# At the crossroad of physiological and pathological invasion: The role of Progesterone-Induced Blocking Factor in trophoblast and tumor invasion

**Ph.D. Theses**

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# INTRODUCTION

Invasiveness is a common feature of first trimester trophoblast and malignant tumors. However, while tumor invasion is a pathological process characterized by uncontrolled invasive behavior and unlimited metastatic capacity, trophoblast invasion is a strictly regulated, physiological event. Trophoblast invasion is restricted in time to the first trimester of pregnancy, and localized in space to the endometrium and the proximal third of the myometrium. The slightest disturbances of the fine tuning of trophoblast invasion may manifest in pathological pregnancies.

## *Regulation of invasion*

Invasion is a multi-step process, involving attachment of the invading cells to extracellular matrix (ECM) components, degradation of the ECM and migration through the eroded connective tissue. Invasion of surrounding tissues is mediated by a set of proteolytic enzymes, among others, matrix metalloproteinases (MMPs). The activity of the MMPs is inhibited by the tissue inhibitors of metalloproteinases (e.g., TIMP-1).

Several enzymes, hormones, cytokines, growth factors and extracellular matrix glycoproteins have been reported to play a role in both trophoblast and tumor invasion, furthermore, the same signaling pathways - i.e. MAPK, FAK, PI3K/Akt, STAT and the Wnt pathways (**Fig.1, 2**) – are involved in this process.

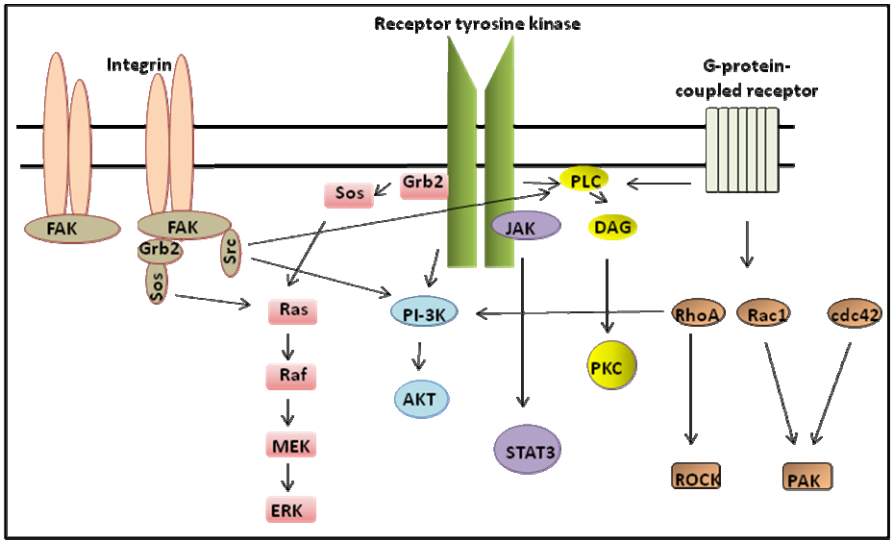


**Figure 1.** Signaling pathways in control of proliferation, differentiation, cell growth, apoptosis, migration and invasion

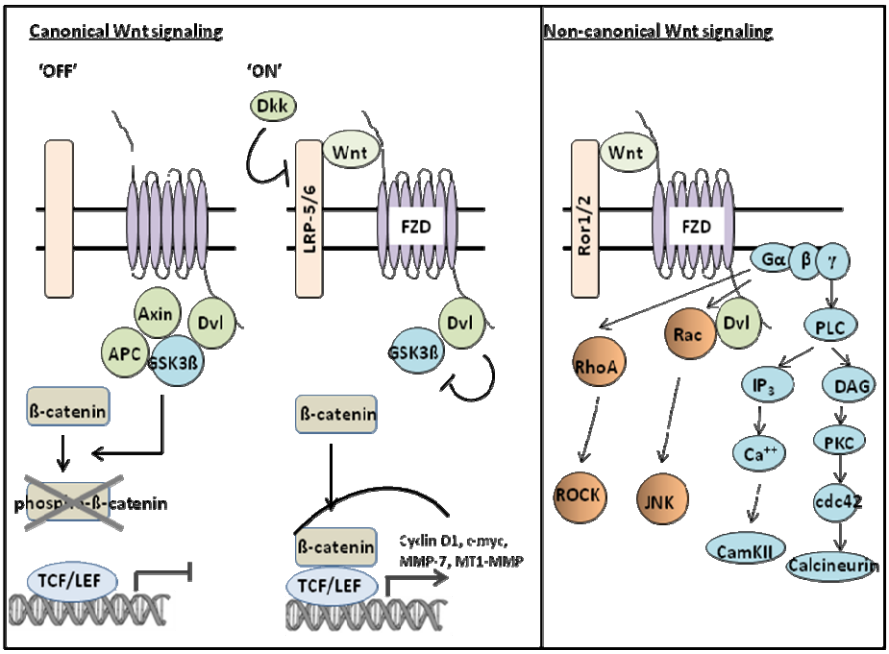


**Figure 2.** Wnt signaling. Canonical Wnt signaling pathway (left panel): In the presence of the Wnt molecule, Dvl inhibits GSK-3β and β-catenin translocates to the nucleus and induces the transcription of the target genes. Two major non-canonical Wnt signaling pathways, the planar cell polarity and Wnt/calcium pathways are shown on the right panel.

### *Progesterone-dependent immunomodulation*

Progesterone is indispensable for the maintenance of pregnancy. A protein named Progesterone-Induced Blocking Factor (PIBF) mediates the immunological effects of progesterone. PIBF supports the pregnancy via inhibiting NK activity, facilitating the production of asymmetric antibodies and altering the Th1/Th2 cytokine balance (**Fig.3**).

PIBF activates the Jak1/STAT6 and PKC/Ca++ - pathways which results in Th2-type cytokine production. PIBF induces SOCS3 which inhibits STAT4 phosphorylation, thus inhibits Th1 response.

Recently it was shown that PIBF is not only produced by pregnant maternal lymphocytes, but it is also present in pregnancy-associated tissues including trophoblast, in rapidly proliferating cells and in a set of malignant tumors.

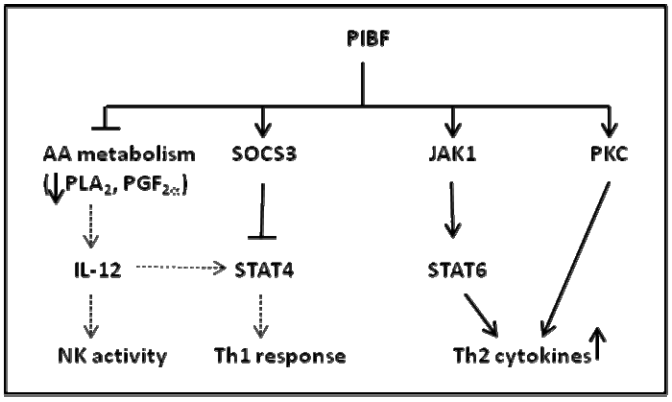


**Figure 3.** PIBF activates the Jak1/STAT6 and PKC/Ca++ - pathways which results in Th2-type cytokine production. PIBF induces SOCS3 which inhibits STAT4 phosphorylation, thus inhibits Th1 response through binding to the IL-12R. PIBF has the capacity to alter the arachidonic acid metabolism, and to inhibit NK activity.

Among others, progesterone and progesterone-induced genes possess a crucial role during implantation via governing trophoblast invasion. Progesterone decreases invasion by reducing MMP-9 and MMP-2 expression in early first trimester trophoblast cells.

The distribution of PIBF within the first trimester decidua coincides with sites of trophoblast invasion showing the strongest PIBF positivity at the extravillous trophoblast.

Various breast tumors overexpress PIBF compared to normal breast tissues. Moreover, MCF-7 mammary carcinoma cells produce PIBF in the absence of progesterone. Human leukemia cell lines have been shown to express PIBF both at mRNA and protein level.

***The above mentioned data raised the question wheather PIBF is involved in regulation of invasion.***

# AIMS

This work aims to investigate the involvement of Progesterone-Induced Blocking Factor (PIBF) in the regulation of invasion and to identify the signaling networks via which it affects the invasive behavior of tumor cells; contributes to the success of implantation during pregnancy by regulating trophoblast invasion and participates in the pathogenesis of trophoblastic diseases.

## I. To confirm the involvement of PIBF in trophoblast and tumor invasion

- To analyse PIBF expression in trophoblast cells with different degrees of invasiveness;
- To investigate the effect of PIBF knock down on trophoblast and tumor invasion.

## II. To analyse PIBF-induced invasion-related signaling pathways

- To characterize the PIBF-receptor;
- To uncover PIBF-induced signaling pathways involved in invasion;
- To investigate the subcellular localization of PIBF;
- To identify the function of nuclear PIBF.

# RESULTS AND DISCUSSION

## 1. The role of PIBF in invasiveness

### *1.1 Expression of PIBF in normal first trimester trophoblast and trophoblastic diseases*

Controlled trophoblast invasion is a key process during human placentation and a prerequisite of successful pregnancy. Another molecule that has been implicated in control of trophoblast invasiveness is leptin; a 16-kDa peptide hormone secreted by adipose tissue, that participates in the regulation of energy homeostasis. Leptin is synthesized by the human placenta and its receptors are expressed in the trophoblast and endometrium during pregnancy. Leptin also promotes the expression of MMP-2 and MMP-9 in cultured human cytotrophoblast cells. Leptin inhibits the secretion of progesterone by cytotrophoblast.

In order to investigate the possible cross-talk between PIBF and leptin in regulation of trophoblast invasion, paraffin embedded sections from healthy first trimester placentae, partial moles, complete moles and choriocarcinomas were analysed by immunohistochemistry. Sections were reacted with PIBF, leptin or leptin-receptor specific antibodies. PIBF was expressed in both normal first trimester villous trophoblast and in partial mole, while PIBF expression was markedly decreased in complete mole and absent in choriocarcinoma. Neither leptin, nor leptin-receptor was detected in partial mole, whereas both of these molecules were present in complete mole and choriocarcinoma.

To confirm the inverse relationship between PIBF and leptin/leptin-receptor expression, PIBF deficient trophoblast cells were generated with siRNA technique and leptin-receptor was detected by Western blotting in normal and PIBF knock down cells. Moreover, leptin expression was tested in the lysates of PIBF-treated cells with a protein array.

Leptin-receptor expression was upregulated in PIBF deficient cells, while leptin expression was decreased in PIBF-treated cells, suggesting that PIBF controls the expression of leptin and its receptor in the trophoblast.

### *1.2 PIBF affects invasion of trophoblast and tumor cell lines by modulating MMP-2 and MMP-9 activity*

To further investigate the possible role of PIBF in regulating invasion, we used trophoblast and tumor cell lines, rendered PIBF deficient by siRNA technique.

To study trophoblast invasion, HTR-8/SVneo cell line was selected which had been generated by transformation of HTR-8 cells with simian virus 40 large T antigen. The primary HTR-8 cultures were obtained after plating and outgrowth of cells from tissue pieces of human first trimester villi. The resulting cell line (HTR-8/Svneo) shares phenotypic properties with the progenitor cells and its proliferation, migration and invasiveness are regulated by the same signaling molecules that modulate extravillous trophoblast cell responses in vitro.

For modelling tumor invasion, the highly invasive fibrosarcoma cell line HT-1080 was selected. Both cell lines express and secrete PIBF and its receptor.

Cell invasion assay was used to determine the invasivity of PIBF knocked down HTR8/SVneo trophoblast- and HT1080 fibrosarcoma cells. Silencing of PIBF increased the invasiveness of HTR8/SVneo cells, and decreased that of the tumor cell lines.

MMPs mediate the invasion of surrounding tissues by degrading extracellular matrix components. MMP-9 and MMP-2 cleave type IV collagen, the main component of basal membranes, thus play a crucial role in trophoblast and tumor invasion. Therefore, cell conditioned media from the invasion assay were subjected to gelatine zymography to measure MMP-2 and MMP-9 activity.

PIBF silencing in trophoblast cells resulted in increased MMP-2 and MMP-9 secretion, while PIBF knock down tumor cells showed reduced MMP-2 and MMP-9 activity.

## 2. PIBF-induced signaling networks and invasive behaviour

### *2.1. Characterization of PIBF-receptor*

#### *2.1.1. PIBF-receptor associates with IL-4Rα*

Earlier we showed that the PIBF-receptor (PIBF-R) associates with the α-chain of the IL-4 receptor for signaling. To further verify the involvement of IL-4Rα in PIBF signaling, IL-4Rα deficient cells were created by siRNA technique. Silencing of IL-4Rα by siRNA

reduced the STAT6 activating effect of PIBF in peripheral blood mononuclear cells, confirming that the α-chain of the IL-4 receptor was indeed indispensable for PIBF signaling.

Confocal experiments were performed to detect co-localization of IL-4Rα and PIBF-R on peripheral blood mononuclear cells. Antibody-induced cross-linking by anti-IL-4Rα and PE-conjugated anti-IgG resulted in co-capping of PIBF-R and IL-4Rα. Ligand-induced molecular aggregation by PIBF-FITC resulted in co-patching of the two binding sites.

These data suggest that PIBF-receptor forms a complex with IL-4Rα and this process is initiated by PIBF binding.

*2.1.2 The PIBF-receptor is a raft-associated, GPI-anchored protein*

The above findings raise the question, why IL-4Rα is needed for PIBF signaling. A plausible explanation would be that the PIBF-receptor has no transmembrane and intracellular domains thus it uses the intracellular tail of IL-4Rα for signal transduction.

Assuming that the PIBF-receptor is a GPI-anchored protein, we digested the putative anchoring-region with phosphatidylinositol-specific phospholipase C (PI-PLC). PIBF-induced phosphorylation of STAT6 molecules was tested by Western blotting in intact and in PI-PLC digested lymphocytes. In PI-PLC digested lymphocytes, PIBF did not induce STAT6 phosphorylation while IL-4 retained its effect, indicating that PIBF-R is indeed a GPI-anchored molecule. Furthermore, capping of IL-4Rα could still be induced in PI-PLC digested lymphocytes but labelling of PIBF-receptor was lost.

GPI-anchored proteins are enriched in the leukocyte membrane within glycosphyngolipid-cholesterol rafts. These submicron domains need cholesterol to function, therefore the hypothesis that receptors of PIBF float in lipid rafts was tested by depletion of cholesterol, using methyl-β-cyclodextrin (MβCD). The STAT6 inducing effect of PIBF was abolished in MβCD-treated lymphocytes. In MβCD treated cells neither PIBF, nor IL-4 was able to Tyr-phosphorylate STAT6, suggesting that not only the PIBF-receptor but also the α chain of the IL-4 receptor might be raft-associated.

### *2.2 PIBF signaling in trophoblast and tumor cells.*

In order to reveal the mechanisms underlying the differential effects of PIBF on trophoblast and tumor invasiveness, we investigated the PIBF-induced signaling pathways in the two cell lines. Thus, effects of PIBF treatment on different signaling molecules were monitored in starved HTR8/SVneo and HT1080 cells by Western blotting.

PIBF treatment resulted in an immediate STAT6 phosphorylation in both cell lines. Moreover, silencing of IL-4Rα by siRNA abrogated the effect of PIBF on STAT6 phosphorylation as well as that on invasion in both cell lines, confirming that the invasion-related signaling of PIBF is indeed initiated by the IL-4Rα/PIBF-R complex. Therefore, we focused on IL-4Rα associated cascades.

IRS, associated with IL-4Rα, can induce Akt and ERK; which have been shown to be involved in invasion and tumorigenesis.

In trophoblast cells PIBF phosphorylated Akt and ERK in a fast, but transient way, whereas, in the tumor cell lines PIBF treatment resulted in sustained and late Akt and ERK activation.

STAT3 is a central molecule in invasion signaling, which has also been implicated both in trophoblast and tumor invasion. Various cytokines and growth factors e.g., EGF, IL-6 activate STAT3 through phosphorylation of tyrosine 705 and serine 727.

In tumor-, but not in trophoblast cells PIBF treatment resulted in late STAT3 Ser and Tyr phosphorylation suggesting an indirect role of PIBF in STAT3 induction.

Wnts are implicated in oocyte implantation and early trophoblast development as well as in the pathogenesis of trophoblastic diseases. Wnts are detectable in the pre-implanting embryo and a shift from non-canonical signaling in the pre-implantation period towards canonical signaling in activated blastocysts was demonstrated during implantation. Dickkopf-related protein-1 (Dkk1), a major secreted Wnt signaling antagonist is up-regulated by progesterone in the endometrium during the implantation window, furthermore, progesterone-dependent induction of Dkk1 inhibits Wnt signaling, suggesting that repression of the pathway plays a role in decidualisation.

Recent data point to a critical role of Wnt5a in malignant progression, but its role is controversial: loss of Wnt5a signaling is related to development of lymphoid malignancies, whereas constitutively active Wnt5a signaling is involved in invasion or metastasis of several cancers.

In our hands, 6 hours incubation with PIBF induced the activation of Wnt5a in HT-1080, but not in HTR8/SVneo cells, thus we analyzed the Wnt5a signaling pathway in the tumor cell line in more detail. After 6h PIBF treatment of fibrosarcoma cells β-catenin levels were reduced and PKCζ as well as PKCδ – the two isoforms which might be involved in Wnt associated signaling processes - were phosphorylated.

Based on the literature, PKCδ can be associated with both Wnt5a signaling and/or apoptosis. Nuclear localisation of PKCδ is required for its apoptotic function while membrane-association of PKCδ implicates its involvement in Wnt5a signaling.

To test the role of PKCδ, sub-cellular localization of phosphorylated PKCδ in PIBF treated HT-1080 cells were followed by confocal microscopy. Phospho-PKCδ was primarily associated with the membrane after 6 h treatment supporting the hypothesis that it plays a role in Wnt5a signaling, rather than apoptosis. The data was further substantiated, as no significant apoptosis was detectable after 6 h PIBF treatment.

Additionally, as PKCζ was also activated by PIBF and PKCζ is a known activator of GSK-3β that marks β-catenin for proteosomal degradation, it was concluded that PIBF induced Wnt5a inhibits the canonical Wnt pathway and blocks gene expression associated with canonical signaling.

Although Wnts are known to affect MMPs via canonical pathways, Wnt5a is capable to induce the expression of certain MMPs via non-canonical pathways and it is related to aggressive behaviour of cancer cells.

To sum up, in trophoblast cells PIBF treatment resulted in fast and transient phosphorylation of Akt and ERK, moreover, PIBF decreased Ser-phosphorylation of STAT3 and reduced intracellular Wnt5a level. In tumor cells, PIBF induced sustained and late phosphorylation of Akt and ERK molecules, late STAT3 phosphorylation and late Wnt5a activation. In tumor cells, PIBF-induced Wnt5a inhibits canonical Wnt signaling and might induce MMPs via non-canonical mechanisms.

### *2.3 PIBF regulated genes*

The late signaling events observed in tumor cells can be attributed to PIBF-induced proteins. To identify PIBF-induced genes, protein arrays for 55 invasion and angiogenesis related molecules were performed on lysates of PIBF knock down and on those of 24 h PIBF-treated trophoblast and fibrosarcoma cell lines. After 24 h PIBF treatment MMP-9 expression

increased in fibrosarcoma- and decreased in trophoblast cells, whereas TIMP-1 – an inhibitor of MMP-9 – was down-regulated in tumor-and up-regulated in trophoblast cells.

Furthermore, silencing of PIBF reduced the expression of FGF-1 and HB-EGF in fibrosarcoma, but not in trophoblast cells. 24 h PIBF treatment resulted in increased expression of FGF-1 and HB-EGF in tumor cells. Either of the above molecules might account for the late signaling.

To confirm that late PIBF signaling was indeed due to gene induction, we investigated the effect of PIBF treatment on STAT3 activation in HB-EGF knock down HTR8/SVneo and HT-1080 cells. In HB-EGF knock down fibrosarcoma cells the STAT3-activating effect of PIBF was reduced in comparison with PIBF-treated control (scrambled) suggesting that late STAT3 activation might have been caused by PIBF-induced HB-EGF.

IL-6 is an important activator of STAT3 signaling pathway. Therefore, secreted IL-6 was measured in the supernatants of PIBF silenced trophoblast and fibrosarcoma cells by cytometric bead array. IL-6 production of PIBF knock down trophoblast cells increased, whereas that of PIBF-silenced HT-1080 cells was reduced.

We also investigated the effect of PIBF knock down on Wnt5a expression. After silencing of PIBF, the levels of Wnt5a decreased in tumor cells and increased in trophoblast cells.

### *2.4 Sub-cellular localization of PIBF in trophoblast and tumor cells*

The full-length PIBF contains leucine-zippers, nuclear localization signals and bZIP motives, which enable the molecule to bind to DNA and regulate gene expression.

To confirm that PIBF enters the nucleus, subcellular localization of PIBF was visualized by confocal microscopy as well as in fractionated cells by Western blotting. PIBF was present in the nucleus of both cell lines, however; the ratio of nuclear versus cytoplasmic PIBF was higher in the tumor, than in the trophoblast cells.

### *2.5 PIBF binds to the promoter of IL-6, EGF, FGF and Wnt5a*

To verify that PIBF is capable to regulate the transcription of the above molecules, chromatin immunoprecipitation (ChIP) was performed with anti-PIBF antibody. This revealed that PIBF has the capacity to bind to the promoter of *IL-6, Wnt-5a, EGF* and *FGF-1* both in trophoblast and fibrosarcoma cells.

However, the protein profile of the protein/DNA complex precipitated by anti-PIBF antibody was different in HTR8/SVneo tophoblast and HT-1080 fibrosarcoma cells: in trophoblast cells the 50 kDa and 67 kDa PIBF isoforms were present in the complex while in fibrosarcoma cells the complex also included the full-length PIBF, suggesting that the composition of the transcription complexes are different in trophoblast and tumor cells and regulate gene expression in a tissue specific manner.

# CONCLUSIONS

These findings provide evidence that PIBF is not only responsible for modulating the immunological effects of progesterone but it also has a vital role in tumor invasion as well as in controlling trophoblast invasion at a physiological level.

Our data allow the assumption that PIBF facilitates invasion in fibrosarcoma cells by activating genes of molecules that initiate invasion signaling (**Fig.4**). The secreted proteins combine with their receptors and induce Akt, ERK and STAT3 phosphorylation which in turn further triggers the expression of invasion promoting molecules, e.g. MMP-9, MMP2.

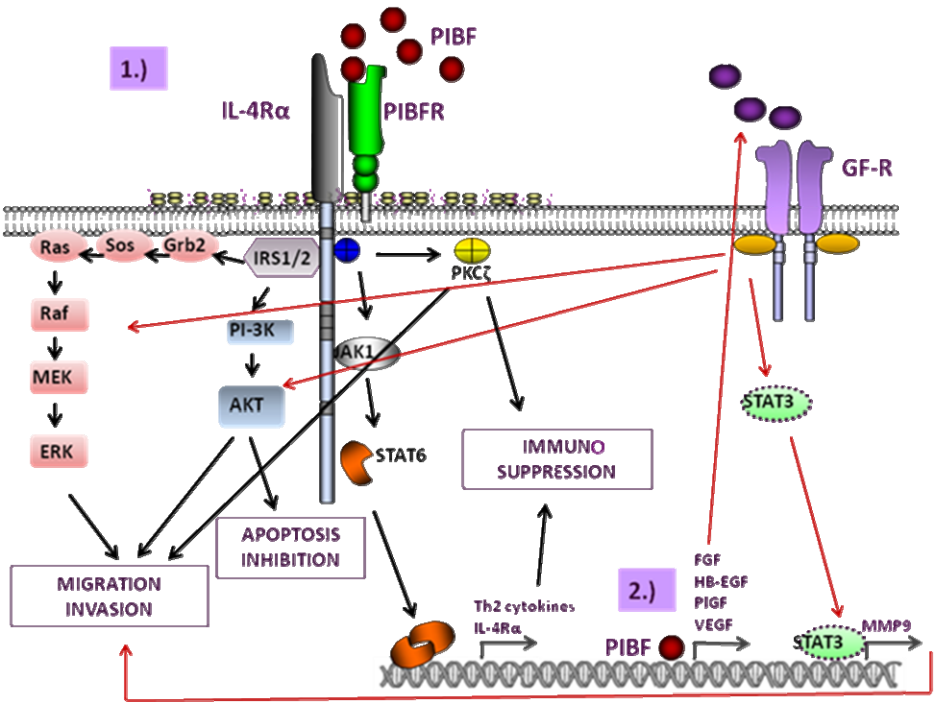


**Figure 4.** Signaling pathways that lead to PIBF-induced invasion in HT-1080 fibrosarcoma cells.

In the trophoblast - by suppressing genes-, PIBF might act as an intrinsic negative regulator of invasiveness (**Fig.5**). This concept gains support by increased expression of Wnt5a and IL-6 in PIBF knock down HTR8/SVneo cells and by PIBF being capable to bind to their promoter. In line with this, in choriocarcinoma, the loss of PIBF results in increased invasive behaviour since the pro-invasive genes that are normally suppressed by PIBF will be transcribed (**Fig.6**).

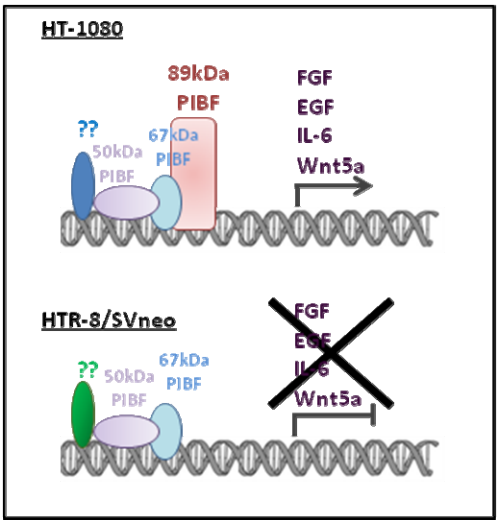


**Figure 5.** PIBF binds to the promoter regions of certain genes. In fibrosarcoma cells PIBF induces *FGF-1, HB-EGF, IL-6* and *Wnt-5a* while in trophoblast cells there is no gene induction.

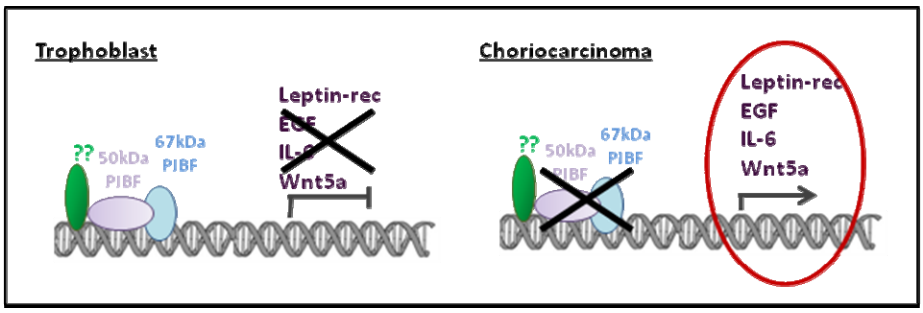


**Figure 6.** In trophoblast cells PIBF might suppress the genes of *leptin-receptor, HB-EGF, Wnt5a* and *IL-6*. In the absence of PIBF-mediated inhibition these genes will be activated.

PIBF binds the promoter of *IL-6, EGF, Wnt5a* and *FGF-1*, however, in fibrosarcoma cells the protein/DNA complex includes the full-length PIBF, in addition to the 50 kDa and 67 kDa PIBF isoforms found in the trophoblast (**Fig.5**). Thus, we hypothesize that the different composition of the DNA-binding PIBF complex might underlie the differential regulation of trophoblast and tumor invasion by PIBF.

## SUMMARY OF THESES

I. In the normal trophoblast → partial mole → complete mole → choriocharcinoma transition there is a shift towards uncontrolled invasive behaviour characterized by gradual loss of PIBF and an increasing leptin as well as leptin-receptor expression, suggesting an inverse relationship between PIBF and leptin/leptin-receptor expression.

The following findings were described in HTR-8/SVneo trophoblast and HT-1080 fibrosarcoma cell lines*:*

II. In the trophoblast cell line, PIBF negatively regulates trophoblast invasion by decreasing the expression of gelatinases (i.e. MMP9-and MMP-2) and inducing TIMP-1 expression. In the tumor cell line, PIBF facilitates invasive behaviour by increasing gelatinase activity and reducing TIMP-1 level.

III. PIBF activates the Akt and ERK pathways via binding to the PIBF-receptor/IL-4Rα heterocomplex in both the trophoblast and tumor cells. In trophoblast cells the effect of PIBF on Akt and ERK phosphorylation is transient, while in the tumor cell line PIBF induces sustained and late activation of these molecules.

IV. In trophoblast cells, PIBF inhibits Wnt5a activation, Ser-phosphorylation of STAT3 and it does not alter Tyr phosphorylation of STAT3. In HT-1080 tumor cells, PIBF induces late activation of Wnt5a and late phosphorylation of STAT3 (both Tyr and Ser) molecules.

V. In HT-1080 cells, PIBF-induced Wnt5a inhibits β-catenin and the canonical Wnt pathway. PIBF-induced Wnt5a might induce the expression of certain MMPs via non-canonical pathways.

VI. PIBF is capable to bind specifically to certain promoter regions, e.g., to the promoter of *Wnt5a, IL-6, FGF-1* and *EGF* in both trophoblast and tumor cells.

VII. In the tumor cell line PIBF activates the genes of molecules, e.g., FGF-1, HB-EGF, IL-6 and Wnt5a that are involved in invasion, while PIBF might suppress the transcription of these genes in trophoblast cells.

VIII. The differential effect of PIBF might be due to the different composition of the protein complex which binds to the promoter region of the above mentioned genes. While in trophoblast cells the promoter-binding complex contains the 50-kDa and 67-kDa PIBF isoforms, in tumor cells the full length PIBF is also included in the transcription complex.

## ACKNOWLEDGEMENTS

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# PUBLICATIONS

***Total impact factor of published papers:*** ***12.759***
***Total impact factor of published abstracts:*** ***43.346***

## Papers Related to Project

**Halasz M**, Berta G, Polgar B, Pongracz JE, Szekeres-Bartho J: Progesterone-Induced Blocking Factor differentially regulates trophoblast and tumor invasion. 2011; [Submitted]

Miko E*, **Halasz M***, Jericevic-Mulac B, Wicherek L, Arck P, Arato G, Skret Magierlo J, Rukavina D, Szekeres-Bartho J: Progesterone-Induced Blocking Factor (PIBF) and trophoblast invasiveness. **Journal of Reproductive Immunology** 2011; 90(1):50-7. Joint first authors: * *(Impact factor: 2.204)*

Szekeres-Bartho J, **Halasz M**, Palkovics T: Progesterone in pregnancy; receptor-ligand interaction and signaling pathways. **Journal of Reproductive Immunology** 2009; 83(1-2): 60-4. *(Impact factor: 2.519)*

Kozma N, **Halasz M**, Polgar B, Poehlmann TG, Markert UR, Palkovics T, Keszei M, Par G, Kiss K, Szeberenyi J, Grama L, Szekeres-Bartho J: Progesterone-Induced Blocking Factor activates STAT6 via binding to a novel IL-4 receptor. **The Journal of Immunology** 2006; 176(2): 819-826. *(Impact factor: 6.293)*

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## Book Chapters Related to Project

Szekeres-Bartho J, Kozma N, **Halasz M**, Polgar B, Miko E, Palkovics T, Szereday L. (2006): Immuno-Endocrine Signals. In: Embryo implantation: From basics to clinics – University Handbook, Eds. Rukavina, D. and Chaouat, G. (Croatia: University of Rijeka), 95-102.

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Szekeres-Bartho, J., **Halasz, M.,** Miko, E., Polgar, B. and Palkovics, T.: PIBF regulates trophoblast invasion. **Journal of Reproductive Immunology** 2010; 86(2): 105. *(Impact factor:2.519)*

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Polgar, B., Marki, A., Halidi, N., **Halasz, M.**, Kozma, N., Palkovics, T. and Szekeres-Bartho, J.: A Molecule of Challenge: Hunting for PIBF Receptor. **American Journal of Reproductive Immunology** 2007; 58(3): 197. *(Impact factor: 2.130)*

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***Oral presentations at international conferences (first author):*** 12 (5)

***Poster presentations on international conferences (first author):*** 13(5)

***Oral presentations on Hungarian conferences (first author):*** 5 (3)

***Poster presentations on Hungarian conferences (first author):*** 1 (1)

## Additional Papers

Par, G., Berki, T., Palinkas, L., Balogh, P., Szereday, L., **Halasz, M.**, Szekeres-Bartho, J., Miseta, A., Hegedus, G., Mozsik, Gy., Hunyadi, B. and Par, A.: Immunology of HCV infection: the causes of impaired cellular immune response and the effect of antiviral treatment. **Orvosi Hetilap** 2006; 147(13): 591-600. (in Hungarian)

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***Additional presentations at international conferences:*** 7
***Additional presentations at Hungarian conferences:*** 4