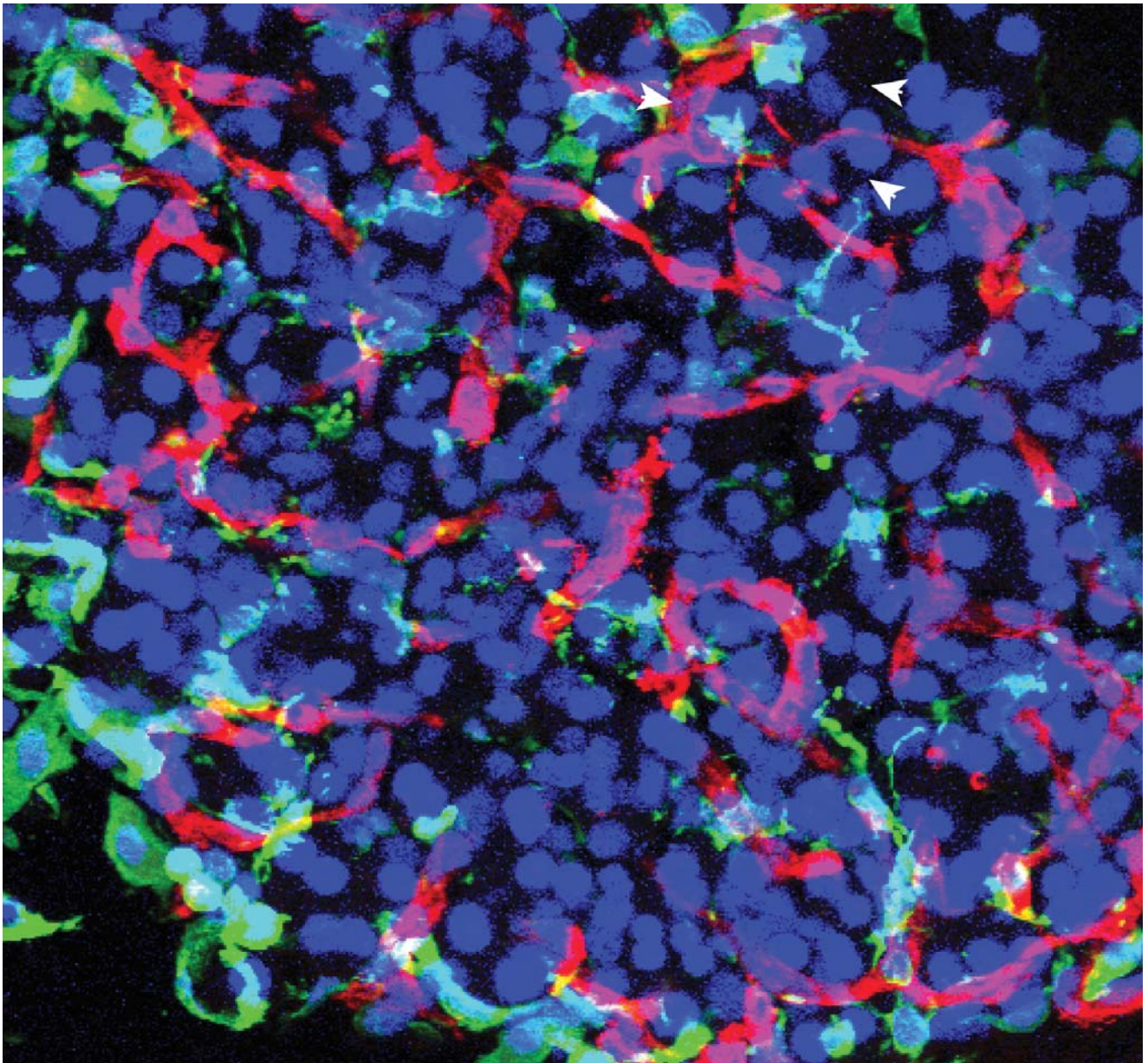


newsletter

NCCR MOLECULAR ONCOLOGY NATIONAL CENTER OF COMPETENCE IN RESEARCH

Contents

Editorial	02
EWS-FLI-1 modulates miRNA145 and SOX2 expression to initiate mesenchymal stem cell reprogramming toward Ewing sarcoma cancer stem cells	03/06
The contribution of myeloid cells to tumor lymphangiogenesis	07/10
Publication Highlights	11
Research Projects	12



Editorial

Dear Colleagues,

In March 2010 the NCCR Molecular Oncology held its annual review panel visit, for the first time at the CHUV campus, where the new center for translational oncology is emerging. Once again the review panel expressed very positive views on the research quality and the increasingly translational orientation of the program. The panel believes that Lausanne has the opportunity to become Switzerland's most influential place in translational cancer research. The panel members strongly urged the involved institutions and leaders to keep this momentum, notably through the recruitment of junior faculty, who could still benefit from the NCCR Molecular Oncology for starting their groups. We reiterate our appreciation to the Swiss Science Foundation for its continuous encouragements and most motivating support.

During the financial crisis of 2009 the Swiss government launched a stimulation program for the Swiss economy with some funding earmarked for the support



Left: Dr. Jürgen Deka, NCCR Associate Director
Right: Prof. Michel Aguet, NCCR Director

of collaborations with Swiss industry within the life sciences. The NCCR Molecular Oncology was able to attract additional funding to launch two new projects with these resources. One of these projects is a collaboration between the group of Jörg Hülsken and the Lausanne-based start-up company Innoxix on the validation of potential diagnostic targets. The second project is a cooperation between the group of Mauro Delorenzi and Novartis with the aim to develop a prognostic lung cancer assay (see project list on the back page). We will report on these projects in a later issue of this newsletter.

In this edition you will find two brief reports on ongoing NCCR projects from the laboratories of Ivan Stamenkovic, University of Lausanne, and Gerhard Christofori, University of Basel, which we hope you will enjoy reading.

Sincerely,
Michel Aguet and Jürgen Deka



▲ **Prof. Ivan Stamenkovic** Professor of Experimental Pathology at the University of Lausanne, and was nominated Vice-Dean for Research of the Faculty of Biology and Medicine in July 2007. Full resume see : www.nccr-oncology.ch

EWS-FLI-1 modulates miRNA145 and SOX2 expression to initiate mesenchymal stem cell reprogramming toward Ewing sarcoma cancer stem cells



▲ **Dr. Nicolò Riggi** obtained his M.D. from the University of Lausanne in 2001. From 2002 he followed the MD-PhD formation in the laboratory of Prof. Ivan Stamenkovic in the Department of Experimental Pathology at the University of Lausanne, and obtained the PhD degree in 2009.

A growing number of malignancies have been shown to contain a subpopulation of cells possessing tumor initiating capability associated with stem cell properties that include expression of embryonic stem cell (ESC) genes and asymmetric division. These cells, termed cancer stem cells (CSC), are believed to be responsible for tumor repopulation, preserving their own numbers through self-renewal, and generating more differentiated progeny that composes the bulk of the tumor. Such cells could, theoretically, arise by at least two mechanisms: transformation of resident stem cells within a tissue that allows them to maintain their « stemness » in the transformed state; or genetic reprogramming of differentiated somatic cells during transformation that endows them with stem cell properties. Recent work has identified a small number of transcription factors normally expressed in ESC, including *OCT4*, *SOX2*, *NANOG*, *C-MYC*, *KLF4* and *LIN28*, that can reprogram fibroblasts and other differentiated cells to acquire pluripotency and become what have been termed human induced pluripotent stem cells (hiPS). Thus, it is conceivable that transformation of primary differentiated or lineage-com-

mitted cells may incur similar reprogramming, and that some degree of reprogramming may even be inherent to transformation itself. In support of these views, loss of the retinoblastoma (*RB*) gene that occurs in a wide variety of malignancies has been shown to cause primary mouse embryonic fibroblasts (MEFs) to undergo reprogramming and acquire CSC properties. In addition, recent observations suggest that two major tumor suppressor genes, *INK4A* and *TP53*, impair efficient iPS generation, consistent with the existence of a relationship between genetic reprogramming and transformation.

Some types of cancer, including a subset of sarcomas and haematopoietic malignancies, can, at least in their early phases, display only a single detectable oncogenic event, usually in the form of a non-random reciprocal chromosomal translocation. Chromosomal translocations that constitute a phenotypic signature of defined tumor types commonly generate a functional fusion gene believed to initiate tumor development. For transformation and subsequent tumor initiation to occur, the corresponding fusion protein should be able to generate



▲ **Dr. Mario Luca Suvà** followed from 2003 the MD-PhD formation in the laboratory of Prof. Ivan Stamenkovic in the Department of Experimental Pathology at the University of Lausanne, and obtained the MD-PhD degree in 2009.

CSC either by exploiting a primary cellular microenvironment that is permissive for expression of its oncogenic properties or by early reprogramming of primary target cells to render them permissive for its subsequent transforming activity.

Ewing sarcoma family tumors (ESFT) provide a malignancy of choice to address this issue because they are associated with a single chromosomal translocation that appears to be the only detectable oncogenic event in as many as 20% of the tumors. Furthermore, ESFT display both mesenchymal and neural crest stem cell features, contain a tumor cell subpopulation with CSC properties and are thought to arise from primary MSC.

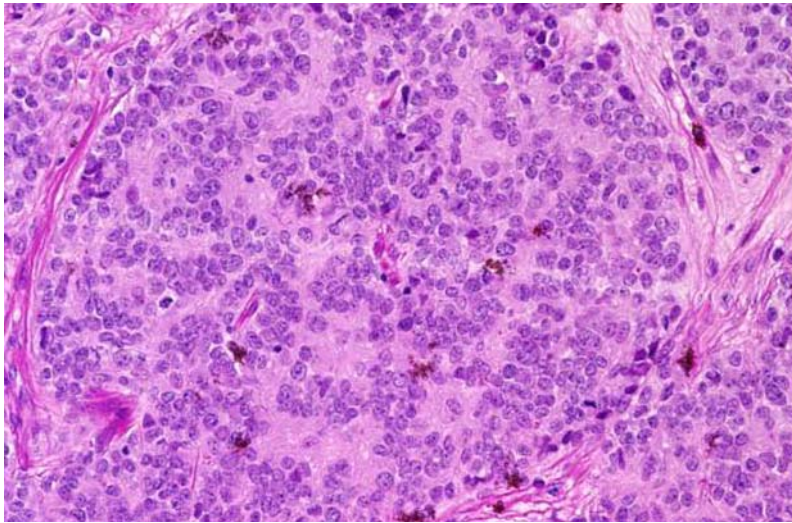
Ewing sarcoma family tumors constitute a highly aggressive bone and soft tissue malignancy of children and young adults associated with a unique chromosomal translocation that generates a fusion protein composed of EWS and a member of the ets transcription factor family. In 90% of cases, the t(11;22) (q24; q12) translocation fuses the 5' end of the *EWS* gene to 3' end of the *FLI-1* gene, giving rise to the EWS-FLI-1 fusion protein where sequences containing the potent EWS transactivation domain are joined to sequences containing the DNA binding domain (DBD) of FLI-1. EWS-FLI1 behaves as an aberrant transcription factor, with both inducer and suppressor activity, that displays distinct target gene specificity from those of its component parts. Expression of the full transcriptional potential of EWS-FLI-1 is highly cell context-dependent as illustrated by its ability to transdifferentiate neuroblastoma and rhabdomyosarcoma cells, but to induce p53-dependent growth arrest and apoptosis in primary human and mouse embryonic fibroblasts. We have previously shown that among primary human cells, only mesenchymal stem cells

(MSC) display permissiveness for stable EWS-FLI-1 expression without undergoing growth arrest, and that in mouse mesenchymal progenitor cells (MPC), expression of EWS-FLI-1 alone is sufficient to induce transformation and development of Ewing sarcoma-like tumors *in vivo*. In adult human MSC, EWS-FLI-1 induces a transcriptome reminiscent of that of Ewing sarcoma, including expression of insulin-like growth factor-1 (*IGF1*), on whose activity ESFT cells are dependent, and the polycomb group gene enhancer of zeste-2 (*EZH2*) that may help maintain stem cell features. We subsequently showed that *IGF1* is a direct target of EWS-FLI-1 in MSC, whereas *EZH2*, which is highly expressed in primary ESFT, plays a central role in the maintenance of Ewing sarcoma cell line tumorigenicity. More recently, we identified ESFT CSC and showed that they constitute about 6-8% of the bulk ESFT population. These cells express the CD133/Prominin-1 stem cell surface marker and represent the tumor initiating population of ESFT. Interestingly, only the CSC population of ESFT retains MSC plasticity *in vitro* and expresses genes implicated in embryonic stem cell maintenance and reprogramming, including *SOX2*, *OCT4* and *NANOG*. A key question then, is how might a single oncogenic event, represented by the chromosomal translocation that generates EWS-FLI-1, produce CSC?

To address this question, it was important to identify cells that constitute the most plausible origin of ESFT. We had previously identified MSC as the most probable cells of origin of ESFT, but we had used only adult MSC whose expression profile upon EWS-FLI-1 expression only partially mimicked that of primary ESFT. We therefore assessed the effects of EWS-FLI-1 expression in human paediatric MSC (hpMSC), given that ESFT are primarily a pae-

diatric/young adult malignancy. We found that hpMSC constitute a far more permissive cellular environment toward EWS-FLI-1 activity than their adult counterparts and display gene expression profile changes that more accurately recapitulate the molecular profile of ESFT. Not only was the level of induction of ESFT-relevant genes higher, but several genes that were not significantly induced in cells cultured in standard medium were strongly upregulated in cells cultured in KO medium. Several of these genes, including *ALK*, *NTRK1*, *PBX1* and *MEIS1* are implicated in transformation and tumorigenesis, and may bear relevance to ESFT pathogenesis. Even more strikingly, hpMSCs underwent genetic reprogramming in response to EWS-FLI-1 reflected by the appearance of a neural crest stem cell molecular phenotype, and the emergence of a subpopulation of cells with CSC features, including cell surface CD133 expression, acquisition of asymmetric division capability and expression of a panel of genes involved in stem cell maintenance and transformation. Interestingly, the emergence of this subpopulation was preceded by the robust induction of the core reprogramming genes *OCT4*, *NANOG* and *SOX2*, driven, in part, by EWS-FLI-1-mediated miRNA145 repression.

MicroRNAs represent a class of small (20-25 nucleotides) non-coding RNAs that are key regulators of numerous cellular events, including the balance between proliferation and differentiation during tumorigenesis and development. They generally inhibit target messenger RNAs by repressing translation or reducing mRNA stability. Among miRNAs that play a major role in supporting stem cell properties, miRNA145 is of particular interest because its expression has recently been shown to inhibit human embryonic stem cell pluripotency and self-re-



Histopathology of Ewing sarcoma showing abundant vascularization and sheets of poorly differentiated cells that form the basis for its inclusion into the small round blue cell tumor category.

newal and to favor lineage-restricted differentiation through repression of *SOX2* and *OCT4*. In hpMSC, *SOX2* may constitute both a direct and indirect EWS-FLI-1 target gene, whose induction along with that of the other core reprogramming factors *OCT4* and *NANOG*, appears to be fine tuned by the suppressive activity of EWS-FLI-1 on the miRNA145 promoter. Thus, modulation of miRNA expression provides an attractive link between stem cell maintenance, induced pluripotency and CSC biology. It is tempting to speculate that the appearance of a subpopulation of hpMSC^{EWS-FLI-1} bearing an ESFT CSC phenotype observed in the present work may be a consequence of the synergy between the direct action of EWS-FLI-1 on stem cell genes and on miRNA-145. Only within the appropriate microenvironment, represented by hpMSC grown in appropriate stem cell medium, can the expression level of the target reprogramming genes attain the putative threshold required to initiate genetic reprogramming of hpMSC into ESFT CSC.

We identified several miRNA145 target sites in the 3' UTR of the

human FLI-1 transcript, which suggests that miRNA145, in addition to modulating *OCT4*, *NANOG* and *SOX2* expression levels after their initial induction by EWS-FLI-1, may be further involved in a feedback regulatory loop controlling EWS-FLI-1 expression itself. Our experiments confirmed the existence of such a reciprocal control mechanism, which seems to provide tight regulation of expression of both partners as well as that of their common target genes. The observation that EWS-FLI-1 constitutes both a miRNA145 transcriptional regulator and one of its direct targets offers new insight into ESFT initiation mechanisms. The requirement for an appropriate microenvironment that allows EWS-FLI-1 to exert its repressing activity on the miRNA145 promoter indicates a link between EWS-FLI-1, miRNA145 and the permissiveness of cells from which ESFT originate. Thus, it is conceivable that initial expression of the fusion protein during the very early steps of ESFT development requires an environment that allows miRNA145 repression, resulting in stabilisation of the EWS-FLI-1 protein and in enhancement of *OCT4*, *NANOG* and *SOX2* expression.

These events could endow the ESFT cell of origin with CSC properties and ultimately result in the conversion of cellular permissiveness for oncogene expression to tumor initiating ability.

The changes in tumorigenic potential and differentiation observed upon miRNA145 expression in ESFT cells may be partially explained by the concomitant reduction in EWS-FLI-1 protein levels. However, changes in expression of one or several EWS-FLI-1/ miRNA145 target genes could also be involved in the appearance of the observed phenotype.

Based on its physiological role in neural stem cell maintenance and differentiation, its participation in genetic reprogramming and its induction by EWS-FLI-1, Sox-2 provides an attractive candidate to explain how expression of EWS-FLI-1 might induce a partial neuroectodermal phenotype in a tumor arising from transformed hpMSC. Consistent with this notion, depletion of *SOX2* in two distinct ESFT cell lines attenuated their neuroectodermal phenotype with a concomitant enhancement of mesenchymal markers. Interestingly, *SOX2* was also observed to participate in ESFT cell proliferation and tumorigenesis, suggesting that among candidate EWS-FLI-1 target genes, it may play a central role in ESFT pathogenesis.

Sox-2 has recently been identified as a lineage-preservation oncogene that is able to promote squamous identity in lung and esophageal squamous cell carcinomas but the mechanism whereby it may promote tumorigenicity in ESFT cells is unclear. However, the ability of Sox-2 to control differentiation may help generate a window of opportunity for the activity of some of the survival and growth-promoting genes induced by EWS-FLI-1. Numerous

candidate EWS-FLI-1 target genes have been proposed to participate in primary cell transformation and maintenance of the resulting tumor cells, but which of these genes are essential for ESFT pathogenesis remains unclear. A plausible scenario is that EWS-FLI-1-mediated transformation requires cooperation between several EWS-FLI-1 target genes that can occur only in a permissive cellular context. HpMSC can provide the microenvironment required for EWS-FLI-1-mediated repression of miRNA-145 and induction of *SOX2*, *OCT4* and other reprogramming genes whose combined activity may ensure maintenance of stem cell properties that facilitate subsequent gen-

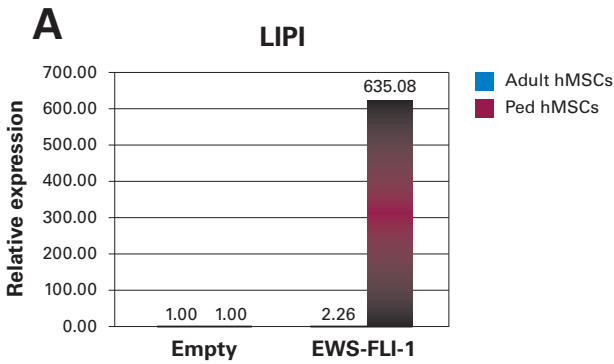
eration of CSC. Concomitant induction of the polycomb group gene product EZH2 may abolish the response to oncogenic stress triggered by overexpression of c-Myc, Alk, or Ntrk1 whereas IGF-1 and possibly other growth factors may secure cell survival. The combined effect of these target gene products may provide a reasonable basis for MSC transformation toward ESFT CSC.

Taken together, our observations indicate that in the appropriate mesenchymal stem cell microenvironment, a single aberrant transcription factor generated by a reciprocal chromosomal translocation is able to induce genetic reprogramming toward a CSC phenotype by com-

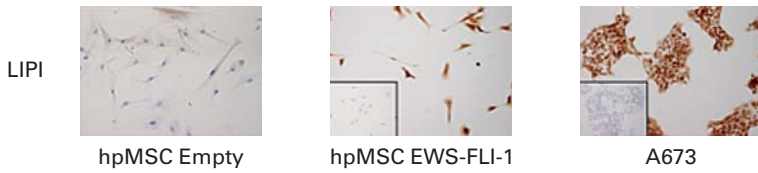
bined action on its target genes and by directly modulating miRNA-145 expression. This work constitutes the first report of a mechanism whereby expression of a single oncogene in a primary cell population generates the features of a cancer stem cell.

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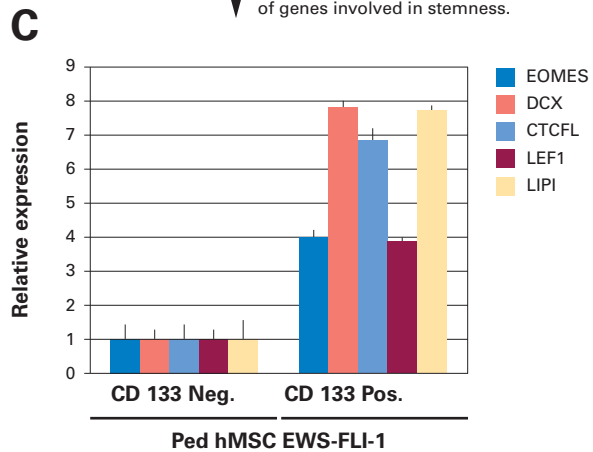
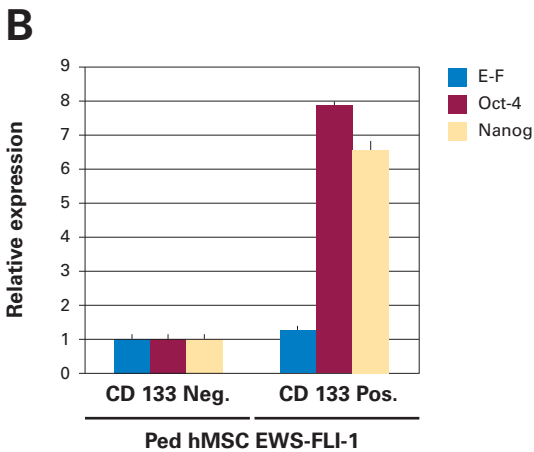


A: EWS-FLI-1 induces expression of the highly specific ESFT gene *LIPI* in hpMSC but not in adult MSC.



B: Real-time PCR analysis of *OCT4*, *NANOG* and *EWS-FLI-1* gene expression levels in CD133 positive and negative hpMSC^{EWS-FLI-1}-derived RNA, showing that the CD133 positive fraction expresses a higher level of *OCT4* and *NANOG*, but a nearly identical *EWS-FLI-1* level.

C: hpMSC^{EWS-FLI-1} CD133 positive fraction express an higher level of the ESFT marker *LIPI*, and of a panel of genes involved in stemness.





▲ **Prof. Gerhard Christofori**

Gerhard Christofori studied Molecular Biology at the University of Heidelberg and received his PhD in the laboratory of Walter Keller at the German Cancer Research Center in Heidelberg and at the Biocenter of the University Basel in 1988. As a postdoctoral fellow he joined the laboratory of Douglas Hanahan at the University of California San Francisco (UCSF) where he began to study the molecular mechanisms of multistage tumor development. In 1994 he started his own research group at the Research Institute of Molecular Pathology (IMP) in Vienna. In 2001, he was appointed Professor of Biochemistry at the Department of Biomedicine of the University of Basel. His group has joined the NCCR Molecular Oncology Program in 2005.

The contribution of myeloid cells to tumor lymphangiogenesis

Numerous experimental evidence and clinical correlation studies have firmly established that tumor-associated lymphangiogenesis, the formation of new lymphatic vessels in the tumor environment, promotes the lymphogenic dissemination of tumor cells and the formation of lymph node metastasis. However, despite major progress in identifying and characterizing the major players regulating tumor lymphangiogenesis, the molecular mechanisms underlying the stochastic onset and the maintenance of lymphangiogenesis during tumor progression in animal models or in cancer patients still remain elusive.

To investigate the cellular and molecular events leading to tumor lymphangiogenesis, we have modulated the expression of angiogenic factors during tumor development in the Rip1Tag2 transgenic mouse model of pancreatic β -cell carcinogenesis and in the MMTV-Neu mouse model of breast cancer. Expression of the lymphangiogenic members of the vascular endothelial growth factor (VEGF) family, VEGF-C and VEGF-D, during β -cell carcinogenesis of double-transgenic Rip1Tag2; Rip1VEGF-C and Rip1Tag2; Rip1-VEGF-D mice results into increased tumor lymphangiogenesis and lymph node metastasis, and in the case of the expression of VEGF-D in lung metastasis (Kopfstein et al., 2007; Mandriota et al., 2001). A similar lymphangiogenic effect with lymphogenic metastasis is observed in transgenic mice that express VEGF-C in in tumors of double-transgenic

MMTV-Neu; MMTV-VEGF-C mice (A. Fantozzi, L. Waldmeier, G. Christofori, unpublished results). These mouse models are suitable experimental systems for the identification of novel markers and potential therapeutic targets of tumor lymphangiogenesis, for the testing of experimental therapeutic approaches against tumor lymphangiogenesis, and for the identification and characterization of surrogate markers for activated lymphangiogenesis.

In the past, we have interfered with the function of angiogenic factors in the mouse models described above, for example by the adenoviral delivery of soluble receptor (trap) constructs or by treatment with pharmacological inhibitors. While VEGF-C and D are well known to exert their lymphangiogenic functions by activating VEGF receptor-3 on lymphatic endothelial cells, soluble VEGF receptor-3 constructs and also neutralizing antibodies to VEGF receptor-3 failed to interfere with lymphangiogenesis in Rip1Tag2 mice expressing VEGF-C or VEGF-D transgenes. Moreover, treatment of these mice with the potent VEGF receptor-inhibitor PTK787, although efficiently repressing hemangiogenesis and with it tumor growth, did not affect tumor lymphangiogenesis (Tammela et al., 2008, Schomber et al., 2008). These results have lead us to hypothesize that lymphangiogenic factors other than VEGF-C or D contribute to tumor lymphangiogenesis. A switch from VEGF- to FGF- or PDGF-mediated blood vessel angiogenesis has been previously

reported by our and other laboratories (Compagni et al., 2000; Casanovas et al., 2005). A similar switch could be at work during tumor lymphangiogenesis, a hypothesis that can be tested by treating mice with combinations of inhibitors against various angiogenic signaling pathways.

Another way by which tumor lymphangiogenesis may escape the inhibition of VEGF receptor signaling may be the recruitment of inflammatory or bone marrow-derived cells (BMDC) to the tumor microenvironment that may provide additional lymphangiogenic factors or directly contribute to lymphangiogenesis. A large number of recent studies have demonstrated that BMDC are recruited to angiogenic sites to support the establishment of new blood vessels. BMDC are typically sub-classified into hema-

topoietic progenitor cells (HPC) and endothelial progenitor cells (EPC). In various tumor models, HPC have been shown to contribute to blood vessel angiogenesis by secreting angiogenic factors and proteases required for the activation of latent forms of angiogenic factors (Cousens et al., 2000; Cursiefen et al., 2004). HPC have also been implicated in the preparation of a pre-metastatic niche in distant organs that are colonized by disseminating cancer cells (Kaplan et al., 2005). EPC on the other hand have been shown to directly integrate into growing blood vessel walls, however, to varying extents ranging from 0 to 50%, and thus raising questions about the functional contribution of EPC in blood vessel angiogenesis in various physiological and pathological conditions (Joyce, 2005). Recently, it has been reported that

also cells of the myeloid lineage are able to differentiate into *bona fide* blood vessel endothelial cells (Bailey et al., 2006).

Only few studies have addressed the role of BMDC in lymphangiogenesis. Myeloid cells present in the murine inflamed conjunctiva can express the lymphatic endothelial specific marker VEGFR-3 and can integrate into lymphatic structures that develop in mouse cornea transplants (Hamrah et al., 2003; Maruyama et al., 2005). In another inflammatory condition, the rejection of human kidney transplants, the increased numbers of lymphatic vessels within the rejected organs contain host-derived lymphatic endothelial cells, suggesting the existence of lymphatic endothelial progenitor cells (Kerjaschki et al., 2006). Another study, employing a cornea angiogenesis model and a syngen-

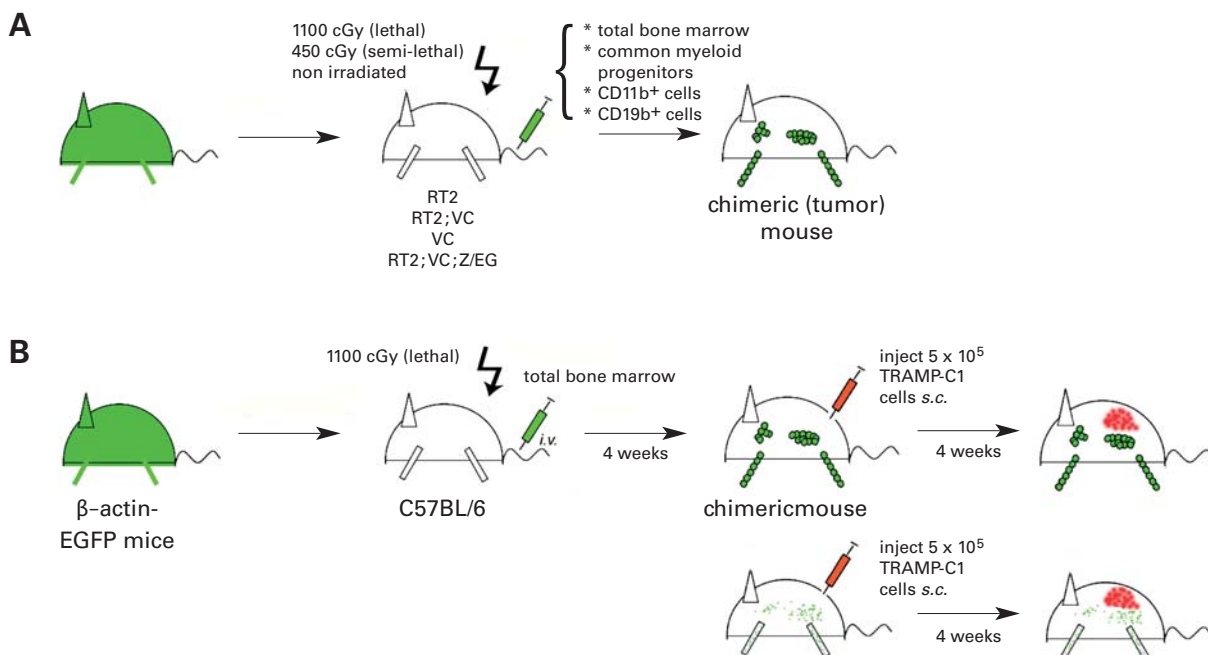


Figure 1. Bone marrow transplantation strategies to assess the contribution of bone marrow-derived cells to tumor lymphangiogenesis.

(A) Bone marrow cells from beta-actin-GFP transgenic mice were transplanted into lethally irradiated mice, as indicated. Semi-lethally irradiated mice were transplanted with FACS-sorted CD11b⁺ myeloid cells, CD19b⁺ B-cells or common myeloid progenitors (CMP) cells. CD11b⁺ myeloid cells were also transferred into non-irradiated mice. After 3-8 weeks mice were sacrificed, engraftment of transplanted bone marrow was evaluated by FACS analysis, and pancreata were analyzed by FACS and confocal microscopy for the presence of GFP⁺ bone marrow-derived cells at the tumor site.

(B) TRAMP-C1 cells were injected into the flank of either C57BL/6 previously reconstituted with bone marrow of beta-actin-GFP transgenic mice or bone marrow of double-transgenic CD11b-Cre;Z/EG mice, and tumors were allowed to grow for 3 to 4 weeks. FACS analysis was used to assess bone marrow reconstitution or Cre recombinase-mediated GFP expression, respectively. Histological sections from TRAMP-C1 tumors were analyzed by confocal microscopy for the presence of GFP⁺ cells.



Group Christofori

From left to right: Mahmut Yilmaz, Gerhard Christofori, Ulrike Hopfer, Miguel Cabrera, Anna Fantozzi, Lorenz Waldmeier, Dorothea Maaß, Chantal Heck, Adrian Zumsteg, Neha Tiwari, Jörg Hagmann, Imke Abrecht, Vanessa Baeriswyl, Lukas Mannhart, Ernesta Fagiani, Helena Antoniadis.

neic fibrosarcoma transplantation model, has also reported the incorporation of BMDC into newly formed lymphatic vessels (Religa et al., 2005).

In close collaboration with the laboratory of Prof. Curzio Rüegg (CePO-UNIL-CHUV, Lausanne, and NCCR Molecular Oncology), Adrian Zumsteg and Vanessa Baeriswyl, two PhD students supported by the NCCR, have assessed whether BMDC are able to contribute to lymphangiogenesis observed in Rip1-Tag2; Rip1VEGF-C double-transgenic mice and in the TRAMP-C1 syngeneic tumor transplantation model of prostate cancer that exhibits high levels of tumor lymphangiogenesis (Zumsteg et al., 2009). Employing transplantation of bone marrow cells from GFP-transgenic mice into lethally irradiated Rip1Tag2; Rip1VEGF-C double-transgenic mice and mice carrying TRAMP-C prostate tumors (Figure 1), most tumors investigated showed a contribution of approximately 3% of (GFP-tagged) bone-marrow-derived cells in their lymphatic vasculature (Figure 2). Transplantation of FAC-sorted macrophages or cells of the myeloid progenitor lineage into Rip1Tag2; Rip1VEGF-C mice also revealed a

significant incorporation of GFP⁺ cells into the lymphatic vasculature. All these cells expressed the lymphatic marker LYVE-1 and a subset of them also Prox-1 (Figure 2). GFP⁺ lymphatic marker-expressing cells could also be identified by FACS analysis indicating that it is individual single cells of myeloid origin that express lymphatic markers. Toge-

ther, these results raise the intriguing possibility that BMDC of the myeloid lineage trans-differentiate into lymphatic endothelial cells. Notably, transplantation of CD19⁺ B cells into Rip1Tag2; VEGF-C mice did not result in any incorporation of transplanted cells into tumor-associated lymphatic vessels. Moreover, transplantation of BMDC into Rip1VEGF-C single-transgenic mice did not result into any incorporation of BMDC into the newly formed lymphatic vessels. These results indicate that only cells of the myeloid lineage incorporate into lymphatic vessels, and they do so only in the context of tumorigenesis.

Genetic lineage tracing experiments confirmed the myeloid origin of the trans-differentiated lymphatic endothelial cells. For example, transgenic mice that specifically expressed Cre-recombinase in the myeloid lineage cells (CD11b-Cre) were crossed to reporter mice that expressed GFP only when Cre-recombinase was present (Z/EG mice). Both mouse lines were also crossed with Rip1Tag2 and Rip1VEGF-C mice to

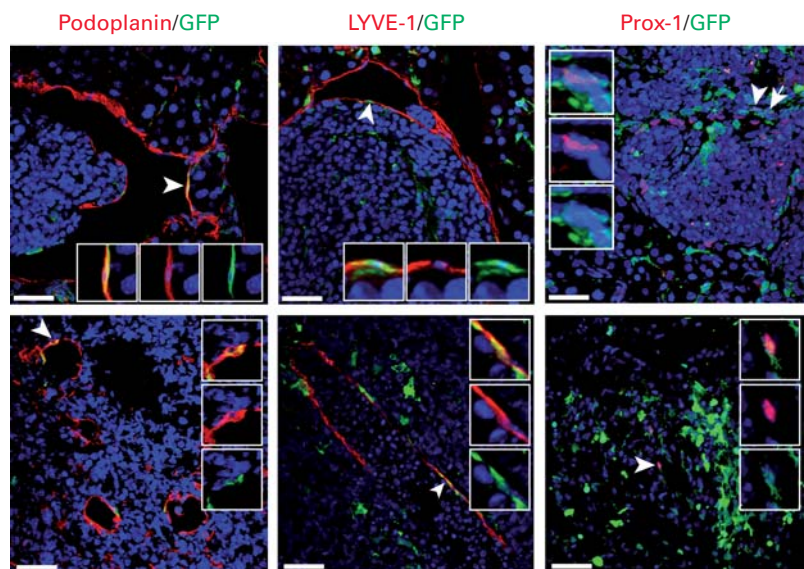


Figure 2.

Bone marrow-derived cells (BMDC) integrate into tumor-associated lymphatic vessels.

Lethally irradiated RT2;VC mice were reconstituted with GFP-labeled bone marrow. 20 μ m histological pancreatic sections were stained for the lymphatic markers Podoplanin, Prox-1, LYVE-1 and for GFP as indicated and analyzed by confocal microscopy. Representative tumor sections per lymphatic marker are shown. Arrowheads indicate double-positive cells for the particular lymphatic marker (red) and for GFP (green) shown in inset magnifications. Insets show merged and individual channels. DAPI stains nuclei (blue). Scale bars: 40 μ m.

obtain quadruple-transgenic mice that express GFP exclusively in the myeloid lineage and develop lymphangiogenic pancreatic tumors. In the resulting composite mice, GFP⁺ cells could be observed incorporated in tumor lymphatic vessels, again indicating that a process of myeloid-lymphatic endothelial cell trans-differentiation had occurred. Such genetically tagged myeloid cells have also been used for bone marrow transplantation into Rip1-Tag2;Rip1VEGF-C double-transgenic mice, again demonstrating the incorporation of myeloid-origin cells into tumor lymphatic vessel. Finally, transplantation of CD11b-Cre bone marrow cells into Rip1Tag2;Rip1-VEGF-C mice carrying the GFP-reporter (Z/EG) gene excluded a process of cellular fusion between myeloid cells and lymphatic endothelial cells as the basis for the integration of BMDC into lymphatic vessels.

In order to determine the functional contribution of macrophages to lymphangiogenesis, we have collaborated with Prof. Reto Schwendener (University of Zürich) to deplete macrophages from Rip1Tag2;Rip1VEGF-C double-transgenic mice by treatment with clodronate-loaded liposomes (Clodrolip; Zeisberger et al., 2006). Treatment with Clodrolip resulted in the depletion of macrophages from the mice and a significant reduction of lymphatic vessel density, indicating that myeloid cells functionally contributed to tumor lymphangiogenesis.

To further address the possibility of trans-differentiation of myeloid cells into lymphatic endothelial cells, Vanessa Baeriswyl has established an *in vitro* assay in which bone marrow-derived cells are first cultured in the presence of M-CSF to differentiate into *bona fide* macrophages (CD11b⁺/F4/80⁺). The cells are then exposed to various growth media, including conditioned medium from VEGF-C-expressing tumor cells, and then analyzed for the ability to ex-

press lymphatic endothelium-specific genes and to form tubes in 3-dimensional growth conditions. In fact, a large number of the macrophages cultured under these conditions begin to form tubes and to

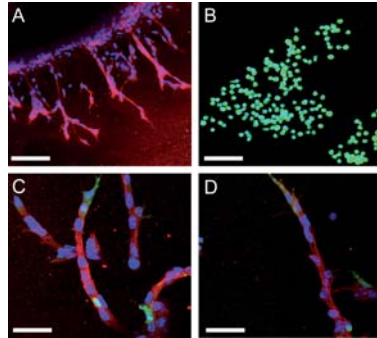


Figure 3. Bone marrow-derived-macrophages form and contribute to lymphatic-like structures *in vitro*.

(A) Immortalized Podoplanin⁺ murine lymphatic endothelial cells (SV-LEC), (B) GFP-labeled bone marrow-derived-macrophages, and (C, D) mixed cultures of macrophages and SV-LEC were seeded in Matrigel. At day 5, cells were stained for Podoplanin (red) and analyzed by confocal microscopy. Mixed cultures demonstrate that bone marrow-derived macrophages contribute to SV-LEC-mediated cord formation: GFP⁺ cells (green) are found integrated into Podoplanin⁺ cord-like structures (C, D). Note the preferential integration of bone marrow-derived macrophages at the tips and branch points of sprouting cord-like structures formed by SV-LEC. DAPI stains nuclei (blue). Scale bars: 100 μm (A, B) and 50 μm (C, D).

express the lymphatic endothelial cell markers podoplanin, LYVE-1, and Prox-1. Notably, when co-cultured with lymphatic endothelial cells the macrophage-derived cells incorporate into tube-like structures preferentially at branch points and tips of sprouting tubes (Figure 3). Time-lapse videomicroscopy indicates that these cells exert a guiding function in extending vascular sprouts and branches. This guiding function may play a critical role in tumor lymphangiogenesis and may explain the fact that interfering with a BMDC incorporation of only 3% into tumor-associated lymphatic vessels substantially represses tumor lymphangiogenesis.

The demonstration that cells of the myeloid lineage are able to trans-differentiate into lymphatic endothelial

cells is intriguing. Apparently, committed progenitor cells and most likely also highly differentiated cells exhibit an unexpected degree of plasticity, a notion that obtains increasing support from a multitude of experiments where differentiated cell types trans-differentiate into other cell types, as well as from the generation of induced pluripotent stem cells (iPS) from somatic, highly differentiated cells. Trans-differentiation, though received with skepticism in the field and not liked by everybody, may establish itself as a frequent cellular process in complex organisms.

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NCCR Publication Highlights

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NCCR Molecular Oncology Research Projects

Cell Signaling in Tumor Development and Metastasis

- Cathrin Brisken _____ The progesterone-Wnt connection and early events in human breast carcinogenesis
- Jörg Hülken _____ Mechanisms controlling tissue homeostasis and their role in cancerogenesis and metastasis
- Ivan Stamenkovic _____ Sarcoma-development and the role of the tumor stroma
- Jürg Tschopp _____ The role of IL -1 in tumorigenesis
- Mauro Delorenzi _____ Large-scale analysis of functional genomics data
- Monika Hegi _____ Epigenetics and gene expression signatures in human glioblastoma and glioma stem like cells and implications for tumor biology and treatment of cancer
- Tatiana Petrova _____ Regulation of colorectal cancer progression
- Michel Aguet _____ BCL9/BCL9L as targets for suppression of cancer stem cells and restoring susceptibility to therapy in colon and other cancers

Tumor Angiogenesis

- Curzio Rüegg _____ Impact of antiangiogenic treatments on tumor evolution and tumor microenvironment
- Gerhard Christofori _____ The molecular regulation of tumor lymphangiogenesis and lymphnode metastasis
- Melody Swartz _____ Role of lymphatic vessels in cancer invasion and metastasis

Tumor Immunity and Cancer Immunotherapy

- Daniel Speiser / _____ Analysis of in vivo differentiation and function, and molecular dissection of antigen specific CD8T cells before and after immunotherapy of melanoma patients
- Nathalie Rufer
- Olivier Michielin _____ Structural design of peptide / MHC and T cell receptor interactions

Economic Stimulus Program

- Mauro Delorenzi _____ Integrative data analysis in development of prognostic lung cancer assay
- Jörg Hülken _____ Cancer stem cells as research and diagnostic targets

Education

- Jean-Pierre Kraehenbuhl _____ Oncology Online : Development of a web-based oncology teaching program