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Signal Transduction in Trophoblast Invasion

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Abstract

During the first trimester of pregnancy, well-differentiated primary cells of the placenta known as trophoblast cells grow in an invasive and destructive fashion similar to malignancies, but limited in space and time. The comparison of trophoblast cells with their malignant counterpart, human choriocarcinoma cells, offers an attractive model to understand the origin or development of malignant growth. Several cytokines and growth factors are known to influence trophoblast migration (e.g. EGF, IGF-2, HGF), proliferation (e.g. leptin, HGF, GM-CSF) and/or invasion (e.g. leukemia inhibitory factor, LIF), each factor utilizing at least one pathway for intracellular signaling in the trophoblast. Two pathways that are crossed especially often mediate the signals of these factors and are simultaneously well established in terms of tumor invasion: the Janus kinase-signal transducers and activators of transcription (Jak-Stat) and receptor-associated tyrosine kinase-mitogen-activated protein kinase (RTK-MAPK) pathways. These two pathways are detrimental for reproduction in general, and in part for placenta development, as a series of knockout experiments demonstrate. Aspects of each pathway are also implicated to be involved in trophoblast invasion, e.g. STAT3 is constitutively activated in invasive first trimester trophoblast cells, and activated ERK is detectable in intermediate trophoblast cells, an invasive phenotype. Interaction at several intersection points between the pathways has been described in several cell systems so that the same would seem to be possible in trophoblast cells. In this review, some of the possible areas of interaction are alluded to.

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Introduction: Trophoblast and Tumor as Models for Comparative Investigation of Signal Transduction

Analogies between the fetus and cancer have frequently been made [1–4]. This is mainly because blastocystic cytotrophoblast cells (CTB) and extravillous

trophoblast cells (EVT), often termed intermediate trophoblast, display highly invasive features especially during implantation and the first trimester of pregnancy. Several aspects of malignant and trophoblastic invasion, migration and proliferation are similar, if not identical. The adhesion molecule and protease profile as well as the underlying autocrine/paracrine dialogue involved in attachment and assault and the method of evasion from host (maternal) immune system are comparable. One chief difference, though, between the two situations is spatial and temporal containment. Invasive growth by EVT is restricted to the decidua of first trimester uteri in healthy pregnancies. Pathological invasion and destruction involved in tumor expansion knows no such boundaries. Another difference is that trophoblast cells, being physiological cells, remain well differentiated, whereas malignant cells are transformed, thus exhibiting signs of dysregulation, especially in respect to survival, proliferation and invasion.

This unique comparability makes EVT an attractive model for investigating intracellular regulatory mechanisms of invasion, particularly because information pertaining to the promotion and demotion of invasion during a normal course of pregnancy is deficient on the signal transduction level.

The fact that trophoblast cells are not only able to implant in other organs, as in ectopic pregnancies [5], but also to act as invasive xenogeneic transplants, e.g. murine renal subcapsular space [6], implies that trophoblast cells themselves greatly contribute to the mechanism of invasion.

Numerous cytokines, often secreted by the trophoblast cell themselves, have been described to influence trophoblast migration, proliferation, differentiation and invasion. Naturally, these cytokines exert their effects through use of one or more signal-transducing pathways (assorted selection of cytokines, table 1). Two pathways seem to be frequented more often: the signal transducer and activator of transcription (Stat)- and mitogen-activated protein kinase (MAPK)-mediated ones. A more detailed description follows.

Janus Kinase-Stat Signal-Transducing Pathway

The Pathway

Cytokine binding leads to receptor aggregation, resulting in the juxtaposition of the Janus kinases (Jaks), which are receptor-associated tyrosine kinases named so because of their two symmetrical kinase-like domains, bearing similarity to the two-headed mythological Roman god, Janus [7]. The Jaks are now able to cross-phosphorylate and activate each other, as well as tyrosine residues on cytoplasmic domains of their respective cytokine receptor, thus leaving behind binding points for phosphotyrosine-binding (PTB) domains or any protein processing Src homology 2 (SH2). These

Table 1. Assorted cytokines, their effects on human trophoblast cells and signal transduction

Mediator	Invasion	Proliferation	Migration	Differentiation	Signal transduction	Effect of signal transduction
EGF (epithelial growth factor)	↑ [58]	↑ [59]	↑ [56]	↑ to syncytium [60]	MAPK, PI3K [56]	Migration [56]
CSF-1 (colony-stimulating factor-1)	– [61]	↑ [61]	?	↑ to syncytium [60]	?	?
VEGF (vasoendothelial growth factor)	↓ [62]	↑ [62]	↓ [62]	↑ to syncytium in CTB [63]	MAPK (in Jeg-3) [64]	Probably invasion (MMP ↑) [64]
PlGF (placental growth factor)	↓ [65]	↑ [65]	↓ [65]	– [63]	MAPK [34]	Uterine Vasculogenesis [34]
IGF-2 (insulin-like growth factor-2)	↑ [66]	– [67]	↑ [68]	?	MAPK [68]	Migration [68]
TGF-β (transforming growth factor-β)	↓ [66]	↓ [69]	↓ [66]	↓ to syncytium [60]	Smad3 [70]	?
Decorin	↓ [66]	↓ [71]	↓ [66]	?	For trophoblast? PKB for endothelial cells [72]	?
IGF-1 (insulin growth factor-1)	↑ [73]	↑ [59]	↑ [74]	↑ to syncytium [75]	?	?
GM-CSF (granulocyte macrophage-colony-stimulating factor)	?	↑ [76]	?	↑ to syncytium [60]	PKC (in Jeg-3, JAR) [77]	Production of ovine IFN-τ [77]
HGF (hepatocyte growth factor)	↑ [78]	↑ [79]	↑ [80]	↑ to syncytium (in mice) [81]	MAPK, PI3K [82]	Motility [82]

Table 1. (continued)

Mediator	Invasion	Proliferation	Migration	Differentiation	Signal transduction	Effect of signal transduction
LIF (leukemia-inhibitory factor)	↑ [19]	↑ [19]	?	↑ indirectly, to syncytium [83]	gp130 [19]	Invasion, proliferation [19]
TNF- α (tumor necrosis factor- α)	↓ [84]	↓ [85]	↓ [84]	↓ to syncytium [86]	NK- κ B [87]	Production of prostaglandins [87]
Leptin	↑ (in mice) [88] ↑ (MMP↑) [89]	↑ (in JAR) [90]	?	↑ to invasive phenotype [89]	Stat (murine oocyte) [91] MAPK in JAR [90] in BeWo [39]	Oocyte maturation [91] Proliferation [39, 90]
IL-10	↓ [92]	?	?	?	?	?
IL-6	↑ [19]	↑ [93]	?	- [86] ↑ (to syncytium) [93]	For trophoblast? gp130 for lymphocytes [94]	?
IL-1 β	↑ [95]	- [96]	↑ [97]	- [98]	For trophoblast? MAPK, NK- κ B (in baboon decidua) [99]	?

include the Stats as well as Ras, phosphatidylinositol 3-kinase (PI3K) and phospholipase C- γ [8].

Upon binding the tyrosine-phosphorylated receptor ligands, the Stats are themselves phosphorylated on specific tyrosine or serine residues by the Jaks, following which the Stats disassociate from the ligand, proceed to form homo- and heterodimers with other phosphorylated Stats and translocate into the nucleus where they bind to specific DNA sequences in the promoter regions of target genes, thus regulating the transcription of these proteins, fulfilling the prophecy implied in their names [9]. Stats also accelerate the transcription of suppressors of cytokine signaling (SOCS), which inhibit the Stat-mediated signal transduction as classical feedback inhibitors [8].

Introducing Stat3

One of the possible regulatory candidates is Stat3. Aberrant activity of phosphorylated, dimerized Stat3 is advocated to be causal for neoplastic cell behavior, e.g. hyperplasia, longevity or invasion, and thus for the malignancy of cells [10]. Indeed, constitutively activated Stat3 has been found in a number of tumors [11].

Stat3 Knockout Model: Relevance for Reproduction?

Evidently, Stat3 has proven to be indispensable for murine pregnancy as it is activated during the early postimplantation period of the mouse and is essential for embryogenesis. Wild-type mouse embryos express Stat3 on the extraembryonic visceral endoderm 7.5 days postcoitum. Concurrent to this, Stat3^{-/-} mice degenerate and die, but can be rescued through substitution with an alternative splice form of Stat3, Stat3 β , in which the C-terminal transactivation domain is replaced with a seven-amino acid extension [12–14].

Furthermore, several cytokines that are relevant to reproductive biology, especially those of the interleukin-6 (IL-6)-type family, can activate the Jak-Stat signal-transducing pathway [15]. The IL-6 receptor consists, amongst others, of the signal-transducing subunit gp130 which is common for all receptors of the IL-6-type family and through which the signal is generated [16, 17].

Stats and Trophoblast Invasiveness

Evidence illustrating that the presence of constitutively activated Stat3 correlates positively with the invasiveness of trophoblast phenotypes or their malignant derivatives, choriocarcinoma cells, has recently been exposed [18]. Here, it was demonstrated that constitutive activation of Stat3 ceased at the same time as loss of invasive properties of trophoblast cells progressively from first trimester to term. However, highly invasive choriocarcinoma cells constitutively expressed activated Stat3 to a much higher degree than the invasive trophoblast phenotype.

A further study indicated that LIF was able to trigger Stat3 activation in Jeg-3 choriocarcinoma cells, a rather low-invasive cell line compared to other choriocarcinoma cells [19]. The higher activation of Stat3 led to higher proliferation and invasion rates, which seems to be due, at least in part, to the suppressed expression of tissue inhibitor of metalloproteinase 1 (TIMP-1) in conjunction with an increased caspase-4 expression. TIMP-1 is linked to the inhibition of metastasis [20] by inhibiting all metalloproteinases (MMPs), but preferentially binding MMP-9 [21], which is considered crucial for CTB invasion [22]. Caspase-4, formerly coined IL-1 β -converting enzyme homologue 2 (ICH-2), enzymatically produces the bioactive form of IL-1 β , as its name implies [23]. The significance of IL-1 β for invasion can be gathered from table 1.

Another noteworthy fact is that four further cytokines or growth factors (GM-CSF, IGF-2, HGF, and IL-6), propagated to mediate their respective effects through tyrosine phosphorylation of Stat3, were also investigated regarding their capacity to activate Stat3 in Jeg-3 choriocarcinoma cells. LIF was the only cytokine capable of triggering Stat3 activity, while the above-mentioned cytokines were negative in this respect [19]. This suggests that these cytokines use alternative pathways to mediate their effects although they all possess the gp130 signal-transducing subunit. This point will be discussed later.

Furthermore, it should be remembered that Jeg-3 cells are transformed trophoblast cells, and serve solely as a model for trophoblast behavior. It remains to be investigated whether this susceptibility towards LIF with the resulting change in proliferation, invasion and protease expression is a manifestation of their transformed state or whether this is indeed a mechanism that physiological trophoblast utilize as well. One study seems to support the idea that trophoblast cells react in the same manner as choriocarcinoma cells in respect to LIF [24]. Stat3 was functionally knocked down in Jeg-3 choriocarcinoma, first trimester and term trophoblast cells via RNAi (RNA interference) and then assessed for invasion with or without LIF supplementation. In invasion assays with control cells, where no Stat3 was knocked down, invasion was significantly elevated through LIF supplementation for all mentioned cell types including the usually noninvasive term trophoblast cells. As to be expected, choriocarcinoma cells were the most and term trophoblast cells the least respondent to LIF stimulation. Upon Stat3 knockdown, invasive capacity of all cells were clearly reduced irrespective of the presence of LIF [24].

Ras Protein-MAPK Signal-Transducing Pathway

The significance of the Ras-MAPK for cellular programs such as proliferation, differentiation, development, survival, transformation and apoptosis has

been maintained in many papers and reviews in the past. In this report excessive details have been omitted in order to achieve clarity. The following contains an outline of some interesting findings as regards certain aspects of reproductive or tumor immunology and biology.

The Pathway

Upon activation of receptor tyrosine kinases (RTK) through specific cytokine-receptor interactions, RTK phosphorylates tyrosine residues on its inner receptor ligands in a fashion reminiscent of the Jaks, as mentioned above. These binding points attract several signaling proteins, such as the Grb-2 (growth-factor-receptor-bound) adaptor protein or SH2-domain-containing tyrosine phosphatases (SHP2), which simultaneously binds to further proteins that ultimately activate monomeric GTPases termed Ras. This sets off a chain reaction resulting finally in the activation of MAPK through consecutive activation of first MAPK kinase kinase (MAPKKK) and then MAPK kinase (MAPKK). The activated MAPK can enter the nucleus and phosphorylate one or more components of a gene regulatory complex (fig. 1), which activates the transcription of a set of immediate early genes (named so because they are so very rapidly turned on in response to extracellular signals). The resulting change in gene expression leads to changes in cell behavior modulating protein activity [as reviewed in 25].

Introducing MAPK

MAPKs are subdivided into four main groups: the classical MAPKs, c-Jun N-terminal kinases (JNKs, often called 'junks', but also referred to as stress-activated protein kinase, SAPK), p38s, and atypical MAPKs. Numerous isoforms of MAPKs, MAPKKs, MAPKKKs and Ras have been identified in mammalian cells (table 2) [26]. Notably, MKK4 has been recognized as a putative tumor suppressor gene in human solid tumors of the breast, prostate and pancreas [27].

MAPK and Reproduction

MAPK and the Oocyte. Since MAPK is pivotal in the cell cycle regulation, it is no surprise that MAPK is also involved in oocyte maturation. Oocytes express the Mos protein during meiotic maturation from the G2 to M phase. MAPK activation through the Mos protein seems to be necessary for activation and stabilization of the M phase-promoting factor, a protein considered to be the master of cell cycle switch. However, Mos protein expression in ectopic, somatic cells results in neoplastic transformation (or uncontrolled G1/S transitions) of these cells [28, 29].

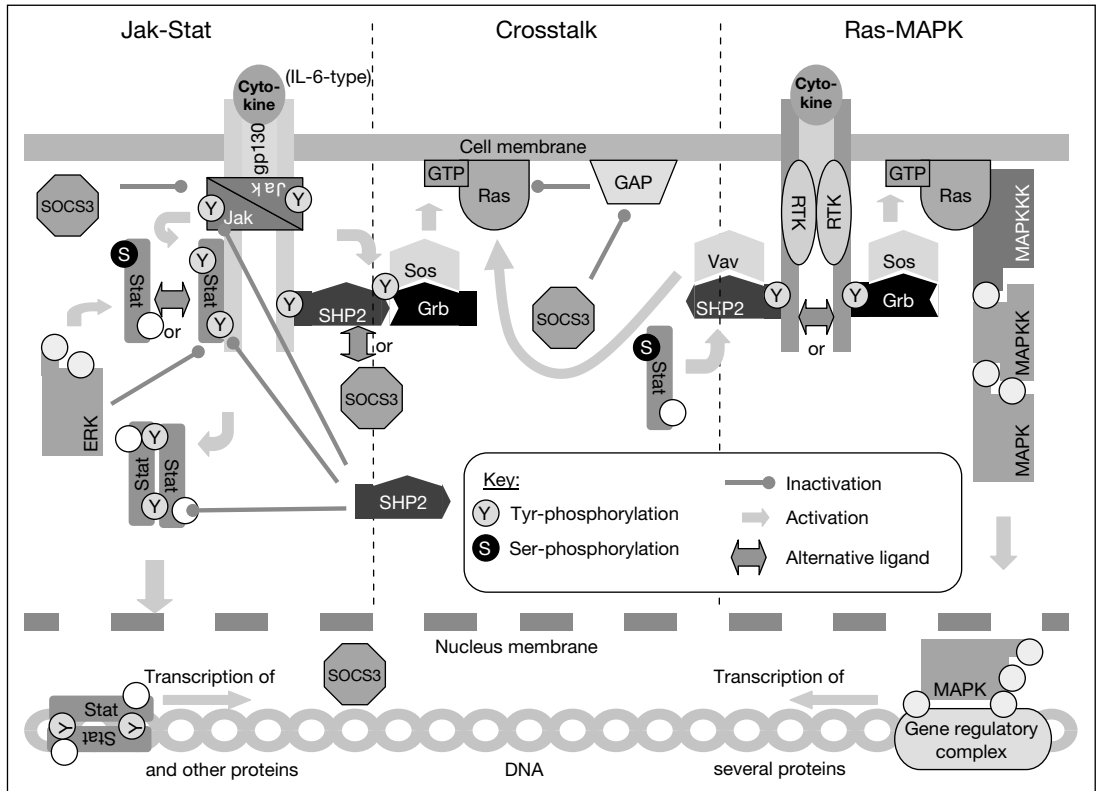


Fig. 1. Simplified animation of the interaction between Jak/Stat and Ras-MAPK pathways as interpreted from the current literature. Cytokine activation of the Jak/Stat pathway leads to transcription of a variety of proteins including SOCS. These regulate the intensity of the signal by binding to Jaks. Activation of the Ras-MAPK pathway occurs via different mechanisms: phosphorylated tyrosine residues on the inner receptor ligands attract Grb-2 or SHP2, which simultaneously bind to further proteins (Vav or Sos, respectively) that ultimately activate Ras. Alternatively, Jak1-phosphorylated SHP2 may act as an adapter by linking Grb-2, which in turn links Sos and activates Ras. Simultaneously, it competes with Stat3 for the tyrosine phosphorylated binding site on the inner receptor ligand. The cross talk between Jak/Stat and Ras-MAPK systems is partly regulated by SOCS, which can compete with SHP2, but which can also block GAP, a deactivator of Ras. On the other hand, ERK, a kinase of the MAPK system, induces Stat serine phosphorylation which may compete with tyrosine phosphorylated Stats. It can also promote the activation of SHP2.

MAPK Knockout Models. Knockout models of various MAPK signal-transducing components in the mouse have led to the perception that MAPK signaling is also important in the development of the placenta. MEKK3, p38 α and ERK5 $-/-$ mice all display defects in angiogenesis and placental formation

Table 2. Major MAPK cascades in mammalian cells [28]

Activators	Growth factors, cytokines	Growth factors, cytokines, stress, TGF- β	Growth factors, cytokines, stress, ceramides
MAPKKK	↓ Raf	↓ MEKK 1-4, MLKs, ASK, TAK1	↓ MEKK 1/4, MLKs, ALS, TAK1
MAPKK	↓ MEK 1/2 (MKK 1/2)	↓ MKK 3/6, MKK4	↓ MKK 4/7
MAPK	↓ ERK 1/2	↓ P38	↓ JNK/SAPK
Substrates	↓ 90 ^{RSK} , MNK 1/2, Ets, Elk1, Myc, Stat 1/3, ER	↓ Hsp 27, PLA2, MNK 1/2, APKA2, Myc, MSK-1, Elk1, ATF-2, Stat1	↓ c-jun, ATF2, Elk1, DPC4, p53, NFAT4
Cell responses	↓ Proliferation, differentiation, development	↓ Proliferation, differentiation, development, inflammation, apoptosis, stress response	↓ Proliferation, differentiation, apoptosis

that lead to embryonic lethality [30–33]. The detrimental placental deviations due to (lack of) the above-mentioned factors point to anomalies especially in placental vasculogenesis. Particularly the labyrinth layer seemed to be greatly reduced in all three investigations; however, p38 α mutants also exhibited a significant alleviation of spongiotrophoblast (the murine correlate to column CTB), which seemed to be due to a higher rate of apoptosis. Development of trophoblast giant cells (the murine correlate to invasive CTB) were apparently not affected. This suggests an independent requirement for p38 α in diploid trophoblast development. The observation that p38 MAPK activation is perceptible in human syncytiotrophoblast in response to PIGF in vitro could support this theory [34].

MAPK and the Endometrium. Trophoblast cells must first penetrate the luminal epithelium of the endometrium before invasion into the endometrial stroma. A line of reasoning valuable to trophoblast invasion is the aspect that luminal endometrial epithelial cells (EEC) and decidualized endometrium play a role in regulating trophoblast invasion. BeWo choriocarcinoma cells that were cocultured with EEC attached to EEC prior to expansion and invasion, which resulted in a significantly higher rate of apoptosis in EEC. As a consequence thereof, p38 MAPK were activated in EEC. On the other hand, the inhibition of p38 MAPK induced the inhibition of both EEC apoptosis as well as trophoblast invasion [35]. Moreover, endometrial stromal cells respond toward IL-1 β with the stimulation of proinflammatory cytokines and an upregulation of p38 MAPK. Inhibition of p38 MAPK and decidualization of EEC through progesterone diminished this IL-1 β -induced response, indicating that decidualization leads to a diminished proinflammatory response to IL-1 β of EEC through attenuation of IL-1 β -induced p38 MAPK activation [36]. This could be of possible relevance for the control of trophoblast invasion.

MAPK in Trophoblast Cells. The information thus far would seem to suggest that MAPK is not involved in trophoblast invasion, or if so, then only indirectly. However, a recent study of expression and activation of MAPK and ERK patterns in human villous trophoblast cells, mostly through immunohistochemistry and in situ hybridization, suggests the contrary. It was demonstrated that villous syncytiotrophoblast cells were negative for total and activated ERKs throughout pregnancy [37]. Villous CTB cells were positive for total ERK throughout pregnancy, but activation of this could be seen only from the 1st to the 12th week of gestation. This activation correlated with c-met (HGF receptor) expression and rate of proliferation. This information agrees well with past studies pointing to the role of HGF in trophoblast proliferation and MAPK in proliferation processes in general. Surprising, though, was the piece of evidence showing that intermediate trophoblast cells (invasive EVT cells) were positive for total and activated ERKs throughout pregnancy. It should be noted that these

cells are not proliferative [38], so that the possibility of linking MAPK activation to intermediate trophoblast invasion is principally possible.

The fact that VEGF could upregulate the expression of MMPs through MAPK activation would reinforce this thought.

Possible Cross Talk?

A Few Examples: Leptin Receptor and gp130

As mentioned earlier, it appears that certain cytokines generate their effects through certain pathways in certain cells, although they feature the capability to transduce their signals through other pathways in other cells. Leptin, for example, is a substance which is able to modulate trophoblast activity, and for which leptin receptors exist on trophoblast cells. The leptin receptor contains a gp130 receptor subunit through which it could potentially transduce its signal to Jak and Stat, but at least in BeWo choriocarcinoma cells, leptin could not enhance Jak2, Stat1 or Stat3 phosphorylation, although Jak2 was constitutively activated. In contrast to this, leptin stimulated cell proliferation and the c-fos gene, an immediate early gene, through the phosphorylation of p42-MAPK [39].

Furthermore, one group generated mice with a COOH-terminal gp130^{ΔStat} 'knockin' mutation, which deleted all Stat-binding sites, in order to define Stat-dependent responses. As in LIF^{-/-} mice, in gp130^{ΔStat} mice blastocysts also failed to implant, and gp130^{ΔStat} mice generally phenocopied LIF or IL-6-deficient mice, with the exception of gastrointestinal ulceration and severe joint disease. It was shown here that this mitogenic hyperresponsiveness of synovial cells in response to the cytokines mentioned was due to sustained gp130-mediated SHP2/Ras/ERK activation, which would normally have been limited through induction of suppressor of cytokine signaling 1 (SOCS1), usually induced through Stat signaling [40].

MAPK-Stat Interactions

These findings indicate that a balance exists between the several transducing pathways and that the regulation thereof takes place through communication between them. The following encompasses an attempt to illustrate recent advances made in defining aspects of signal modulation between the Stat and MAPK systems.

As alluded to earlier, activation of IL-6-type cytokine receptors also leads to MAPK activation, sometimes with the effect of preventing Stat activation. There appear to be a few modes in which this can develop.

Direct Mechanism. One study exposes a direct mechanism of MAPK intervention in the Stat pathway [41]. IL-1 was shown to directly and specifically

inhibit IL-6-induced Jak-Stat signaling through activation of the p38 system independent of protein synthesis (e.g. SOCS).

Tyr⁷⁵⁹: A Common Binding Site. The Stat-binding site Tyr⁷⁵⁹ on gp130 and Tyr⁹⁷⁴ on LIF receptor of certain cells attracts SHP2, which is then phosphorylated by Jak1. SHP2 may now act as an adapter by linking Grb-2, which in turn links Sos (Son of sevenless) and activates Ras (fig. 1). Ras activates the MAPK cascade, as mentioned above. This can be exemplified when Tyr⁷⁵⁹ is mutated or substituted. Here, the MAPK cascade is downregulated, while LIF and IL-6 signaling is increased. However, SHP2 may also act as a phosphatase on Tyr⁷⁵⁹ or any other tyrosine-phosphorylated signaling components, resulting in the deactivation of gp130 and the Jak-Stat signal-transducing pathway (fig. 1). This is made clear by the fact that overexpression of dominant-negative SHP2 mutants also leads to enhanced receptor, Jak, Stat and SHP2 phosphorylation [42].

Phosphorylation of Stat3. Stats can be phosphorylated at several sites in order to be activated. Tyr⁷⁰⁵ is the best-characterized binding site of Stat3 and leads to the effects described earlier (dimerization and activation of transcription). Another possibility for phosphorylation is Ser⁷²⁷ (fig. 1), which occurs at a slower rate than at Tyr⁷⁰⁵ [43]. Unfortunately, to date there is no clear picture of exact cause and effect mechanisms [as reviewed in 42, 44]. The literature mostly indicates an increase in the Stat-induced transcription of target genes upon Ser⁷²⁷ phosphorylation of Stats. However, phosphorylation of Ser⁷²⁷ on Stat3 can decrease Stat3-dependent transcriptional responses. Indeed, Ser⁷²⁷ phosphorylation of Stat3 does not enhance tyrosine phosphorylation of Stat1 or 3, but instead decreases it in some cases. Furthermore, phosphorylation of Ser⁷²⁷ leads to activation of the MAPK system through Vav, Rac, MKK4 and PKC. PKC associates with Stat3 in the nucleus and inhibits Stat3 DNA-binding and transcription activity [45, 46]. Reciprocally, activation of ERK, which has been linked to the negative regulation of Stat3, leads to phosphorylation of Ser⁷²⁷ on Stat3 (fig. 1). HGF stimulated proliferation of human aortic endothelial cells through the ERK-phospho-Ser⁷²⁷-Stat3 pathway [47]. ERK also causes the inhibition of tyrosine phosphorylation, thus inhibiting Stat3-mediated gene transcription [44].

In summary, it can be established that MAPK plays an important role in the phosphorylation of Ser⁷²⁷ residues, but caution is necessary in that serine phosphorylation can occur through several different signal transduction pathways and does not evoke the same effect in every cell.

MAPK and SOCS. Another regulation junction between the two systems can be distinguished in SOCS. On the one hand, tyrosine phosphorylated SOCS (induced through IL-2 or EGF) binds and inactivates the GTPase-activating protein (GAP), which normally inactivates Ras [48]. This ultimately prolongs MAPK activation (fig. 1). Additionally, it could be demonstrated that IFN- γ

stimulation of macrophages infected with the *Listeria* bacteria responded with a p38 MAPK-mediated Ser⁷²⁷ phosphorylation of Stat1 and a higher expression of SOCS3 [49].

Altogether this would indicate a synergistic mechanism between the MAPK pathway and SOCS, but an earlier investigation by Ernst et al. [40] stipulates the contrary as Stat-induced expression of SOCS1 was hypothesized to inhibit MAPK signaling. The possibility that SOCS can inhibit both pathways remains to be explored. SOCS binds with a higher affinity to Tyr⁷⁵⁹ than SHP2 [50], which would suggest just such a mechanism (fig. 1). In addition, overexpression of SOCS3 in human melanoma cell lines could completely abolish activation of both Jak-Stat and Ras-MAPK signaling [51].

Mammalian Target of Rapamycin-MAPK-Stat Interactions

Mammalian target of rapamycin (mTOR), belonging to the phosphoinositide kinase-related kinase family, is involved in cell growth and the cell cycle, control of the cytoskeleton and nutrient transport, protein and RNA stability and transcription and translation [52]. mTOR regulates the rate of protein translation in response to growth factor or mitogenic signals, allowing progression from the G1 to S phase of the cell cycle [53]. In a mouse model it could be demonstrated that disruption of the mTOR gene was implicated with a limited level of trophoblast outgrowth in vitro and postimplantational lethality of homozygotes [54]. Rapamycin treatment of mouse embryos also inhibits trophoblast outgrowth [54]. A deletion of 6 amino acids in the C-terminal part essential for kinase activity of mTOR demonstrated a reduced cell size and a proliferation arrest in early mouse embryos and embryonic stem cells [55]. It has been suggested that mTOR/p70S6K1 is involved in MAP kinase-mediated, but not PI3K-mediated, trophoblast migration in response to EGF since it could be shown that rapamycin inhibited cell migration and p70S6K1 phosphorylation [56]. In neuroblastoma cells mTOR phosphorylates Stat3 at Ser⁷²⁷ by the ciliary neurotrophic factor, which leads to a maximal transcriptional activation together with the Tyr⁷⁰⁵ phosphorylation by Jak kinases [57].

In summary, it is suggested that mTOR, MAPK and Stat pathways interact and potentially can regulate or modulate each other, but depending on which cell and which extracellular signal is being observed, different mechanisms with both synergistic and antagonistic effects are discernable.

Conclusions

Signal transduction in trophoblast invasion, especially in comparison to neoplastic invasion, is an area of reproduction or tumor biology that encourages

further investigation. First, because of the distinct yet similar situations of both types of growths and second, because data, especially concerning trophoblast invasion, is lacking. Particularly two pathways, the Stat- and the MAPK-mediated ones, have been distinguished as being involved in this process. Several studies suggest that these two pathways intersect at the point of modulation, but exact theories are not easily reconciled and instead invite further exploration. Understanding the signal transduction mechanisms of (trophoblast) invasion and their cross talk could assist in clarifying pathologies, such as multiple implantation failure or development of cancer, but also provides impetus for new ideas in respect to therapy.

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