Growth factors, Cytokines and VEGF in human neoplastic and inflammatory pathologies
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Chapter 1

Introduction

VEGF in Nuclear Medicine: Clinical Applications, Future Perspectives and Review of the Literature

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Introduction

The clinical study of human inflammatory and neoplastic pathologies requires an accurate evaluation of tissues microenvironment to better understand the complex mechanisms which may represent the cause of the physiopathological evolution of these diseases. In this perspective immunohistochemistry (IHC) potentially represents an ideal investigative tool which may be very useful in the preliminary approach addressed to the preparation of innovative radiolabeled drug used in nuclear medicine diagnosis and therapy of the above mentioned pathologies. Potential new antigens and/or receptors involved in the progression of tumors or inflammatory conditions, and discovered in sick tissues by IHC, may lead to an interesting selective determination of novel radiolabeled ligands or anti-targets antibodies. For these reasons in this Ph.D. thesis we have tried to establish some intriguing and possibly important correlations between growth factors and/or inflammatory cytokines “immunolabeling” granted by IHC and their potential application in nuclear medicine. The majority of humans neoplasms demonstrates a concrete correlation between factors stimulating vessels’ growth (especially VEGF) and progression of the tumor.

Clinical trials with antiangiogenic drugs revealed their potential against cancer. However, a large percentage of patients still does not benefit from this therapeutic approach highlighting the need of diagnostic tools to non-invasively evaluate and monitor response to therapy. In this perspective the role of Nuclear Medicine remains pivotal, being the discipline that allows the more effective approach to non-invasive lesion detection, especially neoplastic ones, with encouraging results even in advances diseases. It would also allow to predict which patient will likely benefit from antiangiogenic therapy. Reasons for treatment failure may be that low expression of drug’s targets or prevalence of other pathways, therefore, molecular imaging has been explored as a
diagnostic technique of choice. Since the VEGF/VEGFR pathway is the main responsible of tumor angiogenesis, many new drugs target either the soluble ligand or its receptor to inhibit signaling leading to tumor regression. It is currently impossible to evaluate local VEGF or VEGFR levels and their non-invasive measurement in tumors might give insight to the available target for VEGF/VEGFR-dependent antiangiogenic therapies allowing therapy decision making and monitoring of response. Angiogenesis is the process that leads to the formation of new blood vessels, and, if induced by tumors, can also contribute to the growth of the disorganized vasculature able to sustain cancer progression over 2-3 mm and metastasization (1). The events that trigger tumor angiogenesis derive from the interaction between cancer cells and host microenvironment that includes immune cells, connective tissue and soluble factors. Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) are the main contributors to proliferation of endothelial cells, thus representing suitable targets for antiangiogenic therapies (2).

VEGF

Vascular endothelial growth factor (VEGF) is the most important mediator of angiogenesis. It is overexpressed in various tumors, stimulating endothelial cell proliferation and migration, and leading to the formation of new blood vessels from preexisting ones (8). The VEGF family is composed of five glycoproteins (VEGF-A, VEGF-B, VEGF-C, VEGF-D and VEGF-E). VEGF-A is a homodimeric, disulfide-bound glycoprotein, which exists in several isoforms with different numbers of amino acid residues, such as VEGF$_{121}$, VEGF$_{165}$, VEGF$_{189}$ and VEGF$_{206}$. Different VEGF-A isoforms exhibit varying biological properties, such as the ability to bind to cell surface heparin sulfate proteoglycans. VEGF$_{121}$, commonly existing as a homodimer, is freely diffusible without heparin binding. The angiogenic actions of VEGF are mediated primarily via two closely related endothelium-specific receptor tyrosine kinases, Flt-1 (VEGFR1) and Flk-1/KDR
(VEGFR2) (9). Both are largely restricted to vascular endothelial cells and are overexpressed on the endothelium of tumor vasculature, yet they are almost undetectable in the vascular endothelium of adjacent normal tissues (10). All of the VEGF-A isoforms bind to both VEGFR1 and VEGFR2. VEGF and its receptors are overexpressed in a variety of solid tumor biopsy specimens, and overexpression of VEGFR2 or VEGF-A has been considered as a poor prognostic marker in various clinical studies (11-13). Indeed, new vasculature allows tumor cells to grow by supplying nutrients and oxygen, enabling disposal of metabolic waste products and providing a route for metastatic spreading. VEGF production by tumor cells is thought to be regulated by hypoxemia, growth factors signaling, cytokines, and cell differentiation (8).

Given the role of VEGF and VEGFR in several oncological and non oncological diseases, pharmaceutical companies and researchers are deeply involved in developing agents potentially useful in the prevention of VEGF-A binding to its receptors (14), or antibodies blocking VEGFR2 (11) or small molecules that inhibit the kinase activity of VEGFR2 (15, 16) and thereby block growth factor signaling. Indeed, VEGF/VEGFR targeting has already been proved successful in many cancer types (17).

**The VEGF/VEGFR pathway**

The VEGF-VEGFR system is unique in that it consists of a very limited number of molecules that play a central role in angiogenesis. The likely mechanism is that bevacizumab binds to VEGF both soluble and bound to the extracellular matrix and thereby prevents VEGF binding to its receptors, blocking the biologic pathways induced after VEGF binding. Bevacizumab is approved both by the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of metastatic colorectal cancer, non small cell
lung cancer, breast cancer and glioblastoma multiforme in combination with chemotherapy.

VEGF-A and its receptors are the best-characterized signaling pathway in developmental angiogenesis as well as tumor angiogenesis (10). VEGFR2 appears to be the most important receptor in VEGF-induced mitogenesis, angiogenesis, and permeability increase, whereas the role of VEGFR1 in endothelial cell function is less clear (18). During the exponential growth stage, VEGFR expression is highly up-regulated on the newly developed tumor vasculature. Being the naturally existing VEGFR ligand, VEGF₁₂₁ offers several advantages over the synthetic small-molecule VEGFR ligands or anti-VEGFR antibodies, especially as a tracer. It has much higher binding affinity to VEGFR (nanomolar range) than reported peptidic VEGFR inhibitors (submicromolar to micromolar range) (19, 20). If Compared to antibody-based radiopharmaceuticals, VEGF₁₂₁ clears much faster from the blood pool and the non-targeting organs because of its smaller size. Regulation of inflammatory cell recruitment by VEGFR1 appears to be exerted mainly through placental growth factor (PGF). Notably, the expression of PGF is very low under physiological conditions, but it may be strongly upregulated in various cell types by different pathological stimuli such as hypoxia, inflammatory cytokines, or oncogenes (21-23). PGF has recently been regarded as an attractive candidate for anti-angiogenic therapy. Indeed, it has been shown that PGF plays a key role in promoting pathological angiogenesis associated with tumor progression (24) and overexpression of PGF in a mouse melanoma model resulted in increased tumor growth and metastasis (25). Tumor cells may also express VEGFR2, although epithelial and mesenchymal tumor cells typically express VEGFR1 rather than VEGFR2 (26, 27). Nevertheless, increased expression of VEGFR2 on tumor cells has been described for melanoma and hematological malignancies (28). It has been shown that VEGFR2-mediated signaling allowed survival of cancer cells under chronic hypoxic conditions and might contribute to a more aggressive phenotype (29). VEGF-C, VEGFR3 and its involvement in lymphatic endothelial cell proliferation should also be considered for their
potential important role in the pathogenesis of reticulo-endothelial diseases. A soluble form of the VEGFR2 (sVEGFR2) has been also described and it may have important biological roles. sVEGFR2 binds VEGFC and prevents activation of VEGFR3, consequently inhibiting lymphatic endothelial cell proliferation (31). Notably, regulation of sVEGFR2 in advanced metastatic neuroblastoma may promote lymphogenic spread of metastases (32). The expression of VEGFR3 in tumor cells is still controversial (33); however, it has been ascertained that inhibition of VEGFR3 activity arrests tumor vascularization, leading to decreased vascular density in several tumor models (34). The axis VEGF-C/VEGFR3 plays a fundamental role in the tumor microenvironment by promoting the formation of new lymphatic vessels from preexisting ones (35). VEGF-C, produced by neoplastic cells, induces lymphatic endothelial destabilization, resulting in endothelial sprouting as well as leakage and enlargement of the vessels. These modifications induce entry of tumor cells into the lymphatics vessels and further dissemination of metastasis to sentinel lymph nodes (36, 37).

**VEGF and cancer-related inflammation**

Growing evidence supports an important link between chronic inflammation and tumor development. Induction of VEGFR2 expression in tumor cells, and also in intestinal epithelium during colitis, is mediated by the pro-inflammatory cytokine interleukin-6, which is a strong promoter of tumor growth in experimental colitis-associated colon cancer (30). High expression of VEGFs and/or VEGFRs in various tumor biopsy specimens is indicative of poor prognosis for cancer patients (2, 38, 39). Therefore, non-invasive imaging and quantification of VEGFR expression is of relevant importance in cancer patient management. Many strategies have been adopted to block the VEGF/VEGFR signaling pathway for cancer treatment, such as agents that can bind to VEGF-A to prevent its interaction with VEGFRs (bevacizumab, VEGF-trap, etc.) (40,
41) antibodies/antibody fragments that target VEGFR-2 (ramucirumab, CDP791, etc.) (42, 43) and small molecule inhibitors that interrupt the downstream signaling of VEGFR-2 (axitinib, sunitinib, sorafenib, etc.) (44–45). Many of these agents have been approved by the Food and Drug Administration (FDA) for various medical indications in cancer therapy (2, 46).

VEGFR-2 mediates the majority of VEGF-A signaling in the tumor microenvironment including microvascular permeability and endothelial cell proliferation (8, 10). Several agents, including anti-bodies and soluble receptor constructs, have been developed to target the VEGF system. The drug that is currently most widely used in the clinical practice to modulate VEGF-A is the humanized monoclonal antibody. It blocks VEGF-induced endothelial cell proliferation, permeability, and survival, and it inhibits human tumor cell line growth. One of the greatest challenges in bevacizumab therapy is the lack of predictive biomarkers and tools that can predict the efficacy of anti-VEGF therapy (48).

**Anti-VEGF drugs**

Development of anti-angiogenic therapy including anti-VEGF antibodies and VEGF-tyrosine kinase receptors has been a major landmark in cancer therapy leading improvement in survival in several cancers.

The pharmacologic inhibition of angiogenesis via the VEGF pathway is an important therapeutic approach that prevents cancer growth and metastasis formation. In addition to anti-VEGF antibodies, other strategies have been explored and include the blocking of its signaling receptor, receptor tyrosine kinase inhibitors (49–52), and gene therapy approaches, in which the vector produces an antisense molecule or a soluble receptor that acts in a dominant negative manner (53).

Several studies have shown that anti-VEGF treatment, in association with chemotherapy (54) or radiation therapy (55, 56), results in greater anti-tumor
effects than either treatment alone. An issue that is now being debated is the mechanism of such potentiation, and a variety of hypotheses, which are not mutually exclusive, have been put forward. Klement et al. proposed that chemotherapy, especially when delivered at low dose, preferentially damages endothelial cells and the blockade of VEGF blunts a key survival signal for endothelial cells, thereby amplifying the antitumor-cell effects of chemotherapy (54). Jain et al. proposed that antiangiogenic therapy ‘normalizes’ the tumor vasculature, leading to pruning of excessive endothelial cells and perivascular cells, reduction in vessel tortuosity and drop in interstitial pressure and consequent improved oxygenation and delivery of chemotherapy to tumor cells (57). These effects are accompanied by a reduction in permeability of macromolecules (58, 59). Most recently, Willett et al. have shown that VEGF blockade by bevacizumab decreases tumor perfusion, vascular volume, microvascular density, interstitial fluid pressure and the number of viable circulating endothelial and progenitor cells in colorectal cancer patients (60). Surprisingly, these studies have also shown that permeability to small molecules actually increases following VEGF blockade (60).

Bevacizumab has been initially approved for the treatment of metastatic colorectal cancer in combination with intravenous 5-fluorouracil-based chemotherapy (61). Subsequently, bevacizumab has been approved for various indications in nonsquamous cell lung carcinoma (NSCLC), metastatic renal cell carcinoma, and glioblastoma multiforme (62-66). The antitumor activity of bevacizumab is primarily manifested in combination with chemotherapy, except for renal cell carcinoma, where it has shown efficacy as a single agent (67). Presently, bevacizumab is being used in nearly 1000 clinical trials, and despite promising results, its effects in many types of cancer are modest or even irrelevant (68). Furthermore, recent studies have raised the possibility that treatment with bevacizumab may be associated with a more aggressive invasive tumor phenotype, particularly in glioblastoma (69), which is often a greatly vascularized brain tumor. Although the clinical impact of these results is far from clear, it is obvious that antiangiogenic therapy will have to be closely
evaluated depending on disease stage and molecular profile of different patients and tumours.

Preclinical data with anti-VEGFR2 antibodies have demonstrated a reduction in VEGF-induced signaling as well as angiogenesis and primary or metastatic growth in a variety of different tumor models (70 - 73); therefore, the specific, antibody-based blockade of VEGFR2 has also received special attention in clinical trials. Ramucirumab (IMC-1121B; Imclone Systems) is currently being tested in several clinical trials, including breast cancer, gastric cancer, and HCC (74). Basing on preliminary results, this antibody has shown activity in patients previously treated with other antiangiogenic agents, suggesting a more efficient antitumor response by direct targeting of VEGFR2.

Small molecule inhibitors of VEGFR tyrosine kinase activity represent another major approach to blocking VEGF-mediated angiogenesis. Several tyrosine kinase inhibitors have been developed to selectively inhibit VEGFR2, but they have also activity on other VEGFRs and tyrosine kinase receptors, including basic fibroblast growth factor (FGF) receptor, EGFR family members, PDGFR-a, PDGFR-b, c-kit, and Flt3. Sunitinib was approved in 2006 for its clinical use in imatinib-resistant gastrointestinal stromal tumors and advanced metastatic renal cell carcinoma (75, 76), whereas sorafenib received FDA approval for the treatment of metastatic renal cell carcinoma (77) and HCC (78). Sunitinib and sorafenib have shown clinical efficacy as single agents, possibly due to their ability to inhibit multiple RTKs and in particular those regulating tumor angiogenesis. Additional clinical trials aimed to evaluate combinations of sorafenib and sunitinib with different chemotherapeutic agents and other antiangiogenic agents are under evaluation.

There has been a worldwide research program to develop antiangiogenic agents for the treatment of cancer. Many families of antiangiogenic drugs now exist, but their clinical development has been hampered by scarce data concerning the optimal biologically active dose. In addition, although the classical phase I study design focuses on toxicity as an endpoint to establish the maximum tolerated dose, many humanized monoclonal antibodies have no
clinically significant toxicity, which precludes identification of the maximum tolerated dose. Furthermore, biologic dose–response relationships may follow a bell-shaped curve (79) and therefore the maximum tolerated dose may not even be the best dose for clinical applications. To overcome these issues, biologic pharmacodynamic investigations (80) have entered phase I clinical trial design with the goal of establishing the optimum biologically active dose.

Efficacy of anti-VEGF therapy

Antiangiogenic therapies are promising approaches for cancer treatment. However, their systematic application remains problematic because of poor understanding of mechanisms of action and occurrence of resistance (81). Indeed, a significant fraction of patients do not respond to antiangiogenic drugs (82), whereas those who respond have relatively modest benefits, mostly in progression-free survival rather than in overall survival. In addition, a number of significant toxicities have been observed in patients treated with antiangiogenic agents, emphasizing that a careful assessment of the risk-benefit ratio needs to be conducted in individual patients. Despite disease stabilization and increase in the proportion of patients with progression-free survival, tumors eventually become resistant to antiangiogenic agents and relapse (83 - 86).

Antiangiogenic therapy depends on several factors, including the tumor stage, the nature of the tumor vascular bed and the origin and genotype of the neoplastic cells. Tumorigenesis (87), and progression (88) are often associated with a modified expression of different angiogenic factors (88). Advanced human breast cancers may express different pro-angiogenic factors, including VEGF, acidic and basic fibroblast growth factors (aFGF and bFGF), transforming growth factor β1 (TGFβ1), platelet-derived growth factor (PDGF), placental growth factor (PGF) and pleiotrophin (88). The mechanism of action of certain drugs is also different at various stages of tumorigenesis. For example, the
release of VEGF, following the remodelling of the extracellular matrix by matrix metalloproteinase 9 (MMP9), is reported to be a component of the RIP1–Tag2 angiogenic switch (89, 90-93). Inhibition of VEGF is not effective against established β-cell islet tumors (90, 94), and this finding may led to hypothesize that the vasculature matures with increased pericyte coverage, thereby reducing dependence on VEGFR2. The success of targeted therapies, such as trastuzumab (Genentech), is often dependent on the expression of the drug’s target by the tumor (95). Given that bevacizumab is a monoclonal antibody with a well-defined target, it is logical that VEGF expression might predict benefit. However, in retrospective subset analyses, VEGF expression by primary tumors of metastatic, treatment-refractory breast cancers (96) or metastatic colorectal cancers did not predict benefit from the addition of bevacizumab (97). The reasons responsible for this behaviour are not entirely clear. Perhaps VEGF expression by primary tumors is not representative of metastatic disease, but detailed research indicates that they are equivalent (98).

**Imaging of tumor angiogenesis**

Positron emission tomography (PET), very sensitive technique (down to 10-12 molar) and quantitative with superb tissue penetration, has been widely used in clinical oncology for tumor staging and treatment monitoring, where 18F-FDG (fluorodeoxyglucose) was used as the tracer for measuring tumor glucose metabolism (107, 108). High-resolution PET scanners continue to be developed and made available for imaging small animals, improving the capacity for in vivo studies in mice, primates, and humans (Fig. 1 and 2). As already discussed, anti-angiogenic targeted therapies are a promising approach for the treatment of cancer. However, clinical trials showed variable response due to intra- and inter-tumor heterogeneity and non-invasive tools to monitor treatment response and drug efficacy are needed.
Several methods have been developed to image tumor angiogenesis, but there is no general agreement as to which strategy is the most suitable for monitoring anti-angiogenic therapy in single-center and multicenter trials. There is also evidence that angiogenic imaging data may be a useful predictor of response to chemo-radiotherapy, the success of which depends on good perfusion of the tumor (Fig. 3).

Personalized medicine allows to identify the right patient population for the right therapy at the right time, as well as to provide quantitative, non-invasive, and accurate information about the therapeutic responses in real-time. In this scenario nuclear medicine offers several radiopharmaceuticals for “in vivo” imaging of angiogenic markers, but to date, none emerged as a gold standard. As an example, radiolabeled bevacizumab is one of the most studied radiopharmaceuticals since it is able to bind VEGF with high affinity. Indeed, development of a bevacizumab-based imaging agent can play important roles in these aspects, as well as elucidating the function and modulation of VEGF/VEGFR signaling during cancer development/intervention.

**Targeting vascular endothelial growth factor**

Being the most important angiogenic effector and already established therapeutic target, many VEGF-targeting radiopharmaceuticals were developed and studied in vitro and in vivo. In particular, the mAb bevacizumab is one of the most studied radiolabelled anti-VEGF drugs and, to date, it has been labeled with a number of PET isotopes such $^{89}$Zr (109, 110), $^{124}$In (111), $^{86}$Y (112), and $^{64}$Cu (113). In addition, it has also been investigated with various other imaging techniques such as single photon emission computed tomography (SPECT) (114, 115), ultrasound (116), and optical imaging (117, 118). Studies with radiolabeled bevacizumab for imaging tumor angiogenesis were performed in preclinical models proposing that its accumulation in the tumor was due to interactions with the VEGF-A-165 and -189 isoforms,
associated with the tumor cell surface and/or the extracellular matrix (137-139). However, in a clinical study with $^{111}$In-bevacizumab in patients affected by colorectal cancer liver metastases, there was a lack of correlation between radiolabelled bevacizumab uptake and VEGF-A expression in the lesions (140). Authors speculated that the accumulation of the mAb was due to enhanced vascular permeability leading to unspecific uptake in the tumor. This could limit the usefulness of radiolabeled bevacizumab in imaging tumor angiogenesis. However, this radiopharmaceutical showed promising results in many other cancers like breast cancer. Various studies have reported overexpression of VEGF-A in the breast cancer microenvironment, compared with normal breast tissue (142-145). All VEGF-A splice variants are bound by the clinically used monoclonal antibody bevacizumab. When labeled with the PET isotope $^{89}$Zr, it preserves its VEGF-A–binding properties. Thus, tracer dosages of radiolabeled bevacizumab can be used for tumor-specific, whole-body imaging of VEGF-A. In preclinical studies (146, 147) and in a study in renal cell cancer patients (148), we have already shown an excellent tumor-to-background ratio with an optimum at 4 d after tracer injection when using $^{89}$Zr-bevacizumab. $^{89}$Zr-bevacizumab might be potentially valuable for biologic characterization of tumors and for prediction and evaluation of the effect of VEGF-A–targeting therapeutics. VEGF-A is reported in several studies to be over expressed in malignant breast tumors and in ductal carcinoma in situ (145,149), thus covering the full spectrum from early-stage breast cancer to more advanced stages. More frequent VEGF-A staining was found to be related to aggressiveness as assessed by VEGF-A staining in a study with 1,788 breast tumors (145). $^{89}$Zr-bevacizumab PET proved to be able to detect a broad range of VEGF-A expression levels. Quantitative tumor analyses showed a more than 10-fold difference between individual $\text{SUV}_{\text{max}}$ measurements, suggesting large differences in VEGF-A tumor levels between patients, $^{89}$Zr-bevacizumab might be potentially valuable for biologic characterization of tumors and for prediction and evaluation of the effect of VEGF-A–targeting therapeutics.
Because of better and more accurate scatter and attenuation corrections associated with PET, $^{86}\text{Y}$-labeled bevacizumab was developed for imaging VEGF-A tumor angiogenesis and as a surrogate marker for $^{90}\text{Y}$-based RIT (Radioimmunotherapy Trial). The $^{111}\text{In}$ and $^{89}\text{Zr}$-labeled probes have been proposed as surrogate imaging markers for $^{90}\text{Y}$ therapy, however, deviations were observed due to subtle differences in the metalchelate complexes and metabolism (150, 151) highlighting the need for the development of isotopically matched $^{86}\text{Y}$-labeled probes for $^{90}\text{Y}$.

However, $^{86}\text{Y}$ possesses its own set of challenges, in particular, its high positron energy ($\text{E}_{\text{max}} \approx 3.1 \text{ MeV}$) and emission of 1.08 MeV (83% abundance), which can significantly affect the image quality and recovery coefficients due to spurious coincidences. When appropriate corrections are performed, the image quality is greatly improved and is quantifiable (152 – 154).

PET imaging with $^{86}\text{Y}$-CHX-A00-DTPA-bevacizumab may have a useful role in patient selection for bevacizumab-related therapy as it would indicate accessibility of the antibody to VEGF-A target sites. However, $^{86}\text{Y}$-CHX-A00-DTPA-bevacizumab imaging by itself may not predict the response to therapy as it is only indicative of how much bevacizumab reaches the tumor and not the overall tumor microenvironment and the biomolecular characteristics. The primary use of $^{86}\text{Y}$-CHX-A00-DTPA-bevacizumab will be for the selection of patients for $^{90}\text{Y}$-CHX-A00-DTPA-bevacizumab RIT, monitoring of those patients during therapy as well as to provide information for dosimetry calculations (150, 155) To achieve the long-term goal of clinical translation of $^{86}\text{Y}$-CHX-A00-DTPA-bevacizumab, PET/CT and MRI studies are currently being performed with mice bearing orthotopic and disseminated ascites forming colorectal and ovarian tumors.

In conclusion, the utility of $^{86}\text{Y}$-CHX-A00-DTPA-bevacizumab for noninvasive PET imaging of VEGF-A secreting tumors in preclinical models has been demonstrated (156) $^{86}\text{Y}$-CHX-A00-DTPA-bevacizumab may be useful for the assessment of bevacizumab uptake and localization, which may be important for risk stratification, patient screening and appropriate dosage selection.
Ultimately, $^{86}$Y-CHX-A00- DTPA-bevacizumab would serve as a surrogate PET marker for dosimetry and selection of subjects for $^{90}$Y CHX-A00-DTPA-bevacizumab RIT of VEGF-A–secreting cancers (156).

The limiting factor for more general application of imaging with radionuclides is the radiation burden. In a study comparing the risks of radiation-induced cancer from mammography, molecular breast imaging, and positron emitting mammography, the cumulative cancer incidence is 15–30 times higher for positron emission mammography and molecular breast imaging than for mammography (157). The estimated radiation burden of $^{89}$Zr-bevacizumab-PET is 19 mSv per tracer injection, on the basis of extrapolation from $^{111}$In-bevacizumab data and a dosimetry study on $^{89}$Zr-U36, compared with 5.3 mSv for $^{18}$F-FDG PET (158-161).

Besides bevacizumab, other radiolabeled anti-VEGF antibodies such as I-labeled VG76e (121) and HuMV833 (122) have been reported. Phase I trials of the latter revealed that antibody distribution and clearance was quite heterogeneous, not only between and within patients but also between and within individual tumors, which underscored the importance of patient selection to achieve maximum therapeutic effect.

**Targeting VEGF receptor**

In addition to VEGF, VEGFR is another important target for cancer diagnosis and monitoring the therapeutic efficacy of anti-angiogenic therapies. Over the last decade, imaging of VEGFR expression has gained enormous interest not only in cancer but also in many other angiogenesis-related diseases (119, 120). Examination of the tumor in the same animals or cancer patients with both VEGF- and VEGFR-targeted radiopharmaceuticals or fluorescent probes in comparison to IHC expression of VEGFR1 and VEGFR2 in tissue animals (Fig. 4 and 5) or in human neoplastic tissues (Fig. 6 and 7) may give important insights about distribution and expression kinetics of VEGF and VEGFRs during
cancer development and cancer therapy. Substantial effort has been devoted to non-invasive imaging of VEGFR expression in cancer over the last two decades and various agents have been developed for SPECT (125–128), PET (126, 129–133), optical imaging, magnetic resonance imaging (MRI) (134) and ultrasound (US) (135). Because of the high affinity to VEGFRs, VEGF_{121} has emerged as a particularly desirable candidate for tracer development in the literature (123). To avoid significant interference with VEGFR binding, site-specific labeling of VEGF-based proteins has been adopted in many literature reports which typically utilizes a cysteine residue for radiolabeling (126, 136). However, in many of the reported studies, liver and kidney uptake of the tracer was very high (in some cases > 100 percentage of injected dose per gram of tissue [%ID/g]) which significantly hampered the clinical translation/applications of these tracers. The aim is to develop a PET tracer for the imaging of VEGFR expression using lysine tagged recombinant human VEGF_{121} (denoted as K3-VEGF_{121}). The three lysine residues at the N-terminus, far from the VEGFR binding sites, can facilitate radiolabeling without affecting the biological activity and receptor binding. In the design of novel radiotracers, it is important to minimize the radiation dose to normal organs without compromising the imaging characteristics. VEGF121 and its derivatives have been labeled with many PET/SPECT isotopes.

Considered these premises about the importance of IHC in the detection of new potential targets in nuclear medicine management of neoplastic and inflammatory conditions in the 2nd chapter of this thesis are reported the results of a previously published study which correlates the immunohistochemical expression of neurotrophins between meningiomas and normal dura mater. In the 3rd chapter are presented the results of a study performed on human GH-secreting pituitary adenomas to investigate the IHC expression of neurotrophins, their receptors and Ki-67. In the 4th chapter I report the results of an experimental study which investigates the IHC expression of VEGF, TGF-β and PGE_{2} in human pterygium and normal conjunctiva. In the 5th chapter are presented the experimental evidences of IHC expression of some growth
factors, their receptors and markers of proliferation of human osteosarcomas. In the 6th chapter are illustrated the experimental results of a study performed on IHC expression cytokines and growth factors in vestibular schwannomas and normal vestibular nerve. In the 7th chapter are discussed the experimental results of an IHC study on the involvement and the expression of some pro-inflammatory cytokines and growth factors in the pathogenesis of Dupuytren’s contracture. In the 8th chapter are discussed the results of a study (submitted for publication) concerning the tumor uptake of $^{99m}$Tc-HINIC-VEGF$_{165}$ which correlates with VEGF production and not with VEGFR expression. In the chapter 9th are finally discussed the conclusions and the future perspectives deriving from the analysis of the obtained results and from possible applications in the field of the nuclear medicine.
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FIGURE 1. Coronal CT image (A) with clear subcutaneous localization of SKOV-3 tumor (arrow). Fusion of microPET and CT images (B) (168 h after injection) enables adequate quantitative measurement of $^{89}$Zr-bevacizumab in the tumor. FROM: J Nucl Med. 2007;48:1313-1319

FIGURE 2. Coronal planes of microPET images 24 h (A), 72 h (B), and 168 h (C) after injection of $^{89}$Zr-bevacizumab. At 24 h, most uptake is in well-perfused organs. In time, relative uptake in the tumor (arrow) increases. FROM: J Nucl Med. 2007;48:1313-1319
FIGURE 3. (A) In-111-bevacizumab scan: Scintigraphic imaging of a liver metastasis with In-111-bevacizumab. (B) 4-phase CT scan: Imaging of liver metastasis. (C) PET/CT scan: Combined FDG-PET scan and CT scan of the liver lesion. FROM: European Journal of Cancer. 2008;44:1835-1840

FIGURE 4. IHC analysis. Expression of VEGFR1 in mouse subcutaneous tissue implanted and colonized by human HT 29 colon carcinoma cells. VEGFR1 appears heterogeneously distributed between tumor cells. (10x, boxed area: 20x).
FIGURE 5. IHC analysis. Expression of VEGFR2 in mouse subcutaneous tissue implanted and colonized by human HT-29 colon carcinoma cells (10x). VEGFR2 immunoreactivity is present between tumor cells and it is particularly expressed in the wall of the blood vessels. (boxed area: 40x)

FIGURE 6. Immunohistochemical staining for VEGF-A in human glioblastoma (GBM) in a 7-year old patient. Numerous tumor cells show strong cytoplasmic staining for VEGF-A. Human GBM stem-like cells (giant multinucleated cells proliferating) form three typical rings (GBSCs). (40x)
FIGURE 7. Immunohistochemical staining for VEGF-A in a case of nasopharyngeal angiofibroma (age of patient: 14 years). Marked immunoreactivity is visible in the fibrous tissue and in pathological vessels. IHC for VEGF demonstrates expression in more than 50% of stromal cells (boxed area: 40x).