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Featured Article

Yang, Y., et al. (2015). "In vitro induction of human adipose-derived stem cells into lymphatic endothelial-like cells." *Cell Reprogram* 17(1): 69–76.

Abstract

Human adipose-derived stem cells (hADSCs) may provide a suitable number of progenitors for the treatment of lymphatic edema; however, to date the protocols for inducing hADSCs into this tissue type have not been standardized. We wished to investigate the induction of hADSCs into lymphatic endothelial-like cells using vascular endothelial growth factor-C156S (VEGF-C156S) and other growth factors in vitro. hADSCs from healthy adult adipose tissue were purified using enzyme digestion. Differentiation was induced using medium containing VEGF-C156S and bovine fibroblast growth factor (bFGF). Differentiation was confirmed using immunostaining for lymphatic vessel endothelial hyaluronan receptor (LYVE-1) and fms-related tyrosine kinase 4 (FLT-4), two lymphatic endothelial cell markers. The expression levels of LYVE-1, prospero homeobox 1 (PROX-1), and FLT-4 throughout induction were assessed using reverse transcriptase quantitative polymerase chain reaction. hADSCs were successfully obtained by trypsin digest and purification. Flow cytometry showed these cells were similar to mesenchymal stem cells, with a high positive rate of CD13, CD29, CD44, and CD105, and a low positive rate of CD31, CD34, CD45, and HLA-DR. Induction to lymphatic endothelial-like cells was successful, with cells expressing high levels of LYVE-1, PROX-1, and FLT-4. Adipose-derived stem cells can be induced to differentiate into lymphatic endothelial-like cells using a medium containing VEGF-C156S, bFGF, and other growth factors. This population of lymphatic endothelial-like cells may be useful for lymphatic reconstruction in the future.

Commentary

As an alternative to bone marrow-derived mesenchymal stem cells (BM-MSCs), Yang et al aimed to induce differentiation of Human adipose-derived stem cells (hADSCs)

into a lymphatic endothelial cell phenotype. By incubating them with growth factors (e.g. bovine FGF and VEGF-C156S). It has been shown previously that adipose-derived stem cells can differentiate into many cell types. They share many qualities of bone-marrow-derived mesenchymal stem cells, and are readily available by harvesting adult fat tissue.

These authors refined the technique of inducing the transformation to the lymphatic phenotype by using a mutant form of VEGF-C, VEGF-C156s, binding selectively to VEGFR3, modulating lymphangiogenesis. They demonstrated 1) a high yield of hADSCs extractable from adult adipose tissue, 2) qualitative similarities between bone-marrow derived stem cells and adipose-derived stem cells, 3) the pluripotency of these cells, differentiating into adipocytes or osteocytes (and others have shown differentiation into cartilage, muscle and endothelial cells). 4) the selective induction into lymphatic endothelial phenotype by incubation with VEGF-C156s. The authors acknowledge that they only used 3 markers to demonstrate the lymphatic phenotype (LYVE-1, PROX-1 and FLT-4) and that they did not explore the role of lymphatic specific cytokines (inducers and inhibitors). Although these findings must be further developed, the results are promising, and suggest that a readily available source of hADSCs may play a role in promoting lymphatic endothelial cell function, a potential therapy for lymphedema.

Basic Science

- Ayestary, B. and F. Bekara (2015). "Fluorescein sodium fluorescence microscope-integrated lymphangiography for lymphatic supermicrosurgery." *Microsurgery*. Epub.
- Baeyens, N., et al. (2015). "Vascular remodeling is governed by a VEGFR3-dependent fluid shear stress set point." *Elife* 4.
- Chauhan, S. K., et al. (2014). "Corneal Lymphatics: Role in Ocular Inflammation as Inducer and Responder of Adaptive Immunity." *J Clin Cell Immunol* 5.
- Deng, Y., et al. (2015). "Molecular Controls of Lymphatic VEGFR3 Signaling." *Arterioscler Thromb Vasc Biol* 35(2): 421–429.

OBJECTIVES: Vascular endothelial growth factor receptor 3 (VEGFR3) plays important roles both in lymphangiogenesis and angiogenesis. On stimulation by its ligand VEGF-C, VEGFR3 is able to form both homodimers as well as heterodimers with VEGFR2

and activates several downstream signal pathways, including extracellular signal-regulated kinases (ERK) 1/2 and protein kinase B (AKT). Despite certain similarities with VEGFR2, molecular features of VEGFR3 signaling are still largely unknown. **APPROACH AND RESULTS:** Human dermal lymphatic endothelial cells were used to examine VEGF-C-driven activation of signaling. Compared with VEGF-A activation of VEGFR2, VEGF-C-induced VEGFR3 activation led to a more extensive AKT activation, whereas activation of ERK1/2 displayed a distinctly different kinetics. Furthermore, VEGF-C, but not VEGF-A, induced formation of VEGFR3/VEGFR2 complexes. Silencing VEGFR2 or its partner neuropilin 1 specifically abolished VEGF-C-induced AKT but not ERK activation, whereas silencing of neuropilin 2 had little effect on either signaling pathway. Finally, suppression of vascular endothelial phosphotyrosine phosphatase but not other phosphotyrosine phosphatases enhanced VEGF-C-induced activation of both ERK and AKT pathways. Functionally, both ERK and AKT pathways are important for lymphatic endothelial cells migration. **CONCLUSIONS:** VEGF-C activates AKT signaling via formation of VEGFR3/VEGFR2 complex, whereas ERK is activated by VEGFR3 homodimer. Neuropilin 1 and vascular endothelial phosphotyrosine phosphatase are involved in regulation of VEGFR3 signaling.

- Dongaonkar, R. M., et al. (2014). "Mesenteric lymphatic vessels adapt to mesenteric venous hypertension by becoming weaker pumps." *Am J Physiol Regul Integr Comp Physiol*: ajpregu 00196 02014.
- Flaht-Zabost, A., et al. (2014). "Cardiac mouse lymphatics: Developmental and anatomical update." *Anat Rec (Hoboken)* 297(6): 1115–1130.
- Gerner, M. Y., et al. (2015). "Strategically localized dendritic cells promote rapid T cell responses to lymph-borne particulate antigens." *Immunity* 42(1): 172–185.
- Jung, S. J., et al. (2014). "Visualization of the primo vascular system afloat in a lymph duct." *J Acupunct Meridian Stud* 7(6): 337–345.
- Koina, M., et al. (2015). "Evidence For Lymphatics in the Developing and Adult Human Choroid." *Invest Ophthalmol Vis Sci*. Epub.
- Lee, M., et al. (2014). "Transcriptional programs of lymphoid tissue capillary and high endothelium reveal control mechanisms for lymphocyte homing." *Nat Immunol* 15(10): 982–995.

Lymphocytes are recruited from blood by high-endothelial venules (HEVs). We performed transcriptomic analyses and identified molecular signatures that distinguish HEVs from capillary endothelium and that define tissue-specific HEV specialization. Capillaries expressed gene programs for vascular development. HEV-expressed genes showed enrichment for genes encoding molecules involved in immunological defense and lymphocyte migration. We identify capillary and HEV markers and candidate mechanisms for regulated recruitment of lymphocytes, including a lymph node HEV-selective transmembrane mucin; transcriptional control of functionally specialized carbohydrate ligands for lymphocyte L-selectin; HEV expression of

molecules for transendothelial migration; and metabolic programs for lipid mediators of lymphocyte motility and chemotaxis. We also elucidate a carbohydrate-recognition pathway that targets B cells to intestinal lymphoid tissues, defining CD22 as a lectin-homing receptor for mucosal HEVs.

- Liao, S. and P. Y. von der Weid (2014). "Lymphatic system: An active pathway for immune protection." *Semin Cell Dev Biol*. Epub.
- Mahadevan, A., et al. (2014). "The left-right pitx2 pathway drives organ-specific arterial and lymphatic development in the intestine." *Dev Cell* 31(6): 690–706.
- Matsumoto-Okazaki, Y., et al. (2015). "Real-time imaging of interaction between macrophages and lymphatic vessels in an in vitro model to study inflammatory resolution." *J Dermatol Sci* 77(1): 76–79.
- Miyashita, T., et al. (2015). "Prox1 expression in the endolymphatic sac revealed by whole-mount fluorescent imaging of Prox1-GFP transgenic mice." *Biochem Biophys Res Commun* 457(1): 19–22.
- Moghadamrad, S., et al. (2015). "Attenuated portal hypertension in germ-free mice: Function of bacterial flora on the development of mesenteric lymphatic and blood vessels." *Hepatology*. Epub.
- Moriondo, A., et al. (2015). "Diaphragmatic lymphatic vessel behavior during local skeletal muscle contraction." *Am J Physiol Heart Circ Physiol* 308(3): H193–H205.
- Murphy, P. A., et al. (2014). "Constitutively active Notch4 receptor elicits brain arteriovenous malformations through enlargement of capillary-like vessels." *Proc Natl Acad Sci U S A* 111(50): 18007–18012.
- Plog, B. A., et al. (2015). "Biomarkers of Traumatic Injury Are Transported from Brain to Blood via the Glymphatic System." *J Neurosci* 35(2): 518–526.
- Pupulim, L. F., et al. (2015). "Hepatic lymphatics: anatomy and related diseases." *Abdom Imaging*. Epub.
- Rehal, S. and P. Y. von der Weid (2014). "Experimental ileitis alters prostaglandin biosynthesis in mesenteric lymphatic and blood vessels." *Prostaglandins Other Lipid Mediat* 116–117C: 37–48.
- Rohringer, S., et al. (2014). "Molecular and cellular effects of in vitro shockwave treatment on lymphatic endothelial cells." *PLoS One* 9(12): e114806.
- Schindewolf, L., et al. (2014). "VEGF-C improves regeneration and lymphatic reconnection of transplanted autologous lymph node fragments: An animal model for secondary lymphedema treatment." *Immun Inflamm Dis* 2(3): 152–161.

Secondary lymphedema occurs after for example breast cancer surgery and radiation in 20–50% of the patients. Due to the poor outcomes of surgical treatments in the past, the therapy often remains symptomatic. However, avascular transplantation of autologous lymph node fragments (LN-Tx) combined with postoperative injections of vascular endothelial growth factor-C (VEGF-C) emerges as a potential surgical therapy. In this study, adult rats underwent LN-Tx to investigate the following parameters of VEGF-C application: time point, location and dosage. Furthermore, the influences of VEGF-C on lymphatic reconnection and transplant regeneration were analyzed. The reconnection was investigated using intradermally injected blue dye and the regeneration was

evaluated histologically using hematoxylin-eosin (H&E) staining and immunohistochemistry. The higher dosage enhanced the reconnection rates significantly and showed a statistical tendency of improving regeneration. An application on early postoperative days and the injection into the medial thigh improved the reconnection significantly. However, these variables did not affect the regeneration statistically. This study confirms that LN-Tx combined with lymphatic growth factor VEGF-C is a possible approach in the therapy of secondary lymphedema and shows the important role of VEGF-C application parameters.

- Secker, G. A. and N. L. Harvey (2014). "VEGFR signaling during lymphatic vascular development: From progenitor cells to functional vessels." *Dev Dyn*
- Zarkada, G., et al. (2015). "VEGFR3 does not sustain retinal angiogenesis without VEGFR2." *Proc Natl Acad Sci U S A* 112(3): 761–766.

Clinical

- Aggithaya, M. G., et al. (2015). "Yoga for correction of lymphedema's impairment of gait as an adjunct to lymphatic drainage: A pilot observational study." *Int J Yoga* 8(1): 54–61.
- Baulieu, F., et al. (2014). "Serial lymphoscintigraphic findings in a patient with Gorham's disease with lymphedema." *Lymphology* 47(3): 118–122.
- Dellinger, M. T., et al. (2014). "Viewpoints on vessels and vanishing bones in Gorham-Stout disease." *Bone* 63: 47–52.
- Gupta, R., et al. (2014). "Lymphatic manifestations of lymphangioleiomyomatosis." *Lymphology* 47(3): 106–117.

Lymphangioleiomyomatosis (LAM) is a slowly progressive, low grade, metastasizing neoplasm, associated with cellular invasion and cystic destruction of the pulmonary parenchyma. Although the source of LAM cells that infiltrate the lung is unknown, available evidence indicates that the disease spreads primarily through lymphatic channels, often involving abdominal, axial, and retroperitoneal nodes, suggestive of an origin in the pelvis. LAM cells harbor mutations in tuberous sclerosis genes and produce lymphangiogenic growth factors, which facilitate access to and movement through the lymphatic system and likely play an important role in destructive tissue remodeling in the lung. Lymphatic manifestations of LAM include thoracic duct wall invasion, lymphangioleiomyoma formation, chylous fluid collections in the peritoneal, pleural, and pericardial spaces, chyloptysis, chyloporrheal chylometrorrhea, chyle leak from the umbilicus, chylous pulmonary congestion, and lower extremity lymphedema. LAM lesions express lymphangiogenic growth factors VEGF-C and VEGF-D; growth factor receptors, VEGFR-2 and VEGFR-3; and markers LYVE-1 and podoplanin, and are lined with chaotic lymphatic channels. Serum VEGF-D is elevated in 70% of patients with LAM and is a clinically useful diagnostic and prognostic biomarker. Molecular targeted therapy with sirolimus stabilizes lung function, is anti-lymphangiogenic, and is highly effective for the lymphatic and chylous complications of LAM.

Future trials in patients with LAM who have lymphatic manifestations or elevated serum VEGF-D will likely focus on the VEGF-C/VEGF-D/VEGFR-3 axis.

- Hooper, P. J., et al. (2014). "Assessing progress in reducing the at-risk population after 13 years of the global programme to eliminate lymphatic filariasis." *PLoS Negl Trop Dis* 8(11): e3333.
- Jerman, L. F. and A. J. Hey-Cunningham (2015). "The role of the lymphatic system in endometriosis: a comprehensive review of the literature." *Biol Reprod*.
- Khare, P., et al. (2014). "Incidental diagnosis of filariasis in superficial location by fnac: a retrospective study of 10 years." *J Clin Diagn Res* 8(12): FC05–FC08.
- Liu, H., et al. (2014). "The defect of both angiogenesis and lymphangiogenesis is involved in preeclampsia." *Placenta*. Epub.
- Lomauro, K. (2014). "Congenital chylothorax in newborn with trisomy 21." *Georgian Med News*(236): 74–77.
- Lopez-Gutierrez, J. C. and J. A. Tovar (2014). "Chylothorax and chylous ascites: management and pitfalls." *Semin Pediatr Surg* 23(5): 298–302.
- Martel, C., et al. (2014). "Photoacoustic lymphatic imaging with high spatial-temporal resolution." *J Biomed Opt* 19(11): 116009.
- Niimi, K., et al. (2014). "Ultrasonographic findings and the clinical results of treatment for lymphedema." *Ann Vasc Dis* 7(4): 369–375.
- Onishi, T., et al. (2014). "Topical bFGF improves secondary lymphedema through lymphangiogenesis in a rat tail model." *Plast Reconstr Surg Glob Open* 2(8): e196.

BACKGROUND: Secondary lymphedema is a common complication of cancer therapy, but options for treating lymphedema are essentially ineffective and limited. On the contrary, lymphangiogenic growth factors accelerate lymphangiogenesis and improve lymphedema. **METHODS:** Rat tail models of lymphedema were assigned to groups that received either daily topical basic fibroblast growth factor (bFGF) or saline (control) groups. Tail volume was measured, and the function of the lymphatic system was evaluated as the fluorescence intensity of indocyanine green every 3 days. The mRNA levels of vascular endothelial growth factor (VEGF)-C and VEGF-D and the protein levels of VEGF-C were evaluated at postoperative days (PODs) 7, 14, and 28. The subcutaneous and deep areas and lymphatic vessel density were histologically determined at PODs 7, 14, and 28. **RESULTS:** Tail volume was significantly larger in the control than in the bFGF group ($P < 0.05$). The intensity of indocyanine green fluorescence significantly decreased earlier in the bFGF group ($P < 0.05$). The mRNA and protein levels of VEGF-C were upregulated in the bFGF group at POD 14 ($P < 0.01$). Both subcutaneous and deep tissues gradually withered in both groups but more rapidly in the bFGF, than in the control group, reaching statistically significant differences in the subcutaneous and deeper areas at POD 14 ($P < 0.05$). Lymphatic vessel density was significantly higher in the bFGF than in the control group at POD 14 ($P < 0.05$). **CONCLUSIONS:** Topical bFGF induces lymphangiogenesis and improves lymphedema in the rat tail model.

- Ozyurt, A., et al. (2014). "Variable clinical presentation in primary lymphoedema: report of two cases." *Clin Dysmorphol* 23(3): 83–87.
- Quian, Y. P., et al. (2015). "Lymphoscintigraphy for the assessment of autologous stem cell implantation in chronic lymphedema." *Clin Nucl Med* 40(3): 217–219.
- Raica, M., et al. (2015). "Mast cells stimulate lymphangiogenesis in the gingiva of patients with periodontal disease." *In Vivo* 29(1): 29–34.
- Ramaiah, K. D. and E. A. Ottesen (2014). "Progress and impact of 13 years of the global programme to eliminate lymphatic filariasis on reducing the burden of filarial disease." *PLoS Negl Trop Dis* 8(11): e3319.
- Suero Molina, E. J., et al. (2014). "Cerebrospinal fluid leakage in Gorham-Stout disease due to dura mater involvement after progression of an osteolytic lesion in the thoracic spine." *J Neurosurg Spine* 21(6): 956–960.

Patients with Gorham-Stout disease (GSD), a rare disease of poorly understood etiopathophysiology, suffer from progressive osteolysis. Destruction of bone matrix is caused by lymphatic vessels, which can lead to CSF leakage if parts of bony structures adjacent to CSF spaces are involved. So far, fewer than 200 patients have been reported in the literature; only 4 of these patients presented with CSF leakage. The authors report the case of a 30-year-old man with GSD and CSF leakage due to dura mater involvement after progression of an osteolytic lesion in the thoracic spine. Neurosurgical intervention, including dura repair, was needed. Experimental medical therapy with rapamycin was started, leading to disease control for more than 12 months. Progression of GSD can lead to destruction of the meninges, causing CSF leakage. The authors review 4 other cases reported in the literature and discuss therapeutic options.

- Van Kruiningen, H. J., et al. (2014). "Granulomas obstruct lymphatics in all layers of the intestine in Crohn's disease." *APMIS* 122(11): 1125–1129.
- Vybohova, D., et al. (2015). "Quantitative comparison of angiogenesis and lymphangiogenesis in cutaneous lichen planus and psoriasis: Immunohistochemical assessment." *Acta Histochem* 117(1): 20–28.
- Weiss, M., et al. (2015). "Primary lymphedema of the lower limb: The clinical utility of single photon emission computed tomography/CT." *Korean J Radiol* 16(1): 188–195.

OBJECTIVE: The aim of this prospective study was to determine whether the additional use of the single photon emission computed tomography/CT (SPECT/CT) technique improves the diagnostic value of planar lymphoscintigraphy in patients presenting with primary lymph edema of the lower limb. **MATERIALS AND METHODS:** For a defined period of three years (April 2011–April 2014) a total of 34 consecutive patients (28 females; age range, 27–83 years) presenting with swelling of the leg(s) suspicious of (uni- or bilateral, proximal or distal) primary lymphedema were prospectively examined by planar lymphoscintigraphy (lower limbs, n=67) and the tomographic SPECT/CT technique (anatomical sides, n=65). **RESULTS:** In comparison to pathological planar scintigraphic findings, the addition of SPECT/CT provided relevant

additional information regarding the presence of dermal backflow (86%), the anatomical extent of lymphatic disorders (64%), the presence or absence of lymph nodes (46%), and the visualization of lymph vessels (4%). **CONCLUSION:** As an adjunct to planar lymphoscintigraphy, SPECT/CT specifies the anatomical correlation of lymphatic disorders and thus improves assessment of the extent of pathology due to the particular advantages of tomographic separation of overlapping sources. The interpretation of scintigraphic data benefits not only in baseline diagnosis, but also in physiotherapeutic and microsurgical treatments of primary lymphedema.

- Zhang, C. and R. Ma (2015). "Lymphangioma of the foot mimicking melanoma: An uncommon case of a Chinese boy." *J Pediatr Orthop B* 24(2): 159–161.

Oncology

- Bains, S. K., et al. (2015). "A constitutional predisposition to breast cancer-related lymphoedema and effect of axillary lymph node surgery on forearm muscle lymph flow." *Breast* 24(1): 68–74.
- Hos, D., et al. (2014). "Antilymphangiogenic therapy to promote transplant survival and to reduce cancer metastasis: what can we learn from the eye?" *Semin Cell Dev Biol*.

The lymphatic vasculature is - amongst other tasks - essentially involved in inflammation, (auto)immunity, graft rejection and cancer metastasis. The eye is mainly devoid of lymphatic vessels except for its adnexa, the conjunctiva and the limbus. However, several pathologic conditions can result in the secondary ingrowth of lymphatic vessels into physiologically alymphatic parts of the eye such as the cornea or the inner eye. Therefore, the cornea has served as an excellent in vivo model system to study lymphangiogenesis, and findings from such studies have substantially contributed to the understanding of central principles of lymphangiogenesis also with relevance outside the eye. Grafting experiments at the cornea have been extensively used to analyze the role of lymphangiogenesis in transplant immunology. In this regard, we recently demonstrated the crucial role of lymphatic vessels in mediating corneal allograft rejection and could show that antilymphangiogenic therapy increases graft survival. In the field of cancer research, we recently detected tumor-associated lymphangiogenesis in the most common malignant tumors of the eye, such as conjunctival carcinoma and melanoma, and ciliochoroidal melanoma with extraocular extension. These neolymphatics correlate with an increased risk of local recurrence, metastasis and tumor related death, and may offer potential therapeutic targets for the treatment of these tumors. This review will focus on corneal and tumor-associated ocular lymphangiogenesis. First, we will describe common experimentally used corneal lymphangiogenesis models and will recapitulate recent findings regarding the involvement of lymphatic vessels in corneal diseases and transplant immunology. The second part of this article will summarize findings about the participation of tumor-associated

lymphangiogenesis in ocular malignancies and their implications for the development of future therapeutic strategies.

- Ou, J. J., et al. (2015). "Neuropilin-2 mediates lymphangiogenesis of colorectal carcinoma via a VEGFC/VEGFR3 independent signaling." *Cancer Lett* 358(2): 200–209.

Lymphangiogenesis critically contributes to the lymphatic metastasis of colorectal carcinomas (CRCs), but the underlying mechanism of CRC lymphangiogenesis remains largely elusive. We have previously demonstrated that Semaphorin-3F (SEMA3F) is critically involved in CRC metastasis, and the receptor of SEMA3F, neuropilin-2 (NRP2), originally described as an axon guiding chemorepulsant implicated in nerve development, has been suggested in promoting lymphangiogenesis via acting as an obligate co-receptor of VEGFR3 cooperatively enhancing the activity of VEGF-C. Our present study revealed that in colorectal carcinomas, NRP2 expression levels of tumor-associated lymphatic endothelial cells (LECs) are significantly correlated with the density of tumor lymphatic vessels. In vitro, activation of NRP2 in LECs substantially facilitates their migration, sprouting, and tubulogenesis capacity via regulating the rearrangement of cytoskeleton polarity. In vivo model further showed that in the xenografts generated from SEMA3F knockdown CRC cells, NRP2 is substantially activated in tumor-associated LECs, resulting in a significantly increased tumor lymphangiogenesis. Further evidence demonstrated that CRC cell induces the activation of NRP2 in LECs to promote tumor lymphangiogenesis via integrin α 9 β 1/FAK/Erk pathway independent VEGF-C/VEGFR3 signaling. Our study for the first time revealed the novel molecular mechanism of NRP2-mediated-lymphangiogenesis in CRCs, suggesting NRP2 as a potential therapeutic target in preventing lymphatic metastasis of CRCs.

- Yamamoto, T., et al. (2015). "Supermicrosurgical deep lymphatic vessel-to-venous anastomosis for a breast cancer-related arm lymphedema with severe sclerosis of superficial lymphatic vessels." *Microsurgery*. Epub.
- Yoo, K. H., et al. (2015). "Corosolic acid exhibits anti-angiogenic and anti-lymphangiogenic effects on in vitro endothelial cells and on an in vivo CT-26 colon carcinoma animal model." *Phytother Res*. Epub.
- Yu, M., et al. (2015). "Hyaluroan-regulated lymphatic permeability through SIP receptors is crucial for cancer metastasis." *Med Oncol* 32(1): 381.
- Zhang, X. Y. and W. Y. Lu (2014). "Recent advances in lymphatic targeted drug delivery system for tumor metastasis." *Cancer Biol Med* 11(4): 247–254.

The lymphatic system has an important defensive role in the human body. The metastasis of most tumors initially spreads through the surrounding lymphatic tissue and eventually forms lymphatic metastatic tumors; the tumor cells may even transfer to other organs to form other types of tumors. Clinically, lymphatic metastatic tumors develop rapidly. Given the limitations of surgical resection and the low effectiveness of

radiotherapy and chemotherapy, the treatment of lymphatic metastatic tumors remains a great challenge. Lymph node metastasis may lead to the further spread of tumors and may be predictive of the endpoint event. Under these circumstances, novel and effective lymphatic targeted drug delivery systems have been explored to improve the specificity of anticancer drugs to tumor cells in lymph nodes. In this review, we summarize the principles of lymphatic targeted drug delivery and discuss recent advances in the development of lymphatic targeted carriers.

Vascular Anomalies

- Bal, H. S., et al. (2014). "A giant lymphangioma of the body wall in a child: A heavy companion." *BMJ Case Rep* 2014.
- Blei, F. (2015). "Kaposiform Hemangioendothelioma: Therapeutic efficacy for an enigmatic diagnosis." *Pediatr Blood Cancer*.
- Bonifazi, E. and M. Cutrone (2015). "Unusually large patch-type noninvoluting congenital hemangioma of the shoulder: A report of two cases." *Pediatr Dermatol*.

Noninvoluting congenital hemangiomas (NICHs) are vascular tumors that are fully formed at birth and do not regress. Clinically, NICHs persist indefinitely over time. Histologically they are characterized by deep dermal lobules of small vessels with endothelial cells that do not stain with glucose transporter 1 (GLUT1). Two similar cases of unusually large, patch-type NICHs of the shoulder in neonates were observed in the past 20 years in two pediatric dermatology units in Italy. In both cases, histopathology showed lobular collections of small vessels that did not stain with GLUT1. The clinical features of the two cases remained unchanged over a follow-up period of 10 and 4 years, respectively. The large size and flat appearance of the lesions could be due to their early onset during embryogenesis.

- Cao, Y., et al. (2014). "One possible mechanism of pulsed dye laser treatment on infantile hemangioma: Induction of endothelial apoptosis and serum vascular endothelial growth factor (VEGF) level changes." *J Lasers Med Sci* 5(2): 75–81.

INTRODUCTION: Pulsed dye laser (PDL) is an important treatment for superficial infantile hemangioma, but few studies report on its cellular mechanism. The aim of this study was to evaluate alterations of serum vascular endothelial growth factor (VEGF) level in infantile hemangioma (IH) patients after laser treatment and effects of PDL irradiation on human umbilical vein endothelial cells (HUVECs) in vitro, as well as to explore the biomolecular mechanisms and ultrastructure changes of the PDL effect. **METHODS:** 74 children with infant hemangioma including 45 patients in proliferating phase, 18 patients in involuting phase, 11 patients in involuted phase and 10 healthy children were engaged in this study. The plasma VEGF levels of children were measured with the enzymelinked immunosorbent assay (ELISA). 24 hours after, HUVECs cultured in vitro were irradiated with PDL, cell apoptosis, mRNA levels of VEGF, and changes of

ultrastructure were evaluated using flow cytometry, real-time reverse transcriptase polymerase chain reaction (RT-PCR), and transmission electron microscopy, respectively. **RESULTS:** The serum VEGF concentrations in children with proliferating hemangiomas were significantly higher than in patients with involuting/involved hemangiomas and healthy patients. After receiving 3 laser treatments, the plasma VEGF levels of IH patients in proliferating hemangiomas decreased significantly. PDL irradiation could down-regulate VEGF mRNA expression of HUVECs, and increase cell apoptosis rate. **CONCLUSION:** The present study demonstrates that PDL irradiation imparts apoptosis induction effects on HUVECs in vitro. Furthermore, our results suggest that vascular endothelial growth factor may be of particular importance in pathophysiology and PDL treatment of hemangiomas, also serum VEGF levels may be used as an aid in the follow up of IH. This provides valuable evidence of the PDL effect on infantile hemangioma.

- Chamlin, S. L., et al. (2015). "Development and validation of a quality-of-life instrument for infantile hemangiomas (IH-QOL)." *J Invest Dermatol*. Epub.
- Cyrułnik, A. A., et al. (2014). "Kaposiform hemangioendothelioma with Kasabach-Merritt syndrome mistaken for child abuse in a newborn." *Cutis* 93(3): E17–20.
- Díaz-Manzano, J. A., et al. (2014). "Oropharyngeal approach as a surgical alternative for cervical lymphatic malformation with airway compression." *Int J Pediatr Otorhinolaryngol* 78(7): 1176–1178.
- Feigenbaum, D. F., et al. (2014). "Ventral midline blanching in the setting of segmental infantile hemangiomas: Clinical observations and pathogenetic implications." *Pediatr Dermatol*. Epub.
- George, R., et al. (2015). "The delivered promise of prenatal imaging and a challenge to the utility of sildenafil for severe lymphatic malformations." *Int J Pediatr Otorhinolaryngol* 79(2): 89–93.
- Gnarra, M., et al. (2015). "Propranolol and prednisolone combination for the treatment of segmental haemangioma in PHACES syndrome." *Br J Dermatol*. Epub.
- Gul, A., et al. (2015). "A propranolol nonresponsive mass: lymphangioma-mimicking hemangioma." *J Craniofac Surg* 26(1): 327–328.
- Haricharan, R. N., et al. (2014). "Antenatal hemorrhage of a cervical lymphatic malformation presenting as a draining neck mass: An unusual presentation." *J Neonatal Perinatal Med* 7(1): 81–84.
- Hinds, D. F., et al. (2015). "Identification of a pediatric intra-abdominal cystic lymphangioma using point-of-care ultrasonography." *Pediatr Emerg Care* 31(1): 62–64.
- Hosseinzadeh, P., et al. (2015). "Fetoscopic laser ablation of vasa previa for a fetus with a giant cervical lymphatic malformation." *Ultrasound Obstet Gynecol*.
- Hunt, K. M., et al. (2014). "Sirolimus-associated regression of benign lymphangioendothelioma." *J Am Acad Dermatol* 71(5): e221-e-222.
- Janmohamed, S. R., et al. (2014). "Support for the hypoxia theory in the pathogenesis of infantile haemangioma." *Clin Exp Dermatol*. Epub.

BACKGROUND: The pathogenesis of infantile haemangioma (IH) is unknown. Several mechanisms have been proposed, including hypoxia, which triggers up-

regulation and stabilization of hypoxia-inducible factor (HIF)1 α . HIF1 α stimulates downstream transcription of target genes that enhance angiogenesis. **AIM:** To identify possible involvement of hypoxia in the pathogenesis of IH, as hypoxia signalling constitutes a potential therapeutic target. **METHODS:** IH tissue samples collected during the period 1991–2011 (preserved in paraffin wax) were immunohistochemically analysed for HIF1 α and the known HIF1 α targets: BCL2/adenovirus E1B kD-interacting protein family member 3 (BNIP3), carbon anhydrase (CA)-IX, glucose transporter (GLUT)-1, phosphorylated protein kinase B (pAKT), phosphorylated S6 protein (pS6) and vascular endothelial growth factor (VEGF). Four observers independently assessed the findings. **RESULTS:** Of the 10 IH samples, 2 appeared to be in the growth phase. In all samples, GLUT-1, BNIP3, pAKT and VEGF were positive, CA-IX was weakly positive, and HIF1 α was negative. pS6 was positive in 9/10 cases and negative in 1/10. **CONCLUSIONS:** Several factors implicated in hypoxia-induced angiogenesis may be involved in IH development. However, the small sample size and retrospective approach of the study preclude definitive conclusions. Prospective studies are needed to conclusively determine which of the factors involved in the (hypoxia) cascade are required for an IH to grow, and could thus be a possible target of drugs for IH treatment.

- Keppeler-Noreuil, K. M., et al. (2015). "PIK3CA-related overgrowth spectrum (PROS): Diagnostic and testing eligibility criteria, differential diagnosis, and evaluation." *Am J Med Genet A* 167(2): 287–295.

Somatic activating mutations in the phosphatidylinositol-3-kinase/AKT/mTOR pathway underlie heterogeneous segmental overgrowth phenotypes. Because of the extreme differences among patients, we sought to characterize the phenotypic spectrum associated with different genotypes and mutation burdens, including a better understanding of associated complications and natural history. Historically, the clinical diagnoses in patients with PIK3CA activating mutations have included Fibroadipose hyperplasia or Overgrowth (FAO), Hemihyperplasia Multiple Lipomatosis (HHML), Congenital Lipomatous Overgrowth, Vascular Malformations, Epidermal Nevi, Scoliosis/Skeletal and Spinal (CLOVES) syndrome, macrodactyly, Fibroadipose Infiltrating Lipomatosis, and the related megalencephaly syndromes, Megalencephaly-Capillary Malformation (MCAP or M-CM) and Dysplastic Megalencephaly (DMEG). A workshop was convened at the National Institutes of Health (NIH) to discuss and develop a consensus document regarding diagnosis and treatment of patients with PIK3CA-associated somatic overgrowth disorders. Participants in the workshop included a group of researchers from several institutions who have been studying these disorders and have published their findings, as well as representatives from patient-advocacy and support groups. The umbrella term of "PIK3CA-Related Overgrowth Spectrum

(PROS)" was agreed upon to encompass both the known and emerging clinical entities associated with somatic PIK3CA mutations including, macrodactyly, FAO, HHML, CLOVES, and related megalencephaly conditions. Key clinical diagnostic features and criteria for testing were proposed, and testing approaches summarized. Preliminary recommendations for a uniform approach to assessment of overgrowth and molecular diagnostic testing were determined. Future areas to address include the surgical management of overgrowth tissue and vascular anomalies, the optimal approach to thrombosis risk, and the testing of potential pharmacologic therapies. (c) 2014 Wiley Periodicals, Inc.

Kim, E. J., et al. (2015). "Maternal and perinatal factors of importance for occurrence and severity of infantile haemangioma." *Acta Derm Venereol*. Epub.

Kim, K. J., et al. (2014). "Role of pigment epithelium-derived factor in the involution of hemangioma: autocrine growth inhibition of hemangioma-derived endothelial cells." *Biochem Biophys Res Commun* 454(2): 282–288.

Hemangioma is a benign tumor derived from abnormal blood vessel growth. Unlike other vascular tumor counterparts, a hemangioma is known to proliferate during its early stage but it is followed by a stage of involution where regression of the tumor occurs. The critical onset leading to the involution of hemangioma is currently not well understood. This study focused on the molecular identities of the involution of hemangioma. We demonstrated that a soluble factor released from the involuting phase of hemangioma-derived endothelial cells (HemECs) and identified pigment epithelium-derived factor (PEDF) as an anti-angiogenic factor that was associated with the growth inhibition of the involuting HemECs. The growth inhibition of the involuting HemECs was reversed by suppression of PEDF in the involuting HemECs. Furthermore, we found that PEDF was more up-regulated in the involuting phase of hemangioma tissues than in the proliferating or the involuted. Taken together, we propose that PEDF accelerates the involution of hemangioma by growth inhibition of HemECs in an autocrine manner. The regulatory mechanism of PEDF expression could be a potential therapeutic target to treat hemangiomas.

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Marques, L., et al. (2015). "Topical rapamycin combined with pulsed dye laser in the treatment of capillary vascular malformations in Sturge-Weber syndrome: phase II, randomized, double-blind, intraindividual placebo-controlled clinical trial." *J Am Acad Dermatol* 72(1): 151–158 e151.

BACKGROUND: Sturge-Weber syndrome (SWS) is characterized by port-wine stains (PWS) affecting the face, eyes, and central nervous system. Pulsed dye laser (PDL) is the standard treatment for PWS. Unfortunately, recurrence is frequent because of reformation and reperfusion of blood vessels. **OBJECTIVE:** We sought to assess the clinical efficacy of topical rapamycin combined with PDL in PWS of patients with SWS. **METHODS:** We conducted a phase II, randomized, double-blind, intraindividual placebo-controlled, clinical trial. We recruited 23 patients with SWS and facial PWS (12 women; median age 33 years, age range 17–65 years) from the University Clinic of Navarra, Spain. Four interventions were evaluated: placebo, PDL + placebo, rapamycin, and PDL + rapamycin. Clinical and histologic responses were evaluated using a chromatographic computerized system, spectrometry, and histologic analyses at 6, 12, and 18 weeks after the intervention. **RESULTS:** PDL + rapamycin yielded the lowest digital photographic image score and the lowest percentage of vessels in histologic analysis, and showed a statistically significant improvement compared with the other interventions. The treatment was generally well tolerated. **LIMITATIONS:** PDL was only applied to the lateral parts of the PWS area. **CONCLUSION:** Topical rapamycin associated with PDL seems to be an effective treatment for PWS in patients with SWS.

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Troilius Rubin, A., et al. (2015). "The heredity of port-wine stains: investigation of families without a RASA1 mutation." *J Cosmet Laser Ther*: 1–13.

Background: The prevalence of capillary malformations, also known as port-wine stains (PWS), is 0.3%. Familial segregation can occur. The capillary malformation-arteriovenous malformation (CM-AVM)

phenotype is caused by mutations in the *RASA1* gene. In PWS familial cases, the inheritance is considered to be autosomal dominant with variable penetrance. Objective: Investigation of the heredity of PWS among patients who attended the vascular anomaly section at the department of dermatology in Malmö, Southern Sweden, between 1993–2004 and to study the involvement of the *RASA1* gene in patients with a positive family history of PWS. Subjects and methods: A total of 254 patients were examined and given a questionnaire regarding family history of PWS. The first group of 175 patients (109 females and 66 males) reported a negative family history. The other group of 65 patients (46 fe-

males and 19 males) reported a positive family history (50% parents or brothers and sisters). Results: The heredity of PWS was 27% (65/240). Twenty-one patients with a positive family history and relatives had no CM-AVM phenotype for mutations in the *RASA1* gene. Conclusion: PWS may have a stronger heredity component than earlier reported and inheritance should be considered when counselling a patient. *RASA1* mutations do not explain the PWS in our patients.

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