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Angiogenic factors in peritoneal adhesion formation

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Abstract Abdominal surgery is considered as the leading cause of peritoneal adhesions and almost universally as adhesiogenic. Peritoneal injury at the time of surgery initiates an inflammatory reaction determining fibrin deposition on the wound surface. Depending on the balance between the different components of the plasminogen system, this fibrin can be either lysed, leading to normal peritoneal healing, or organised, serving as a scaffold for fibroblast ingrowth, extracellular matrix deposition and angiogenesis, leading to adhesion formation. The mechanism underlying the predisposition to form adhesions in some patients and in some specific anatomic sites and not in others after similar surgical procedures remains unknown. In spite of the many attempts proposed over the years for reducing the incidence of adhesion formation, peritoneal adhesions remain a major clinical problem, inducing intestinal obstruction, pelvic pain, female infertility and difficulties at the time of re-operation. The available evidence indicates that understanding the adhesion formation process at the molecular level is essential for developing successful strategies for preventing adhesions. Fortunately, the advancement in molecular biology during the last years has led to the identification of many molecules with the potential of regulating inflammatory and immune responses, tissue remodelling and angiogenesis, key events in peritoneal healing and adhe-

sion formation. This review focuses on the role of angiogenesis and angiogenic factors in peritoneal adhesion formation.

Keywords Peritoneum · Adhesions · Angiogenesis · Vascular endothelial growth factor · Hypoxia inducible factors · Laparoscopy · CO₂

Definition and aetiology of peritoneal adhesions

Adhesions are pathological bonds between surfaces within body cavities. These bonds can be a thin film of connective tissue, a thick fibrous bridge containing blood vessels and nerve tissue, or a direct contact between two organ surfaces [1]. Adhesions can be found in abdominal, pericardial, pleural, uterine and joint cavities, and in the chamber of the eyes. Adhesions in the abdominal cavity are also known as peritoneal adhesions because the peritoneum is always involved.

Peritoneal adhesions may be classified, according to the aetiology, as congenital or acquired, which in turn can be classified as postinflammatory or postoperative [2]. Abdominal surgery is the most common cause of adhesions, 70–85% of all adhesions being attributed to previous surgery. On the other hand, surgery has been documented as almost universally adhesiogenic, the reported incidence of adhesions in patients undergoing surgery being between 55 and 100% [3].

Among postoperative adhesions, different processes can be distinguished [4]:

- Adhesions type 1 or de novo adhesion formation: adhesions formed at sites that did not have adhesions previously.
 - Type 1A: no previous operative procedures at the site of adhesions.
 - Type 1B: previous operative procedures at the site of adhesions.
- Adhesions type 2 or adhesion reformation: adhesions formed at sites where adhesiolysis was performed.

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Type 2A: no operative procedures at the site of adhesions besides adhesiolysis.

Type 2B: other operative procedures at the site of adhesions besides adhesiolysis.

Clinical significance of peritoneal adhesions

Depending on their location and structure, adhesions may remain silent or cause clinically important complications such as intestinal obstruction, chronic pelvic pain, female infertility and difficulties at the time of re-operation.

Intestinal obstruction is the most serious complication of peritoneal adhesions as it can be life threatening due to strangulation. Adhesions are the leading cause of intestinal obstruction in the Western world, accounting for more than 40% of all cases of intestinal obstruction and for 60–70% of those involving the small bowel [2].

Adhesion formation is a major cause of chronic pelvic pain and it has been reported as the primary cause in some 25% of patients with chronic pelvic pain. It was suggested that pelvic pain is a consequence of the restricted organ mobility imposed by adhesions. After adhesiolysis, a relief of symptoms has been consistently reported. From a clinical point of view, however, the relation between adhesions and chronic pelvic pain is unclear since their association does not necessarily imply a causal relationship. Indeed, it was demonstrated that a large number of infertility patients with adhesions do not experience pelvic pain [5].

Peritoneal adhesions are well recognised as a cause of female infertility. The proposed mechanism of infertility is that adhesions restrict the sweeping of the fimbria over the ovary. Periadnexal adhesions were found in some 20–30% of infertile women and marked increases in pregnancy rates were reported after adhesiolysis [6].

Adhesions increase the technical difficulty for surgeries, increasing the difficulty of accessing the abdomen and/or the operation site, the complication rates, the anaesthesia, operating and recovery time, the use of surgical materials and the need for blood transfusion. Therefore, the magnitude of adhesions related disorders (ARD) is larger than could be anticipated and is better illustrated by the reports showing that hospital readmission for ARD rival the number of hip replacements, heart bypass or appendix surgeries, that 35% of women having open gynaecologic surgery are readmitted 1.9 times in 10 years for operation due to adhesions or complicated by adhesions, and that the estimated annual cost for ARD in the USA is 1.3 billion US\$ [7].

Pathogenesis of peritoneal adhesions

The peritoneum is one of the largest organs in humans with a surface of some 10.000 cm². It serves to minimise friction and facilitate free movement of abdominal viscera, to resist and localise infections and to store fat, especially in the

greater omentum. It forms a closed sac in males and an open sac in females, lining the abdominal walls (parietal peritoneum) and the viscera (visceral peritoneum). It is composed of a continuous layer of mesothelial cells and a layer of loose connective tissue [8].

Peritoneal mesothelial cells are highly differentiated, as are pleural and pericardial mesothelial cells, and their apical surface contain abundant long microvilli that increase the functional surface of the peritoneum for absorption and secretion. Mesothelial cells are connected to one another by desmosomes and very loosely attached to the underlining basement membrane. The connective tissue is composed of bundles of collagenous and elastic fibres oriented in different directions and a rich network of blood and lymphatic vessels. Interspersed among these fibres and vessels, there are poorly differentiated epithelioid-like cells, similar to fibroblasts, macrophages, mast cells and fat cells [8].

The intact peritoneal cavity contains 3–50 ml of fluid with a pH of 7.5–8.0 and with a significant buffering capacity. The peritoneal fluid (PF) contains plasma proteins, including a large amount of fibrinogen, and a variety of free-floating cells, including macrophages, lymphocytes, eosinophils, mast cells and desquamated mesothelial cells [8].

Peritoneal injury, due to surgery, infection or irritation, initiates an inflammatory reaction that increases all components of the PF, i.e. proteins and cells, generating a fibrinous exudate and the formation of fibrin [9]. Fibrin formation is the result of the activation of the coagulation cascade, which includes two pathways, i.e. the contact factor or intrinsic pathway and the tissue factor or extrinsic pathway. Activation of these pathways transforms prothrombin (Factor II) into thrombin (Factor IIa) via the common pathway. Thrombin then triggers the conversion of fibrinogen into monomers of fibrin, which interact with each other and polymerise. The initially soluble polymer becomes insoluble by some coagulation factors such as Factor XIIIa and is deposited on the wound surface [9]. Within this fibrinous exudate, polymorphonuclears (PMN), macrophages, fibroblasts and mesothelial cells migrate, proliferate and/or differentiate. During the first two post-operative days, a large number of PMN enter and, in the absence of infection, depart within 3–4 days. Macrophages increase in number and change their functions, becoming the most important component of the leukocyte population after day 5. They phagocytose more accurately, have greater respiratory burst activity and secrete a variety of substances including cytokines and growth factors that recruit new mesothelial cells onto the injury surface. Mesothelial cells migrate, form islands throughout the injured area and proliferate in order to cover the denuded area. This healing process is different from that occurring in the skin because the entire surface becomes epithelialised simultaneously from the islands of mesothelial cells and not gradually from the borders. Therefore, it is irrespective of the size of the injury and is complete in 5–7 days [8].

These cells release a variety of substances including plasminogen system components, arachidonic acid metab-

olites, reactive oxygen species (ROS), cytokines and growth factors such as interleukins (IL), tumour necrosis factor α (TNF- α), transforming growth factors α and β (TGF- α and TGF- β), which modulate the process of peritoneal healing and adhesion formation at different stages [9, 10].

This fibrinous exudate and fibrin deposition is an essential part of normal tissue repair, but its complete resolution is required to restore the preoperative conditions. The degradation of fibrin is regulated by the plasminogen system. In this system, the inactive proenzyme plasminogen is converted into active plasmin by plasminogen activators (PAs), which are inhibited by plasminogen activator inhibitors (PAIs) [11]. Plasminogen is a glycoprotein synthesised in the liver that is abundant in almost all tissues. It is the inactive precursor of plasmin, a serine protease that is highly effective in the degradation of fibrin into fibrin degradation products (FDP) and that has a role in other stages of tissue repair such as extracellular matrix (ECM) degradation, [12] activation of proenzymes of the matrix metalloprotease (MMP) family [13] and activation of growth factors [14]. The principal activator of plasminogen is the serine protease tissue-type PA (tPA), which is expressed in endothelial cells, mesothelial cells and macrophages. tPA has a high affinity for fibrin and binds to a specific receptor, which exposes a strong plasminogen-binding site on the surface of the fibrin molecule. Therefore, in the presence of fibrin the activation rate of plasminogen is strikingly enhanced, whereas in the absence of fibrin, tPA is a poor activator of plasminogen [15, 16]. This results in higher plasminogen activation on the sites where it is required, whereas systemic activation is prevented. The other activator of plasminogen is the serine protease urokinase-type PA (uPA). The properties of uPA differ from those of tPA as it lacks high-affinity binding for fibrin and thus the increased activity in the presence of fibrin. Therefore, uPA is limited in its capacity to activate plasminogen [17].

The action of the PAs is counteracted by PAI-1 and PAI-2 through the formation of inactive complexes. The most potent inhibitor of tPA and uPA is the glycoprotein PAI-1, which is expressed in endothelial cells, mesothelial cells, macrophages, platelets and fibroblasts. The glycoprotein PAI-2 is a relatively poor inhibitor of tPA and uPA and is expressed in mesothelial cells, macrophages and epithelial cells. The role of other PAIs, i.e. PAI-3 and protease nexin 1, and plasmin inhibitors, i.e. α 2-macroglobulin, α 2-antiplasmin and α 1-antitrypsin, in peritoneal fibrinolysis remains unknown.

The balance between fibrin deposition and degradation is critical in determining normal peritoneal healing or adhesion formation. If fibrin is completely degraded, normal peritoneal healing will occur. In contrast, if fibrin is not completely degraded, it will serve as a scaffold for fibroblasts and capillary ingrowth. Indeed, fibroblast will invade the fibrin matrix and ECM will be produced and deposited. The ECM can be completely degraded by MMPs, leading to normal healing. However, if this process

is inhibited by tissue inhibitors of MMPs (TIMPs), peritoneal adhesions will be formed.

Angiogenesis, angiogenic factors and adhesion formation

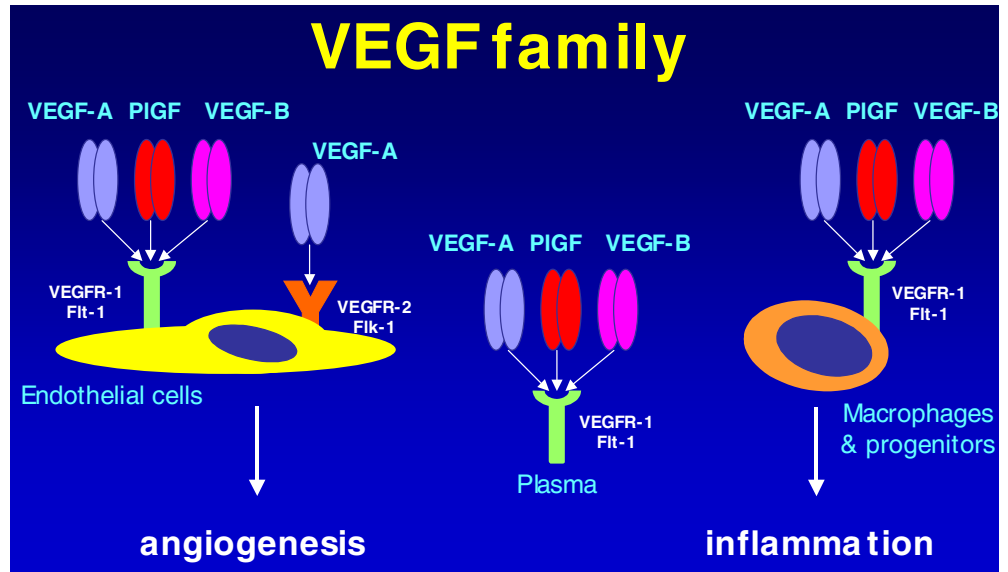
The formation of new blood vessels on peritoneal adhesions has been universally claimed to be important and supported by animal data demonstrating increasing vascularisation over days [18]. The details of the angiogenesis process in peritoneal adhesion formation remains, however, largely unexplored.

Angiogenesis, the formation of new blood vessels extending from existing vessels, occurs when the distance between cells and the nearest capillary exceeds an efficient diffusion range for maintaining an adequate supply of oxygen and nutrients to cells. This process is regulated by cellular hypoxia through the modulation of angiogenic factors and their inhibitors.

Angiogenesis is a self-limited and strictly controlled process that occurs in a sequential manner, involving degradation of the vascular basement membrane and interstitial matrix, migration and proliferation of endothelial cells and finally tubulogenesis and formation of capillary loops. The proteolytic enzymes production such as MMPs and PAs, in response to angiogenic factors is fundamental for all stages of angiogenesis, i.e. degradation of perivascular matrix and tissue stroma, migration and proliferation of endothelial cells. Since these proteases are produced in inactive forms and must become activated to initiate their actions, their activities are regulated by naturally occurring physiological inhibitors, i.e. TIMPs and PAIs. Cytokines and growth factors such as IL 1, IL 8, TNF- α , vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), TGF- α , TGF- β , platelet-derived growth factor (PDGF) are considered as angiogenic factors due to their ability to regulate the expression of MMPs, PAs and their inhibitors and to modulate endothelial cell migration and proliferation [19].

VEGF, the most potent known angiogenic factor, is a family that includes VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PlGF). These factors are transcribed from single genes and processed by alternative splicing into different isoforms. VEGF-A, also known as VEGF, is processed into four isoforms in humans (VEGF-A₁₂₁, VEGF-A₁₆₅, VEGF-A₁₈₉ and VEGF-A₂₀₆) and three in mice (VEGF-A₁₂₀, VEGF-A₁₆₄ and VEGF-A₁₈₈). VEGF-B is processed into two isoforms in humans and two in mice (VEGF-B₁₆₇ and VEGF-B₁₈₆), whereas PlGF is processed into three isoforms in humans (PlGF-1, PlGF-2 and PlGF-3) and one in mice (PlGF-2). These factors bind to two high-affinity transmembrane tyrosine kinase receptors with 7 immunoglobulin-like extracellular domains and a kinase intracellular domain, i.e. VEGFR-1/Flt-1 (for VEGF-A, VEGF-B and PlGF) and VEGFR-2/Flk-1 (for VEGF-A). These receptors are selectively but not exclusively expressed on endothelial cells. A truncated

Fig. 1 Vascular endothelial growth factor family



soluble form of VEGFR-1, resulting from alternative splicing and retaining its binding activity, is present in serum. VEGFR-1 is, unlike VEGFR-2, also expressed on inflammatory cells. Therefore, VEGF-A, VEGF-B and PIGF can stimulate inflammation in addition to angiogenesis (Fig. 1) [20–23].

Because of the presence of VEGF in endothelial cells of blood vessels supplying pelvic adhesions, a key role for VEGF in angiogenesis during adhesion formation has been suggested [24]. This observation was supported by studies in rats demonstrating up-regulation of VEGF₁₈₈ and VEGF₁₂₀ during early stages of peritoneal healing and down-regulation of VEGF₁₆₄ 24–48 h following open surgery [25], suggesting a compensatory mechanism to regulated angiogenesis in order to provide nutrients and oxygen to the injured tissues. The role of VEGF is also supported by the reduction of adhesion formation after

treatment with antibodies against VEGF in an open surgery mouse model [26].

The role of VEGF-A, VEGF-B and PIGF in adhesion formation after laparoscopic surgery has been addressed in studies using wild type mice (i.e. VEGF-A^{+/+}, VEGF-B^{+/+}, PIGF^{+/+}), transgenic mice and monoclonal antibodies. Adhesions were induced during laparoscopy and scored after 7 days during laparotomy. Since adhesions increase with the duration of the pneumoperitoneum and the insufflation pressure [27, 28], the CO₂ pneumoperitoneum was maintained at 14 mmHg for the minimum time needed to induce the lesions (10 min) or for a longer period (60 min) to evaluate “basal adhesions” and “pneumoperitoneum-enhanced adhesions”, respectively [29]. In all control groups, 60 min of pneumoperitoneum increased adhesion formation. In transgenic mice for VEGF-A, (i.e. deficient for VEGF-A₁₂₀ and VEGF-A₁₈₈ and expressing

Fig. 2 Role of VEGF-A in adhesion formation. Proportion of basal adhesions (10 min of pneumoperitoneum) and pneumoperitoneum-enhanced adhesions (60 min of pneumoperitoneum) in wild-type mice (VEGF-A^{+/+}) and transgenic mice deficient for VEGF-A₁₂₀ and for VEGF-A₁₈₈ isoforms and expressing exclusively VEGF-A₁₆₄ isoform (VEGF-A^{164/164}). Means±SE are indicated

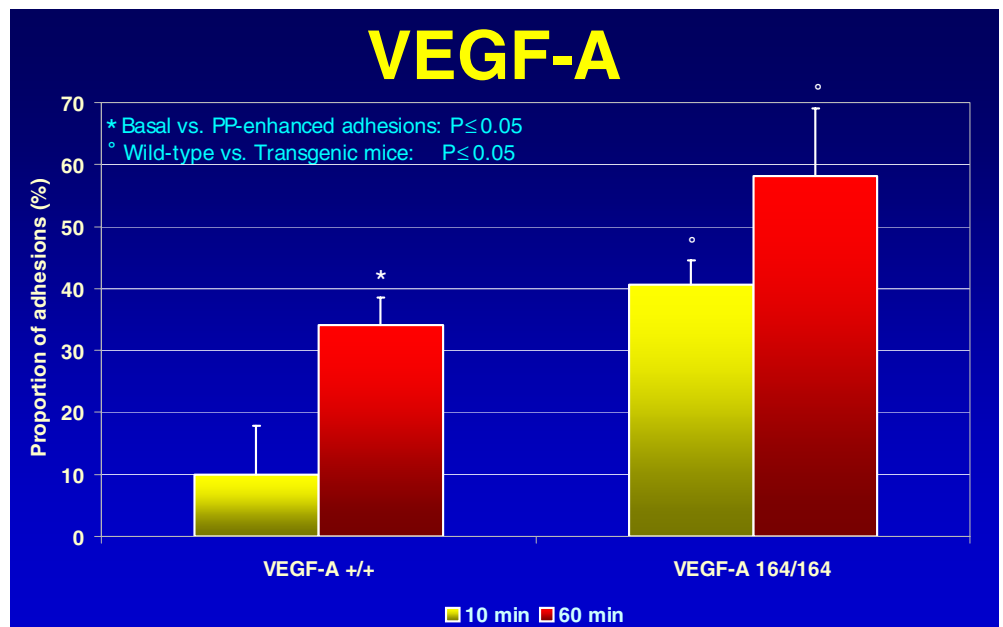
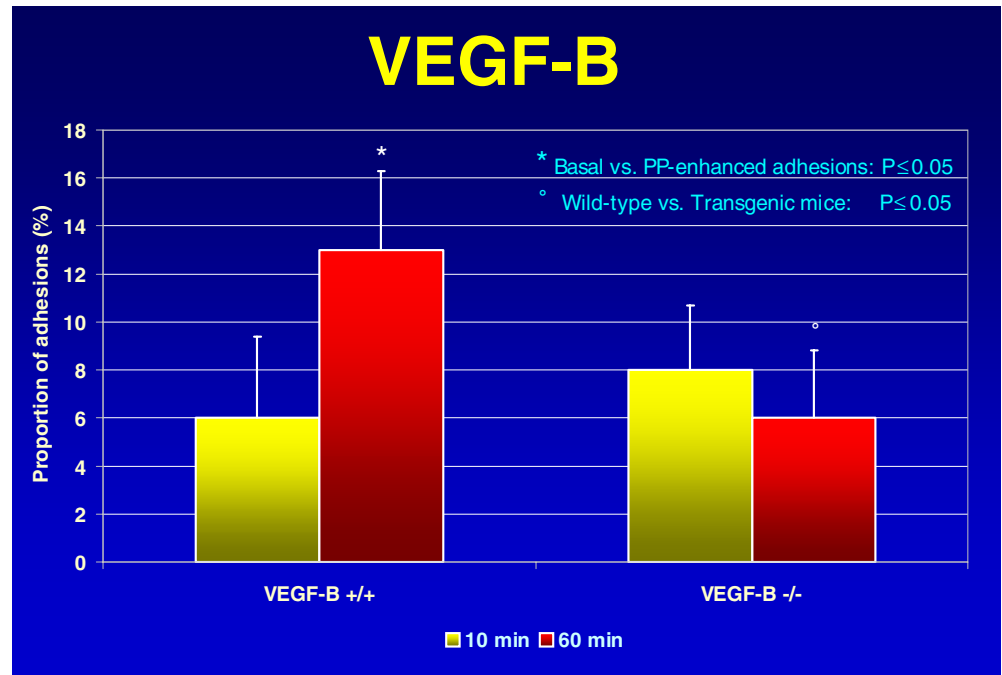


Fig. 3 Role of VEGF-B in adhesion formation. Proportion of basal adhesions (10 min of pneumoperitoneum) and pneumoperitoneum-enhanced adhesions (60 min of pneumoperitoneum) in wild-type mice (VEGF-B^{+/+}) and transgenic mice deficient for VEGF-B (VEGF-B^{-/-}). Means±SE are indicated



exclusively VEGF-A₁₆₄: VEGF-A^{164/164}, basal adhesions were higher than in VEGF-A^{+/+} mice, the pneumoperitoneum slightly increased adhesions, and “pneumoperitoneum-enhanced adhesions” were higher than in VEGF-A^{+/+} mice (Fig. 2) [30]. In mice deficient for VEGF-B (VEGF-B^{-/-}), “basal adhesions” were similar than in VEGF-B^{+/+} mice and the pneumoperitoneum did not increase adhesions, “pneumoperitoneum-enhanced adhesions” being therefore lower than in VEGF-B^{+/+} mice (Fig. 3) [30]. In mice deficient for PIGF (PIGF^{-/-}), basal adhesions were slightly lower than in PIGF^{+/+} mice and the pneumoperitoneum did not increase adhesions,

pneumoperitoneum-enhanced adhesions being therefore lower than in PIGF^{+/+} (Fig. 4) [30]. The role of PIGF was confirmed by using monoclonal antibodies with different neutralising capacities of the binding of PIGF to its receptor. In mice treated with neutralising antibodies, basal adhesions were lower than in the control groups and the pneumoperitoneum did not increase adhesions, pneumoperitoneum-enhanced adhesions being therefore lower than in the control groups [30] (Fig. 5).

The role of the common receptor of VEGF-A, VEGF-B and PIGF, i.e. VEGF-R1, was evaluated by using mono-

Fig. 4 Role of PIGF in adhesion formation. Proportion of basal adhesions (10 min of pneumoperitoneum) and pneumoperitoneum-enhanced adhesions (60 min of pneumoperitoneum) in wild-type mice (PIGF^{+/+}) and transgenic mice deficient for PIGF (PIGF^{-/-}). Means±SE are indicated

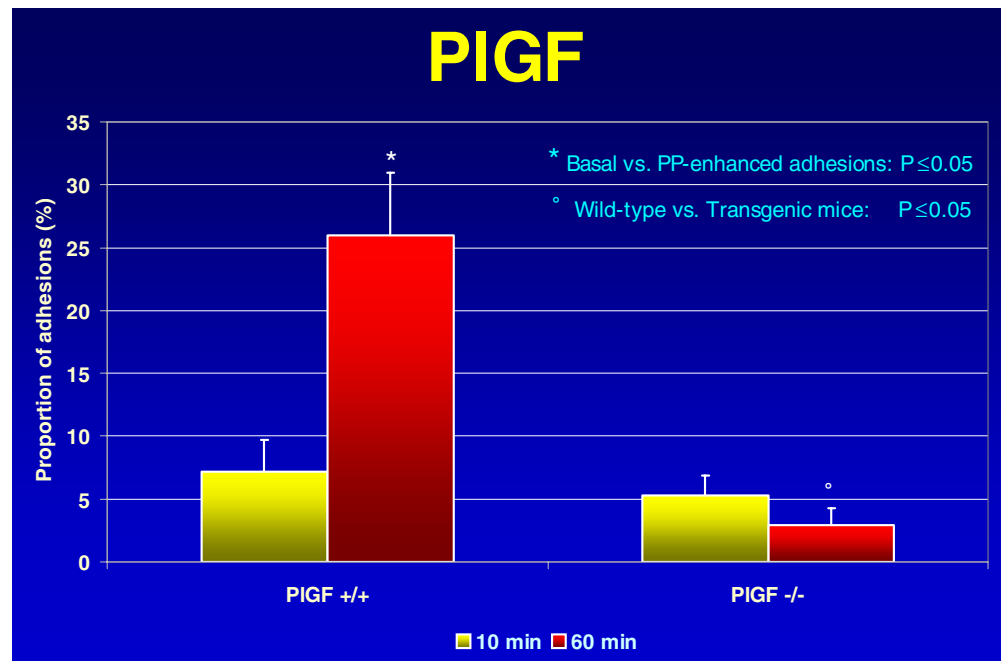
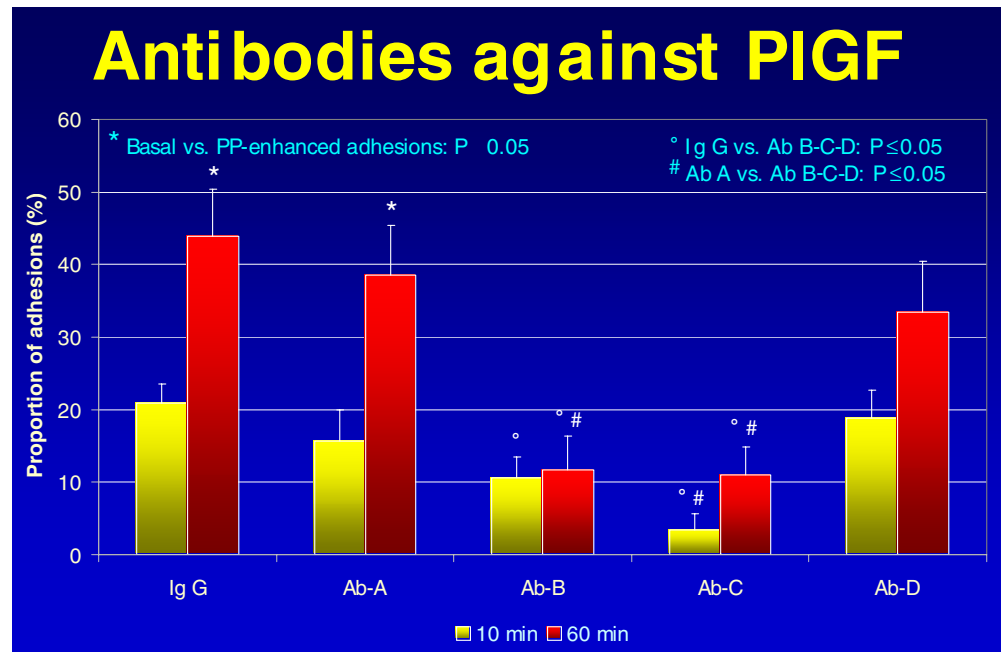


Fig. 5 Role of antibodies against PIGF in adhesion formation. Proportion of basal adhesions (10 min of pneumoperitoneum) and pneumoperitoneum-enhanced adhesions (60 min of pneumoperitoneum) in wild-type mice treated with IgG or with PIGF antibodies with different neutralising capacity according to their ability to inhibit the binding of PIGF to VEGFR-1 (Ab A: no neutralising, Ab B: neutralising, Ab C: neutralising, Ab D: semi-neutralising). Means±SE are indicated



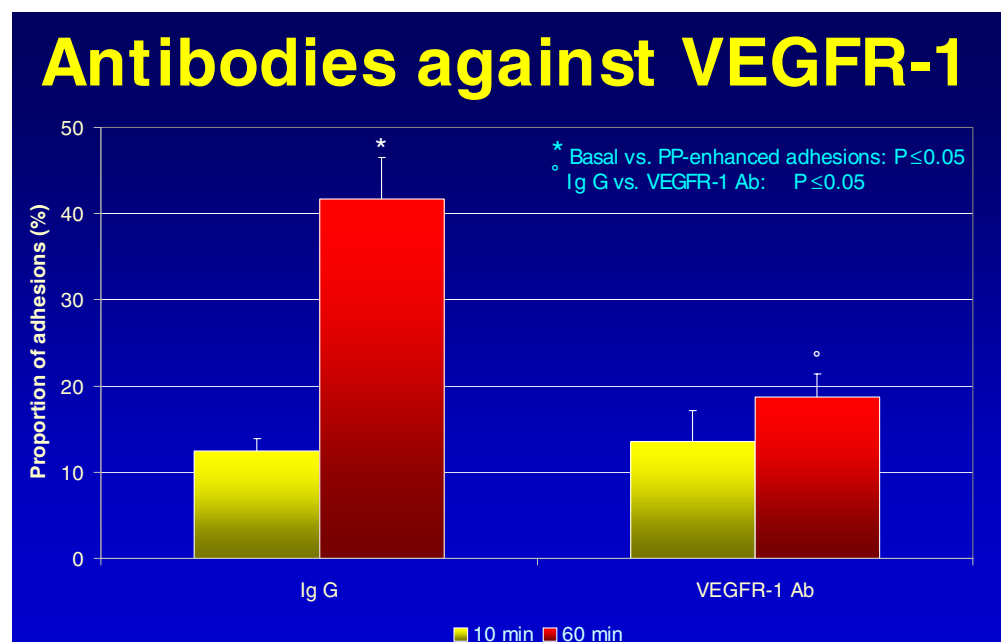
clonal antibodies against VEGFR-1. In the control group, i.e. IgG-treated mice, pneumoperitoneum increased adhesions. In VEGFR-1 antibodies-treated mice, basal adhesions were similar than in IgG-treated mice and the pneumoperitoneum did not increase adhesions, pneumoperitoneum-enhanced adhesions being therefore lower than in IgG-treated mice (Fig. 6) [31].

For basal adhesions the data clearly demonstrate a role for VEGF- A_{164} , whereas for pneumoperitoneum-enhanced adhesions, the data indicate that the pneumoperitoneum increases adhesions through VEGF-B and PIGF up-regulation and probably also through VEGF- A_{164} up-regulation. Indeed, pneumoperitoneum-enhanced adhesions is

absent in VEGF- $B^{-/-}$ and PIGF- $^{-/-}$ mice because the pneumoperitoneum cannot up-regulate these nonexistent factors. This is fully consistent with the observations in mice treated with PIGF antibodies. The only slight increase in adhesions following 60 min of pneumoperitoneum in VEGF- $A^{164/164}$ mice does not rule out VEGF- A_{164} up-regulation because adhesion formation could already be near maximal due to the over-expression of this factor.

Since PIGF, VEGF-A and VEGF-B have a common receptor, i.e. VEGFR-1, and since antibodies against VEGFR-1 prevent pneumoperitoneum-enhanced adhesions, the data indicate that the effects of the VEGF family are mediated to a large extent by this receptor. This is

Fig. 6 Role of antibodies against VEGFR-1 in adhesion formation. Proportion of basal adhesions (10 min of pneumoperitoneum) and pneumoperitoneum-enhanced adhesions (60 min of pneumoperitoneum) in wild-type mice treated with IgG or with VEGFR-1 antibodies. Means±SE are indicated



supported by the recently reported reduction of peritoneal fibrosis after soluble VEGFR-1 gene transfer in mice [32], since this isoform, by retaining its binding capacity, reduces the binding of the ligands to the functional cellular receptors. As VEGFR-1 is expressed on endothelial cells and on inflammatory cells, it remains unclear whether these effects are mainly related to stimulation of angiogenesis and/or inflammation.

Several mechanisms have been proposed for VEGF-driven angiogenesis [21, 33]. VEGF-A induces angiogenesis by activating VEGFR-2, while VEGFR-1 might function as an inert “decoy” regulating the availability of VEGF-A to activate VEGFR-2. PIGF stimulates angiogenesis by several mechanisms. First, PIGF displaces VEGF-A from VEGFR-1, increasing the fraction of VEGF-A available to activate VEGFR-2. Second, PIGF up-regulates the expression of VEGF-A. Third, PIGF transmits its own intracellular angiogenic signals through VEGFR-1. Fourth, PIGF activates receptor cross-talk between VEGFR-1 and VEGFR-2, enhancing VEGFR-2-driven angiogenesis. Fifth, PIGF forms heterodimers with VEGF-A. On the other hand, VEGF-driven inflammation is mediated by VEGFR-1 by increasing mobilisation of bone marrow-derived myeloid progenitors into peripheral blood, by increasing myeloid cell differentiation, mobilisation and activation, and by increasing cytokines production by macrophages [21, 36].

Regardless of the main mechanism of action of VEGF, the available data point to peritoneal hypoxia as the trigger factor. The hypoxic response is not restricted to specific specialised cell types and a general similar mechanism might act in a variety of cell types. Most mammalian cells can respond to alterations in oxygen levels by increasing or decreasing the expression of specific genes [34, 35]. The hypoxic regulation of many of these genes takes place at both transcriptional and post-transcriptional levels. The transcriptional regulation is mediated by transcription factors known as hypoxia inducible factors (HIFs) [36–38]. Since VEGF is up-regulated by hypoxia through HIFs and since HIFs have a well-known role in angiogenesis [39], a role for these factors in adhesion formation can be postulated.

HIFs are nuclear proteins that bind to hypoxia response elements (HRE) in the promoter or enhancer regions of hypoxia inducible genes, activating gene transcription in response to hypoxia [43]. HIFs are members of the basic helix-loop-helix (bHLH) periodic (Per) aryl hydrocarbon receptor nuclear translocator (ARNT) single-minded (Sim) (PAS) domain protein family. Several proteins have been identified in this bHLH-PAS family that belong to the α or β classes. Each member of the α class form a stable heterodimer with a member of the β class. Whereas β class members are constitutively expressed in a ubiquitous or a tissue-specific way, α class members are often inducible by environmental stimuli such as light or hypoxia [40]. HIF-1 is composed of HIF-1 α and HIF-1 β subunits [41–43], whereas HIF-2 is composed of HIF-2 α and HIF-1 β subunits [44]. HIF-1 α and HIF-2 α , the specific hypoxia-regulated subunits, are structurally very similar and share

the same heterodimerisation partner. Therefore, both HIF-1 and HIF-2 have a high similarity in structure and regulatory domains and are able to bind to the same HRE of target genes (Fig. 7).

The specific role of HIFs in adhesion formation was evaluated in mice partially deficient for HIF-1 α or HIF-2 α using the model previously described. While, in the control groups, 60 min of pneumoperitoneum increased adhesions, in the transgenic mice this effect was not observed, pneumoperitoneum-enhanced adhesions being therefore nonexistent (Figs. 8 and 9) [45]. These observations are consistent with the effects of the VEGF family [30, 34] and are also supported by the absence of pneumoperitoneum-enhanced adhesions in mice deficient for PAI-1 [29], since PAI-1 is up-regulated by hypoxia through HIF-1 α [46].

Angiogenesis not only depends on the angiogenic factors but also on the availability of their inhibitors. Among the angiogenic suppressors are TGF- β , TNF- α , interferons, collagen synthesis modifiers, protamine, cyclosporine, hyaluronic acid, thrombospondin, angiostatin and endogenous oestrogen metabolites [19]. Since peritoneal vascular endothelial cells contain receptors for ovarian steroids [60], these steroids can potentially regulate peritoneal healing and adhesion formation related angiogenesis.

Antiangiogenic therapy for peritoneal adhesion prevention

Although antiangiogenic therapy is widely used in other fields such as cancer, to the best of our knowledge, only recently have antiangiogenic agents been used for evaluating their efficacy for peritoneal adhesion prevention. The angiogenesis inhibitor TNP-4 [34], an analogue of fumagillin secreted by the fungus *Aspergillus fumigatus*, has been shown to reduce peritoneal adhesions and to delay vascular ingrowth in a laparotomy mouse model [48]. However, side effects of the drug such as neurotoxicity and delayed wound healing, precluded further investigations of TNP-470.

Enzymes involved in the transformation of the arachidonic acid as the first step in the prostaglandin synthesis

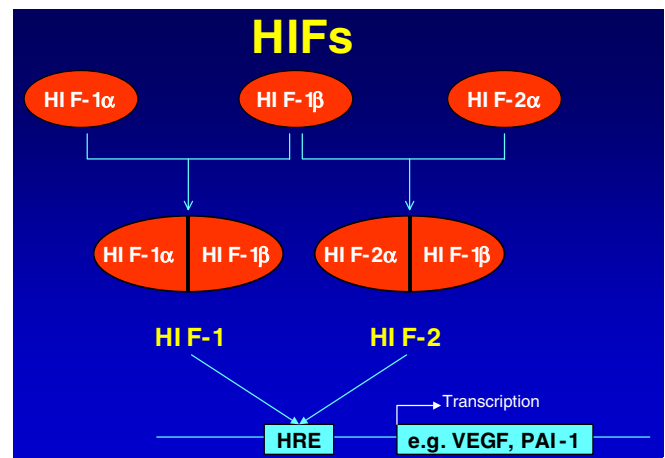
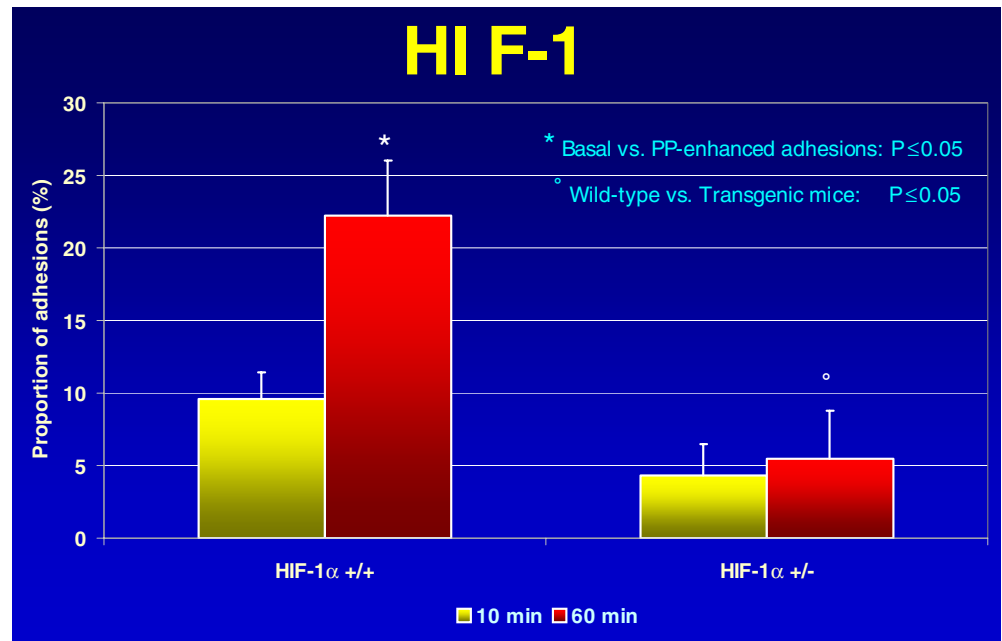


Fig. 7 Hypoxia inducible factors

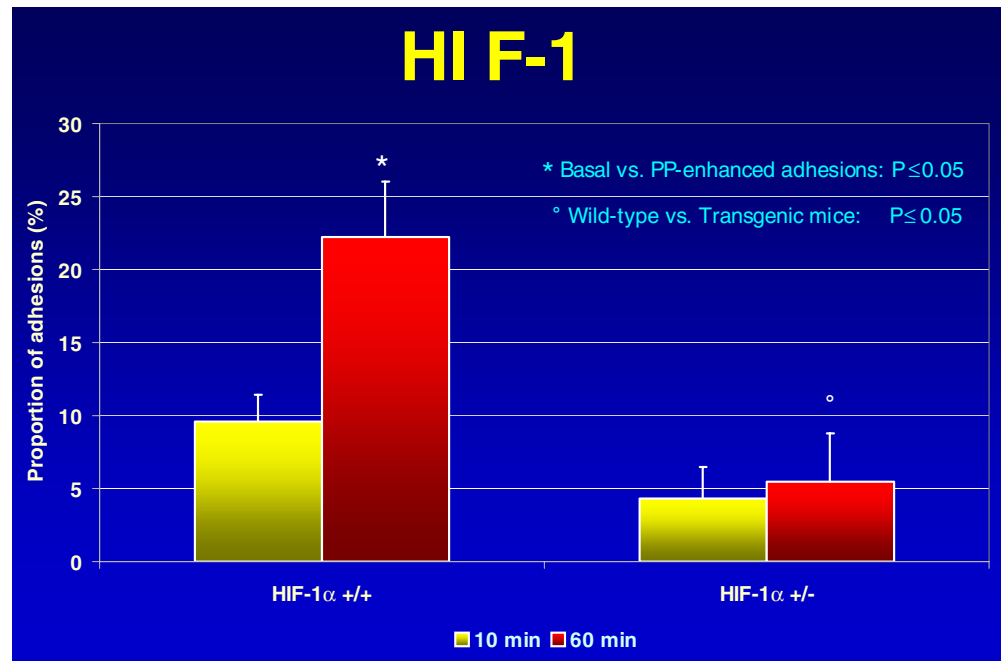
Fig. 8 Role of HIF-1 in adhesion formation. Proportion of basal adhesions (10 min of pneumoperitoneum) and pneumoperitoneum-enhanced adhesions (60 min of pneumoperitoneum) in wild-type mice (HIF-1 α ^{+/+}) and transgenic mice partially deficient for HIF-1 α (HIF-1 α ^{+/-}). Means \pm SE are indicated



pathway, cyclooxygenase-1 and 2 (COX-1 and COX-2), were also evaluated for adhesion prevention. In contrast to COX-1, which is expressed on endothelial cells of normal blood vessels, COX-2 is present on new angiogenic endothelial cells [49], as well as in fibroblasts associated with surgical adhesions [50]. Therefore, COX-2 inhibitors have the potential of reducing angiogenesis and adhesion formation. In a laparotomy mouse model, in which adhesions were created by rubbing the cecum and by a silicone patch attached to the abdominal wall, animals were treated with the selective COX-2 agents, celecoxib or rofecoxib, and the nonspecific COX inhibitors, aspirin, naproxen, ibuprofen or indomethacin. Animals treated with

selective and nonselective COX-2 inhibitors, except aspirin, had significantly fewer adhesions than control animals. Celecoxib produced a maximal reduction in adhesion formation compared with rofecoxib and the nonselective COX-2 inhibitors. Adhesions from mice treated with celecoxib had reduced microvessel density, suggesting inhibition of peritoneal adhesions through an antiangiogenic mechanism [51]. These observations were further supported by the reduced human fibroblast expression of VEGF after *in vitro* treatment with another COX-2 inhibitor, i.e. NS-358, and by stimulation of aerobic metabolism with dichloroacetic acid [52].

Fig. 9 Role of HIF-2 in adhesion formation. Proportion of basal adhesions (10 min of pneumoperitoneum) and pneumoperitoneum-enhanced adhesions (60 min of pneumoperitoneum) in wild-type mice (HIF-2 α ^{+/+}) and transgenic mice partially deficient for HIF-2 α (HIF-2 α ^{+/-}). Means \pm SE are indicated



Although it was withdrawn from the market for the teratogenic side effects, the antiangiogenesis inhibitor thalidomide was shown to reduce adhesions formation after colonic anastomosis in rabbits [53]. The antiangiogenic agent tamoxifen, however, did not show any beneficial effect for reducing adhesion formation in an ileo-ileal anastomosis rat model, although no adverse effects on wound or anastomotic healing were reported [54]. As mentioned before, mice data clearly demonstrate a reduction in adhesion formation after treatment with antibodies against VEGF [55], PlGF [30] or VEGFR-1 [31] opening new alternatives for adhesion prevention in humans.

Conclusions

Peritoneal adhesions, induced by infection, inflammation or surgery, are a leading cause of pelvic pain, intestinal obstruction and female infertility and cause increasing difficulties at the time of re-operation as well as increasing medical costs. It remains unknown why peritoneal wounds heal without adhesions in some patients, whereas in others, severe adhesions are formed from seemingly equal procedures, and why adhesions can develop in one surgical site and not in others in the same patient.

These observations, together with the failure of the many strategies developed over the years to prevent or at least to reduce peritoneal adhesions, clearly highlight the importance of understanding adhesion formation at the molecular level. Fortunately, we have witnessed, during the past decade, extraordinary advances in molecular biology that have led to the identification of many molecules, e.g. cytokines, growth factors, chemokines and proteases, with the potential of regulating inflammatory response, tissue remodelling and angiogenesis—events that are central to normal wound healing and to tissue fibrosis. However, the roles of all these molecules, specifically in the peritoneal biology and in the adhesion formation process, remain speculative to a large extent. Recently a specific adhesion phenotype has been reported, describing the substantial differences between the adhesion peritoneum and the apparently normal adjacent peritoneum [57], an observation that may be crucial for prevention of adhesion reformation (type 2 adhesions).

In addition to the cellular players, molecules and processes involved in postoperative adhesion formation, we have reported the importance of taking into account the potential effect of the local environment, i.e. CO₂ pneumoperitoneum for laparoscopy and air for laparotomy, to fully understand the intrinsic mechanisms involved. These observations should not be underestimated since environment-related factors such as hypoxia [28–57], hyperoxia [58, 59], desiccation and hypothermia [60, 61] could modulate every stage of the adhesion formation process in different ways. Indeed, we have initially postulated that the CO₂ pneumoperitoneum induces peritoneal hypoxia by compressing the capillary flow at the time of insufflation, which could enhance the formation of adhesions. This hypothesis was supported by the increase in adhesion

formation with the duration of the pneumoperitoneum and the insufflation pressure using both CO₂ and helium pneumoperitoneum and by the decrease in adhesion formation observed after adding 2–4% of oxygen to any of the two insufflation gases [27, 28]. It was also confirmed by the absence of pneumoperitoneum-enhanced adhesions in mice deficient for the genes encoding for factors regulated by hypoxia such as HIFs [54], VEGF [30] and PAI-1 [29]. The important role of hypoxia in adhesion formation was further supported by a series of in vitro studies demonstrating increased expression of many adhesiogenic factors produced by fibroblasts cultured under hypoxic conditions [62–79].

Since angiogenesis is one of the essential steps in adhesion formation, and since it is mainly regulated by hypoxia, all these data together indicate that antiangiogenic measurements, either by preventing hypoxia and thus angiogenesis or by using antiangiogenic drugs, could be an alternative for prevention of peritoneal adhesions.

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