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ANGIOGENESIS MARKERS IN GYNECOLOGICAL TUMOURS AND PATENTS FOR ANTI-ANGIOGENIC APPROACH: REVIEW

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ABSTRACT

The formation of a tumor-associated vascular network is an important step in understanding the stages of tumor progression. This review aims to highlight the main markers of induction, proliferation and inhibition of angiogenesis, as well as the quantification of microvessel density, correlated with preclinical and clinical research in gynecologic cancers. Studies show that in the most advanced cases of gynecological cancers, biomarkers such as VEGF (Vascular Endothelial Growth Factor), MMP (Matrix Metalloproteinase), CD105 (Endoglin), TIMP (tissue inhibitors of metalloproteinases) and VASH (Vasohibin) are more expressed compared to healthy individuals. Continuous evaluation of these biomarkers in cancer cases could serve in the future as a basis for development of new therapeutic approaches, leading to a good response to cancer treatment, and thus increase survival of cancer patients.

Keywords: Angiogenesis, biomarkers, Endoglin, gynecologic cancer, microvessel density, VEGF.

INTRODUCTION

The process whereby cancer cells arise from normal cells is called tumorigenesis and occurs in several stages. These include the accumulative mutations that lead to proto-oncogenes activation and then inactivation of tumor suppressor genes. The progression and/or regression, and the future of tumor cells, however, depend directly upon the generation of a favorable environment. The so called tumor stroma, where there is a mutual relationship between tumor cells and resident cells in the stroma, such as fibroblasts and endothelial cells [1].

The understanding of the formation of a tumor-associated vascular network is an important step in understanding the process of tumor progression until the generation of metastasis. The importance of angiogenesis process for tumor progression was showed by studies on the monoclonal antibody against the VEGF, the first anti-angiogenesis drug for humans, and so there is an increasing interest in the study of vascularization of tumors [2].

Several cellular markers are used to investigate this process of angiogenesis in different stages of tumoral neovascularization. This review highlights the chief markers (Table 1)[3-23]

for induction of angiogenesis (VEGF, and MMPs), markers available for endothelial proliferation and quantification of microvessel density (CD105) and angiogenesis inhibitors (TIMP and VASH), as well as reviewing the patents available in the literature regarding the applicability of these markers in preclinical and clinical anti-angiogenesis drugs research in gynecological cancer.

TUMOR ANGIOGENESIS

Vasculogenesis and angiogenesis are the terms used for the process of formation of new blood vessels. The first term is the process of blood vessel formation during embryonic stage, and takes its origin from the angioblasts. The second term is the process that occurs during the post-embryonic stage when the formation of new blood vessels arises from pre-existing blood vessels [24, 25].

In many physiological processes vascularization is an important step, such as in embryo development, the female menstrual cycle, tissue repair processes, as well as in growth and differentiation of tissues[26-28]. However, when in pathological conditions (ischemic, inflammatory and neoplastic diseases) this process is called neoangiogenesis [29-31].

In case of tumors, the observation of angiogenesis was done over a century ago, but the hypothesis of a tumor producing angiogenic substances has only been made in 1968, by Folkman. He proposed that tumor growth and metastasis depend upon angiogenesis, and that blocking this process could be a strategy to inhibit tumor progression [26, 32].

Several mutations in proto-oncogenes and tumor suppressor genes lead to uncontrolled production of growth factors, including angiogenic factors. For example, mutations of the RAS and p53 genes increase the expression of VEGF. Associated to VEGF in induction angiogenesis there is the angiopoietins (ANG), that show two isoforms (ANG-1 e ANG-2), however, this growth factor is still not fully known, since in certain circumstances act as a pro-angiogenic factor in another cases act as antagonist[33, 34]. Another factor that not fully understood, platelet derived growth factor (PDGF), which presents in its family five isoforms (PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC and PDGF-DD) capable of binding to three receptors (PDGFR- α , PDGFR- β , and PDGFR- α/β)[35]. The PDGF / PDGFR interaction is important for cell growth, chemotaxis, pressure control of the interstitial fluids and modulation of tumor stroma including pericytes and fibroblasts (reviewed by Madsen et al., 2012)[36], but in gynecological cancer still not clear the functions. For these reasons these factors were not highlighted in this review.

Other factors that can affect tumor growth from the vasculature are the condition of vascular permeability, ie matrix metalloproteinases, that also influence the infiltration of immune cells (eg, monocytes, mast cells and lymphocytes), as well as agents promoting the production of angiogenic factors, ie CD105, or inhibit the proliferation of endothelial cells (TIMPs and VASH) [27,31, 37-40].

Vascular Endothelial Growth Factor (VEGF)

The term "VEGF" refers to the dimeric glycoprotein composed of four isoforms VEGF-A, VEGF-B, VEGF-C and VEGF-D [6,7]. VEGF is produced by most cells in the body, but is upregulated by hypoxia. To perform their actions VEGF family members bind to three tyrosine kinases receptors, VEGFR1, VEGFR2 and VEGFR3. Regarding the action in the neovascularization process during in vivo assays, the performance of VEGF-A is mainly linked to the activation pathway linked to the VEGFR2 receptor [6,7].

Associated with the VEGF receptors, there is a co-receptor, neuropilin with two isoforms (NRP-1 and NRP-2) which are involved in cellular regulation and exhibits high affinity to VEGF and thus leading to their activation pathways[41,42]. There are reports that the NRP-1 regulates the functions of endothelial cells independents of relationship VEGF/VEGFR2[43,44].

VEGF is produced in the tumor environment under hypoxia, mainly by endothelial cells (EC) and infiltrating myeloid cells, such as tumor-associated macrophages (TAM). Tumor

angiogenesis is commonly associated with an immunosuppressive phenotype of macrophages, 'M2' who are pro-angiogenic [45, 46]. Other infiltrating cells such as neutrophils may also play a role in tumor angiogenesis [47, 48].

VEGF in breast cancer

Considering the role of VEGF, several studies have investigated the behavior of this glycoprotein as a biomarker for tumor development; It is under investigation as a marker for the induction and progression of angiogenesis in gynecological tumors (Table 2). Xu *et al* (2013) [49] evaluated breast cancers and benign breast tumors correlating the expression of VEGF by immunohistochemical and imaging techniques [computer tomography (CT)] for three aspects: 1) blood flow 2) vessel volume and 3) permeability of the surface at the site of tumor development. The results demonstrated that in patients with invasive breast cancer there is higher expression of VEGF and a significant positive correlation for the variables assessed by tomography compared to cases of benign breast tumors.

VEGF in ovarian cancer

The investigation of VEGF is not limited to the study of breast tumors, since this marker is overexpressed in 94% of cases of ovarian tumors, and its receptor VEGFR2 is overexpressed in 82% of primary ovarian cancer cases and recurrent cases [51, 52]. The VEGF-related ovarian tumors can be exemplified by the study of Lawicki *et al.* (2013) [53] which showed high levels of VEGF in cases of ovarian cancer compared to controls (samples from benign and normal ovary samples). It was concluded that the synthesis of VEGF could be a therapeutic target. The study of Tsoi *et al.* (2013) [54] corroborates to this aspect, since their results suggest that anti-VEGF therapy is effective in inhibiting the growth of experimentally induced ovarian tumors and found a reduction of microvascular density and cell proliferation via the VEGFR2-MAPK.

VEGF in cervical cancer

Cancer of the cervix is the most occurring gynecological cancer, with tumor stage, lymph node involvement and histological type as prognostic factors. However, little is known about prognostic and / or predictive significance of angiogenesis in cervical cancer (Table 2). In order to investigate the pathways of angiogenesis, especially in cervical intraepithelial lesions, the studies of Cimpean *et al.* (2012) [57] and Stepan *et al.* (2012) [58] suggest active angiogenesis for progression of cervical neoplasia and explains in part, the early presence of lymph node metastasis in cervical cancer. Another important point, correlated with VEGF, evidenced by these authors is that Prox1 expression (Molecular regulator factor with a key role in the performance of lymphatic endothelial cells) in lymphatic vessels, but also in the venous endothelial cells and stromal cells are involved in the tumor angiogenic process. It is suggested that different mechanisms exist for the development of lymphatic vessels during the progression of cervical neoplasia. In the study of Chen *et al* (2013)[56] it is emphasized that one of the pathways that VEGF-C acts in promoting metastasis of cervical carcinoma cells would be through the activation of FAK protein and activation of the pathway Flt-4/c-Src. These proteins are associated with intracellular signal transduction and proliferation of vascular permeability.

Matrix Metalloproteinase (MMP)

Matrix metalloproteinases (MMPs) are endopeptidases with proteolytic activity for collagen during degradation of extracellular membrane (ECM) proteins[8]. The MMP family includes around 28 members, which represent an important class of endopeptidases. Matrix metalloproteinases, particularly MMP-2 and MMP-9 play an important role in the development of the tumors, invasion of tumors and metastasizing. The no presence of balance between MMPs and their inhibitors could facilitate tumor progression[8].

MMP in breast cancer

Some studies showed the importance of investigating MMPs 2 and 9 in gynecological cancers (table 2). In a study conducted by Xu *et al* (2013)[49], MMP-2 expression, assessed by immunohistochemistry, was correlated with the surface of vascular permeability assessed by CT

scan. It was possible to find that comparing volunteers with breast cancer and benign breast tumor, patients with breast cancer showed higher values for MMP-2 and positive correlation and significant with the increase of vascular permeability. Other reports in the literature emphasize that the expression of MMP-2 is linked to poorly differentiated breast tumors [59, 60].

MMP in ovarian cancer

In regard to the action of MMP 2 and 9 in ovarian cancers there is a primary role of MMP-2 over MMP-9 [61,62]. In the study conducted by Su *et al.* (2013)[63], on the influence of MMP-2 and Id1 (inhibitor of DNA binding / differentiation 1) on endothelial progenitor cells, they showed that the expression of Id1 and MMP -2 were increased in endothelial progenitor cells isolated from patients with ovarian cancer compared to cells of healthy individuals. These results were associated with an increase in angiogenesis-related signaling pathways PI3K / Akt and NF- κ B / MMP-2.

MMP in cervical cancer

Highlighting the expression of MMP-9 in cervical cancer, Li *et al* (2012)[64] correlated the expression of MMP with clinicopathological aspects and prognosis. The expression of MMP-9 was increased in cases of cervical cancer correlated with stromal invasion, FIGO classification, lymph node metastasis, and vascular invasion, showing that MMP-9 is an independent prognostic factor, which can be a potential target for diagnostic and even therapeutic interventions of cervical cancer.

Measuring Microvessel desity (by CD34 and CD31) and Endoglin (CD 105)

Endoglin (CD105) is a homodimeric cell surface glycoprotein that acts as an auxiliary receptor for the TGF beta receptor complex; CD34; cell surface glycoprotein involved in adhesion and CD31 is a platelet endothelial cell adhesion molecule (PECAM-1).CD105 is a protein, homodimer of molecular weight 180 kDa, and the gene for this protein is located on chromosome 9, this CD105 is expressed on the cell surface[66]. CD105 (Endoglin) is an important marker in angiogenesis, as it is essential for the proliferation of endothelial cells and stimulates the angiogenic active phase; The protein (CD105) is expressed on the cell surface [14, 66, 67]. Several functions of CD105 are likely to be associated with the TGF- β pathways [15]. CD34 is a cell surface glycoprotein involved in adhesion, CD31 is Platelet endothelial cell adhesion molecule (PECAM-1).

CD105, CD34 and CD31 in breast cancer

Studies with gynecological tumors (Summarized in Table 2) used labeling with CD105 and correlated it as a predictive factor for prognosis, measurement of microvascular density and evaluation of therapeutic methods. In this regard, the study of Dales *et al.* (2003) [69] suggests that the presence of CD105 in patients with breast cancer shows clinical relevance for the prognosis. In addition, the immunostaining of CD105 could be considered an important associate factor for selecting patients, wich could improve the antiangiogenesis therapy, using mAb (monoclonal antibody) α -CD105. The fact that angiogenesis is an important event for tumor growth and metastasis generation implies that the level of angiogenesis [for example, micro vessel density (MVD)] also has value to be assessed as a prognostic factor [70, 71]. The extension of new blood vessels in invasive breast cancer has an important link with metastasis to axillary lymph nodes [71-73]. Together with CD105 analysis, the quantification of the MVD in tumors can be done by staining the tissue with pan-endothelial antibodies such as CD34, CD31 and von Willebrand factor [74]. However, these markers are used when the aim is to distinguish preexisting vessels of new blood vessels. In this context, human tumors - including breast cancer - were found to highly express CD105 in endothelial cells of tumor blood vessels, but were not detectable or weakly staining in vessels from normal tissues [75, 76]. In summary, MVD, such as evaluate using a mAb to CD105, was an independent predictor of prognosis in cases of breast cancer, but this assessment not association when used CD34, a marker of preexisting vessels. The capacity to quantitatively differentiate between tumor neoangiogenesis and preexisting vessels could be a key proceeding in the evaluation of tumoral vascularization [74].

CD105 in ovarian cancer

The expression of CD105 in ovarian carcinomas followed the same patterns as evaluated in breast cancer (and uterine cervix cancer), and is associated with progression of lesions, generation of metastases and worse survival rates; which can be evidenced in the study of Bock *et al.* (2011)[78]. They concluded that the blocking of CD105 could be used for anti-angiogenic therapy. Following this line of thought, studies of Rosen *et al.* (2012) [79] and Ziebarth *et al.* (2012) [80] showed that anti-CD105 therapy has good results in ovarian carcinomas.

CD105 in cervical cancer

One of the ways to assess cervical tumor development would be via the pattern of tumor vascularization. There are numerous studies evaluating the immunological behavior of cervical intraepithelial neoplasia (CIN) and invasive cervical carcinomas [81-83], there are, also, reports in the literature describing the expression of CD105 in different degrees of cervical injury. In this sense a study developed by Stepan *et al.* (2012)[58], which evaluated the expression of CD105 in squamous cell carcinomas of the cervix and premalignant lesions (CIN), showed that in both malignant and premalignant lesions microvessel density was increased. Cases of high-grade lesions (CIN III) compared to cases of CIN I and II, and when comparing the cases of poorly differentiated carcinomas with cases of well-differentiated carcinomas.

Tissue inhibitors of metalloproteinases (TIMPs) and Vasohibin (VASH)

TIMPs

The levels of cytokines and growth factors regulate the expression of MMPs and their activity is regulated by pro-enzyme activation after their secretion or inhibited by their endogenous inhibitor [16]. Tissue inhibitors of matrix metalloproteinases (TIMPs) provide an inhibition in the activity of MMPs. Four metalloproteinase inhibitors have been characterized so far, referred to as TIMP-1, TIMP-2, TIMP-3 and TIMP-4. Among these, TIMP-1 and TIMP-2 have been characterized most extensively, followed by TIMP-3 [16]. Each TIMP has specificity for inhibiting a certain MMP. TIMP-1 presents inhibitory activity towards MMP-9, but not in all times, as reported by Leifler *et al.* (2013)[18], where TIMP-1 in breast tumor cells was unable to inhibit the action of MMP-9. Study performed by Pal *et al.* (2014) [86] revealed in cellular line of breast tumor (MCF-7), the expression of integrins influenced the synthesis of MMP-9, and inhibited the activity of TIMP. Thus, an imbalance between the synthesis of MMPs and TIMPs is present in the tumor progression and emergence of metastasis. As reported by Cardeal *et al.* (2012) [87] wherein the deregulation of MMP-2 and MMP-9 and TIMP-2 were presents in cases of cervical carcinomas by influence of E7 protein of HPV16.

In ovarian tumors there are differing reports of the action of TIMPs in the prognosis, for example, study conducted by Brun *et al.* (2012) [88] reported that the expression of MMP-9 and TIMP-2 did not affect the survival in patients with advanced ovarian cancer. However, Halon *et al.* (2012) [89] showed positive correlation in increase TIMP-2 expression in the stroma and tumor compartment with better survival rate of patients with ovarian cancer.

Regarding TIMP-3, it has no inhibitory effect on MMP, but on VEGF. The study of Qi *et al.* (2013)[17] found that TIMP-3 peptides are effective inhibitors of angiogenesis by blocking VEGF binding to the VEGFR2 receptor and has a potential to be used therapeutically in diseases with increased neovascularization. Although the action and behavior of the TIMPs are not yet clearly understood, the literature suggests that TIMPs are promising targets for angiogenic studies.

VASH

Endothelial cells (ECs) can produce stimulators as well as inhibitors of angiogenesis and have a system of self-regulation or feedback. In this sense, tests with cDNA microarray analysis were performed to detect VEGF-inducible genes in ECs [19,23,90], and functional *in vitro* assays were performed for angiogenesis [19], resulting in the isolation of one gene with

anti-angiogenic activity. The gene was entitled as vasohibin (VASH), but its anti-angiogenic activity *in vivo* has not yet been confirmed.

Since these proteins were discovered, the intention to verify their role in the vascularization process was present. A subcutaneous model of angiogenesis revealed VASH1 expression in non-proliferating ECs in distant branching, but not in newly formed blood vessels of a vascular root. Opposite to VASH1, VASH2 is expressed priority in mononuclear cells and in front of infiltrating sprouting [91].

In study of Yoshinaga *et al* (2011)[92] related that the expression ratio of vasohibin in the normal cervical epithelium was significantly lower than that of Squamous Cell Carcinoma (SCC) and Adenocarcinoma. For normal cervical epithelium, Carcinoma *in situ*, and SCC, there was a moderate correlation between the expression percentage of vasohibin and the expression percentage of VEGFR-2.

Takahashi and Cols (2012)[93], in a study with SKOV-3 and DISS, two representative human serous adenocarcinoma cell lines, proceeded with a knockdown of VASH2 and showed little effect on the proliferation of cancer cells *in vitro* but notably inhibited tumor growth, peritoneal dissemination, and tumor angiogenesis in a murine xenograft model. Indicating that VASH2 expressed in serous ovarian carcinoma cells promoted tumor growth and peritoneal dissemination by promoting angiogenesis. These studies suggests that VASH would be a clinically relevant biomarker with predictive value in the prognosis of cancer patients.

PATENTS ON ANGIOGENESIS MARKERS: THERAPEUTIC TARGETS AND PREDICTIONS

Based on the knowledge of angiogenesis markers with respect to induction, proliferation and inhibition as well as markers for quantification of microvessel density, it is important to review the prospects of these markers as therapeutic targets for cancer treatment. We therefore reviewed recent patents on the issue (Table 3).

Patents on VEGF

One of the great promise in the treatment of cancer has been the discovery of the anti-VEGF monoclonal antibody, known as. Avastin (generic name is bevacizumab). The use of avastin was placed in doubt its effectiveness in cases of metastatic breast cancer, by presenting a higher efficiency in case of higher levels of VEGF-A, and has known that other subtypes have important angiogenic potential, this drug presents with limitations, which enables the search for new and more effective antibodies. Despite its efficacy bevacizumab has been associated with significant risk of cardiovascular complications, such as hypertension, cardiac ischemia, and congestive heart failure facts that makes the search for new anti-angiogenic therapies even more relevant [94].

Regarding to use the inhibition of VEGF as a therapeutic target, Zhou *et al.*, 2010[95], published a patent [US20100331250] about a fusion protein, called SR1, which would be able to block the actions of VEGF. In this patent the authors describe that in nude BALB/c mice injected with colon cancer cells (LS174T), treatment with SR1 was more effective than Avastin (bevacizumab) (Roche 3615286HK0508.1070). Another anti-VEGF antibody was published in the patent [US20110159009] deposited by Fuh *et al.* (2011) [96]. They found that treatment with anti-VEGF (B20-4.1.1) was effective in reducing the tumor size, in an assay using lung (A549) and breast (MDA-MB-231) tumor cells. In the same context, Baca *et al.* (2013) [97] patented a humanized antibody against VEGF and its variant [US20130058927]. This antibody variant showed strong binding to VEGF and thereby inhibits the action of VEGF of induction and proliferation of endothelial cell in *in vitro* assays and inhibits tumor growth *in vivo*.

Another aspect to be analyzed for therapy with anti-VEGF antibodies would be the combination with another monoclonal antibody. The recent patent [US2013/0183292] of Friess *et al.* (2013)[98] provides a treatment for HER2 positive patients, using a combination of anti-HER2 antibody (Trastuzumab) and anti-VEGF (bevacizumab - Avastin). This combination therapy gives a satisfactory response with regard to a significant reduction of tumor volume in animals inoculated with a strain of human breast cancer cells (KPL-4). The scheme of anti-

HER2 for 63 days followed by administration of anti-VEGF was the most effective combination. This combination also showed significant effects when the presence of tumor DNA in the liver was assessed, resulting in lower rates of liver metastases.

To take effect, , members of the VEGF family (Table 1) bind to three tyrosine kinases receptors, VEGFR1, VEGFR2 and VEGFR3. The patents of Alitalo and Makinen (2015) [US 8,940,695 B2] [99] and [US 2015/0141622A1] [100], demonstrated that VEGF-C overexpression interacting with VEGFR-3 in MCF-7 mammary tumors was able to induce a strong and specific tumor growth associated with lymphatic vessels, but does not have major effects on tumor angiogenesis. In this patent Alitalo and Makinen invented a fusion anti-Flt4 (anti-VEGFR-3), able to block VEGF-3 pathway this way inhibiting tumor metastasis.

Patents on MMPs

The matrix metalloproteinases (MMPs), secreted in various diseases including cancer, have also been researched. If MMPs are blocked, the tumors ability to metastasize diminishes, as well as the tumors angiogenic activity, which is reduced. Devy *et al* (2011)[101] [WO2011028883] reported that MMPs are binding proteins (eg, antibodies, peptides and Kunitz domain proteins). These proteins have the ability to bind to MMP-2 and MMP-9 and thus inhibiting them. Therefore, in cases of tumors (e.g., breast cancer), the use of single doses of anti-MMP and/or other anti-cancer agents prevented or reduced the risk of developing tumors, as well as inhibited angiogenesis and reduced the extracellular membrane degradation at the tumor site.

In the patent of Freskos *et al.* (2014)[102] [US2014/0271465 A1], reported one compound conjugates of MMP inhibitors linked metal chelators that were used for diagnosis and treatment of cancer. More specifically in inhibit the MMP-3, MMP-9 and MMP-14, Bissell *et al.* (2015)[103], in your patent [US 2015/0079071 A1], reported a invention relevant to the field of diagnosis and prognostic methods of human cancers, specially breast cancer. Targeting sites of MMPs such as noncatalytic components, this way antibodies for bind in integrin- β 1 and HSP90 β may give more effectiveness in inhibit degradation of extracellular matrix.

Patents on CD105

CD105, also called Endoglin has been a focus of therapeutic interest since Seon *et al.* (1999)[104] reported using an anti-endoglin antibody capable of acting as an anti-angiogenic agent in tumors and diseases related to angiogenesis (patent [5928641]). In the same context, Seon *et al.* (2007)[105] showed, in their patent [US20070071761], the use of combined chemotherapy and anti-endoglin sequentially or concomitantly, resulting in satisfactory effects in the decrease of tumor vascularization.

Babcook *et al.* (2010) [106], concerning the patent [WO2010032059], and Gazit-Bornstein *et al.*, (2010)[107] on the patent [US20100196398], reported a ligand against CD105 as a therapeutic agent. Herewith, they created a fully human antibody directed against CD105, and subject to study diseases associated with the activity and / or overexpression of CD105 as well as its use as a diagnostic marker.

The patent published in 2012 by Guidi *et al.* [108] [WO2012149412] exemplified a combined therapy of an anti-CD105 antibody (huRH105-1) with bevacizumab in an ovarian cancer model (A2780 cell line). A combination therapy with greater efficiency compared to isolated therapies. Confirming this patent, Theuer *et al.* (2012)[109] [US20120244147] showed satisfactory results of the combination of an anti-endoglin chimeric antibody in association with bevacizumab in different types of gynecologic cancers in animals and humans. They reported reduction of angiogenesis such as "sprouting", increased survival rates and decreased time of tumor progression.

When reviewing the patents and their findings, they confirm the importance of angiogenic markers in general tumors, but deeper investigation is needed in gynecological tumors. These markers may assist in the diagnosis, prognosis and establishment of new guidelines in the therapy of various types of tumors.

CURRENT & FUTURE DEVELOPMENTS

This brief review discusses some of the markers that were indicated to verify the markers involved in the angiogenic process, factors that promote and inhibit angiogenesis as well as markers to quantify neovascularization Fig. (1).

Studies using such biomarkers propose a continuous evaluation of the various cases of gynecological cancers, since the increased expression of VEGF and MMPs are linked with the presence of tumors, and the generation of metastasis and overexpression of CD105 are correlated with increased tumor progression. This characterization and monitoring may serve as a basis for future prognostic, could be implemented in new detection techniques as seen in the patent of Autelitano *et al.* (2015)[110] [US2015/0025810A1] that reported a new assay for diagnosing of presence or risk of having some gynecological cancer, its subtypes and stage, observing biomarkers. This way, enabling better characterization and development of new therapeutic approaches, leading to reduction of tumors, or even elimination, and thereby increases the survival rate of cancer patients.

CONFLICT OF INTEREST

There is no conflict of interest to report.

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Table 1. Description of markers of angiogenesis process with subdivision and functions.

Markers	Subdivision	Function	Ref
Vascular Endothelial Growth Factor (VEGF)	VEGF-A VEGF-B VEGF-C VEGF-D Receptors VEGFR1 VEGFR2 VEGFR3 Co-receptors NRP-1 NRP-2	↑ Function of endothelial cells (ECs)	Leung <i>et al.</i> , 1989[3] Keck <i>et al.</i> , 1989[4] Kim <i>et al.</i> , 2012[5] Yang <i>et al.</i> , 2012[6] Claesson-Welsh; Welsh, 2013[7]
			Gross; LaPiere, 1962[8] Woessner, 1991[9] Werb <i>et al.</i> , 1996[10] Zeng <i>et al.</i> , 1999[11] Murnane <i>et al.</i> , 2009[12]
Matrix Metalloproteinases (MMP)	MMP-2 MMP-9	Degradation of basal membrane (↑ Vascular Permeability)	Fonsatti <i>et al.</i> , 2003[13] Nassiri <i>et al.</i> , 2011[14] Levi <i>et al.</i> , 2011[15]
Endoglin (CD105)	---	Interaction in TGF- β pathway for proliferation and new ECs differentiation.	Groblewska <i>et al.</i> , 2012[16] Qi <i>et al.</i> , 2013[17] Leifler <i>et al.</i> , 2013[18]
Tissue Inhibitors of Metalloproteinases (TIMPs)	TIMP-1 TIMP-2 TIMP-3 TIMP-4	Inhibiting the action of MMPs	Watanabe <i>et al.</i> , 2004[19] Shimizu <i>et al.</i> , 2005[20] Shibuya <i>et al.</i> , 2006[21] Kern <i>et al.</i> , 2008[22] Sato, 2013[23]
Vasohibin (VASH)	VASH-1 VASH-2	Functional blocking of VEGF	

Note: (ECs) Endothelial cells

Table 2. Changes of expressions of markers of angiogenesis in cases of gynecological tumors.

Ref	Localization	Status of Marker
Vascular Endothelial Growth Factor (VEGF)		
Qi <i>et al.</i> , 2013[17] Xu <i>et al.</i> , 2013[49] Saporano <i>et al.</i> , 2013[50]	Breast	<p>↑ VEGF in cases of genetic alterations for BRCA1/2</p> <p>Blocking of VEGFR2 = less rate of proliferation and migration of ECs (<i>in vitro</i>)</p> <p>↑ VEGF in breast cancer compared to benign breast tumors and positive correlation with blood flow, vessel volume and permeability assessment by CT.</p>
Lawicki <i>et al.</i> , 2013[45]	Ovary	<p>↑ VEGF in cases of tumors vs controls</p> <p>Blocking VEGF = ↓ tumors/ ↓ MVD/ ↓ proliferation</p>
Biedka <i>et al.</i> , 2012[55] Chen <i>et al.</i> , 2013[56]	Uterine Cervix	<p>↑ VEGF-A/VEGF-C associated to progression of cervical lesion</p> <p>↑ VEGFR3 in cases with more advanced of cervical lesions</p> <p>VEGF-C promoting metastasis of cervical carcinoma cells by activation of FAK and flt4/c-Src</p>
Matrix Metalloproteinases (MMP)		
Pellikainen <i>et al.</i> , 2004[60]	Breast	<p>↑ MMP-2/MMP-9 associated with expression of AP-2 and HER-2</p> <p>↓ survival of hormone sensitive tumors</p>
Schmalefeldt <i>et al.</i> , 2001[61] Kamel <i>et al.</i> , 2010[62]	Ovary	<p>↑ MMP-2 and ↓ MMP-9</p> <p>↑ MMP-2 = tumoral proliferation, invasion and metastasis</p> <p>↑ MMP-1/MMP-9 in invasive carcinomas</p>
Li <i>et al.</i> , 2012[64] Ryzhakova <i>et al.</i> , 2013[65]	Uterine Cervix	<p>MMP-2 without influences in cervical tumors</p> <p>↑ MMP-9 associated with metastasis in lymph nodes</p>
Endoglin (CD105)		
Mishra <i>et al.</i> , 2012[68]	Breast	<p>↑ CD105 associated with androgen receptors in cases of poorer prognosis and generation of metastasis</p>
Taskiran <i>et al.</i> , 2006[77] Bock <i>et al.</i> , 2011[78]	Ovary	<p>↑ CD105 = progression of lesions.</p> <p>Generation of metastasis and poorer survival rate</p>
Cimpean <i>et al.</i> , 2009[84] Zijlmans <i>et al.</i> , 2009[85]	Uterine Cervix	<p>↑ CD105 associated with progression of cervical lesions and ↑ generation of metastasis</p>

Table 03 - Patents related to angiogenesis and anti-angiogenic drugs.

Authors	Number of Patent	Title
Zhou <i>et al.</i> , 2010[95]	US2010/0331250A1	Anti-angiogenesisfusionproteins
Fuh <i>et al.</i> , 2011[96]	US2011/0159009A1	Anti-VEGFantibodies
Baca <i>et al.</i> , 2013[97]	US2013/0058927A1	Anti-VEGFantibodies
Friess <i>et al.</i> , 2013[98]	US2013/0183292A1	Tumor therapywithananti-VEGFantibody
Alitalo & Makinen, 2015[99]	US8940695 B2	Flt4 (VEGFR-3) as a target for tumor imaging and anti-tumor therapy
Alitalo & Makinen, 2015[100]	US2015/0141622A1	Flt4 (VEGFR-3) as a target for tumor imaging and anti-tumor therapy
Devy <i>et al.</i> , 2011[101]	WO2011/028883A2	Metalloproteinase 9 andmetalloproteinase 2 bindingproteins
Freskos <i>et al.</i> , 2014[102]	US2014/0271465 A1	Matrix metalloprotease (MMP) targeted agents for imaging and therapy
Bissell <i>et al.</i> , 2015[103]	US2015/0079071 A1	Non-catalytic domain targets in matrix metalloprotease proteins for cancer therapies
Seon, 1999[104]	5,928,641	Anti-endoglin monoclonal antibodies and their use in antiangiogenic therapy
Seon, 2007[105]	US2007/0071761A1	Method for increasing the efficacy of anti-tumor agents by anti-endoglin antibody
Babcook <i>et al.</i> , 2010[106]	WO2010/032059A2	Targeted binding agents directedto CD105 and uses thereof
Gazit-Bornstein <i>et al.</i> , 2010[107]	US2010/0196398A1	Targeted binding agents directedto CD105 and uses thereof
Guidi <i>et al.</i> , 2012[108]	WO2012/149412A2	Anti-endoglin (CD105) antibodies, immunoconjugates, and uses thereof
Theuer <i>et al.</i> , 2012[109]	US2012/0244147	Combination therapy of cancer with anti-endoglin antibodies and anti-VEGF agents
Autelitano <i>et al.</i> (2015)[110]	US2015/0025810A1	Assay to detect a gynecological condition

Figure 1. Representation of markers of angiogenesis for induction of angiogenesis (VEGF), tumor progression and invasion (CD105, MMP-2 and MMP-9), inhibition of angiogenesis (TIMPs and VASH) and markers used for quantification of microvessel density (CD31, CD34 and CD105).