Src (IC_{50} = 1.0 μM), c-Src (IC_{50} = 5 nM) and FynT (IC_{50} = 600 nM) [86]. PP2 inhibits c-Src (IC_{50} = 5 nM), lck (IC_{50} = 4 nM), Fyn (IC_{50} = 5 nM), and Hck (IC_{50} = 5 nM). PP2 also inhibits the activation of focal adhesion kinase (FAK) [87]. PP3 or [4-Amino-7-phenylpyrazol[3,4-d]pyrimidine] is a negative control for the SFK inhibitor PP2 [88]. Genistein or [4′,5,7-Trihydroxyisoflavone] is a broad range tyrosine kinase inhibitor. All these SFK inhibitors have been used to demonstrate the implication of SFK in villous trophoblasts differentiation [24].

Role of SFK in Villous Trophoblasts Differentiation

During rat trophoblast GC differentiation, Src, Yes and Lyn are activated and play different roles. Src and Yes are activated in proliferating and differentiating GC. Lyn is activated only in differentiating GC and shows a differentiation-dependent accumulation [89]. In human placenta, c-Src mRNA expression enhances during the first trimester of gestation and decreases thereafter, suggesting a role in early trophoblast development [90].

Daoud *et al.* demonstrated that human villous trophoblast expressed all members of SFK and some of them are expressed as different splice variants [24]. In primary villous trophoblast cells culture, no difference in mRNA expression profile of Fyn, Hck and Lyn was observed, while Fgr, Lck, Src, and Yes shown an increase of their mRNA expression during differentiation. In addition, Src was rapidly phosphorylated at Tyr-416 and dephosphorylated at Tyr-527 after FBS addition. Surprisingly, inhibition of SFK by PP2 or herbimycin A have different effects on villous trophoblast differentiation. Genistein and herbimycin A inhibited morphological and biochemical differentiation, whereas PP2 increases hCG and hPL secretion without affecting cells fusion. Therefore, PP2 plays different role on biochemical and morphological villous trophoblast differentiation: It stimulates the first and inhibits the second. These results showed that SFK play different roles in trophoblast differentiation, probably depending on specific SFK activation.

SFK and MAPK Pathways Interaction

It seems clear that SFK and MAPK members are involved in trophoblast cells differentiation. The SFK members can stimulate MAPK, resulting in cell survival, division, differentiation, and gene transcription regulation [80]. In human primary villous trophoblast cells culture, treatment

with PP2 for three days increases ERK1/2 and p38 phosphorylation independently of Src activation. When cells are starved overnight and then challenged with PP2, p38 acti-vation is increased, while ERK1/2 activation remains unchanged. Moreover, when cells were preincubated with PP2 for two hours and then challenged with FBS, PP2 had no effect on ERK1/2, while it increased p38 phosphorylation. Those results suggest that the activation of ERK1/2 by FBS is not regulates by Src. Another hypothesis to explain the regulation of MAPK by PP2 is that Src acts as a repressor for ERK1/2 and p38, and that the inhibition of Src by PP2 supresses its repressive effect on ERK1/2 and p38 [91]. Several others factors can interfere between SFK and MAPK. One on these factor is the FAK. It is well known that SFK can phosphorylate FAK which is very important for cell adhesion, spreading, migration, shape, and differentiation [80]. Moreover, implication of FAK in placental implantation, and cytotrophoblast migration, invasion and differentiation has been reported [92].

SFK and FAK Pathways Interaction

The FAK family contains two proteins, FAK1 and FAK2. FAK1 plays a major role in cell adhesion and migration, and is mainly regulated by the integrins [93]. FAK2 is a cytoplasmic PTK which is involved in calcium-induced regulation [94].

FAK activation is characterized by a phosphorylation cascade of many tyrosine residues. First, Tyr 397 is phosphorylated and represents the activation state of the protein. Then, different members of SFK interact with FAK and phosphorylate many of its tyrosine residues in the kinase domain (Tyr 407, 576 and 577) and the C-terminal region (Tyr 861 and 925). The phosphorylation of Tyr 576 and 577 by Src increases the kinase activity of FAK and generates new binding sites for other ligand-effectors [95,96].

In human placenta, FAK and paxillin, a focal adhesion-associated protein, are highly expressed between the 5^{th} and 8^{th} weeks of gestation, particularly in vCTB and evCTB, and dramatically decrease after 10 to 12 weeks of gestation. Activated FAK, phosphorylated on Tyr-397, interacts with α5 integrin and matrix metalloproteinase-2 (MMP2) in evCTB. Studies with antisense FAK oligonucleotides have shown dramatic reduction of trophoblast outgrowth with drastic reduction in cell proliferation and MMP2 activity. In light of these results, FAK seems a key kinase in early trophoblast differentiation and in the regulation of trophoblast proliferation and migration during early placental development [97].

In human villous trophoblast cells culture, FAK1 expression increases in a time-dependent manner. Neither FBS nor PP2 have an effect on rapid transient FAK1 activation in these cells. However, in cells treated 24 h or more, herbimycin A or PP2 inhibits FAK1 phosphorylation at many sites (Tyr 397 and 576/577). This difference between transient rapid (0–60 min) and long term (day 2–day 4) activation of FAK1 might be due to differences between signals responsible for FAK activation. Integrins might be responsible of rapid FAK activation while SFK might be responsible for long term FAK activation. Thus, FBS-independent Src activation may induce FAK1 activation, which results on cells adhesion and spreading via MAPK-dependent or -independent pathways [91].

SFK and C-Terminal Src Kinase (Csk) Pathways Interaction

SFK are inactivated by phosphorylation of Tyr-527 by Csk and Csk-homologous kinase (CHK). *In vitro*, both phosphorylate the inhibitory C-terminal tyrosine of several SFK members, including c-Src, Lck, Fyn, and Lyn. Csk has a ubiquitous expression while the expression of CHK is limited to neuronal and hematopoietic cells [98,99]. A loss of Csk expression results in a decrease in the abundance of the Src and Fyn proteins, which could be restored by reintroducing catalytically active Csk. The effect of Csk on Src expression is not due to an increase in Src mRNA, but to a stabilization of the Src protein [100].

In human primary villous trophoblast cells culture, Csk and all isoforms of CHK are expressed. During villous trophoblast differentiation, mRNA expression of Csk increases in a time-dependent manner while its protein expression remains unchanged. Treatment of vCTB with PP2 increases Csk protein levels which are associated with an increase in Src protein levels. This correlation between Src and Csk protein expression is important for a regulated Src activation and stabilization [91].

Growth Factors Regulation

The MAPK signalling cascades are initiated by various stimuli as growth factors, cytokines, hormones, extracellular matrix adhesion and cell-to-cell contact. Depending on the stimulus, MAPK signalling pathways have different effects on cells. For example, SKF-activated MAPK signalling pathway can pre-vent villous trophoblasts differentiation stimulated by fibro-blasts growth factor 4 (FGF4) [101]. FGF4, via a tyrosine phosphatase Shp2, is required to repress differentiation and ensure vCTB stem cells renewal. FGF4 causes FGF receptor 2 (FGFR2) phosphorylation in trophoblast stem cells, followed by the formation of a Grb2/FRS2α/Shp2 complex that activates ERK2 via SFK. This signalling pathway inhibits the proapoptotic protein Bim and the apoptosis of the trophoblast stem cells [102] and their differentiation [103]. On the other hand, EGF is known to induce vCTB differentiation [27]. EGF-stimulated villous trophoblast cells fusion and hCG secretion requires p38 activation [104].

Downstream Events

p38 Regulates PPARγ in Villous Trophoblast

The peroxisome proliferator-activated receptors (PPAR) are members of the nuclear hormone receptor superfamily of ligand-dependent transcription factors. Three subtypes have been identified, PPARα, β and γ. In human placenta, all PPAR subtypes are expressed and have various functions [105,106]. They regulate placental fatty acid metabolism [106,107]. They are also involved in evCTB invasion and vCTB differentiation [105,106]. Treatments of human primary villous trophoblast cells with specific PPARγ ligands stimulated hCG secretion and vCTB differentiation [105,108]. However, in the same model, PPARα and PPARβ activation have no effects on hCG secretion [109].

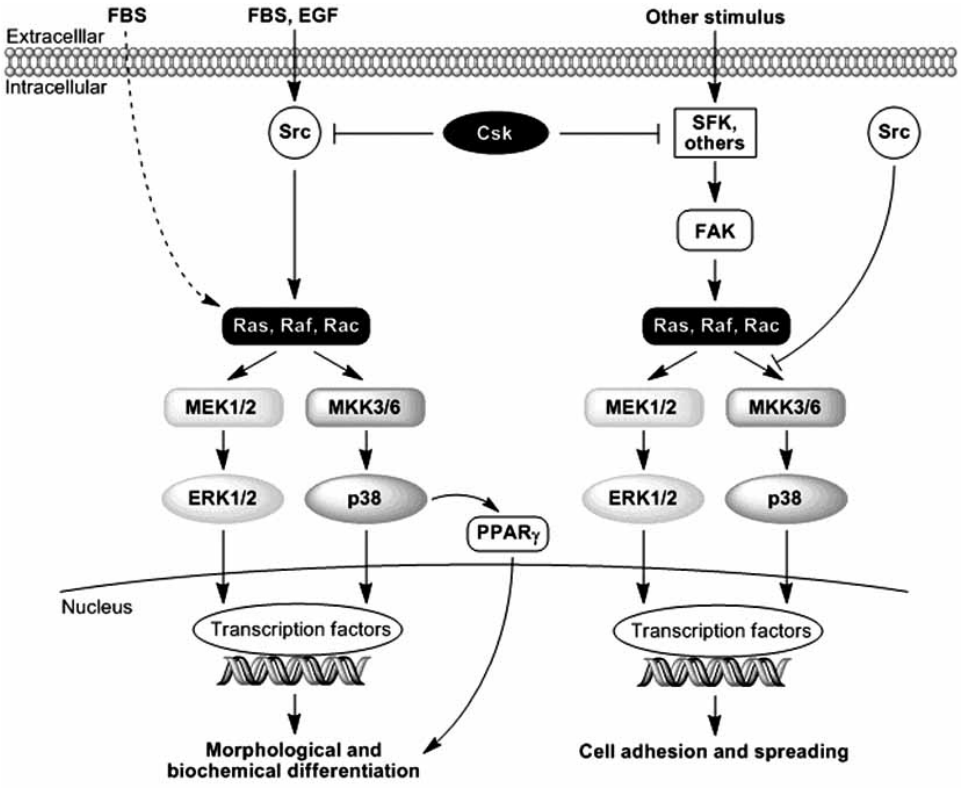

Fig. (6). Schematic presentation of signal transduction pathways involved in villous trophoblast differentiation. The pathways are activated by hormones or growth factors fixation with their receptors, leading to phosphorylation of kinases and finally to villous trophoblast differentiation. Abbrevation: FBS: Fetal serum bovin; EGF: Epidermal growth factor; MEK: MAPK/ERK kinase; MKK: MAPK kinase; ERK: extracellular signal-regulated kinases; PPAR: peroxisome proliferator-activated receptors; Csk: C-terminal Src kinase; SFK: Src family kinase; FAK: focal adhesion kinase.

Similarities between the p38α-null mice and the PPARγ-null mice in placental abnormal development and vascularization suggest a cross talk between those signalling pathways [76,110]. In human primary villous trophoblast cells, the p38 inhibitors SB203580 and SB202190 decrease the transcriptional activity of PPARγ and the PPARγ-stimulated hCG production. Moreover, inhibition of p38 reduces expression of PPARγ protein without any effect on mRNA expression [111]. These data indicate that p38 regulates PPARγ expression and activity in human term villous trophoblast cells.

CONCLUSION

Tightly controlled continuous formation of STB is a requisite for the integrity of the placental barrier and pregnancy well-being. In this review, we show that MAPK signalling pathways play a key role in villous trophoblasts morphological and biochemical differentiation. Hence, dysregulation of MAPK pathways could results in aberrant STB formation and, by consequent, to obstetric complications. Indeed, a study reports that the activation of the JNK transcription factors, c-Jun and Rac1, are significantly lower in placentas from women with preeclampsia and HELLP syndrome compared to normotensive women. Moreover, p38 phosphorylation is significantly higher in placentas of women with preeclampsia than with HELLP syndrome while ERK1/2 activity shows no difference between both groups [112]. The role of each member of the MAPK family is complex (Fig. (**6**)). Better understanding of the role and mechanisms of action of the MAPK in villous trophoblasts differentiation will help to guide the pharmaceutical/chemical industry to develop new drugs and therapeutic and/or diagnostic avenues for the obstetric diseases associated with an alteration in villous trophoblast differentiation, such as preeclampsia and HELLP syndrome.

ABBREVIATIONS

ATF-2	=	Activating transcription factor 2
Blk	=	B lymphoid tyrosine kinase
Cdk	=	Cyclin-dependent kinase
CHK	=	Csk-homologous kinase
Csk	=	C-terminal Src kinase
C-terminal	=	Carboxy-terminal
EGF	=	Epidermal growth factor
ERK	=	Extracellular signal-regulated kinases
evCTB	=	Extravillous cytotrophoblast
FAK	=	Focal adhesion kinase
FC	=	Fetal capillaries.
FGF4	=	Fibroblasts growth factor 4

FGFR = FGF receptor

FRS2α = FGFR Substrate 2 α

Fgr = Gardner-Rasheed feline sarcoma viral (v-fgr) oncogene homolog

FBS = Fetal bovin serum

GC = Trophoblast giant cells

hCG = Human chorionic gonadotropin

Hck = Hemopoietic cell kinase

HELLP = Hemolysis, elevated liver enzymes, and low platelet count

hPL = Human placental lactogen

IC_{50} = Concentration of an inhibitor that is required for 50% inhibition

IVS = Intervillous space

JNK = c-Jun NH2-terminal kinase

Lck = Lymphocyte specific protein tyrosine kinase

Lyn = v-yes-1 Yamaguchi sarcoma viral related oncogene homolog

MAPK = Mitogen-activated protein kinases

MBP = Phosphorylation of myelin basic protein

MEK = MAPK/ERK kinase

MEKK = MAPK/ERK kinase kinase

MKK = MAPK kinase

MMP2 = Matrix metalloproteinase-2

N-terminal = Amino-terminal

PD98059 = 2-(2-Amino-3-methoxyphenyl)-4H-1-benzopyran-4-one;

pGH = Placental growth hormone

PKC = Protein kinase C

PP1 = 4-Amino-1-tert-butyl-3-(1′-naphthyl) pyrazolo[3,4-d] pyrimidine

PP2 = 4-Amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo[3,4-d] pyrimidine

PP3 = 4-Amino-7-phenylpyrazol[3,4-d]pyrimidine

PPAR = Peroxisome proliferator-activated receptors

PTK = Protein tyrosine kinase

RNA = Ribonucleic acid

SA = Uterine spiral arterie

SB202190 = 4-(4-Fluorophenyl)-2-(4-hydroxyphenyl)-5-(4-pyridyl)1H-imidazole

SB203580 = [4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1H-imidazole]

SEK = SAP (stress-activated protein) /ERK kinase-1

SFK = Src family kinase

Src = v-Src Sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian)

STB = Syncytiotrophoblast

Tyr = Tyrosine

U0126 = 1,4-Diamino-2,3-dicyano-1,4-bis(2-aminophenylthio) butadiene

vCTB = Villous cytotrophoblast

UV = Ultra-violet

Yes = v-yes-1 Yamaguchi sarcoma viral oncogene homolog

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