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GASPARE ASELLI AND THE DISCOVERY OF CHILIFEROUS VESSELS

A summary of the historical-medical report by BRUNO ZANOBIO

On the path traced by Andreas Vesal (1514-1564) the 16th Century marked the triumph, partly thanks to Italian scholars, of "new anatomy," which was, however, somewhat "static," being based essentially on the dissection of corpses. The 17th Century saw the beginning of anatomic experimentation as the organism began to be considered as a machine that functions according to precise mathematical laws and mechanisms; this led to the birth of the "animated anatomy" and vivisection.

Characteristic of this century was the tendency of scholars to form scientific societies for the sake of conducting more fruitful experimental researches. In July 1622 a group of five Milanese doctors, led undeniably, by the associative spirit, asked Gaspare Aselli (Cremona 1581–ca. Milan 1625) to participate in their vivisection experiments.

Aselli, who had completed his medical studies in Pavia and worked as a proto-surgeon for the Spanish army in Italy (a position he held until his appointment in 1624 as professor of anatomy at the University of Pavia), was already well known for his expertise in conducting anatomic-experimental researches.

In Milan, on July 23, 1622, therefore, in the presence of the abovementioned doctors, he proceeded to vivisect a dog in order to show them the recurrent nerves. After this demonstration, he opened the abdominal cavity to show also the movement of the diaphragm. As he pushed down the intestinal mass and the stomach there suddenly appeared numerous tiny white threads that proceeded from the mesentery and intestines, and which he had never seen before. Once he was sure that these were not nerves, after careful thought he took a suitable scalpel and cut through one of the threads, which immediately gave out a white, milky liquid, in front of the astonished doctors' the unexpected sight caused Aselli to shout out using the famous exclamation "Eureka!"

The dog, however, died immediately, and Aselli was unable to continue his experiment. The next day Aselli repeated the experiment on another dog, but was disappointed to find no trace of the tiny vessels he had seen the day before. After careful consideration he remembered that, while the first dog had been well fed, the second was thin and underfed. Thus, then, was the explanation, which Aselli was to confirm in subsequent experiments on other animals.

The manuscript describing the discovery of chiliferous vessels is kept in the History Museum of the University of Pavia, while the printed book entitled De Lucubris sive Lucrècis venis Quarto Volumen Metcalcoamii genere Novo Invento Gasp. Aselli appeared posthumously in 1627, thanks to the good offices of Alessandro Tadino and Senator Settala, two of the doctors who had witnessed the discovery of the fourth type of vessels—the meseric chiliferous vessels, joining the three known types (arteries, veins and nerves), which, as we know, were in those days believed to have vascular structures. The book was illustrated with plates that were created with the technique of polychrome wood-engraving, used here for the first and last time and consequently earning it a special place in the history of printing.

In the description of his find, Aselli makes a number of errors that will be explained, from a critical historical point of view, in the lecture, which will examine them in relation to the anatomical-physiological knowledge of the period. The demonstration of blood circulation, in fact, was only published later (William Harvey, 1628).

The discovery of Aselli encountered various difficulties before it was fully recognized, being firmly supported by some and strongly opposed by others. The first demonstration of chiliferous vessels in humans was performed several years later in 1654. A number of reports of great historical-medical interest were written on the distinction between chiliferous and lymphatic vessels (e.g. Thomas Bartholin, Olof Rudbeck Senior), of the 19th Century, Italians, moreover, mention must be made especially of Paolo Mascagni and Bartolomeo Panizza.

GASPARE ASELLI E LA SCOPERTA DEI VASI CHILIFERI

Sulla strada maestra tracciata da Andreas Vesal (1514-1564) il Cinquecento vede il trionfo; pure 6' opera di studiosi italiani, della "nuova anatomia," peraltro piuttosto "statica," essendo fondata essenzialmente sulla dissection del cadavere.

Nel Seicento si passa all'esperimento anatomico. Vennero considerati l'organismo come macchina a funzione secondo precise leggi matematiche e meccaniche, da cui nasce la "anatomia animata" comportando la vivisezione degli animali. Caratteristica setecentesca è la tendenza degli studiosi a riunirsi in società scientifiche, onde potere più fruttuosamente condurre le ricerche sperimentali.

Certamente animati da spirito associativo, nel luglio del 1622, cinque medici milanesi chiesero a Gaspare Aselli (Cremona 1581–ca. Milano 1625) di assistere ai suoi esperimenti di vivisezione. Aselli compì i suoi studi medici a Pavia, protraggendo degli esercizi sporgenti in Italia (carica che conservò sino alla nomina nel 1624 professore di anatomia all'università di Pavia), era già noto allora per la sua competenza nella conduzione di ricerche anatomiche-sperimentali.

A Milano il 23 luglio 1622, aderendo alla richiesta dei medici suddetti, procedeva alla loro presenza alla vivisezione di
Di Alessandro Tofano e Simona Sanna, due del medico che aveva assistito alla scoperta del corpo, furono di causa. I osservatori, come i chirurghi che lavorano al fianco di questi esperti, erano impegnati nell’osservare le alterazioni, se ne occupavano nel momento del proseguire dell’esame, per quanto possibile.

In definitiva il corpo di Asclepio, in cui si trovavano i segni di una celebrazione, venne esaminato ed esaminate con attenzione e dedizione. La relazione onoraria, che il comandante dei relatori, nonostante le ore di notte, si era peraltro adeguata, si concluse con le parole finali: "La scoperta dell'Achille ha riscosso un nuovo interesse nella storia, e i suoi risultati potrebbero portare alla conoscenza di nuove strutture e di nuove possibilità di studio."

La nota storica è stata redatta dal professor William Harvey, medico italiano, che ha giocato un ruolo importante nella storia della medicina. Harvey è stato un esponente della medicina che ha lavorato per la progettazione di nuovi sistemi di studio e per la meticolosità nella documentazione dei propri ricerche. La sua cura, ed è stato colto da una preziosa chiarezza, ha permesso di dedicare nuove attenzioni alla conoscenza dei segni del tempo e alla documentazione dei dati."
ABSORBING LYMPHATIC VESSEL: LEUKOCYTE AND NEOPLASTIC CELL TRANSENDOTHELIAL MIGRATION MECHANISMS

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Lymphatic microcirculation is prevalently composed by the Apparatus Lymphaticus Periphericus Absorbers (ALPA), which plays a basic role in preserving tissue homeostasis, in directing 50% of fluids and plasma proteins back to the bloodstream, in the immune response and represents the main pathway for the metastatic spread of tumour cells.

Firstly, we consider the organization and fine structure of lymphatic canalization in order to define the morphofunctional aspects of lymphatic vascular system, which is conveniently divided in two sectors: 1) lymphatic vessels with high and medium absorbent capacity (ALPA vessels and first tract of precollectors); 2) lymphatic vessels with prevalent flowing and conduction function (lymph node and postlymph nodal collectors, main trunks and thoracic duct).

In the second part, we investigate the mechanisms regulating the transmigration of fluids, micro- and macromolecules and cells from the extravascular matrix into the lumen of lymphatic vessels. In this connection, the formation of intraendothelial channels of the ALPA vessel wall, in an interendothelial contacts-independent way, is explained. These channels are dynamic and unpermanence elements, whose numerical density changes during physiologic (dehydration) and pathologic (lymphedema) metabolic conditions. For a comparison with the lymphatic vessel, we consider models employed for blood vessels studying the role of adhesion molecules in leucocytes extravasation through high endothelial venules (HEV) of aggregate lymph nodes. Furthermore, the mechanisms of lymphocyte transendothelial migration from the interstitial matrix into the lumen of ALPA vessel, which occurs for the peculiar plasticity of endothelial lymphatic cells, are studied by means of ultrastructural serial sections and three-dimensional reconstructions.

In the third part, we evaluate the distribution and characterization of ALPA vessels (using transmission electron microscopy and D2-40 and LYVE-1 endothelial markers) in the tumour mass of some experimental tumours [such as T84 adenocarcinoma, B16 melanoma and transgenic mice prostate adenocarcinoma (TRAMP)]. Besides, we analyse our original findings concerning the mechanisms which provide for the diffusion and transendothelial migration of tumour cells into the lumen of lymphatic vessels. In T84 cell line-inoculated mice, lymphatic vessels are only detectable in the peritumoral connective tissue. They show a monolayer endothelium lacking a continuous basal membrane and interendothelial gaps. Tumour cells with an invasive phenotype migrate in cords from the extravascular matrix of peritumoral connective tissue towards the lymphatic vessel; once arrived on the abluminal surface of the vessel, they take firm adhesion with the abluminal endothelial wall. Afterwards, ultrastructural and three-dimensional approaches show tumour cell probed into the lumen of the lymphatic vessels through an intraendothelial channel similar to that described by Azzali (1989, 2003) in human and other mammals ALPA lymphatic vessels.

In B16 melanoma-inoculated nude mice, the subcutaneous tumour mass and gut metastases display lymphatic vessels only in the peripheral portion. Tumour cells with invasive phenotype, distributed in a diffused way within the extracellular matrix, after a firm adhesion to the abluminal endothelial wall migrate inside the ALPA lymphatic vessel in the same way of cells found in T84 adenocarcinoma-inoculated mice. These vessels do not present any ultrastructural degradation of the endothelial wall, nor modifications of interendothelial junctions.

In TRAMP transgenic mice, the prostate tumour mass, as the metastases in seminal vesicles and liver, show mostly collapsed ALPA lymphatic vessels in the middle and peripheral portions. They are formed by a continuous endothelial wall with irregular profile, lacking ultrastructural degradations, with prevalent interdigitating and overlapping interendothelial contacts and rare end to end contacts. The endothelial cells of these vessels present a non-nuclear cytoplasm with clear or electron dense matrix. Cells with an invasive phenotype, positive for SV-40 marker, are diffused in the extravascular matrix, and migrate individually towards the ALPA vessel; once reached the vessel, after a early stage of adhesion to the endothelial cell, these cells transmigrate into the lumen of the lymphatic vessel by an canalicular formation formed independently of interendothelial junctions. The latter are intact, with the plasma membranes of cytoplasmic extensions between adjacent cells fixed by tight and gap junctions. These ultrastructural and three-dimensional aspects emphasize a transmigration mechanism similar to that found in lymphatic vessels within B16 melanoma and T84 adenocarcinoma.

With regard to cell transendothelial migration processes, hypotheses are advanced on the roles of VEGF-C and VEGF-D growth factors and other members of this family in intraendothelial channels induction and organization, as well as of adhesion molecules responsible of tumour cell-endothelial cell interactions.

Interpretations are made concerning the following items: a) the role of ALPA lymph vessel endothelial cells in cell chemotraction and transendothelial migration; b) tumour cell transmigration in existing lymphatic vessels, rather than in newly formed ones after VEGF growth factors overexpression; c) possible modifications in the structure and function of ALPA vessel endothelium in tumours, if compared with those present in normal tissues.
On the whole, our morphological, ultrastructural and three-dimensional findings show the spatial arrangement of lymphatic vessels with high absorptive capacity in lymphatic vascular system and the plastic properties of endothelial cells in forming intraendothelial canaliculare formation, and bring elucidations also on the still unclear mechanisms regulating fluids, macromolecules and cells transport into ALPA lymphatic vessels. Future investigations shall provide concrete contributions to the delicate molecular mechanisms controlling the interactions between tumour cell and endothelial cell of ALPA (lymphatic vessel), in order to allow prospects and therapeutic strategies for increasing the diffusion of fluids (lymphedema) and for limiting or preventing the metastatic spread of tumour cells.

**VASO LINFAFICO ASSORBENTE: MECCANISMI DI MIGRAZIONE/ TRASENDOTELIALE DEL LEUCOCITI A E DELLA CELLULA TUMORALE.**

Il microcircolo linfatico è prevalentemente costituito dall’ap parato linfatico periferico assorbente (ALPA) il quale gioca un ruolo importante nel mantenere l’omocisiostasi periferica nel fondare il ritorno al sangue del 50% dei fluidi e delle proteine plasmatiche, nelle funzioni di difesa dell’organismo e nel con tribuire la porta principale per la diffusione metastatica delle cellule tumorali. La prima parte della lettura viene presa in considerazione la organizzazione e la fine struttura della vascolatura linfatica del sistema vascolare linfatico, il quale viene opportunamente distinto in due settori: 1) vasi linfatici ad alta e media capacità assorbente (vaso ALPA e tratto iniziale del vaso precollaterale) e 2) vasi linfatici a prevalente funzione di scorrimento e di conduzione (vasi collaterali pre-e postfissionali, grossi tronchi e dotti linfatici). La seconda parte, particolare attenzione viene riservata ai meccanismi che provengono alla transmigrazione dalla matrice extravasale all’interno del lume dei vasi linfatici dei fluidi, delle micro e macromolecole e delle cellule. Al riguardo viene sottolineata l’organizzazione della parete endoteliale del vaso ALPA di canalicoli endoteliadi che si formano in modo del tutto indipendente dai contatti interendoteliali. Detti canalicoli sono elementi che non permangono la cui densità numerica varia con stadi metabolicici fisiologici (disidratazione) e patologici (insufficienza). Vengono presi in considerazione, al fine di una comparazione con il vaso linfatico, modelli impiegati per il vaso sanguigno circa il ruolo delle molecole di adesione nella extravasazione dei leucociti a livello delle vene postcapillari (HEV) dei linfodioli aggregati. Così pure, mediante feltini sertate ultrastrutturali e la loro ricostruzione tridimensionale, sono stati studiati i meccanismi della migrazione transendotheliale del linfocito dalla matrice intestinale all’interno del vaso linfatico ALPA che si realizza in virtù della peculiarità plasticità della cellula endoteliale linfatica. Nella terza parte viene presa in considerazione la distribuzione e la identificazione dei vasi linfatici ALPA (microscopio elettronico a trasmissione ed i markers D2-40 e LYVE-1) nella massa tumorale di tumori sierocitomi adenocarcinoma T84, melanoma B16 e adenocarcinoma della prostata di topi transgenici (TRAMP). Inoltre sono valutati gli originali referiti circa i meccanismi che il tumoraggio nella diffusione e nella migrazione transendotheliale della cellula tumorale all’interno del lume del vaso ALPA. Nei topi inoculari con la linfa cellulare T84 i vasi linfatici risultano rilevabili solamente a livello del tessuto connettivo peritumorale. Essi si presentano caratterizzati da un endotelio monostratificato privo di membrana basale continua e di aperture interendotheliali. Le cellule tumorali con fenotipo invasivo migrano sotto forma di un agglo merato cordoniforme dalla matrice extravasale del connettivo peritumorale verso il vaso linfatico, e una volta raggiunta la superficie abilumina dell’endothelio il vaso contraggono stretta adesione con la parete endoteliale abiluminata. Dopo di che, i quadri ultrastrutturali e tridimensionali mostrano la cellula tumorale trasmigrata nel lume del vaso linfatico attraverso una formazione canalicolare simile a quella descritta nei vasi linfatici ALPA dell’uomo e di numerosi mammiferi da Azzali (1989, 2003). Nei topi inoculari con melanoma B16 la massa tumorale sottocutanea e quella delle metastasi intestinali, lascia rilevare presenza di vasi linfatici solo nella sua porzione più periferica. Le cellule tumorali trasformate nel fenotipo invasivo distribuite in modo diffuso nella matrice extravasale, dopo stretta adesione alla parete abiluminata endoteliale migrano all’interno del vaso linfatico ALPA seguendo le stesse modalità rilevate nei topi inoculati con T84. Detti vasi non presentano segni di degradazione ultrastrutturale della parete endoteliale e così pure modificazioni delle giunture interendotheliali. Nei topi transgenici TRAMP la massa tumorale della prosta ta, come quella delle metastasi a livello delle vescichette semi nalì e del legato, presenta nella porzione media e periferica vasi linfatici ALPA per lo più collettivi. Essi sono costituiti da una parete endoteliale continua a profilo irregolare del tutto priva di degradazioni ultrastrutturali con costanti interendotheliali per lo più di tipo interdigitating e overlapping, mentre raro sono le end to end. Le cellule endoteliali di detti vasi sono caratterizzate da citoplasma non nucleare provvisto di matrice chiaro o elettrooscura. Le cellule con fenotipo invasivo, positive al marker SV40, si distribuiscono in modo diffuso nella matrice extravasale e migrano in modo individuale verso il vaso linfatico ALPA raggiungendo il quale, dopo una prima fase di adesione con la cellula abiluminata, trasmigrano nel lume del vaso linfatico attraverso una formazione canalicolare che si forma in modo del tutto indipendente dalle giunture interendotheliali. Queste ultime si presentano immo dificate con le membrane delle deposizioni citoplasamiche di cellule endoteliali adiacenti fissate in modo costante da tight gap junctions. Detti quadri ultrastrutturali e tridimensionali ottenuti sottocutaneo un meccanismo transmigratorio molto simile a quello rilevato nei vasi linfatici del melanoma B16 e dell’adenocarcinoma T84. Riguardo al processo migratorio transendotheliale delle cellule, sono progettate ipotesi favorevoli a un meccanismo di crescita VEGF-C e VEGF-D e altri membri di questa vasta famiglia circa la induzione e la organizzazione delle formazioni canalicolari, così pure quello delle mole-
cole di adesione responsabili delle interazioni “cellula tumorale-cellula endoteliale”.

Vengono formulate interpretazioni circa: a) il ruolo svolto dalle cellule endoteliali del vaso ALPA riguardo la chemiotrauzione e la migrazione transendoteliale delle cellule; b) la transmigrazione delle cellule cancerogene nei vasi linfatici già esistenti piuttosto che in nuovi vasi linfatici generati dalla sovraespressione dei fattori di crescita VEGF, c) le eventuali modificazioni della struttura e della funzione dell’endotelio linfatico nei vasi ALPA dei tumor rispetto a quella dei vasi dei tessuti normali. 

Nel complesso i reperti morfologici, ultrastrutturali e endimensionali da noi illustrati consentono di sottolineare l’individualizzazione spaziale nel sistema vascolare linfatico del vaso linfatico ad alta capacità assorbente e le proprietà plasme di cellularità, portando anche un contributo chiarificatore sui meccanismi, ancora non del tutto chiari, che presiedono al trasporto all’interno del vaso linfatico ALPA dei fluidi, delle macromolecole ed in particolare molecole delle cellule. Compiuti di future ricerche il fornire concetti contributi sui delicati meccanismi molecolari che presiedono alle interazioni cellula tumorale-cellula endoteliale del vaso linfatico ALPA al fine di consentire prospettive e strategie terapeutiche per incrementare la diffusione di liquidi linfedermat e per limitare o impedire la disseminazione metastatica delle cellule cancerogene.

REFERENCES
Molecular Pathways for Lymphangiogenesis and Their Role in Human Disease

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Abstract: Lymphangiogenesis is a critical process in the development of the lymphatic vascular system. It plays a key role in the growth and dissemination of lymph node metastases in several human cancers. The process of lymphangiogenesis is complex and involves the coordinated interaction of several molecular and cellular mechanisms. Understanding these pathways is essential for the development of novel therapeutic strategies to target lymphangiogenesis and thereby improve the treatment of cancer.

Keywords: Lymphangiogenesis, Lymphatic vessels, Lymph node metastases, Cancer treatment, Molecular pathways.

Introduction

Lymphangiogenesis is the growth of lymphatic vessels from pre-existing lymphatic networks. It is a dynamic and complex process that is critical for normal development and the maintenance of homeostasis in the body. In addition to its normal physiological functions, lymphangiogenesis also plays a key role in several pathological conditions, including cancer metastasis, wound healing, and inflammatory diseases.

Mechanisms of Lymphangiogenesis

The process of lymphangiogenesis involves the coordinated interaction of several molecular and cellular mechanisms. One of the key molecular mechanisms involved in lymphangiogenesis is the activation of the lymphangiogenic transcription factor, VEGFR3 (also known as Flk-1). VEGFR3 is expressed on lymphatic endothelial cells and plays a crucial role in the proliferation and survival of these cells.

Another key player in the lymphangiogenic process is the VEGF-C and VEGF-D ligands. These ligands bind to VEGFR3 and activate the downstream signaling pathways that lead to the expansion of lymphatic vessels. VEGF-C and VEGF-D are also produced by tumor cells and are thought to be involved in the lymphangiogenesis that occurs in the process of cancer metastasis.

Therapeutic Targets for Lymphangiogenesis

Our growing understanding of the molecular pathways that control lymphangiogenesis has led to the identification of several potential therapeutic targets. These targets include VEGFR3, VEGF-C, and VEGF-D. Agents that inhibit the activity of these molecules have the potential to block lymphangiogenesis and thereby prevent the growth and dissemination of cancer metastases.

Conclusion

Lymphangiogenesis is a critical process in the development of the lymphatic vascular system. It plays a key role in the growth and dissemination of lymph node metastases in several human cancers. Understanding the molecular pathways involved in lymphangiogenesis is essential for the development of novel therapeutic strategies to target this process and thereby improve the treatment of cancer.

References


NUCLEAR MEDICINE IN THE STUDY OF LYMPHATIC DISORDERS

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Nuclear medicine techniques allowing the study of the lymphatic system diseases include:
- the lymphoscintigraphic investigations (LySc) using 99mTc-labeled colloids,
- the investigations with Positron Emission Tomography (PET) after administration of various tracers.

In the past, the lymphoscintigraphic investigations using 99mTc-labeled colloids were used to demonstrate the metastatic invasion of several lymph node groups (with prognostic implications as for instance in breast cancer with the lymphoscintigraphic investigations of the internal mammary nodes) and/or to define the spatial localization of lymph nodes to be irradiated (the internal mammary nodes in breast cancer). Actually, they are used in the management of the ominous situations, primary or secondary, and have largely proven their interest in these indications. Now, they are also and more and more used to demonstrate in vivo the lymph nodes’ draining one tumour bearing area and at risk to be invaded by metastatic cells (the so-called “sentinel lymph nodes”). Today, quite all types of cancer are concerned by this application: cutaneous tumours as melanomas or Merkel’s ones, mammary tumours, head and neck ones, prostatic tumours.

If LySc allow to study directly the lymphatic vessels and lymph nodes, the PET techniques after intravenous administration of various tracers on the other hand allow to demonstrate the metabolic activity of primary lymph node diseases (lymphoma) and/or the presence in the lymph nodes of metabolically active secondary metastatic disease.
RADIATION THERAPY IN DOGS WITH SPONTANEOUS VASCULAR ENDOTHelial GROWTH FACTOR EXPRESSION

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VEGF promotes angiogenesis, endothelial cell survival and vessel maintenance of immature vessels. VEGF also has a significant role in the development of tumors, and VEGF expression is associated with the aggressiveness of different tumors. VEGF is actively secreted from tumor cells, and its concentration is increased in tumors with a high mitotic rate. The high plasma levels of VEGF in dogs showed a significantly higher overall survival and a lower rate of relapse compared to dogs with low VEGF concentrations. A clinically useful tool for the prediction of clinical outcome in dogs with tumors is the measurement of VEGF concentrations.

The significance of VEGF in the formation of tumors is further evident from the finding that VEGF expression is upregulated in response to hypoxic conditions, which is the main cause of tumor growth factor production. Hypoxia is a major factor contributing to the development of tumors, and VEGF is one of the key factors involved in hypoxia-induced neovascularization.

The overall objective of this study was to investigate the role of VEGF in the development of canine vascular tumors and its potential as a prognostic marker. The study included 50 dogs with vascular tumors, and VEGF concentrations were measured in the plasma samples of these dogs at the time of diagnosis. The results of this study indicated that VEGF concentrations were significantly higher in dogs with tumors compared to dogs without tumors.

In conclusion, the measurement of VEGF concentrations in the plasma of dogs can be a useful tool for the prediction of clinical outcome in dogs with vascular tumors. Further studies are needed to investigate the potential of VEGF as a therapeutic target for the treatment of vascular tumors in dogs.
VEGF. Low levels of plasma VEGF were found in dogs with epithel, a benign tumor with a minimal metastatic potential. In contrast, high plasma VEGF levels were found in more aggressive tumors with a high metastatic potential, such as oral melanoma, carcinoma, and osteosarcoma. These data indicate a close relationship between plasma VEGF and aggressiveness of the tumor. Secondly, the effect of fractionated RT on VEGF levels in the plasma of tumor-bearing dogs was analyzed and a tendency for increased plasma VEGF level in curatively treated dogs was found. These results are in correspondence with the in vitro study, where a low radiation dose resulted in pronounced activation of the VEGF expression. VEGF concentrations immediately before and after dose application did not differ.

In human medicine, an association between high serum or plasma VEGF level and a shortened survival time was seen. Interestingly, high preirradiation plasma VEGF levels in dogs with spontaneous tumors also resulted in a shortened survival time. This was seen in curatively and palliatively treated dogs. This prediction was not dependent on tumor histology or tumor stage. Additionally, we found lowest mean plasma VEGF levels in dogs with a complete response to therapy in both treatment groups. Correspondingly, palliatively treated dogs with a low plasma VEGF level that responded to therapy also had a significantly longer time to treatment failure and a longer life span.

In conclusion, a close relationship between plasma VEGF and the aggressiveness of the tumor was proven and plasma VEGF is a good prognostic indicator for dog patients receiving radiation therapy.
frame C. In all patients we performed, for 2 intensive weeks a
tailored physical treatment (respiratory gymnastics, M.L.D.,
sequential presurgery, head-and-shoulders isometric gymnastics).
In the first clinical stage we observed all normal echogenic frame.
The in second clinical stage 63 cases of frame A and 8 of frame
B in the third clinical stage 9 frame A, 48 frame B and 9 frame C;
in the fourth clinical stage 11 frame B and 9 frame C in the fifth
clinical stage only 4 frame C. There was a substantial coincidence
between the echographic frame and the clinical stage.
In all patients with echographic frame A we observed a remark-
able decrease, after treatment, of supra-fascial tissue thickness.
In 31% of patients we saw a regression to normal frame.
In the patients with echographic frame B we observed, after
treatment, a decrease of hypo-echogenic component (corre-
sponding to the fluid component of oedema); in 24.5% a regres-
sion to the previous clinical stage.
In all cases the echographic frame was unaltered after treatment.
In the patients with echographic frame C was observed a very
low decrease of hypo-echogenic component of supra-fascial
thickness.
In 24.7% it is visible a regression to the previous clinical stage.
In all cases an unaltered echographic frame was observed. There
are the echographic frame modifications in function of the treat-
ments.
The echographic frame of lymphedema can help us also for the
prognosis. In fact the echographic frame A can regress to the
normal frame. The complete reversibility is not possible for the
other echographic frames (B and C).
Under the clinical point of view the reversibility is completely
possible for the frame A, partially for the frame B and very low
for the echographic frame C.
In conclusion we can assert that the high resolution echography
allows us to confirm the diagnosis of lymphedema, the monitor-
ing of the results for the treatment and to provide also the prog-
nostic indications.
Computing Tomography shows some important informations
about the tissue characteristics, both of the supra-fascial compart-
ment and of the sub-fascial one.
The study aimed 220 patients suffering from lymphedema
(86 with primary kind, 134 with secondary kind), age ranging
between 12 and 83 years.
The study focused above all on the main signal present in
supra-fascial compartment of the limb at various level of it.
The values strongly negative (-70, -80) was corresponding to
the fat tissue; the values ranging between -30 and -15 repres-
ented the main water component; The values over +50, +60 testified
the presence of tissue perifascial oedema.
To respect the quality-factor we accepted and examined only the
images with ‘‘standard-deviation’’ within 10.
The parameters evaluated was:
- modification of the thickness of supra and sub-fascial compart-
ment at various level of the limb;
- the main tissue component of supra-fascial tissue at various
level;
- the assessment of the main articulations of the limb;
- the toposis of various musculous component of the limb.
In this way the computing tomography permit to address the
therapeutical approach (general and particular) on all the
anatomical component to treat with the proper rehabilitative
protocol: CPT+drugs (when it was present the combined
presence of water and perifascial oedema super-fascial tissular
component: 37% of subjects); CPT+drugs+microsurgery
(when it was present the diffuse supra-fascial tissular compo-
ent: 37% of subjects); CPT+drassion (in subject with pre-
valing suprafascial fat component: 16% of patients).
In conclusion the Computing Tomography permit to define
the various diagnostic aspects of lymphedema and the choice of
the tailored therapeutical protocol; the exam is important also to
the monitoring of the illness.
Also the Magnetic Resonance, when indicated associated with
the injection of the contrast liquid, can help us to define the
ultrasturctural features of the tissue, above all of the supra-fas-
cial thickness.
The Lymphography, today, is indicated for the morphological
study of some cases of chilipertoneous, chilidromus, or in
tumoral secondary kind of lymph-stasis.
All these instrumental techniques favourize the diagnosis and
permit to us to define the clinical approach; each of them give us
some important informations to realize the proper tailored reha-
bilitative project in lymphedema patient.

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The lymphatic system, lymphangiogenesis, lymphatic vessels, lymphatic, lymphocytes, and lymph nodes are all involved in the immune system. The lymphatic system is responsible for the movement of lymph from the tissues to the bloodstream. Lymphangiogenesis is the process of new lymphatic vessel formation.

Several disorders, such as lymphedema and lymphatic malformations, are associated with abnormalities in lymphatic vessel development and function. The molecular mechanisms involved in lymphangiogenesis are not fully understood, but recent studies have identified several key regulators that play a role in this process.

One such regulator is the transcription factor FOXC2, which is essential for lymphatic vessel development. Mutations in FOXC2 have been identified in patients with lymphatic malformations. Other key regulators include the transcription factors SOX18 and FOXC2, which interact with one another to control lymphatic vessel development.

In addition to genetic factors, environmental factors such as inflammation and tissue damage can also affect lymphangiogenesis. For example, inflammation can stimulate the production of proangiogenic factors that promote lymphatic vessel formation.

The understanding of lymphangiogenesis and its role in various diseases is crucial for the development of targeted therapies. Future research will likely shed light on the complex interplay between genetic and environmental factors in the regulation of lymphatic vessel development.
DISCUSSION

Very few attempts have been made in the past to isolate lymphatic endothelial cells (LEC), mainly using lymphatic from vascular tumors (Mancaidi, 1991, Weninger 1999). Due to the lack of specific markers, a stable phenotype in culture and the histogenetic origin of this expanded population could not be defined. But the recent identification of novel lymphatic markers such Podoplanin, a transmembrane mucoprotein (Matsuai et al., 1999), hyaluronidase receptor LYVE-1 (Banerji et al., 1999), VEGFR-3 (Jussila et al. 2002), transcription factor PROX-1 (Perrota et al., 2002) and D2-40, recognizing an O-linked sialoglycoprotein (Kahn et al, 2002) as made possible. Furthermore, the identification of specific lymphatic growth factors, like VEGF-C, and substrates recently has made possible the isolation and growth of LECs from derma by using two different markers: podoplanin and VEGFR-3 (Mäkinen, Kriehuber). Here we described for the first time the isolation of LECs from palatine tonsil and thoracic duct using a mAb recognizing the O-linked sialoglycoprotein D2-40, specifically expressed on LECs, PT as source of LECs are extremely useful because is easy to obtain as discarded material of therapeutic surgical procedures and because of their dimension, weight and richness in lymphatic capillaries. Generation of LEC cultures required tissue dispersion, removal of non adherent cells after 12-18 h of plating, immune preselection with UEA-1-coated magnetic beads followed by a second selection with magnetic beads coated with D2-40 mAb. It is known that LECs directly interact in vitro with the extracellular matrix (Gerli et al., 2000) and indeed both PT-LEC and TD-LEC were successfully propagated on collagen and fibronectin coated flasks in the presence of EGM added with VEGF-C. However, LECs cultured on collagen and fibronectin adhere better and proliferate more efficiently than LECs grown on culture dishes coated with collagen alone (Grimet et al. 1981, Kleinman et al. 1981), according with data that fibronectin provides additional signal for adhesion, survival and proliferation of LECs. PT-LECs and TDL-LECs were morphologically similar to BECs, although their shape appeared more elongated. Most of the known blood vascular markers, such as CD31, vWF and UEA-1, are present at lower levels or almost absent, like KDR, in agreement with previous studies performed with LECs derived from derma (Kriehuber et al., 2001). Also lymphatic markers are differentially represented. PT-LECs were strongly stained by D2-40 mAb, almost all express Prox-1 and only few element were LYVE-1* and Podoplanin* while almost all TD-LECs in cultures equally expressed all the lymphatic markers tested. Furthermore, stimulation of PT and TD-LEC culture with the specific lymphatic endothelial growth factor VEGF-C induce proliferation on both PT and TD-LEC culture but not of HUVEC indicating the presence of functional VEGFR-3 molecules during all the period of cultures on the LECs surface. As suggested by Podgrabinska et al. (2002) the different markers expression reflects the existence of LECs with different phenotypes in lymphatic macro- and microvessels, that results in different biological activities and functions. This hypothesis is supported by studies performed on BECs, that exhibit phenotypic and functional differences depending on their origin (adult versus foetal), anatomic localization, and vessels size (large versus capillary vessels) (Turner et al., 1987; Page et al., 1992), and that show different functions. In conclusion, our results collectively show that palatine tonsils are an important and easy to get source for isolating human lymphatic micro vessel ECs, Culture of PT and TD-LEC in the presence of specific growth factors and extracellular matrices was possible without losing their differentiated properties and functional activity. PT-LECs and TD-LECs may then represent new tools for investigating genetic, phenotypic and functional diversity between macro- and microvessels derived LECs isolated from different organs and tissues. Furthermore, our work will contribute to determine some of the characteristics of LECs, to understand their specialized lymphatic functions, to analyze their role in tumor lymphatic metastatic dissemination and to analyze their capability in supporting virus replication as already demonstrated for BECs (Lathley et al., 1996; Ricona et al., 2001; Caruso et al., 2002).

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ASPECTS OF PREVENTION, DIAGNOSIS AND SURGICAL THERAPY OF LYMPHEDEMA SECONDARY TO CANCER DISEASES

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Introduction
Chronic secondary lymphedema is a frequent complication of surgical treatment of malignant neoplasms, especially when radiotherapy follows.
The secondary lymphedema of the upper limb (post-mastectomy lymphedema) has an incidence in patients who underwent axillary lymphadenectomy for breast cancer, between 5 to 25%, up to 40% after radiotherapeutic treatment.

Clinical Experience and Methods
We studied 60 patients with breast cancer. Patients were enrolled between April 1992 and June 1994, and treated in different Centers, besides ours, according to generally accepted oncological protocols. The inclusion criteria were the presence of invasive breast cancer (T1-T2 tumors), and a treatment plan that included axillary lymphadenectomy and irradiation.
The patients were divided into two groups of 25 each, comparable for age, sex, pathology and treatment, and followed up to 5 years after operation (18 lymphedemias and 32 mastectomy) for breast cancer (39 ductal and 11 lobular carcinomas). The number of excised lymphnodes varied from 12 to 26 (average 14), Radiation therapy was performed from the 3rd to the 6th week, post-operatively.

One group of 25 patients was controlled only clinically (physical examination, water volume) at 1-3-6 months and 1-3-5 years from breast cancer treatment. The other group of 25 patients was followed also by lymphatic scintigraphy performed pre-operatively and after 1-3-6 months and 1-3-5 years from operation.

In the first group followed only clinically, lymphedema appeared in 9 patients after a period variable from 1 week to 2 years, with highest incidence between 3 and 6 months (time coincident with radiotherapy). We considered lymphedemas also at the very first stages (Ib-II), with a volumetric difference of 150 ml at least.

In the second group of 25 patients, lymphoscintigraphy showed pre-operative lymphatic circulatory alterations in 4 cases (reduced tracer transit capacity, tracer dispersion, decreased lymphodermal axillary capitation, absence of defined way). Post-operatively, lymphatic impairment was found in 22 patients (5 cases after 1 month, other 3 cases after 3 months, 6 more at 6 months, other 5 at 1 year and, finally, 3 more after 3 years). In the remaining 3 patients, followed up to 5 years, lymphoscintigraphy did not point out any lymphatic alteration. All 22 patients underwent physical-rehabilitative preventive procedures:

1. For 2 weeks every month, for 3 months: manual lymphatic drainage (MLD) for half an hour followed by moderate péréistaltic mechanical lymph drainage (30-40 min/hr) for an hour, and again MLD for half an hour.
2. After this treatment a functional elastic multilayer bandaging was used.
3. During the 2 weeks free of treatment the patients wore only an elastic garment.
4. The entire cycle was repeated another time for other 3 months.

This preventive therapeutic protocol allowed to have a clinically evident arm lymphedema only in 2 patients. These, who were not enough responsive to combined physical therapies (edema regression did not last long and there was the worsening of the edema), underwent early derivative lymphaticovenous microsurgery (at Ib and II stages)

Microsurgery allowed to bring about a complete long term edema regression. The patency of lymphatic-veinous anastomosis was proved by lymphatic scintigraphy also a long time after microsurgical operation the last recent control was at 4 years from microsurgery so far.

Results
The comparison of the two groups of 25 patients proved a statistically significant difference in the appearance of arm secondary lymphedema (p<0.01, using Fisher’s exact test).

We can say, then, that the diagnostic and therapeutic above mentioned preventive procedures allow to reduce the incidence rate of lymphedema significantly, in comparison with patients who did not undergo this protocol of prevention.

Discussion and Conclusions
To this purpose, we do believe that to confirm this finding, it would be useful to conduct a multicenter study of the preventive protocol under the guidelines and the supervision of the ESL.
Due to the rapidity of lymphedema once it is developed, prevention and prompt medical intervention are extremely important. The earlier treatment begins, the better the appearance of lymphedema. Techniques of massaging, pneumatic compression, and exercise have been reported, both in the immediate post-mastectomy period and at various intervals post-operatively. The immediate post-operative use of these techniques may be helpful in reducing the risk of lymphedema.

To reduce the incidence of lymphedema, a review of the literature has been performed to identify the most appropriate time for use of these techniques. The immediate post-operative use of these techniques appears to be the most effective in reducing the incidence of lymphedema.

In conclusion, the prevention of lymphedema is a vital concern in the treatment of cancer patients. The use of techniques such as massage, compression, and exercise may be helpful in reducing the risk of lymphedema. However, the optimal timing and duration of these techniques are still under investigation. More research is needed to determine the most effective methods for preventing lymphedema in cancer patients.
SURGICAL PLANNING AND SURGERY IN THE ANIMAL ONCOLOGICAL PATIENT

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Surgery is the most important modality of treatment for most localized tumours since it results in a higher rate of cure than all other modalities. However, for most neoplasms, a multimodality approach is more often capable of reaching the best results in terms of disease free period and overall survival. Surgery should not always be extremely aggressive if other therapeutic tools (i.e., chemotherapy and/or radiotherapy) have shown to be effective for the control of that tumour. These ancillary treatments can be used postoperatively (neoadjuvant), preoperatively (adjunctive) or intraoperatively.

Cytology and histology are standard techniques to reach diagnosis in oncology. Diagnosis implies the primary tumour has been identified and that it has not already spread regionally and/or systemically. Diagnostic imaging techniques should be used properly to determine the extent and size of the primary tumour, mainly if surgical excision is going to be performed. Based on the standard biological-clinical behaviour of that tumour, metastasis is carefully looked for at the level of the regional lymph nodes, lungs, and/or other sites. If enlarged, regional lymph nodes are submitted to multiple fine-needle aspirates and/or biopsy but metastasis, even if present, may be missed. If endocardiac, surgical exploration may be indicated. Concerning lungs, metastasis evaluation implies right and left lateral and ventrodorsal views; in selected cases, a CT-scan may be useful. Bronchoalveolar lavage may be of some help for tumours (e.g., melanomas and some carcinomas) that have already reached a critical size; however, if metastasis is not demonstrated, doubt still remains. Other sites are evaluated depending on the standard clinical biology of that tumour (e.g., bone metastasis in case of melanoma, carcinoma, osteosarcoma, etc.). Attention is also driven to specific paraneoplastic syndromes that may accompany the neoplastic growth.

Some aspects must be kept in mind when a tumour surgical excision is planned: 1) knowledge of the standard biological behaviour of that tumour; 2) the first surgery has more probabilities to be successful; 3) resection of the tumour with uniform wide margins all around it may also result in the removal of the corresponding bone below the tumour; 4) this implies a correct planning of the aggressive surgery and reconstruction of the body part. The latter is better performed keeping in mind the surgeon’s individual skills, all techniques of reconstruction, and the consequent functional deficit caused by the surgery performed. In general, limitations are mainly related to the probability of getting a definitive cure or, at least, to prolong dramatically higher survival, preventing or restraining the metastatic spread; the latter is usually reached by the appropriate use of an adjuvant chemotherapeutic regimen.

Local recurrence is controlled through the application of a local treatment such as radiation therapy. In order to decide if a second surgery and/or adjuvant radiotherapy is indicated, infiltration of surgical margins has to be confirmed. Therefore, it should be emphasized the importance of identification of margins through the application of sumers or, more typically, through the application of ink to all the bed where the suspicion of tumoral infiltration is real. Finally, a main concern in the oncological patient is, apart from the age of the animal that is not a limiting parameter if clinical conditions are good, a good quality of life of these patients after treatment.

Types of surgery in oncology and resection margins

Surgery may be classified as:

1) diagnostic: it is applied when less invasive procedures are not diagnostic. The goal is to get a sample of tissue to submit to cytology and histology. It is referred to as “incisional biopsy” (wedge of tissue obtained in an area easily removable in the subsequent resection of the tumour) and “excisional biopsy” (e.g., exploratory thoracotomy or laparotomy, or removal of cutaneous lesions surely benign);

2) cytoreductive (debunking): Inoperability may depend both on the type of growth (predominant infiltrative pattern) and tumour location (neck, axial skeleton, great vessels, nerves, etc., i.e., parts that cannot be radically excised without compromising some vital functions or life). Inoperable tumours may be debunked but some other therapeutic tools have proved to be effective in controlling their growth (radiation, chemotherap-

y); 3) curative: it provides an “en bloc” excision of the tumour with margins of 1-4 cm of normal tissue all around it; sometimes, depending on the neoplastic location, this is realized removing also the corresponding bone (mandiblectomy, maxillectomy, amputation, partial or total scapulectomy, pelvico-

my, limb salvage, rib resection, etc.);

4) palliative: it is rarely applied. It may be used if a real postoperative improvement of the quality of life of the animal is expected (e.g., pericardectomy for tumours of the heart base). Classification of surgical resection margins reflects all these different types of surgeries:

a) intracapsular: the mass is removed in pieces. This techni-

que should be reserved only to benign lesions and it is obvi-

ously contraindicated in case of malignant tumours but in some situations (e.g. malignancies extending in the nose, middle ear, spinal cord, etc.) the surgeon is forced to remove the tumour in such a way (“debunking” or cytoreductive). Some adjuvant treatment is imperative since the tumour is macro-

scopically still present;
localized in the mouth and relatively benign when they grow on the flank; canine oral squamous-cell carcinomas tend to be more benign when localized rostrally, more malignant when localized aborally.

to some extent, a worse prognosis may arise from clinical complications such as stenosis, compression, hemorrhages, infections, functional deficits, etc caused by the neoplastic growth.

- Clinical staging of the tumour: a specific TNM clinical staging is established through a meticulous diagnostic work-up. Universally applied both in human and veterinary oncology, it facilitates the exchange of information among centres, and comparison of results obtained through different treatment protocols. The TNM system is based on: a) T: extension of the primary tumour; b) N: regional lymph nodes status; c) M: presence or absence of distant metastases, mainly in lungs. Other factors are: d) F: it is referred to as histopathological extent (e.g. in the thickness of the wall of a cavity organ); e) G: it expresses the malignancy grade (low, medium, high); f) L: it expresses the tendency of the tumour to invade lymphatics; g) V: it expresses the tendency of the tumour to invade veins.

- Disease-related factors: such as the paraneoplastic syndromes (hypoglycemia, hypercalcemia, hyperthyroidism, fever, anemia, leukocytosis, DIC, gammapathies, degranulation effects of malignant mast cells, hypercalcemic osteopathy, cachexia, neurologic manifestations, etc. They can cause more morbidity (and eventually mortality) than the primary tumour.

- Chemosensitivity / Radiosensitivity: the first is influenced by tumoral cellular heterogeneity and growth rate. Concerning radiation, tumours that were considered in the past as typically radioresistant may now be approached through a combination of surgery and radiotherapy with a dramatic improvement of control and overall survival (e.g. soft tissue sarcomas, nasal tumours, etc.).

- Patient-related prognostic factors

- Pre-existing diseases: cardiopathies, liver and renal diseases, etc may make prognosis worse since they may limit the possibility of planning any treatment.

- Immunosuppressive treatments: in general, prolonged administrations of steroids facilitate tumour growth. This should be avoided if the intention is to cure the animal unless the tumour is a lymphoproliferative disorder (combination chemotherapy). In case no treatment is attempted, steroids may improve the quality of life in terminal patients, with the awareness of a shorter survival.

SELECTED REFERENCES


During oncologic surgery, the draining lymph nodes are usually removed for staging, although the same incision can be used for a second approach.

Laparoscopy and thoracoscopy have introduced even the possibility to obtain a complete search of the abdominal and thoracic cavity.

CT and MRI are the most widely used diagnostic tests and are usually performed in the same session. The procedure can be performed under general anesthesia and the patient can be awake and responsive. After a complete exploration, the more significant findings are discussed.

A technique that is called laparoscopic needle biopsy is also used to check the real value of medical intervention.

Lymphangiography is usually performed on small draining lymph nodes that the surgeon has removed during surgery. A lymphogram is performed to determine the size and shape of the nodes.

The lymph node is X-rayed after it has been filled with contrast dye. This will allow the surgeon to determine the size and shape of the nodes and whether they are cancerous.
STATE OF THE ART ON LYMPHATIC CANCER METASTASIS IN DOMESTIC ANIMALS

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LYMPHATIC FUNCTION
The lymphatic circulation transports interstitial tissue fluid, macromolecules and cells back into the blood circulation. The lymphatic system begins with initial finger-shaped lymphatic vessels consisting of valueless lymphatic capillaries and precollectors with valves. The precollectors become collectors and transport the lymph to the so-called lymph stems. These vessels lead into the right and left lymphatic duct and into the jugular trunk of the head and neck region. At the end they join into the blood circulation at the junction point of the jugular and subclavian veins.

LYMPHATIC SYSTEM AND CANCER
The lymphatic system is one of the primary routes for the neoplastic metastasis. Neoplastic cells spread via lymphatic vessels to the regional lymph nodes which play an important role in diagnosis, staging, and therapy. Epithelial malignant cells often metastasize to the regional lymph node and their presence are an important prognostic factor. There is a consequential anatomic progression of neoplastic cells from the original mass to the regional lymph node via lymphatic capillaries and collecting trunks. Once neoplastic cells are established in the sentinel lymph node further metastasis to lymph nodes occurs and then via lymphatico-venous connections to the general systemic circulation. Direct hematogenous metastasis may occur without regional lymph node involvement.

Lymphatic vessels containing clusters of neoplastic cells are often observed at the periphery of malignant neoplasia, but no evidence of metastasis has been observed in intratumoral ones.

Recent studies reported intratumoral lymphatic vessels in some neoplasia but it is not still clear if they are preexisting lymphatic vessels or the expression of lymphangiogenesis inside the neoplasia. The significance of preexisting peritumoral lymphatics as a route for neoplastic cell metastasis is clear, but it is not yet clear whether neoplasia can stimulate lymphangiogenesis and whether neoplastic metastasis is mediated by molecular activation of the lymphatic system.

Morphological transendothelial migration of neoplastic cell remain unknown and a transcellular passage, macrophage via, or channel-like path, polynucleated leukocytes via, have been hypothesized. Recently, several novel molecules have been identified that allow a more precise distinction between lymphatic and blood vascular endothelium.

MOLECULAR REGULATION OF TUMOR LYMPHANGIOGENESIS AND LYMPHATIC METASTASIS
These include VEGFR-3 (FLT-4), the receptor for the vascular endothelial growth factors VEGF-C and VEGF-D in lymph nodes and the homeobox gene product Prox-1 that is involved in regulating development of the lymphatic system. A novel hyaluronan receptor termed LYVE-1 has been shown to be restricted to lymphatic vessels in normal tissue and associated with tumors (1,3,10). Vascular endothelial growth factor-C (VEGF-C), a member of the VEGF family of growth factors stimulates lymphangiogenesis in addition to angiogenesis. The specific effects of VEGF-C on lymphangiogenesis depend on its proteolytic processing. The mature form of human VEGF-C stimulates both VEGF-R2 and VEGF-R3 and can therefore stimulate both angiogenesis and lymphangiogenesis, whereas the partially processed form preferentially binds and activates VEGF-R3 and specifically stimulates lymphangiogenesis. Another recent study demonstrated the important role of VEGF-D in tumor lymphangiogenesis and metastasis. VEGF-C may induce the proliferation of lymphatic vessels in the stroma of primary gastric carcinomas. CD44v5 and CD44v6 positive, via activation of VEGF-C, expressed on the endothelial cells of lymphatic vessels.

CD44 AND METASTASIS
Active migration of tumor cells in extracellular matrix (ECM) is a prerequisite for tumor-cell invasion and metastasis. Specific membrane glycoproteins termed cell adhesion molecules, in addition to their basic role in cell-cell contact or cell-matrix interaction, were recently shown to be involved in more complex intracellular events, such as cell motility and gene transcription. CD44 is one of the adhesion molecules, and importance with respect to tumor-cell invasion and metastasis has become increasingly clear. CD44 is a type I transmembrane protein and functions as the major cellular adhesion molecule for hyaluronic acid (HA), a component of ECM. The extracellular domain (recombinant) of CD44 interacts with ECM, and the intracellular domain associates with the actin cytoskeleton via binding to ERM (ezrin, radixin, and moesin) proteins. Thus, CD44 is an important mediator in regulating interaction between ECM and the intracellular actin cytoskeleton. CD44 is expressed in most human and dog cell types and is implicated in a
CD44 HOMOLOGOUS ISOFORMS v6-5 IN PANCcancer: A METASTATIC INDEX

CD44 is a cell surface glycoprotein that plays a role in cell-cell and cell-matrix interactions. It consists of six isoforms: CD44v6, CD44v5-v6, CD44v3, CD44v7-8, CD44v9, and CD44v10. These isoforms are generated by alternative splicing of the CD44 mRNA.


GALACTOSIDASE IN BENGAL CARCINOMA

GALACTOSIDASE expression in DCC44.5

DCC44.5 is a novel gastric cancer cell line that has been established from a patient with gastric cancer. It is characterized by high expression of CD44v6, which is associated with increased cell motility and invasiveness.


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LYMPHATIC AND NEOPLASTIC METASTASIS: THE NEW ANATOMICAL CONCEPTS OF THE STUDY OF THE LYMPHATIC DRAINAGE OF VISCERAS EXPLAIN THE INVOLVEMENT OF REGIONAL NODES BY CANCER. A PERSONAL EXPERIENCE UPON 1000 CADAVERIES INJECTIONS

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After 30 years of studying the lymphatic vessels on human beings we injected more than 1000 cadavers for the interpretation of lymphatic metastasis on gastric, pancreatic, thyroid, oesophagus cancers.

For this injections we used special dyes (non vital staining dyes): coloured cedar oil according to Papanutiades or a personal technique using China wood oil. We found the different roads from the initial sub-serous lymphatic vessels towards the regional lymph-nodes and have described them like the classical authors.

But we had essentially insisted upon the fact that anatomical study of lymphatic drainage of corpus is a non sense because it concerns cadavers without life and physiology.
Transmural Pressure In Initial Subpleural Lymphatics During Spontaneous Or Mechanical Ventilation

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Introduction: The importance of maintaining lymphatic function in tissue injury, which determines the lymphatic cycle of external compression/expansion of the lymphatic vessels, is well documented. The effect of mechanical ventilation on lymphatic vessel function is not fully understood. In this study, we investigated the role of mechanical ventilation on lymphatic vessel function during initial subpleural lymphatic drainage.

Methods: The role played by the mechanical ventilation on lymphatic vessel function was evaluated using an open-chest preparation. Lymphatic vessels were studied in situ using digital high-resolution imaging. Axial auscultation was performed at different levels of the thoracic cavity during normal and mechanical ventilation. The effect of ventilation on lymphatic vessel function was assessed by measuring the lymphatic vessel diameter and lymphatic perfusion during spontaneous breathing.

Results: During mechanical ventilation, the lymphatic vessel diameter decreased significantly compared to spontaneous ventilation. The lymphatic perfusion also decreased during mechanical ventilation compared to spontaneous ventilation.

Conclusion: Our findings suggest that mechanical ventilation may alter lymphatic vessel function, potentially affecting lymphatic drainage from the thoracic cavity. This highlights the importance of monitoring lymphatic function in patients undergoing mechanical ventilation.
EMILINs EXPRESSION IN LYMPHATIC SYSTEM

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Key words: EMILINs, Lymphatic endothelial cells, Lymphangioma.

Introduction: EMILINs (Elastin Microfibril Interface Located proteins) are a family of extracellular matrix proteins characterized by a peculiar domain structure, including a N-terminal cysteine-rich domain, called the EMI-domain, followed by a coiled-coil domain and a G1Q-like domain. (Colombatti A et al., 2000). The first isolated member of the family is EMILIN1, a glycoprotein particularly abundant in aorta and present in connective tissue of a wide array of organs mainly in association with elastic fibers (Dolianna R et al., 1999). The family also includes EMILIN2 (Dolianna R et al., 2001), Multimerin1, a protein secreted by endothelial cells (EC) and platelets (Hayward CF et al., 1995) and Multimerin2, a pan-EC surface glycoprotein (Christian S. et al., 2001).

KO mice for EMILIN1 gene have been already established and they display mild elastogenesis and vascular cell defects (Zanetti M et al., 2003). However, at the present the precise function of EMILINs is unknown. Interestingly in a comparative microarray analysis of gene expression profiles of lymphatic endothelial cells (LEC) and vascular endothelial cells (EC), EMILIN1 has been found to be selectively and abundantly expressed in LECs. (Podgrabinska et al., 2002).

Accordingly to this finding, we hypothesized an involvement of EMILINs in the lymphangiogenesis. To investigate a possible role of EMILINs in this context we analysed induced lymphangiomas in wild type and EMILIN1 KO mice.

Materials and Methods: CD1 wildtype and EMILIN1 KO mice were intraperitoneally injected with incomplete Freund’s adjuvant for the lymphangioma induction and LECs were isolated from the tumor masses (Manca et al., 1999). The LECs expression of EMILINs was detected by RT-PCR. Immunofluorescence (IF) techniques on cultivated LECs and on cryostate tumor sections were applied to study the in vitro production of ECM proteins and to visualize the distribution of EMILINs in lymphatic vessels.

Results: the RT-PCR analysis reveal that LECs express the EMILIN1 and EMILIN2 mRNA, whereas Multimerin1 mRNA expression is not detected. The data about EMILIN1 mRNA expression is supported by the IF results. In cryostate sections EMILIN1 surrounds lymphatic vessels, visualized by a double staining with an anti LYVE-1 antibody and in the inner tumor mass EMILIN1 is overexpressed. Interestingly Multimerin2 stains selectively vascular EC. Whereas there is no significant difference in the development and in the size of the lymphangiomas induced in wild type and EMILIN1 KO mice, preliminary data show that the lymphatic tumor vascularization seems to be different in the two types of mice.

Conclusion: The strictly association between EMILIN1
THE EVALUATION OF LYMPHOGRAPHY IN THE ABDOMINAL PATHOLOGY

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Key words: nodus lymphaticus

Introduction
On the level of pelvis minor and abdomen can be present changes in lymphatic system on the basis of diseases of different etiology. The reason for these changes can be inborn defects, parasitic, neoplastic diseases. On the other hand the lymphatic system can cause changes in the surrounding structures and in the main vessels. The changes in the lymphatic system can demonstrate itself in the blockade of lymphatic vessels, insufficient achaustic filling, and also in the leaking of contrast medium out of the lumen of the vessels.

Material and methods
116 patients were examined mainly with onological diagnosis but also some patients who demonstrated diseases of unknown etiology. These patients were examined by USG, CT/MR, but the final diagnosis was stated only after lymphographic examination. We carried out biplanal lymphography by J.B. Kinmonth the patients were given 16 ml of contrast medium Lipiodol U/P into a prepared lymphatic vessel in dorsum pedis. X-ray pictures were taken during the examination and after 24 hours in different projections.

Results
In patients with neoplastic and inflammatory processes we could see blockades of lymphatic vessels changes in the structure of nodes, pressure and infiltrative changes in main vessels. In patients with inborn anomalies were found eg lymphovenous junctures or leaking of contrast medium out of lymphatic vessels.

Discussion
For the correct interpretation of the X-ray picture and stating the correct diagnosis is necessary to have a thorough knowledge of the normal anatomy of the lymphatic system. Although these days the classic lymphography is a less used method, in some cases it helps to prove those changes which other modern non-invasive methods are not able to prove.
ASPECTS OF THE IMAGISTIC PARTICULARITIES INducted
BY THE PHLEBOEDEMA ON THE FLABBY PARTS AT THE
LEVEL OF THE INFERIOR MEMBERS

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Key words: Phlebedema, computerized tomographie, magnetic resonance images

The purpose of the research: there has been followed the identifi-
cation of the imagistic particularities at the patients with
phleboedema.

Material, method: The study was imagined and practiced at 21
patients with PTS (post-thrombosis syndrome), at 14 patients
with hydrostatic varis (HIV) and at 8 patients with profound
acute thrombophlebitis (APAT). There have been followed the
modifications on the CT scan and MRI generated by the alter-
ations in the conjunctive tissue of the inferior members in the
conditions of the venous haemodynamic modifications gener-
ated by the venous insufficiency (VI).

Concomitantly, there have been evaluated also the composi-
tion and the evolution of the edema liquid.

Results: CT scan and MRI have emphasized 2 groups of
images at the level of the conjunctive tissue: tigre images
present in the hypoderm are characteristic to the acute thombo-
ophlebitis. Honey comb images have not been identified at the
patients with PTS (rule at patients with phleboedema). In the
hypoderm of these patients there have been emphasized uni-
form structures (hyperdermatosclerosis after the suppression of
the fat tissue) and under aponevrose calcifications.

The macromolecules (proteins- lipids) have been in low per-
centage in the edema liquid of the patients with PTS i 1/10,
respectively. 1/6 of the serum values.

Conclusions: These results can facilitate the understanding and
the completion of the aspects and physiopathologic knowledge
, especially in the PTS case in the presence of some vast
beaches of uniform structured (hyperdermatosclerosis), without
the necessity of the introduction of these explorations in the
diagnosis algorithm.
ROLE OF TRANSFORMING GROWTH FACTOR-BETA (TGF-β), IN TUMOR METASTASIS

TGF-β is a member of the TGF superfamily and plays a significant role in various biological processes such as cell proliferation, differentiation, and apoptosis. In the context of tumor metastasis, TGF-β has been linked to the development and progression of cancer through its ability to stimulate migration and invasion of tumor cells. TGF-β signaling can also modulate the behavior of immune cells, influencing their ability to combat or promote tumor growth. Understanding the role of TGF-β in tumor metastasis is crucial for developing targeted therapies to inhibit its activity and prevent the spread of cancer.
THE ROLE OF VEGF AND ITS RECEPTOR KDR IN PROMOTING TUMOR ANGIogenesis IN FELINE AND CANINE MAMMARY CARCINOMAS: A PRELIMINARY STUDY OF AUTOCRINE AND PARACRINE LOOPS

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Introduction: angiogenesis is regulated by specific angiogenic factors and their receptors in primary breast cancer; this phenomenon has shown to be of predictive value in primary breast cancer. Vascular endothelial growth factor (VEGF) and its receptor KDR constitute an important angiogenic pathway which is up-regulated in several human solid tumours. We therefore evaluated the angiogenic process in feline and canine mammary carcinomas and its prognostic potential. Materials and methods: twenty-nine samples of canine and fortyeight of feline mammary tumours were investigated. The subjects were surgically treated and submitted to a two-year follow-up. The tissue samples were investigated by immunohistochemistry to determine the expression of VEGF, of its receptor KDR and for the quantification of the microvessel density (MVD). These variables were related to some relevant clinicopathological parameters and to overall survival (OS). VEGF and KDR expression were evaluated in epithelial, stromal and endothelial compartments in order to identify autocrine and/or paracrine loops. Results: in dogs an increased VEGF expression did not show any statistical correlation with the clinicopathological parameters examined and was not correlated to a poorer prognosis. MVD was found to be significantly correlated to the histologic type (p<0.04), age (p<0.01), tumour grading (p<0.02), and to the OS (p<0.01) in cats: VEGF expression was significantly correlated to tumour grading (p<0.01) and OS (p<0.03), while no significant associations were found between MVD and the other parameters. VEGF and KDR were found to be detected on the epithelial, and/or endothelial and/or stromal cells of the carcinomas in both species, suggesting indications for some possible autocrine and paracrine loops. Conclusion: our results encourage further studies on the possible prognostic role of VEGF and MVD in canine and feline mammary tumours and on the role of growth factors and their receptors in promoting tumour proliferation and an “angiogenic shift”. The VEGF/KDR system may play a role in malignant transformation and tumor progression.
THE EFFECT OF HUMAN SKIN LYMPH ON TUMOR CELL PROLIFERATION

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Key words: lymph; tumor; metastases; cytokines

Contemporary attention on tumor proliferation has been focused on lymphangiogenesis mediated by angiogenic products of tumor cells. Neglected has been the humoral environment tumor cells grow. Both primary and metastatic cells proliferate, at least in the initial stage, in tissue fluid (lymph) characteristic for the tissue in which they are located. The aim of this study was to examine the effect of normal human skin lymph on proliferation of rapidly growing, established, lymphoid and non-lymphoid human tumor cell lines which either grow or metastasize in skin and subcutaneous tissue. Human skin lymph displayed both inhibitory and stimulatory effects on proliferation of the examined tumor cell lines of lymphoid and non-lymphoid origin, both in short-term and long-term cultures. The effect was characteristic for a particular tumor cell line. Low lymph concentrations (5 and/or 20%) stimulated colony formation in a soft agar of most of the examined cell lines: B cell lines: Rael by 39.6%, Raji by 30.0% and Balm by 197.4%; T cell lines: CCRF-CEM by 82.3%; CCRF-HSB by 204.4%; JURKA-T by 72.6% and MOL-T by 95.2%; melanoma: FMEX by 24.9% and LOX by 58.8%; sarcoma SAO-2 by 37.2% and dermoid origin: HT3 by 52.2%. High lymph concentrations (80%) inhibited the growth of B cell lines: Rael by 29.5% and KM3 by 51.1%; T cell lines: CCRF-CEM by 19.6% and CCRF-HSB by 23.7%; melanoma: FMEX by 8.8%; sarcoma: OHS by 60.5% and GAOS-2 by 24.8% and dermoid origin: HT3 by 42.8% and HACAT by 47.5%; whereas-stimulated B cell lines: Balm by 33% and Raji by 10.9%; T cell lines: Molt-4 by 35.2% and JURKA-T by 50.3% and melanoma cell line: LOX by 665.9%. The inhibitory effect of skin lymph was often more pronounced (as for FMEX and OHS tumor cells) when tumor cells were cultured in a serum-free system in a Waymouth medium. No significant differences between the results of short-term cultures and soft-agar colony formation cultures were observed. Preincubation of tumor cells with lymph demonstrated that the growth regulatory lymph factor(s) might be absorbed by tumor cells. Our results indicate that the in vivo tumor growth in its tissue-specific humoral environment may have different kinetics than in the in vitro standard culture media.
LYMPHANGIOGENESIS IN MAMMARY TUMOURS OF THE CAT ASSESSED BY VEGFR-3 EXPRESSION

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Key words: lymphangiogenesis, cat, mammary tumor, VEGFR-3

Introduction. Lymphatics play a crucial role in the formation of metastasis, and only recently were found specific markers, such as VEGFR-3 (Vascular Endothelial Growth Factor Receptor-3) that has been employed in this study. It is found in both lymphatic and blood vessels during embryogenesis, and only in lymphatics after birth. This study is aimed to verify if lymphangiogenesis develops (de novo or from pre-existing lymphatic vessels) in the intratumoral/extra-tumoral/ET/stroma of a series of feline mammary tumours.

Materials and Methods. The samples were 6 cases of normal mammary gland (NMG), 10 benign (BN) and 32 malignant (MN) neoplasms, all formalin-fixed and paraffin-wax embedded. Malignancies were graded into non-infiltrating (stage II) and infiltrating with stromal invasion (stage II) or lymphatic or blood emboli and/or regional lymph node metastases (stage II). More 4 th sections from the same samples were immunohistochemically (IH) stained with a laminin/VEGFR-3 double stain. Lymphatics, assessed as all vessels negative for laminin and expressing or non-expressing VEGFR-3, were counted in 10 intratumoral/extra-tumoral/ET/EM fields. Counts included VEGFR-3 positive, VEGFR-3 negative and total (the sum of both) lymph vessels.

Results. A red intracytoplasmatic staining revealed the anti-VEGFR-3. IH positivity, and a sub-endothelial or sub-epithelial (perpendicular and parallel) brown staining the anti-laminin positivity. In NMG, BF and MT, the positive or negative total VEGFR-3 lymphatic vessels had a significantly higher number in the ET/EM vs ET/IM fields (Spearman test, P<0.01 for all comparisons). Comparing IM with IT fields, no difference was detected in the number of lymphatic vessels, whereas there was a significantly higher number of total and VEGFR-3 negative (Spearman test, P=0.05), but not of VEGFR-3 positive lymphatics (Spearman test, P=0.26) in ET vs EM fields. No difference emerged comparing IT or ET counts among the 3 histological grades in MT, except for IT VEGFR-3 positive lymphatic vessels in stage II carcinomas vs stage II and I (Spearman test, P<0.05), which were more numerous in the former.

Discussion. The significantly higher number of lymphatic vessels in the ET vs IM stroma is likely to reflect the expansive growth of the tumour and the concentration of vessels in the extraparenchymal areas. This is enforced by the significant increase in VEGFR-3 positive and total but not VEGFR-3 positive lymphatics in ET vs EM fields. In spite of an extremely limited lymphangiogenesis, early metastases of mammary carcinomas are located in the regional lymph node.
LYMPHANGIOLOPHOTROPHISM OF MAMMARY CARCINOMAS OF THE CAT: ROLE OF VEGF-C AND CD-44

INTRODUCTION

Tumour spreading through the lymphatic system is a common and important feature in human mammary carcinomas. It is well known that the transformation of normal breast tissue to tumour is driven by multiple factors, including genetic and environmental changes. The lymphatic system plays a crucial role in the spread of cancer cells, and understanding its role in this process is essential for developing effective treatment strategies.

METHODS

This study aimed to investigate the role of VEGF-C and CD-44 in the lymphangiogenesis of mammary carcinomas of the cat. A panel of antibodies against VEGF-C and CD-44 was used to examine the expression of these proteins in a series of mammary tumours. Immunohistochemical analysis was performed on formalin-fixed, paraffin-embedded tissue specimens.

RESULTS

The results showed that VEGF-C and CD-44 were expressed in the tumour cells, and their expression correlated with the lymphangiogenesis of the tumours. The expression of these proteins was higher in invasive and metastatic tumours compared to non-invasive ones.

DISCUSSION

The findings of this study suggest that VEGF-C and CD-44 play a significant role in the lymphangiogenesis of mammary carcinomas of the cat. Understanding the mechanisms behind this process could lead to the development of novel therapeutic strategies for breast cancer.

CONCLUSION

Further studies are needed to elucidate the role of VEGF-C and CD-44 in the lymphangiogenesis of mammary carcinomas of the cat, and to develop targeted therapies for this disease.

Abbreviations: VEGF-C: Vascular Endothelial Growth Factor C; CD-44: Cluster of Differentiation 44

Keywords: lymphangiogenesis, cat, mammary tumor, VEGF-C, CD-44
ENDOTHELIAL AREA AND MICROVASCULAR DENSITY IN A CANINE NON-HODGKIN’S LYMPHOMA: AN INTERSPECIES MODEL OF TUMOR ANGIOGENESIS

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Key words: endothelial area, microvessel density, dog lymphoma

Introduction
Experimental and clinical data indicate that tumor progression is associated with angiogenesis and that increase in microves
sel density (MVD) is associated with a poor prognosis, haematological malignancies. No data have been published con
cerning the relationship between angiogenesis and malignancy grade in canine non-Hodgkin’s lymphoma. In this study we have evaluated this relationship in a series of 45 cases of canine non-Hodgkin’s lymphoma.

Materials and Methods
Canine NHL were selected and classified according to a mod
ified Kiel classification. Briefly, 6-micrometer thick serial tissue
sections were incubated with a rabbit polyclonal antibody anti factor VIII-related antigen (Dako, Glostrup, Denmark). The bound antibody was visualized by using a biotinylated secondary antibody, an avidin-biotin peroxidase complex, and 3-amino-9-ethylcarbazole. Differences in both MVD and EA between low-grade and high-grade canine NHL groups was assessed by 1-Student test. correlation between MVD and EA was tested by Pearson test.

Results
MVD mean was significantly higher in high-grade (23±9 s.d.
at 400X and 8±2 s.d. at 1000X) than in low-grade (12±4 s.d. at 400X and 3±2 s.d. at 1000X) canine NHL. Significant differences in EA means were found between low-grade (78.33±10.27±2.2 ± 20.62 s.d. at 400X and 25.68±10.27×2 ± 7.65 s.d. at 1000X) and high-grade (116.29±10.27×2 ± 28.21 s.d. at 400X and 41.89±10.27×2 ± 6.41 s.d. at 1000X) canine NHL. The correlation analysis between MVD and EA in global canine NHL series was significant at 400X and 1000X (r=0.74; p=0.002; and r=0.73; p=0.002 respectively).

Discussion
Several reports have been published showing that angiogene
tis is an important pathway in the development and progres
sion of human NHL. However, to our knowledge no data have been published regarding the correlation between angiogenic index and the degree of malignancy of canine NHL. In the present study we found that MVD and EA significantly corre
tate with each other and that both paralleled with the degree of malignancy of canine NHL. Similarly to human NHL, increased angiogenesis might play a crucial role in the trans
tion from low-grade to high-grade canine NHL and could explain the biological aggressiveness and metastatic capacity already demonstrated for high-grade canine NHL. On these data we suggest that canine NHL could represent an interest
ing spontaneous tumor model to study the angiogenesis as an interspecies pathway of tumoral malignancy and biological aggressiveness.
INTRODUCTION: The inflammatory lesions of lymphatic vessels and nodes often denote a good prognosis for patients with a variety of neoplastic diseases. In the last 5 years, magnetic resonance lymphography and lymphangiography have been developed as two different methods to examine the lymphatic system with either positive or negative contrast agents. The purpose of this study was to evaluate the effectiveness of the two methods and their ability to compare lymphangiographic findings with direct magnetic resonance lymphography.

MATERIALS AND METHODS: We studied three rabbits with lymphangiography, which were injected subcutaneously with 0.1 ml of a solution of 5% (w/v) blue dye in saline at the flank. Magnetic resonance lymphography was performed in all rabbits after they received the injection. The rabbits were divided into two groups of six rabbits each.

GROUP 1. Lymphangiography was performed using the direct lymphography technique. The lymph nodes were injected with contrast agent, and the enhancement of lymph nodes was observed. The rabbits were sacrificed 30 minutes after injection.

GROUP 2. Magnetic resonance lymphography was performed using the direct magnetic resonance lymphography technique. The lymph nodes were injected with contrast agent, and the enhancement of lymph nodes was observed. The rabbits were sacrificed 30 minutes after injection.

RESULTS: The results of both lymphangiography and magnetic resonance lymphography were almost identical. After the injection, the rabbits were divided into two groups of six rabbits each. The results of lymphangiography were compared with the results of magnetic resonance lymphography. In comparison with rabbits without lymphangiography, the rabbits with lymphangiography showed a statistically significant difference. The results of magnetic resonance lymphography were more accurate than the results of lymphangiography.

CONCLUSION: Magnetic resonance lymphography is a good contrast agent for the examination of lymphatic system. The results of magnetic resonance lymphography were more accurate than the results of lymphangiography. The use of magnetic resonance lymphography in the examination of lymphatic system is highly recommended.
LACK OF EVIDENCE OF LYMPHANGIOGENESIS IN CANINE AND BOVINE ISLET BETA CELL CARCINOMAS

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Key words: islet beta cell carcinoma, bovine, canine, lymphangiogenesis, metastases

INTRODUCTION. Islet beta cell carcinomas are uncommon tumors in animals. Despite both normal and neoplastic endocrine tissue is very rich in haematic vascular supply, lymphatic metastases to regional lymph nodes (duodenal, hepatic and mesenteric) are more frequent than blood born hepatic ones. This fact highlight the role of vascular lymphatic system in metastatic spread of beta cell carcinomas. One of the most exciting animal model for the study of tumor lymphangiogenesis is the double transgenic mice ripVEGF-C/ripVEGFR3-2, in which VEGF-C-induced lymphangiogenesis renders locally invasive islet cell tumors lymphangiogenesis and metastatic (Meadnet al., 2001). The hypothesis is that metastatic behavior is enhanced by extensive intratumoral lymphatic neoangiogenesis, as in human melanomas and squamous cell carcinomas.

The aim of this work is to investigate presence of preformed and newly formed lymphatic vessels in canine and bovine islet beta cell carcinomas.

MATERIALS AND METHODS. 30 islet beta cell carcinomas (25 from cattle and 5 from dogs) have been retrospectively evaluated by light and electron microscopy. Immunohistochemical evaluation of lymphatic vessels has been performed using D2-40 antibody and antibodies against LYVE-1, Podoplanin, Prox-1, Laminin and VEGFR-3.

RESULTS. In all the bovine cases examined, and in the three canine tumors in which a complete necropsy was performed, there was metastatic spread to regional lymph nodes and liver; in 4 bovine cases metastases were present exclusively in lymph nodes. Histologically the tumors were characterized by well developed intratumoral vascularization, with vascular lacunae, and a peritumoral network of thin walled capillaries, in some of which neoplastic emboli were observed. Prox-1 and Podoplanin, did not react at all with the tested tissues. LYVE-1 staining was faint and inconstant, while D2-40 was non specific, marking also haematic vessels, identified by their erythrocyte content. VEGFR-3 was non reactive in bovine tumors but stained some canine specimens. Double labelling with VEGFR-3 and Laminin did not allow definitive distinction between lymphatic and haematic vessels in canine cases. Electron microscopy revealed thin vessels around and inside the tumor mass, but was inconclusive in differentiating between lymphatic and haematic vessels, as none of the features typical of lymphatic endothelium (i.e. absent basal membrane, cell fenestraions) were observed.

DISCUSSION. Several markers for lymphatic endothelium have been identified in human medicine. Unfortunately none of these have been definitively tested in animal species, and reports are anecdotic. Based on the results of this study, none of the antibody tested is a reliable tool to identify both preformed and newly formed lymphatic vessels in formalin-fixed paraffin-embedded canine and bovine tissues. This could be due to species-specific differences, lack of cross-reactivity with human antibodies, or on the processing techniques, that have not been standardized for retrospective archive material. Thus the role of lymphatic system in the metastatic spread of canine and bovine islet beta cell carcinomas can't be assessed, even if the evidence of neoplastic emboli in thin-walled erythrocyte-free peritumoral vessels, and the presence of constant nodal metastases, is strongly suggestive of a lymphatic dissemination that can involve peritumoral preformed capillaries as well as intratumoral newly formed vessels. Further studies are needed to confirm intratumoral lymphangiogenesis in animal pancreatic endocrine tumors, as recently confirmed in humans (Sipos et al., 2004)

ENDOTHELIN-CONVERTING ENZYME-1 (ECE-1) EXPRESSION AND LYMPHATIC VESSELS DISTRIBUTION IN HUMAN COLORECTAL CANCER

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Key words: Colorectal cancer, lymphatic vessels, endothelin-converting enzyme-1 (ECE-1)

INTRODUCTION
The endothelin-converting enzyme-1 (ECE-1) is a metalloproteinase that is involved in the cleavage of big endothelin-1 to generate active endothelin-1 in vivo. It is thought that high levels of ECE-1 expression are associated with lymphatic vessel formation and tumor invasion. In this study, we aimed to investigate the expression of ECE-1 and its role in the formation of lymphatic vessels in human colorectal cancer.

RESULTS
We found that ECE-1 was localized in the lumens of lymphatic vessels and in the endothelial cells, suggesting an association between ECE-1 and lymphatic vessel formation. The expression of ECE-1 was higher in tumors with a more advanced stage of invasion.

CONCLUSIONS
Our findings suggest that ECE-1 plays a role in the formation of lymphatic vessels in colorectal cancer. The identification of potential therapeutic targets for ECE-1 could provide new strategies for the treatment of colorectal cancer.

MATERIALS AND METHODS
Colorectal cancer specimens were fixed in Bouin’s solution and embedded in paraffin. The sections were stained with alkaline phosphatase to detect lymphatic vessels, and the expression of ECE-1 was evaluated using immunohistochemistry.

REFERENCES

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METASTATIC POTENTIAL OF A NEWLY ESTABLISHED TRANSPLANTABLE RAT MAMMARY TUMOR CELL LINE

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Key words: rat mammary cancer, cell line, animal model

In many animal models of human mammary cancer, primary tumors show no tendency to metastasize or suffer from other considerable drawbacks. We therefore established from a female DA/Han rat an estrogen-receptor-positive mammary tumor, which had been induced by a single intragastric dose of 20 mg dimethyl-benzearacene. We further processed the tumor, thus resulting in a single cell suspension which was then, at a cell count of 1 x 10^6 cells/animal, subjected to three subsequent in vivo passages in female, syngenic, adult animals. Parallel in vitro cultures were performed in phenol-red free special mammary epithelial cell growth medium designed to suppress fibroblast growth. After varying cultivation periods, when the cells had reached a uniform appearance, 1 x 10^6 cells/animal were transferred onto recipients as well. Starting at 12 days after subcutaneous inoculation, tumors became palpable. In intact female animals, tumors grew steadily over a period of up to 28 days. After this observation period, tumor sizes frequently necessitated that animals had to be killed for ethical reasons. As soon as subcutaneous transplantation had reproducibly been performed from in vitro cultured cells, we tested in a follow-up experiment for metastatic potential of the cells by injecting either 1 x 10^4 or 1 x 10^5 cells/animal intravenously into groups of 5 female DA/Han rats each. 28 days after intravenous injection of 1 x 10^5 cells, metastases of 3-5 mm diameter were found in lungs and regional lymph nodes of experimental animals. When administered to OVX vs. NOVX animals, it became obvious, that tumors grew considerably retarded in OVX animals. Reduced tumor growth under OVX conditions, compared to intact animals, was evident in primary tumors as well as in the rate of formation of lung metastases.

Cells were characterized from in vitro culture and in vivo ectopic tumors. Whole RNA was extracted and RT-PCR was performed according to standard procedures using commercially available kits and published primer sequences. With RT-PCR we tested primary tumor specimen for the presence of mRNA for androgen receptor (AR), estrogen receptor (ER) and cyclin D1 (CyD1) as proliferation marker. Interestingly, only mRNA for AR and CyD1 could be detected. With the here presented cell line, designated 03/664, we have a tool for investigating influences on the metastatic potential of a hormone responsive mammary tumor. Absence of ER mRNA in the primary tumor, isolated after in vivo passages, would correspond to human breast cancer after several cycles of anti-estrogenic therapy. There, tumors often become refractory to the anti-hormonal drug and show an ER-negative phenotype. Further characterization, both in vitro and in vivo, is currently performed, in order to test how various growth parameters of 03/664 cells react to standard and experimental anti-neoplastic drugs.
SURGERY IN TREATMENT OF LYMPHEDEMA: A NEW SUCCESSFUL METHOD

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Key words: Lymphedema, Modified Homann

Objective: To review common treatment methods of lymphedema and to evaluate the effectiveness of surgery in the treatment of lymphedema, with emphasis on the modified Homann method.

Method: A case study was conducted on 50 patients with lymphedema referred to the surgery department of the hospital with a diagnosis of lymphedema. The patients were divided into two groups: Group A underwent the modified Homann method, and Group B underwent the conventional method. Both groups were followed up for 6 months post-surgery.

Results: The modified Homann method showed significantly better results than the conventional method in terms of symptom improvement and lymphedema reduction. The modified Homann method was also associated with fewer complications and a shorter hospital stay.

Conclusion: The modified Homann method is a promising surgical approach for the treatment of lymphedema. It is recommended as a viable alternative to conventional surgical methods, particularly for patients with severe lymphedema and those with a high risk of complications.