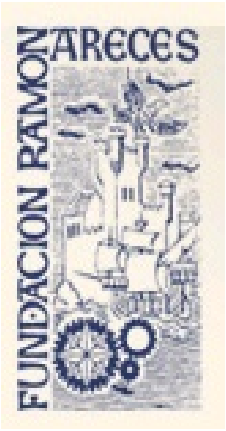
International Symposium

Angiogenesis and cancer: from basic mechanisms to therapeutic applications



Simposio Internacional/International Symposium

Angiogénesis y cáncer: de los mecanismos básicos a las aplicaciones terapéuticas

Angiogenesis and cancer: from basic mechanisms to therapeutic applications

UNIVERSIDAD DE MÁLAGA

Coordina: Ana Rodríguez Quesada
Depto. de Biología Molecular y Bioquímica. Universidad de Málaga.
Secretaría técnica: Ángel Luis García Ponce

Málaga, 12-13 November, 2008
Edificio del Rectorado de la Universidad de Málaga
Avda Cervantes 2, Málaga

Edificio del Rectorado de la Universidad de Málaga/Rectorado Building

Vista de la Alcazaba desde la terraza del edificio del Rectorado
La Alcazaba, from the Rectorado Building

PROGRAMA / PROGRAM

Día 1: 12 de noviembre de 2008/Day 1: November 12, 2008

8:30-9:15 Entrega de documentación.

9:15-9:45 Presentación del simposio
Welcome and opening remarks

9:45 Sesión 1: Mecanismos moleculares de la angiogénesis (I)
Session 1: Molecular mechanisms of angiogenesis (I)
Chairpeople: Alicia Arroyo, Agnes Noel

9:45-10:30
Ralf H Adams
Vascular Development Laboratory, Cancer Research UK London Research Institute
Regulación del fenotipo endotelial angiogénico
Regulation of the angiogenic endothelial phenotype

10:30-11:15
Julián Aragonés López
Servicio de Immunología. Hospital de la Princesa. Departamento de Medicina, Facultad de Medicina, Universidad Autónoma de Madrid
Factores inducidos por hipoxia (HIF) y cáncer
Hypoxia-inducible factors (HIF) in tumor biology

11:15-11:45 Descanso/Break

11:45 Sesión 2: Mecanismos moleculares de la angiogénesis (II)
Session 2: Molecular mechanisms of angiogenesis (II)
Chairpeople: Ramón Muñoz-Chápuli, Sagrario Ortega

11:45-12:30
Carmelo Bernabeu
Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas (CSIC), Madrid
Endoglina, un receptor del factor de crecimiento transformante-beta implicado en angiogénesis y tumorogénesis
Endoglin, a receptor of the transforming growth factor involved in angiogenesis and tumorogenesis

12:30-13:15
Agnes Noel
Laboratory of Tumor and Development Biology, Centre de Recherche en Cancérologie Expérimentale (CRCE), University of Liège, Liege, Bélgica

Papel de las metaloproteasas de matriz extracelular (MMP) en la angiogénesis
Role of MMP in angiogenesis

DESCANSO/BREAK

15:15 Sesión 3: Mecanismos moleculares de la angiogénesis (III)
Session 3: Molecular mechanisms of angiogenesis (III)
Chairpeople: Ralf Adams, Carmelo Bernabeu

15:15-16:00
Peter Carmeliet.
Vesalius Research Center Leuven, Bélgica.
Pro y anti-angiogénesis: de los estudios genéticos al potencial terapéutico
Pro- and anti-angiogenesis : from genetic insights to therapeutic potential

16:00-16:45
Juan Carlos Rodríguez Manzaneque
Centro de Genómica e Investigación Oncológica (GENYO). Granada.
ADAMTS1: una metaloproteasa de matriz con propiedades antiangiogénicas.
ADAMTS1: a matrix metalloprotease with angioinhibitory properties.

16:45-17:30
Alicia García Arroyo.
Matrix Metalloproteinase Unit, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid.
Más allá de las MT-MMPs: Nuevos moduladores de la respuesta angiogénica
Beyond MT-MMPs: new molecular players of the angiogenic response

17:30-18:30 Posters Session

Día 2: 13 de noviembre de 2008/Day 2: November 13, 2008

9:00 Sesión 4: Modelos experimentales de la angiogénesis
Session 4:Experimental models enlighten angiogenesis understanding and ease drug discovery
Chairpeople: Andreas Bilfalvi, Juan Carlos Rodríguez-Manzanequel

9:00-9:45
Miguel Angel Medina
Dpto. de Biología Molecular y Bioquímica, Universidad de Málaga
Ensayos de angiogénesis
In vitro and in vivo assays of angiogenesis

9:45-10:30
Sagrario Ortega
Transgenic mice unit. Centro Nacional de Investigaciones Oncológicas (CNIO). Madrid
Modelos de ratones transgénicos para monitorizar y estudiar la angiogénesis tumoral in vivo.
Knockin mouse models to monitor and to study tumor angiogenesis in vivo.

10:30-11:15
Gabriele Bergers
University of California at San Francisco.
Mecanismos de neovascularización y adaptación a la terapia antiangiogénica en modelos murinos de cáncer.
Mechanisms of neovascularization and adaptation to antiangiogenic therapy in mouse models of cancer.

11:15-11:45 Descanso/Break

11:45 Sesión 5: Nuevos conceptos-nuevas estrategias terapéuticas (I)
Session 5: New concepts rendering new therapeutic strategies (I)
Chairpeople: Dan Duda, Miguel Ángel Medina

11:45-12:30
Emilio Alba.
Servicio de Oncología Médica, Hospital Universitario Virgen de la Victoria, Málaga.
Quimioterapia metronómica: ¿enseñando nuevos trucos a los viejos fármacos?
Metronomic chemotherapy: teaching old drugs new tricks?

12:30-13:15
Andreas Bikfalvi
INSERM E113 'Molecular Angiogenesis Laboratory', Université Bordeaux I, Talence, Francia.
Nuevas ideas en la regulación e inhibición de la angiogénesis tumoral
New insights in the regulation and inhibition of tumor angiogenesis

DESCANSO/BREAK

15:15 Sesión 6: Nuevos conceptos - nuevas estrategias terapéuticas (II)
Session 6: New concepts yielding new therapeutic strategies (II)
Chairpeople: Emilio Alba, Gabriele Bergers

15:15-16:00
Dan Duda
Department of Radiation Oncology, Harvard Medical School, EEUU
Papel de las células endoteliales progenitoras circulantes en la angiogénesis del adulto
Role of circulating progenitor cells in adult angiogenesis

16:00-16:45
Ramón Muñoz-Chápuli Oriol
Dpto. de Biología Animal, Universidad de Málaga.
Endotelio y angiogénesis. Una perspectiva evolutiva
Endothelium and angiogenesis: An evolutionary perspective

16:45-17:30
Ana Rodríguez Quesada
Dpto. de Biología Molecular y Bioquímica, Universidad de Málaga.
Presente y futuro de las terapias antiangiogénicas
Current and future of pharmacological intervention of angiogenesis

17:30 Conclusiones finales/Closing remarks

Lecture abstracts

Regulación del fenotipo endotelial angiogénico

Regulation of the angiogenic endothelial phenotype

Ralf H Adams

Director, Department Tissue Morphogenesis

Professor at the University of Münster

Max-Planck-Institute for Molecular Biomedicine

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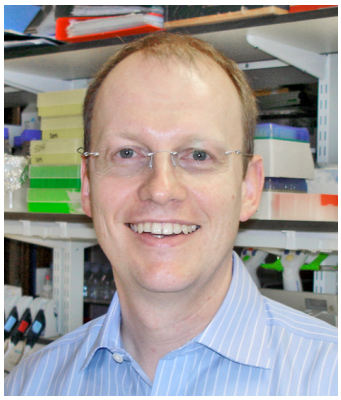
Blood vessels form a highly organised hierarchical network throughout the vertebrate body, integrate functionally into very different tissue environments and remain remarkably adaptable to changing local requirements. The angiogenic programme controls much of the growth of the vasculature, which allows a remarkable expansion of the vascular network without compromising blood circulation or tissue access to oxygen and nutrients. Angiogenesis involves a wide range of cellular processes such as proliferation, sprouting, migration, adhesion, the formation of endothelial cell junctions or the recruitment of mural cells, i.e., pericytes (PCs) and vascular smooth muscle cells (vSMCs).

The Notch signalling pathway has been recently implicated in arteriovenous differentiation and the regulation of vessel sprouting and branching. Inhibition of Notch signalling mediated by the ligand Delta-like 4 (Dll4) leads to the loss of arterial identity and increased numbers of tip cells and enhanced vessel branching. Our results show that besides Dll4, the Notch ligand Jagged1 is also expressed in the developing vasculature in a pattern that partially overlaps with Dll4. To address the role of Jagged1 in angiogenesis, we have generated endothelial cell-specific and inducible gain-of-function and loss-of-function mutants. Characterisation of the mutant vasculature in the embryonic dermis and the postnatal retina revealed that Jagged1 is a critical regulator of endothelial sprouting and branching.

We conclude that the endothelial angiogenic phenotype is controlled by the balance between Dll4 and Jagged1 and that the activity of both Notch ligands is essential for normal vascular morphogenesis.

Prof. Dr. Ralf H. Adams



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1987-1992	Studies in Biochemistry, University of Bayreuth
1996	Graduation Dr. phil. nat., MPI for Brain Research and Johann Wolfgang Goethe-University Frankfurt
1996-2000	Postdoc with Dr. Rüdiger Klein, EMBL Heidelberg
2000-2005	Group Leader at the Cancer Research UK London Research Institute, London
2000	Werner Risau Memorial Award
2000-2003	EMBO Young Investigator Programme
2005-2008	Senior Group Leader at the London Research Institute
Since 2007	Director department 'Tissue Morphogenesis' at the Max-Planck-Institute for Molecular Biomedicine in Münster
	Professor at the University of Münster

Selected references

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Factores inducidos por hipoxia (HIF) y cáncer

Hypoxia-inducible factors (HIF) in tumor biology

Julián Aragonés López

Servicio de Immunología. Hospital de la Princesa. Departamento de Medicina, Facultad de Medicina, Universidad Autónoma de Madrid

Oxygen sensors (PHD-1, -2 and -3) as well as hypoxia-inducible transcription factors HIF-1α and HIF-2α play an important role in hypoxic tumor regions. HIF-1α and HIF-2α increase the expression of angiogenic factors (i.e VEGFa), which in turn increase tumor vessel density. This response is aimed to organize blood conduction in order to facilitate oxygen and nutrient supply. However, the biology of PHDs & HIF is beyond angiogenesis. In this regard, loss of PHD1, without altering angiogenesis, offers acute protection of myofibers against lethal oxygen deprivation (i.e. ischemia). This is achieved by lowering oxygen consumption in skeletal muscle and reprogramming the glucose metabolism from oxidative to a more anaerobic ATP production. A similar protective metabolic mechanisms driven by PHD1 sensor could also be exploited in tumoral cells to increase their tolerance to recurrent oxygen fluctuations in the inner core of the tumors. These effects could resemble, in some extent, those occurring in the ischemic microenviroment.

Dr. Julián Aragonés López

Mi trayectoria científica se ha centrado fundamentalmente en entender la respuesta biológica a las fluctuaciones en el aporte de oxigeno mediada por los sensores de oxigeno PHD-1,-2 and -3 así como los factores de trancripción HIFs. Esta fluctuaciones se han visto implicadas en numerosos procesos biológicos (crecimiento tumoral, isquemia...) y esta atrayendo más y más el interés de la comunidad internacional. Durante mi periodo predoctoral en el grupo de M. O. de Landázuri desentrañamos algunos de los mecanismos intracelulares (que implicaban segundos mensajeros lipídicos - DAG y PA) que gobiernan la regulación de los factores HIFs. Después de la lectura de mi tesis en Febrero 2000, continué tres años más en el mismo laboratorio para terminar con la publicación de esta información. En Febrero del 2003, marcha al grupo del Dr Peter Carmeliet, un grupo de contrastada relevancia internacional el angiogénesis así como en el estudio de la respuesta biológica a la hipoxia. En este sentido alli generamos los ratones deficientes los sensores de oxigeno PHD-1, PHD-2 y PHD-3. En concreto recientemente publicamos en Nature Genetics que la ausencia de PHD1 produce un programa de metabolismo anaerobio que protege los tejidos frente a episodios de isquemia aguda lo cual ha reforzado mas el valor clínico de los sensores de oxigeno como dianas terapéuticas. Actualmente disfruto de un contrato Ramón y Cajal y sigo adelante en este campo de la biología del oxigeno.

Endoglina, un receptor del factor de crecimiento transformante-beta implicado en angiogénesis y tumorogénesis

Endoglin, a receptor of the transforming growth factor involved in angiogenesis and tumorogenesis

Carmelo Bernabeu

Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas (CSIC), Madrid

Endoglin is an auxiliary transforming growth factor-beta (TGF-β) receptor expressed in endothelial cells that regulates angiogenesis, vascular remodelling and cardiovascular development. Endoglin binds different TGF-β family members including TGF-β1, TGF-β3, activin-A, BMP-7, BMP-9 and BMP-10, all of which signal through the activation of type I receptors leading to the mobilization and nuclear translocation of the Smad transcription factor family. In endothelial cells, endoglin potentiates the type I TGF-β receptor ALK1 signaling, which triggers phosphorylation of Smad1/5, leading to enhanced cellular proliferation and migration, hallmarks of the active phase of angiogenesis. Mutations in the endoglin gene give rise to the Hereditary Hemorrhagic Telangiectasia type 1 (HHT1), an autosomal dominant vascular disorder characterized by telangiectases in skin and mucosa and arteriovenous malformations in lung, liver and brain. Endoglin-null (eng-/-) mice die at mid-gestation from defective angiogenesis and severe cardiovascular abnormalities, while Eng heterozygous mice have normal life spans, but are predisposed to develop HHT-like vascular abnormalities. Supporting the role of endoglin in vascular homeostasis, endothelial nitric oxide synthase (eNOS) expression is reduced, and NO synthesis is impaired in eng heterozygous mice, and a soluble form of endoglin has been linked to preeclampsia, a systemic hypertension occurring in pregnant women. Several lines of investigation have underlined the important role of endoglin in cancer. First, endoglin is a marker of tumour neoangiogenesis with prognostic significance in several types of cancer, for example in breast cancer. Also, endoglin-haploinsufficiency leads to reduced tumour growth and angiogenesis in mice and antibodies anti-endoglin have been used to target and destroy the tumour vasculature in animal models. More recently, it has been shown that endoglin is expressed in tumour cells and behaves as a suppressor of malignancy during the late stages of mouse skin carcinogenesis. Here, we will also review novel molecular mechanisms of endoglin that are relevant in the context of angiogenesis and cancer.

Dr. Carmelo Bernabeu

Carmelo Bernabeu is Research Professor working at the Spanish Research Council. He obtained his Ph.D. degree in Biochemistry at the Autonomous University in Madrid (1977), where he was appointed Lecturer in the Microbiology department (1977-79). Then he moved to the University of California in Los Angeles (UCLA) to work on the ribosome structure using immune electron microscopy (1980-1981). After two years, he moved to Harvard Medical School in Boston, where he worked in Immunology on the human major histocompatibility complex (1982-1983). In 1985 he was appointed Staff scientist at the Spanish Research Council, where he is currently working as a Research Professor. He has coauthored more than 150 publications in international scientific journals, is Section Editor of the journal BBA Molecular Basis of Disease and Chair of the Medical and Scientific Advisory Board of the Hereditary Hemorrhagic Telangiectasia Foundation International. His current research interests include the study of vascular pathology, namely the rare disease known as Hereditary Hemorrhagic Telangiectasia and the two major genes involved endoglin and ALK1, their roles as transforming growth factor receptors in endothelial cells, and their involvement in angiogenesis and vascular remodelling.

Papel de las metaloproteasas de matriz extracelular (MMP) en la angiogénesis
Role of MMP in angiogenesis

Agnès Noël

Laboratory of Tumor and Development Biology, University of Liège, Sart-Tilman B23, Groupe Interdisciplinaire de Génoprotéomique Appliquée (GIGA-Cancer), University of Liège, Sart Tilman B23, B-4000 Liège, Belgium.

Blood and lymphatic vessels play crucial roles in promoting tumour growth and metastasis. Early lymph node metastasis is a common clinical finding in many human cancers, and it is associated with aggressive disease and poor prognosis. Angiogenesis, the formation of new blood vessels from a pre-existing vascular network has been the focus of extensive research and this led to the successful development of anti-angiogenic therapies. In tumors, angiogenesis is reinforced by vasculogenesis, the recruitment and functional incorporation of bone marrow-derived cells into the newly forming vessel. Despite its implication in cancer progression, the lymphatic system has until recently been overshadowed by the greater emphasis placed on the blood vascular system, and the lack of appropriate markers, as well as adequate in vivo and in vitro experimental models. A substantial body of evidence indicates that matrix metalloproteinases (MMPs) regulate endothelial cell migration and activation during angiogenesis. Their contribution in blood vessel architecture and during lymphangiogenesis is less known. Emerging concepts on the contribution of membrane type MMPs and MMP2 in the host response to tumor implantation, (lymp)angiogenesis and in metastatic dissemination will be discussed.

Dr. Agnès Noël

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Pro y anti-angiogénesis: de los estudios genéticos al potencial terapéutico

Pro- and anti-angiogenesis : from genetic insights to therapeutic potential

Peter Carmeliet.

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More than 500 years ago, the Belgian anatomist A. Vesalius recognized remarkable similarities in the patterning of vessels and nerves; often, vessels and nerves even track alongside each other. Recent genetic studies revealed that vessels and nerves share many more common principles and signals for navigation, proliferation and survival than previously suspected. For instance, gene inactivation studies in mice and zebrafish showed that axon guidance signals such as netrins, semaphorins, ephrins and slits regulate angioblast and vessel navigation. Ephrins have also been implicated in arterio-venous cell fate specification. Conversely, neural stem cells proliferate and differentiate in vascular niches in the central nervous system.

Prototypic angiogenic factors such as VEGF and its family members VEGF-B and VEGF-C control neurogenesis and affect oligodendrocyte proliferation, independently of their angiogenic activity. VEGF also regulates axon and neuron guidance. In addition, disregulation of the neuro-vascular link contributes to various disorders. For instance, axon guidance signals co-determine the angiogenic switch in tumors and regulate cancer cell survival. Genetic studies in mice further showed that low VEGF levels or loss of VEGF in motoneurons cause motoneuron degeneration such as characteristically observed in amyotrophic lateral sclerosis (ALS), also termed Lou Gehrig's disease. Apart from regulating neural perfusion, VEGF also has a critical neurotrophic activity for motoneurons and, thus, when VEGF levels are insufficient, impaired neural perfusion and deprivation of neuroprotective signals may lead to motoneuron degeneration. Furthermore, overexpression of a VEGF receptor transgene in motoneurons prolonged the survival of the mutant SOD1G93A mouse model of ALS. Restoring the neuro-vascular deficit may also offer novel therapeutic opportunities. Indeed, delivery of VEGF, either via gene transfer or via intracerebroventricular administration of recombinant VEGF, protected motoneurons against degeneration and prolonged the survival of a SOD1G93A mouse and rat model of ALS. Apart from effects on the motoneuron cell body, VEGF also preserves axon integrity and innervation of the neuromuscular junction. Other VEGF family

members seem to have similar neuroprotective properties. Recent studies further suggest that a disregulation of the neuro-vascular link in general and of VEGF in particular may be implicated in other neurodegenerative disorders such as Alzheimer's disease as well. The next coming years promise to become an exciting journey to further unravel the molecular basis and explore the therapeutic potential of the neuro-vascular link.

Dr. Peter Carmeliet

Peter Carmeliet holds a M.D. degree (1984) and Ph.D. degree in Medicine (1989) from the University of Leuven, Belgium, and is currently Professor of Medicine and Adjunct Director of the Vesalius Research Center (VRC) of the Flanders Interuniversity Institute for Biotechnology (VIB-3) at the University of Leuven, Belgium. He has a joint appointment as Professor at the Cardiovascular Research Institute Maastricht, Maastricht, The Netherlands; is visiting Professor at the University of Dartmouth, USA, and assists in teaching the Course on Angiogenesis at the Harvard Medical School, USA. He has research interests in unraveling the molecular basis of angiogenesis, lymphangiogenesis, hemostasis, neurodegeneration and neurovascular processes in health and disease, using mouse, zebrafish, Xenopus and human genetics and functional genomics. These insights are then used to provide novel 'proof-of-principle' treatment strategies.

He has received numerous scientific awards including the Interbrew-Baillet Latour Prize (jointly with Désiré Collen), the Francqui Prize, Belgium; Outstanding Investigator Award, Intl Soc Heart Research; unrestricted Bristol-Myers-Squibb grant, USA; elected member of EMBO; Nobel Forum Lecture, invited by Nobel Committee. He is a member of the editorial board of Cancer Cell, Circulation Research, ATVB, Trends Cardiovascular Medicine, and an advisor for Nature Cancer Reviews.

ADAMTS1: una metaloproteasa de matriz con propiedades antiangiogénicas.

ADAMTS1: a matrix metalloprotease with angioinhibitory properties.

Juan Carlos Rodríguez Manzaneque

Centro de Genómica e Investigación Oncológica (GENYO). Granada.

Proteolysis of extracellular matrix components appears as a critical factor for maintenance of tissue architecture and proper functionality. In the context of tumor microenvironment, the alteration of this proteolytic balance can be crucial for malignancy and bad prognosis. Major advances have been obtained regarding the characterization of proteases implicated in these processes, although the identity of functional protease-substrate partners is still an ongoing scientific challenge. ADAMTS1 (a disintegrin and metalloprotease with thrombospondin motifs) is an extracellular metalloproteinase known to participate in a variety of biological processes including inflammation, angiogenesis and development. Its role in cancer has also been highlighted although the specific mechanisms have not been fully disclosed. Using distinct methods we have identified various components of the extracellular milieu as targets of the action of this protease, including the inhibitor Tissue Factor Pathway Inhibitor-2 (TFPI-2), the transmembrane proteoglycan syndecan-4, and the basement membrane glycoproteins nidogen-1 and nidogen-2. Our studies reveal an important role of ADAMTS1 in various cellular events such as migration and adhesion. Additional studies in a fibrosarcoma cellular model showed the relevance of these substrate-protease partners for the development of vasculogenic-like networks, rich in matrix components. This phenomenon, first characterized for aggressive melanoma cells and named vasculogenic mimicry, illustrates a paradigm of tumor cell plasticity. With these results, our main objective was to study the implication of the extracellular protease ADAMTS1 in the formation of pseudo-vascular channels in both melanoma and sarcoma models. First, we demonstrated mRNA and protein expression of ADAMTS1 in aggressive Ewing sarcoma and various melanoma cell lines, that formed vascular-like channels in 3D-cultures. We also studied the presence of specific substrates of ADAMTS1 in aggressive cell lines with consequences in its location in the extracellular matrix (ECM). These phenomena also happened in xenografts derived from vasculogenic mimicry positive cells. Indeed, we performed xenograft assays and we observed that ADAMTS1 overexpression altered tumor growth rate in a different manner depending of the presence of specific substrates. Indeed, the presence of ADAMTS1 was

associated with the appearance of vascular-like structures and the overexpression of vasculogenic mimicry-related genes, such as VE-Cadherin, and various recognized markers. Our work appears in accordance with further reports that suggest the essential role of ECM remodelling for tumor plasticity. Extracellular proteases and their substrates may be involved in the vasculogenic mimicry phenomena and this knowledge is relevant for the improvement of drug treatments to overcome antiangiogenic resistance.

Dr. Juan Carlos Rodríguez-Manzaneque

After his PhD degree at the Universidad Complutense de Madrid (1996), Juan Carlos Rodríguez-Manzaneque joined the laboratory of Dr. Iruela-Arispe at the Beth Israel Deaconess Medical Center/Harvard Medical School (Boston, USA) to study physiological angiogenesis and the characterization of endogenous inhibitors of this process. He continued his postdoctoral training in UCLA (Los Angeles, USA) concluding the characterization of members of a recently identified family of extracellular metalloproteases, named ADAMTS. He also studied in a tumor-prone mouse model the relevance of proteolysis within the extracellular tumor microenvironment. Since June 2002, he leaded the Angiogenesis Group at the Vall d'Hebron Research Institute (Barcelona), where he initiated his current research on the role of proteases during extracellular remodeling. His group has identified new substrates of ADAMTS proteases that revealed new properties for these molecules during tumor neovascularization. This last year he established his group at the new Andalusian Center for Genomics and Oncology (GENYO) in Granada.

Más allá de las MT-MMPs: Nuevos moduladores de la respuesta angiogénica

Beyond MT-MMPs: new molecular players of the angiogenic response

Alicia García Arroyo.

Matrix Metalloproteinase Unit, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid.

Angiogenesis can be defined from a simplistic point of view as the formation of new capillaries from pre-existing vessels. This process may take place by the remodelling of an immature vascular network during the embryonic development or by sprouting of capillaries from pre-existing vessels in pathologies such as tumorigenesis or chronic inflammatory disorders. For the proper formation of functional capillaries it is required a perfect coordination of the action of pro- and anti-angiogenic factors; these would act regulating membrane receptors and signalling cascades that will modulate the interactions of endothelial cells with other endothelial cells and with the extracellular matrix, the endothelial cell migration, invasion and proliferation, the assembly into new capillary tubes, and finally the stabilization by recruitment of accessory cells to the vessel wall. There is a large variety of molecules involved in this process; among them it is worth to highlight the relevant role of matrix metalloproteinases (MMP) and in particular of the membrane-anchored MMP called MT1-MMP.

The expression and activity of MT1-MMP is fine-tuned regulated in a spatial-temporal manner during the migration and activation of endothelial cells induced by angiogenic factors such as the chemokine MCP-1/CCL2, the nitric oxide and the PgE2. Moreover, MT1-MMP is required for efficient migration and invasion by endothelial cells as well as for capillary tube formation both in vitro and in vivo; this requirement has been demonstrated by our group by blocking MT1-MMP activity with neutralizing antibodies and by using MT1-MMP-deficient mice.

EMMPRIN, also known as basigin or CD147, is a relevant molecule in the regulation of MMP expression within the tumor context. However, its putative role in the angiogenic response has not been characterized yet. EMMPRIN is expressed in endothelial cells, it can regulate MMP expression in other cellular contexts, EMMPRIN soluble forms can be produced by MT1-MMP processing, and EMMPRIN can associate to caveolin-1, structural protein of caveolae (membrane microdomains abundant in endothelial cells that are key for MT1-MMP internalization and activity);

therefore, we have proposed to investigate the regulation and function of EMMPRIN in endothelial cells during the angiogeneic response. The results obtained will be shown and discussed during the Symposium.

Dr. Alicia García Arroyo

Alicia G. Arroyo se licenció en 1989 en Medicina y Cirugía por la Universidad Complutense de Madrid iniciando su formación científica como estudiante en el Centro de Investigaciones Biológicas (CSIC). Posteriormente, se especializó en Inmunología en el Hospital de la Princesa (Madrid) donde también realizó su tesis doctoral (Universidad Autónoma de Madrid, 1994) sobre nuevos mecanismos de regulación de los receptores de adhesión celular integrinas en leucocitos humanos bajo la dirección del Dr. F. Sánchez-Madrid. En 1995 se incorporó al laboratorio del Dr. R.O. Hynes (MIT, Cambridge, USA) donde analizó la función in vivo de la subfamilia de receptores de adhesión integrinas $\alpha 4$ en hematopoyesis e inflamación empleando modelos de ratones deficientes o quiméricos para dichos receptores. En el año 1999 formó su grupo de investigación en el Hospital Universitario de la Princesa (como contratado FIS, Ramón y Cajal y Científico Titular del CSIC) donde inició sus estudios sobre la regulación de las metaloproteinasas de matriz extracelular en angiogénesis e inflamación. Se incorporó como Jefe de Grupo al Centro Nacional de Investigaciones Cardiovasculares en Diciembre de 2003.

Ensayos de angiogénesis

In vitro and in vivo assays of angiogenesis

Miguel Ángel Medina Torres

Dpto. de Biología Molecular y Bioquímica, Facultad de Ciencias, Universidad de Málaga

Our group is actively involved in the search for new modulators of angiogenesis. To screen, identify, characterize and evaluate modulators of angiogenesis, currently there are available different in vitro and in vivo assays. There are also assays for the follow-up of angiogenesis modulators in the clinical setting for either diagnostic or prognostic purposes. The currently utilized preclinical assays are not equivalent in terms of efficacy or relevance to human disease. In fact, some of them have significance for screening, while others are mainly used in studies of dosage-effects, structure/function and the evaluation of combined effects. This report will summarize the main available angiogenesis assays. Furthermore, their specific utilities will be illustrated by showing some selected examples of our own research involving the identification and characterization of new antiangiogenic compounds.

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Dr. Miguel Ángel Medina Torres

Licenciado (1985) y Doctor (1989) en Ciencias Biológicas por la Universidad de Málaga. Profesor Titular de Bioquímica y Biología Molecular de la Universidad de Málaga desde 1995. *Estancias en centros de investigación*: Mc Gill University (Montreal, Canadá), Max Planck Institut für Ernhärungsphysiologie (Dortmund, Alemania), Universität Heidelberg (Alemania) y Max Planck Institut für biophysikalisches Chemie (Göttingen, Alemania). *Producción científica:* Más de 150 artículos de investigación, 6 patentes,16 capítulos de libro, un libro, dirección de seis tesis doctorales, 13 tesinas de licenciatura y 1 de máster. *Producción divulgativa:* 25 artículos y 16 capítulos de libro.

Modelos de ratones transgénicos para monitorizar y estudiar la angiogénesis tumoral in vivo.

Knockin mouse models to monitor and to study tumor angiogenesis in vivo.

Sagrario Ortega

Unidad de Ratones Transgénicos. Programa de Biotecnología. Centro Nacional de Investigaciones Oncológicas (CNIO). Madrid

Genetic mouse models to study angiogenesis in normal development and in disease are an essential tool for the understanding of the molecular mechanisms that control this process but also for the discovery of new therapeutic targets and for the development of more effective therapies. Lymphatic vessels and lymphangiogenesis play very important roles in tissue development and homeostasis and are involved in pathological processes such as lymphedema, inflammation and cancer. Despite of its relevance, still much remains to know about the molecular mechanisms that control the formation of new lymphatic vessels as well as their implication in tumour expansion and spread. The identification of specific lymphatic endothelial markers has facilitated the study of lymphatic vessel development and lymphangiogenesis in the last few years. VEGFR3 (flt4) is considered to be a good marker of lymphatic endothelium and VEGFR-3-meditated signalling is one of the main pathways that promote formation of new lymphatic vessels. During mouse development VEGFR3 is initially expressed in endothelial cells of developing blood vessels, but its expression becomes restricted to lymphatic endothelial cells after midgestation where it persists in adult lymphatic vessels. Expression of VEGFR3 has also been detected in adult fenestrated blood vessel endothelia and in some tumour-associated blood vessels, as well as in some inflammatory cells.

To better characterize the process of lymphangiogenesis we have generated a reporter mouse line in which an IRES-EGFP-luciferase cassette has been targeted into the 3´UTR of the flt4 gene by homologous recombination. This bicistronic reporter leads to the concomitant expression of both the traceable EGFP-luciferase fusion and the VEGFR3 protein under the endogenous transcriptional regulation of the flt4 locus. Luciferase and/or EGFP expression can be independently monitored by different imaging techniques and they faithfully recapitulate endogenous VEGFR3 expression. Therefore we have developed a mouse model to monitor in vivo physiological and pathological processes in which VEGFR3 expressing cells are involved and that will facilitate the study of lymphatic development as well as

pathological processes such as inflammation, and tumor-associated lymphangiogenesis.

Using a similar strategy we have also generated a knockin mouse model to express the tamoxifen-inducible Cre recombinase (CreERT2) under the endogenous control of the flt4 locus. This mouse line allows the conditional activation or inactivation of any given floxed allele specifically in lymphatic endothelial cells and to study the genetic control of lymphatic vessels development and function.

Dr. Sagrario Ortega

Sagrario Ortega obtained her PhD in Chemistry in 1987 at the Universidad Complutense de Madrid. From 1987 to 1990 she was a Fulbright scholar at Merck Sharp and Dohme Research Laboratories (Rahway NJ, USA) where she studied fibroblast growth factors (FGFs), their function in the control of cell proliferation and their therapeutical applications. In 1992 she joined the NYU Medical Center (New York, USA) as a research associate where she studied the in vivo function of members of the FGF family through the generation of knockout mice and she was in charge of the Gene Targeting and ES cell Culture Facility. In September 1998 she joined the CNIO as a staff scientist and since 2002 she is also Head of the Transgenic Mice Unit at the CNIO. She is currently working in the generation of mouse models to study angiogenesis and lymphoangiogenesis in vivo and how these processes contribute to tumour growth and expansion.

Mecanismos de neovascularización y adaptación a la terapia antiangiogénica en modelos murinos de cáncer.

Mechanisms of neovascularization and adaptation to antiangiogenic therapy in mouse models of cancer.

Gabriele Bergers

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New blood vessels in tumors are predominantly derived from the existing vasculature by a process referred to as angiogenesis. Based on a wealth of studies from different laboratories over the last decade, there has now been increasing appreciation that tumor neovascularization is also supported by the mobilization and recruitment of bone marrow-derived circulating vascular progenitor cells and proangiogenic myeloid support cells to generate a new tumor vasculature. Although the functional significance of endothelial progenitor cells (EPC) and pericyte progenitors (PPC) as major contributors to tumor vessel formation is controversial due to their extremely variable and in general rather low frequencies of incorporation into tumor vessels, there is more evidence that innate bone marrow-derived cells (BMDC) contribute to the formation and maintenance of tumor vessels by providing crucial pro-angiogenic and angiogenesis modulating factors. The most prominent myeloid subpopulations include tumor-associated macrophages (TAM) and more immature monocytic cells including Tie2+ monocytes (TEM), VEGFR1+ CXCR4+ hemangiocytes and CD11b+Gr1+ myeloid-derived suppressive cells (MDSC). In addition, neutrophils, mast cells and dendritic cells have also been described to be able to support neovascularization. Intra-tumoral hypoxia appears to be a pivotal driving force in recruitment of bone marrow-derived progenitors and myeloid support cells because it induces various proangiogenic growth factors including VEGF, PIGF and SDF-1α that besides activating residing endothelial cells can also regulate BMDC influx to sites of vascular remodeling in the tumor. Recent results suggest that BMDC appear to not only be instrumental for neovascularization of nascent tumors but may also be critical in the context of antiangiogenic therapy. Notably, there are reports suggesting that anti-angiogenesis-induced hypoxia can elicit BMDC recruitment and thereby

foster an adaptive mechanism that enables tumors to overcome hypoxia and induce re-neovascularization.

Congruent with this notion, there are data emerging from several laboratories that adaptation of tumors to antiangiogenic inhibitors targeting the VEGF signaling pathway might be manifested in at least four distinct adaptive mechanisms: 1) activation/up-regulation of alternative proangiogenic signaling pathways within the tumor and/or 2) recruitment of bone marrow-derived pro-angiogenic cells, both of which can avert the necessity of VEGF signaling, thereby effecting a re-initiation of tumor angiogenesis; as well as, 3) increased pericyte coverage of the tumor vasculature, to support its integrity; and 4) activation and enhancement of invasive pathways to provide access to normal tissue vasculature without the necessity to induce reneovascularization. While the transitory efficacy of the VEGF pathway inhibitors might be considered as disappointing, the results must be evaluated in the context of the current standards of care for most of the major human cancers, which typically have transitory efficacy, inevitable resistance, and common toxicity and poor quality of life. Angiogenesis inhibitors, despite their evident limitations, still represent an important milestone in cancer therapeutics, where they are becoming components of standard-or-care therapy, for example for colorectal and renal cancers. The growing state of knowledge about their effects and efficacy, and about the existence and mechanistic basis for adaptive-evasive resistance presents a future of opportunity for improving and sustaining the benefits of anti-angiogenic therapy.

Dr. Gabriele Bergers

Gabriele Bergers is an Associate Professor in the Department of Neurological Surgery at the University of California, San Francisco, a principal investigator of the UCSF Brain Tumor Research Center, and holds the Neill H. and Linda S. Brownstein Chair in Brain Tumor Research. After obtaining her Ph.D. in Molecular Genetics from the Institute for Molecular Pathology in Vienna, Austria. Dr. Bergers began studying the interactions between tumor cells and blood vessels to regulate neovascularization, tumor growth, and invasion in transgenic and orthotopic mouse models of cancer at UCSF. Her laboratory is further engaged in translational research aimed at revealing adaptation mechanisms in relapsed tumors during the course of antiangiogenic therapy.

Quimioterapia metronómica: ¿enseñando nuevos trucos a los viejos fármacos?

Metronomic chemotherapy: teaching old drugs new tricks?

Emilio Alba

Servicio de Oncología Médica, Hospital Universitario Virgen de la Victoria, Málaga.

The year 1991 marked a radical change in the definition of cancer therapeutic target: instead of the tumor cell, the genetically stable, proliferating immature endothelium of the tumoral stroma was suggested as the primary target. This proposal was a milestone in the development of anti-angiogenic treatments in cancer. Several agents targeting angiogenesis have already been developed, and some are now commercially available. Interestingly, classical chemotherapy has also shown an anti-angiogenic activity in preclinical models, both in vitro and in vivo. This activity is particularly significant when the drugs are administered continuously at low doses. This observation paved the way for the development of the metronomic chemotherapy concept, i.e. the administration of low doses of cytotoxic drugs at shorter time intervals, for long periods and without interruptions. Several mechanisms have been proposed to account for its anti-angiogenic effects: the inhibition of the endothelial cell migration and proliferation; the activation of a paracrine-endocrine loop involving thrombospondin-1 and the reduction of the number of circulating endothelial and progenitor cells. In general, improvements in clinical outcomes have not been dramatic although, in metastatic breast cancer, metronomic chemotherapy still constitutes a reliable option in selected patients, with the great advantage of negligible toxicity. This success marks the beginning of the potential application of metronomic chemotherapy used alone or in combination with other strategies.

Dr. Emilio Alba Conejo

Licenciado en Medicina por la Universidad de Malaga en 1981. Doctor en Medicina por la Universidad de Malaga en 1988, con una tesis sobre factores pronosticos en cancer de mama operable. Residencia en Oncología Médica (1983-1987) en el Hospital de la Santa Cruz y San Pablo de Barcelona. Ha sido Presidente de la Sociedad Andaluza de Cancerología (SAC) y Vicepresidente de la Sociedad Española de Oncología Médica (SEOM). Miembro de la ESMO (European Society of Medical Oncology) y de la ASCO (American Society of Clinical Oncology). Miembro de la Junta Directiva del GEICAM (Grupo Español para la Investigación y Tratamiento del Cancer de Mama). Autor de mas de 100 publicaciones en revistas internacionales y numerosas comunicaciones en congresos Internacionales. Actualmente es Jefe del servicio de Oncología Medica del Hospital Universitario Virgen de la Victoria de Malaga y Profesor Titular de Oncología de la Universidad de Malaga.

Nuevas ideas en la regulación e inhibición de la angiogénesis tumoral

New insights in the regulation and inhibition of tumor angiogenesis

Andreas Bikfalvi

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Angiogenesis is promoted by several cellular stress factors including hypoxia, hypoglycemia, or amino acid deprivation. The major pathway regulating the response to hypoxia is the hypoxia-inducble factor

(HIF) system. However, we have recently found that another pathway is critical for angiogenesis promotion the unfolded protein response (UPR). We have demonstrated that UPR is critical in promoting the upregulation of angiogenesis factors and the down-regulation of angiogenesis inhibitors. Using orthtotopic glioma models we furher evidenced an increase of invasive behaviour of glioma cells when UPR is inhibited. Thus, it seems that UPR is critical for angiogenesis induction and tumor cell invasion. We also developped a chicken model for developmental, wound and tumor angiogenesis and demonstated its usefulness for omics studies.

Finally, we have studied extensively the ELR-negative chemokine CXCL4 and CXCL4L1 and demonstrated critical differences in their biochemical and biological properties.

Dr. Andreas Bikfalvi

Andreas Bikfalvi is a trained MD, Ph.D. who obtained his Medical Degree at the University of Bretagne Occidentale (Brest, Brittany, France) and carried out residency in Haematology and Oncology at the University of Kiel Germany). He then did his PHD in Paris at INSERM followed by post-doctoral training at INSERM in Paris and at the New York University Medical Center. In 1995, he became Professor in cell and molecular biology at the university of Bordeaux. He is the head of the INSERM angiogenesis laboratory at the university of Bordeaux.

His current research is focussing on angiogenesis factors and inhibitors, the role of the stress response in tumor angiogenesis and on new angiogenesis models for omics studies.

Papel de las células endoteliales progenitoras circulantes en la angiogénesis del adulto

Role of circulating progenitor cells in adult angiogenesis

Dan G. Duda, DMD, PhD

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The function of circulating progenitors in blood vessel assembly is currently unclear. We characterized progenitors among bone marrow-derived cells by flow cytometry, and established their function in tumors by intravital and confocal microscopy in bone marrow transplant models. In addition, we used high-resolution imaging to capture sequential changes in circulating progenitors as they formed capillary walls in lungs. We show that bone marrow-derived cells can function as endothelial as well as perivascular cell precursors. In cancer patients, blockade of angiogenic and inflammatory pathways modulated the number of circulating progenitors, and this modulation associated with treatment outcome. Collectively, these studies suggest that circulating progenitors may play a role in neovascularization and tumor growth and response to targeted therapies.

Dr. Dan G. Duda

Dan G. Duda received his PhD in Medical Science from Tohoku University in Sendai, Japan in 2001, and pursued post-doctoral studies at Massachusetts General Hospital (MGH) in Boston, USA. Previously, he received a DMD degree from the University of Medicine of Iasi, Romania in 1993. Currently, Dr. Duda is an Assistant Professor of Radiation Oncology at MGH and Harvard Medical School, and is teaching in graduate programs at MGH and Massachusetts Institute of Technology. Dr. Duda's expertise lies in the area of tumor neovascularization and the role of bone marrow-derived cells in tumor progression and treatment. In addition, he is actively involved in multi-institutional translational studies of antiangiogenic therapy approaches to cancer. He has received several awards from the AACR, the Cancer Research Institute and other organizations. His research is currently supported by grants from the US National Cancer Institute.

Endotelio y angiogénesis. Una perspectiva evolutiva

Endothelium and angiogenesis: An evolutionary perspective

Ramón Muñoz-Chápuli Oriol

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Circulatory systems of vertebrate and invertebrate metazoans are very different. Large vessels of invertebrates are constituted of spaces and lacunae located between the basement membranes of endodermal and mesodermal epithelia, and they lack an endothelial cells. There is no phylogenetic theory about how endothelial cells arose in vertebrates, and the relationship between the process of angiogenic growth of vertebrate and invertebrate vessels (i.e. involving endothelium or not) is still obscure. We have recently proposed that endothelial cells originated from a type of specialized blood cells, called amoebocytes, that adhere to the basement membrane in invertebrate vessels. The transition between amoebocytes and endothelium involved the acquisition of an epithelial phenotype. We suggest that immunological cooperation was the earliest function of these protoendothelial cells. Furthermore, their ability to transiently recover the migratory, invasive phenotype of amoebocytes (i.e., the angiogenic phenotype) allowed for vascular growth from the original visceral areas to the well-developed somatic areas of vertebrates (especially the tail, head, and neural tube). We also hypothesize that pericytes and smooth muscle cells derived from myoepithelial cells detached from the coelomic lining. As the origin of blood cells in invertebrates is probably coelomic, our model relates the origin of all the elements of the circulatory system with the coelomic wall. We have collected from the literature a number of comparative and developmental data supporting this hypothesis, for example the localization of the vascular endothelial growth factor receptor-2 ortholog in hemocytes of Drosophila or the fact that endothelial cells differentiate from circulating progenitors in adult vertebrates. The recognition of the endothelial cells as a highly specialized type of blood cells might provide new avenues for the understanding of phenomena such as angiogenesis, vasculogenesis and inflammation.

Dr. Ramón Muñoz-Chápuli Oriol

Ramón Muñoz-Chápuli is Professor of Animal Biology in the University of Malaga. He is the leader of the Cardiovascular Development and Angiogenesis Lab. He has worked since 1990 in issues related with cardiovascular development, mainly the origin, differentiation and fate of the cells derived from the embryonic epicardium, the epicardial/myocardial interactions and the development of the coronary vessels. Recently he has become interested in Evo-Devo issues, such as the evolutionary origin of the vertebrate endothelium, and the origin of the epicardium as a derivative of an ancestral heart-kidney connection. He has also collaborated with Ana Rodríguez Quesada and Miguel Ángel Medina group in the characterization of new angiogenesis modulators.

Presente y futuro de las terapias antiangiogénicas

Current and future of pharmacological intervention of angiogenesis

Ana Rodríguez Quesada

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Angiogenesis inhibition has been proposed as a general strategy to fight cancer. However, in spite of the promising preclinical results, a first generation of antiangiogenic compounds yielded poor results in clinical trials. Conceptual errors and mistakes in the design of trials and in the definition of clinical endpoints could account for these negative results. The initial pessimism about the usefulness of the antiangiogenic therapeutic approach for cancer, transformed into an increasing interest in the development of antiangiogenic compounds after the first clinical approval of an antiangiogenic therapy. The anti-vascular endothelial growth factor (VEGF) antibody bevacizumab has been approved for use in combination with chemotherapy for the treatment of metastatic colorectal and non-small cell lung cancer patients. However, no survival benefit has been obtained in anti VEGF monotherapy trials, probably due to the high complexity of tumor angiogenesis regulation. The discovery and pharmacological development of future generations of angiogenesis inhibitors will benefit from further advances in the understanding of the mechanisms involved in human angiogenesis regulation, exerted by multiple factors secreted by tumor cells and their surrounding host stromal cells, modulated by extracellular matrix and controlled by multiple complementary, overlapping and independent pathways. In fact, monotherapies with antiangiogenic compounds could be more useful as adjuvant treatments in situations of minimal residual disease following either cytoreductive surgery or cytotoxic treatment, slowing the progression of premalignant lesions and reducing the risk of developing invasive tumors. Growing evidence advises the use of multitargeted approaches to reach an effective inhibition of tumor angiogenesis, “orchestration” arising as an attractive concept in antiangiogenesis. However, toxicity of combination treatments, could hardly be predicted and could limit their duration, the high cost of new molecular targeted drugs and reluctance to collaboration between different pharmaceutical companies could greatly difficult the combined use of several “antiangiogenic instruments”. Reductions in the cost treatments derived from expenditure cap strategies, the development of biomarkers that can inform dosing, initial drug choice, emerging resistance and

second-line treatments, or the use of metronomic chemotherapy will help to control the economic impact of the new and promising therapeutic alternatives.

Dr. Ana Rodríguez Quesada

After her PhD degree at the University of Málaga, Ana Rodríguez Quesada joined the Research Department of Antibióticos S.A. (León, Spain) as a staff scientist to work in the search of new therapeutic compounds. This work was continued after her incorporation to the Research Department of Pharmacia-Antibióticos Farma (Madrid, Spain). After this seven-years experience in the pharmaceutical industry, she incorporated to the Molecular Biology and Biochemistry Department at the University of Málaga, where she is an Assistant professor since 1996. Her laboratory is mainly devoted to the search and characterization of new inhibitors of angiogenesis. This work is carried out thanks to the collaboration with pharmaceutical companies and research groups from Spanish and foreign universities.

Communications

P01

CIRCULATING MARKERS OF ANGIOGENESIS, COAGULATION AND INFLAMMATION IN PATIENTS WITH GLIOBLASTOMA

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The median survival of patients with glioblastoma (GB) is below one year. In these patients, tumor progression is related to angiogenesis, a process in which the vascular endothelial growth factor (VEGF-A) and its receptor VEGFR-1 are involved. Objectives: To determine the presurgical levels of circulating markers of angiogenesis, coagulation and inflammation, in patients with GB. Patients and methods: 30 patients and 60 healthy controls were studied. Blood levels of VEGF-A, soluble VEGFR-1 (sVEGFR-1) and thrombospondin-1 (TSP-1) were assessed as angiogenesis markers. Furthermore, prothrombin fragment 1+2 (F1+2), tissue factor (TF), endogenous thrombin generation (ETG), interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), fibrinogen (Fg), sialic acid (SA), and C-reactive protein (CRP) were determined. The statistical significance of the differences between means was evaluated using the Student's t-test. The correlation study was made using the Spearman's test. Results: A significant increase in VEGF-A levels (245±180 vs 123±120, $p<0,001$) was found in GB patients. However, no differences were found in levels of sVEGF-A and TSP-1 between patients and controls. Levels of F1+2 were significantly increased in GB patients (0,42±0,59 vs 0,17±0.04 nmol/L, $p<0,001$), whereas TF and ETG values were not modified. A statistically significant increase in levels of all inflammatory markers was found in GB patients as compared with healthy controls. The correlation study shows a positive association between VEGF-A and inflammation markers (Fb and SA, $r=0,65$-$0,51$; $p<0,01$). Conclusion: In patients with GB, an increase of angiogenesis is observed, and it correlates with an increase of inflammation.

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P02

ISOLATION, PROLIFERATION AND DIFFERENTIATION OF ENDOTHELIAL PROGENITOR CELLS OBTAINED FROM LIPOASPIRATES WITH VASCULAR REGENERATION CAPACITY

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According to 2005 data from WHO cardiovascular diseases (ischemic heart disease and stroke) are the principal cause of death, representing 30% of all deaths in the world. Due to limitations in the use or efficiency of current treatments, in recent years new therapies based on the regenerative capacity of stem cells are being tested. In 1997, Asahara et al. identified a subpopulation of "Putative Endothelial Cell" (EPC) from the CD34 + fraction of stem cells isolated from human peripheral blood. These EPC were able to migrate and participate in postnatal neovascularization in angiogenesis and/or vasculogenesis processes. They also have the ability to act in the endogenous response of the cardiovascular pathological processes.

The aim of our study was to optimize the conditions of isolation and proliferation of EPC from human adipose tissue, as well as functional and phenotypic characterization of the cells obtained. We performed an enzymatic digestion of lipoaspirates from patients undergoing liposuction, and isolated the adherent cells which were able to form colonies of three-dimensional appearance in selective media. Cells were characterized by flow cytometry with mesenchymal stem cells (CD 90, CD 73, CD 105), hematopoietic and endothelial markers (CD45, CD34, CD31, CD133, and KDR CXCR4). Subsequently, cells were grown on Matrigel and showed three-dimensional mesh formation with characteristic tubular and vessel like appearance.

Our results indicate that human lipoaspirates could be an important source of EPCs able to form vascular structures with a potential use in cardiovascular regenerative therapy.

P03

TSP1 EXPRESSION IS ASSOCIATED TO BASAL PHENOTYPE OF BREAST CARCINOMA.

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Background.- Thrombospondin- 1 (TSP-1) is a known antiangiogenic factor that inhibits tumour growth and metastasis in animals. However, its clinical relevance in breast cancer is equivocal. Some date suggest that TSP-1 expression could be associated with poor prognosis.

The aim of this study is to determine any associations between TSP-1 and prognostic factors as well as with the basal/luminal phenotype of breast cancer.

Method.- Paraffin-embedded tumour from 266 cases of breast cancer patients were used to make a tissue-microarray (TMA). Consecutive sections of TMA were stained immunohistochemically for ER, PR, HER2, p53, Ck18, Ck19, Ck5.6, Ck14, and TSP-1. Basal subtype of breast carcinoma was defined by positivity of Ck5,6 and/or Ck14, and luminal subtype by positivity of CK18 and/or Ck19.

Results.- A basal phenotype was found in 10% of the cases. RE and RP expression directly correlated with luminal subtype and inversely with basal one ($p<0.0001$). TSP-1showed stromal stain in 78 cases (29,3%) and was positive in tumour cells in 20 cases (7,5%). TSP-1 stain was seen in most of the basal carcinomas reaching statistical signification ($p=0.01$). In patients with affected lymph nodes, stromal expression of TSP-1 was associated with high Ki-67 ($p=0.01$) and positive HER2 ($p=0.05$), while cellular expression was associated with high grade ($p=0.01$).

Conclusions.- Our results suggest that TSP-1 may play a major role in angiogenesis of basal subtype of breast cancer. On the other hand, TSP-1 expression is associated with factors of poor prognosis in breast carcinoma with positive lymph nodes

P04

PTK 787/Z222485, A POTENT INHIBITOR OF VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR TYROSINE KINASE PHOSPHORYLATION, MIGHT CONSTITUTE A NEW EFFECTIVE THERAPY TO ACUTE MYELOID LEUKEMIA.

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Acute Myeloid Leukemia (AML) is a disease with a poor prognosis. Angiogenesis plays an important role in the pathogenesis of this disease. It has been demonstrated that AML cells express the vascular endothelial growth factor (VEGF) as well as kinase domain-containing receptors, VEGFR1/ Flt-1 and VEGFR2/KDR, resulting in an autocrine pathway for cell survival.

Vatalanib (PTK787/ZK 222584) is a new oral antiangiogenic molecule that inhibits all known vascular endothelial growth factor receptors, the platelet-derived growth factor receptor tyrosine kinase and the c-kit protein tyrosine kinase.

Objective: To investigate the role of the VEGFRs inhibitor PTK787/ZK222584 on cell proliferation, survival and angiogenesis in AML cells, and the effect of the combined treatment with a chemotherapeutic drug, idarrubicin.

Material and methods: Four AML cell lines (MV4-11, MOLM-13, NB4 and THP-1) were treated with PTK787/ZK, given alone or combined with idarrubicin for 24 and 48 hours. Then, cell apoptosis was analyzed by flow cytometry, and cell proliferation was detected using an XTT colorimetric assay. The effects on the activation of VEGFRs and several intracellular pathways (ERK, Akt and STAT5) were studied by western blot. The VEGF levels in the cellular supernatants were evaluated by ELISA assay.

Results: PTK787/ZK decreased VEGF levels and inhibited the VEGFR phosphorylation in a dose-dependent manner in the AML cells showing FLT3/ITD mutation. Both drugs induced inhibition of cell proliferation and apoptosis. Moreover, the addition of Idarubicin to the treatment with PTK787 promoted more apoptosis and inhibition of cell proliferation than each compound administered separately.

In conclusion, PTK787 combined with Idarubicin achieved a better therapeutic efficacy than chemotherapy alone in AML patients showing FLT3/ITD mutations, preventing the activation of angiogenic process.

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P05

THE ROLE OF CARCINOEMBRYONIC ANTIGEN-RELATED CELL ADHESION MOLECULE-1 (CEACAM1) IN ANGIOGENESIS AND LYMPHANGIOGENESIS

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As well as angiogenesis describes the sprouting of new blood vessels from pre-existing ones, lymphangiogenesis is defined as the development of lymphatics from pre-existing lymphatic vasculature. Intensive research during the last few years has evidenced, that not only angiogenesis but also lymphangiogenesis is needed for tumor growth and metastasis. In both processes, which are regulated by a balance between activators and inhibitors, cell adhesion molecules play also an important role. We previously showed that CEACAM1 is expressed in the newly formed immature blood vessels of angiogenic tissues such as in tumors, but not in quiescent blood vessels of normal human tissues. Recently, we demostrated that CEACAM1 is also up-regulated in endothelial cells of lymphatics of early tumor stages, even prior to its appearance in vascular endothelial cells (EC). Furthermore, it has been reported that CEACAM1 is down-regulated in some tumors such as colorectal and prostate carcinomas. Mimicking this epithelial down-regulation we demonstrated that CEACAM1 gene silencing in human bladder and prostate cancer cell lines increases the expression of VEGF-A, -C and –D. Inversely, our studies evidence that CEACAM1 overexpression in ECs induces angiogenesis via up-regulation of angiogenic and lymphangiogenic factors. Taken together, these findings suggest that CEACAM1 can exhibit inverse effects regarding angiogenesis/lymphangiogenesis by discriminating between activating different signaling cascades in endothelia and epithelia respectively. Strategies to either conserve the epithelial CEACAM1 or to target endothelial CEACAM1 might be useful for an antiangiogenic and antilymphangiogenic therapy of bladder and prostate cancer.

P06

INHIBITION OF POLY(-ADP-RIBOSE)-POLYMERASE-1 DECREASES ANGIOGENIC CAPACITY OF THE ENDOTHELIAL CELL

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Poly (ADP-ribose) polymerase (PARP) is a nuclear enzyme that participates in the regulation of DNA repair, gene transcription, genomic stability, cell cycle progression, cell death, and chromatin function. Poly (ADP-ribosylation) of different nuclear acceptors by PARP-1 is an early event when a single strand DNA lesion is produced. Nonetheless, recent results from our group in a model of epidermal carcinogenesis, have shown that PARP-1 is also able to modulate the trans-activation of multiple genes involved in tumor progression, among them, genes involved in tumor response to hypoxia and angiogenesis. These is the case of the hypoxia inducing factor (HIF-1). A vast majority of human cancer overexpress HIF-1 and the failure of different therapeutical regimes has been correlated with its overexpression, leading to the inability of tumor cells to induce apoptosis and favoring tumor progression through increased angiogenesis (mainly, by increased trans-activation of VEGF). In this study we focalised on the ability of a PARP inhibitor as new therapeutical tools against tumor angiogenesis. Inhibition of PARP delays endothelial cell migration, and strongly inhibit tube formation and in vivo angiogenesis and the release of VEGF-a. An ungoing proteomic analysis has revealed some important proteins are downregulated after PARP inhibition. Globally, these results suggest that inhibition of PARP might have important consequences in limiting angiogenesis during tumor development.

P07

HUMAN SMOOTH MUSCLE CELLS PROMOTE MIGRATION AND STABILIZATION OF MICROVASCULAR ENDOTHELIAL CELLS

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Background: Angiogenesis plays a fundamental role in the progression of many pathologic processes, ranging from cancer to rheumatoid arthritis. During the early stage of angiogenesis, new capillaries sprout from pre-existing vessels. As they mature, microvessels acquire a coating of mural cells (smooth muscle cells/pericytes), which are critical for the development and maintenance of the developing vasculature. However, the role of smooth muscle cells in blood vessel development remains to be defined. Our purpose was to understand the mechanisms underlying this complex sprouting & stabilization process in order to identify possible targets for more efficient antiangiogenic therapeutic strategies.

Methods: Human coronary smooth muscle cells (SMC), obtained by the explant technique from human coronary arteries (heart transplant surgery), and microvascular endothelial cells (HMEC-1) were mixed and then plated together onto basement membrane reconstructed gel (Matrigel). To discriminate each cell type, cells were labeled with two different fluorescent membrane dyes, PKH2 and PKH26. Analysis of capillary-like network formation was performed by time lapse video microscopy (Leica TCS SP2-AOBS). Chemotactic migration of cells was measured in a modified Boyden chamber. Experimental data is expressed as means of three independent experiments performed in triplicate.

Results: Direct contact between ECS and SMC was clearly observed by confocal microscopy in order to form capillary-like structures. SMCs promote early migration and differentiation of ECs.

Conclusions: Our data indicate that SMC not only stabilize ECs, but play a key role in organizing cell-cell contacts and forming capillary-like networks.

P08

EFFECT OF ESTROGENS (RALOXIFEN) ON ENDOTHELIAL CELLS

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Hereditary haemorrhagic telangiectasia (HHT), or Rendu- Osler-Weber syndrome, is an autosomal dominant vascular disease. This is manifested as epistaxis, mucocutaneous and gastrointestinal telangiectases, and arteriovenous malformations. There are two main HHT types, type 1 and type 2 which are caused by mutations in *Endoglin* and *ALK-1* genes. Both genes code for proteins involved in the TGFβ-signalling pathway. It is generally accepted that Endoglin or ALK1 haploinsufficiency is the origin for the pathogeny of the disease. Some patients show severe epistaxis which notably interfere with their quality of life. Therefore, the knowledge of drugs able to increase the transcriptional activity of the promoters of those genes is essential to propose therapies for HHT. The efficacy of estrogens, in particular raloxifene, was assessed in postmenopausal women diagnosed of osteoporosis. The study is being currently conducted with HHT women in the Spanish Hospital of reference for the disease, Sierrallana. In parallel, we have carried out a study to unravel the molecular mechanisms involved in the therapeutic effects of Raloxifene. According to our results, this drug increases the expression of ENG and ALK1 at the endothelial cell surface, and the origin of the effect is at the transcriptional level. Therefore, we may conclude that the treatment with estrogen may decrease nosebleeds, consequently improving the quality of life in HHT patients.

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P09

THE SHORT ISOFORM OF ENDOGLIN IS INDUCED DURING THE SENESCENCE OF ENDOTHELIAL CELLS

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Senescence of endothelial cells (ECs) may contribute to age-associated cardiovascular diseases, including atherosclerosis and hypertension. The functional and gene expression changes associated with cellular senescence are poorly understood. Here we have analyzed the expression, during EC senescence, of two different isoforms (L, long; S, short) of Endoglin, an auxiliary TGF-β receptor involved in vascular remodelling and angiogenesis. As evidenced by RT-PCR, the S/L ratio of Endoglin isoforms was increased during senescence of human ECs in vitro as well as during aging of mice in vascularized tissues. Next, the effect of S-Endoglin protein on the TGF-β receptor complex was studied. As revealed by co-immunoprecipitation assays, S-Endoglin was able to interact with both TGF-β type I receptors, ALK5 and ALK1, although the interaction with ALK5 was stronger than with ALK1. S-Endoglin conferred a lower proliferation rate to ECs and behaved differently than L-Endoglin in relation to TGF-β-responsive reporters with ALK1 or ALK5 specificities, mimicking the behaviour of the endothelial senescence markers Id1 and PAI-1. In situ hybridization studies demonstrated the expression of S-Endoglin in the endothelium from human arteries. Taken together, these results suggest that S-Endoglin is induced during endothelial senescence and may contribute to age-dependent vascular pathology.

P10

ACTIVATION OF ANGIOGENICAL PROCESS BY TRANSCRIPTIONAL REGULATION OF ALK1 AFTER VASCULAR INJURY MEDIATED BY KLF6

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Activin like kinase receptor type 1 (ALK1) is a type I TGFβ receptor predominantly expressed in endothelial cells and activates angiogenesis after stimuli like vascular injury. Because little is known about its transcriptional regulation, we performed an *in silico* analysis over a fragment of 1235 bp of genomic DNA containing the theoretical transcription start site and part of the transcribed not translated first exon. We have found lack of TAATA and CAAT boxes, and multiple G/C rich regions containing consensus for Sp1. We have demonstrated that basal transcriptional activity of ALK1 promoter is null in absence of Sp1, and increasing amounts of Sp1 remarkably transactivate it. KLF6 is a transcription factor related with vascular repair in endothelium, and transactivates other genes of the TGFβ signalling pathway. We find that overexpression of KLF6 is able to transactivate ALK1 promoter by synergic cooperation with Sp1 in HEK293T. Using a wound healing *in vitro* model in HUVECs (*human umbilical vein endothelial cells*) we have detected that the levels of ALK1 mRNA and protein are upregulated in membrane after 2 hours of injury. By chromatin immunoprecipitaton experiments we have demonstrated the binding of KLF6 and Sp1 on the promoter, showing stronger binding after wound healing. Moreover, total RNA of liver tissue from KLF6+/- mice showed decreased levels of ALK1 mRNA compared with their KLF6+/+ siblings. These data provide evidence that injury-induced KLF6 and preexisting Sp1 may cooperate potentiating ALK1 expression in vascular repair.

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P11

ANTITUMORAL EFFECT OF STATINS IN AN ANIMAL MODEL OF BREAST CANCER

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Statins are a group of inhibitors of the 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase used extensively in medical practice because of their ability to reduce cardiovascular mortality and stroke. By blocking cholesterol biosynthesis, statins decrease also the pool of intermediate isoprenoid metabolites, such as farnesyl pyrophosphate (FPP) or geranylgeranyl pyrophosphate (GGPP), which are required for the activation of multiples GTPases. Impairment of these signaling proteins allows statins to affect actin-based cytoskeletal remodeling involved in cell motility, induction of apoptosis or antigen presentation and lymphocyte activation among other effects. Therefore it has been recognized that statins are pleiotropic drugs with anti-inflammatory and immunomodulatory properties. Although retrospective studies in patients indicate that statins treatment does not increase tumor incidence and even protects from tumor development, the extent to which each of the pleiotropic activities of statins contributes to these effects is not well understood. To this end, we have analyzed the effect of lovastatin in a murine model of spontaneous mammary tumor, the MMTV-neu transgenic mouse. In this model, 50% of female mice develop mammary tumors by 205 days and a median of 2 mammary tumors per mice arise. Administration of lovastatin to tumor-bearing transgenic mice slow down the growth of the pre-existing tumors and also it reduces the final number of tumors. The tumor weight reduction seems not to be a consequence of changes in proliferation and/or apoptosis of the tumoral cells. Instead we found differences in the number and phenotype of vessels by CD31 staining of endothelial cells and by FITC-lectin labeling of vessels *in vivo* besides with an altered association of perycytes analyzed by staining with nestin and SMA. We have also analyzed the level of hypoxia in our tumoral samples by the detection of the hypoxia marker pimonidazole previously administered to the mice. The possible relationship between the effects of lovastatin in angiogenesis and hypoxia is under investigation in order to understand the mechanism leading to the decrease in tumoral growth.

P12

FRAGMENT BASED CLICK CHEMISTRY LIGANDS FOR DEVELOPMENT OF POTENT ORGANIC KDR INHIBITORS

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VEGFR-2 activation is considered to be one of the main pathways leading to endothelial cells division, migration, and differentiation. Activation of endothelial cells can causes angiogenesis (new blood vessel formation from pre-existing ones) and consequently progression of tumour and its metastasis. Recently first two organic inhibitors (sunitinib L-malate and sorafenib tozylate) have been approved by FDA for clinical treatment of cancer patients. Successful clinical applications of them underline high importance for development of other oral inhibitors of tumour driven neovascularisation. Our research is focused on designing and synthesizing oxazole and urea fragment based ligands for focused “Click chemistry” library. We assume that by 1,3-dipolar cycloaddition of proper azides and alkynes ligands chosen via *in Silico* modelling will lead to development of highly potent KDR inhibitors.

In our announcement synthesis of designed derivatives of oxazole and arylureas that are promising inputs for *in situ* “Click chemistry” development will be described.

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P13

USE OF LATRUNCULINS AND DERIVATIVES AS ANGIOGENESIS INHIBITORS

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Angiogenesis is a highly regulated process by which new capillaries are generated by sprouting pre-existing microvessels. Uncontrolled angiogenesis is the major contributor to a number of diseases including cancer, acute macular degeneration and psoriasis, among others. The use of angiogenesis inhibitors has been proposed as a new strategy for the treatment of those angiogenesis-dependent diseases.

The development of new blood vessels is a complex multi-step process. Endothelial cells resting in the parent vessels are activated by an angiogenic signal and stimulated to synthesize and release degradative enzymes allowing endothelial cells to migrate, proliferate and finally differentiate to give rise to capillary tubules. Any of these steps may be a potential target for pharmacological intervention.

In the course of a blind screening of new inhibitors of angiogenesis from marine origin, latrunculin B, a macrolide isolated from the Red Sea sponge *Negombata magnifica*, was selected by means of its capability to inhibit endothelial tubulogenesis in vitro at non toxic concentrations. Several semisynthetic analogues of latrunculin B were also selected in our screening, and their antiangiogenic activity was characterized by means of a number of assays resembling the angiogenesis process.

Our results show that latrunculin derivatives inhibit proliferation, differentiation and migration of endothelial cells *in vitro*, as well as they inhibit angiogenesis *in vivo* in the chick chorioallantoic membrane. Taken together, these data indicate that these compounds inhibit several essential steps of the angiogenic process. This work underscores the possibility of utilizing compounds derived from marine organisms as potential new sources of angiogenesis inhibitors that could be used for the treatment of angiogenesis-related malignancies.

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P14

IB05204, A DICHLOROPYRIDODITHIENOTRIAZINE, INHIBITS ANGIOGENESIS *IN VITRO* AND *IN VIVO*

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In the course of a blind screening program for inhibitors of angiogenesis, IB05204 (4,8-dichloro-12-phenylpyrido[5',6':4'',5'';3',2':4,5]dithieno[3'',2'' d':3,2 d]-1,2,3-ditriazine) was selected for its ability to inhibit endothelial tubule-like network formation on Matrigel. IB05204 inhibits the *in vivo* angiogenesis in the chorioallantoic membrane (CAM) and the mouse Matrigel plug assays. Antiangiogenic activity seems to be highly dependent on the chloro substituents because their renoval results in a complete loss of the *in vitro* inhibitory activity of endothelial differentiation and *in vivo* antiangiogenic activity in CAM assay. Although IB05204 inhibits the growth of endothelial and tumor cells in culture, its antiangiogenic activity seems to be mainly dependent on the prevention of endothelial capillary-like tube formation and inhibition of endothelial migration because these effects are recorded at lower concentrations. IB05204 treatment inhibits matrix metalloproteinase-2 (MMP-2) production in endothelial and tumor cells, down-regulates endothelial cyclooxygenase-2 expression, and represses phosphorylation of endothelial Akt in response to serum stimulation, suggesting that IB05204 interferes with molecular mechanisms of cell migration and survival. IB05204 induces apoptosis in endothelial cells through cytochrome *c* release and caspase activation.

Data here shown altogether indicate that IB05204 is a compound that interferes with several key steps of angiogenesis, making it a promising drug for further evaluation in the treatment of angiogenesis-related pathologies.

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P15

INHIBITION OF ANGIOGENESIS RELATED GENES BY EGCG IN HUMAN MONOCYTES

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Monocytes/macrophages are an important population of immune inflammatory cells implicated in several physiological and pathological processes. Migration and adhesion are essential for monocytes to carry out their functions. Our group demonstrated previously that epigallocatechin-3-gallate (EGCG), the major bioactive component of green tea and a compound described as antiallergic and anti-inflammatory, decreased migration and adhesion in the human mast cell line HMC-1. EGCG decreased the expression of some angiogenesis related genes, including some integrins and the monocyte chemotactic protein-1 (MCP-1) in HMC-1, decreasing their abiliy of monocyte recruitment. In this work, we demonstrated that EGCG inhibited the human monocyte cell line THP-1 adhesion to fibronectin and the expression of activated integrin beta1, one of the main integrins involved in adhesion to fibronectin and required for monocyte migration. EGCG also inhibited the secretion of MCP-1 by THP-1 and the expression of CCR2, the specific receptor for MCP-1, in the surface of the monocytes. Furthermore, THP-1 migration ability in response to MCP-1 was also decreased by EGCG. These inhibitory effects of EGCG on monocyte functionality show the ability of EGCG to inhibit inflammatory dependent processes such as angiogenesis.

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P16

KAHWEOL, A DITERPENE PRESENT IN COFFEE, EXHIBITS MODULATORY EFFECTS ON ANGIOGENESIS AND CANCER

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Kahweol is a diterpene present in considerable amounts in coffee. Epidemiological studies associate unfiltered coffee drinking with lower incidence of colon and liver cancer. On the other hand, its preventive effects against oxidative stress and DNA damage are well documented.

Angiogenesis is described as one of the hallmarks of cancer and it is required for both tumor progression and metastasis. Our group is actively involved in the identification of new angiogenesis modulators from natural sources.

In this study, the potential modulatory effects of kahweol on angiogenesis are analysed by studying its effects on cell proliferation, extracellular matrix degradative activity, migration, formation of tubule-like structures and apoptosis. Furthermore, its antiinflammatory potential is studied focusing on its effects on cyclooxygenase 2 (COX-2).

Our results show that kahweol inhibits endothelial cell proliferation, although this effect does not seem to be specific for endothelial cells. However, kahweol seems to exert a pro-apoptotic effect specifically on breast and colon cancer cells. On the other hand, kahweol clearly inhibits endothelial cell "differentiation" to tubule-like structures. Furthermore, kahweol inhibits endothelial cell migration and affects their capacity to remodel extracellular matrix. Finally, kahweol inhibits COX-2 expression in endothelial cells, therefore increasing its interest as a potential antitumor agent.

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P17

ROSEMARY AS A NEW SOURCE OF ANTIANGIOGENIC COMPOUNDS

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Since Folkman's hypothesis that tumour growth was angiogenesis dependent, it has become clear that interfering with and /or preventing angiogenesis is an attractive therapeutic approach to the treatment of angiogenesis-dependent diseases.

The development of new blood vessels requires the activation of endothelial cells by an angiogenic signal, the synthesis and release of degradative enzymes that will allow endothelial cells to migrate and invade, and endothelial cell proliferation and differentiation to give rise to capillary tubules. Any of these steps may be a potential target for pharmacological intervention.

Carnosol and carnosic acid are phenolic diterpenes present in Rosmarinus officinalis (Rosemary), both of which possess anti-oxidant, anti-inflammatory anti-proliferative, anti-tumorigenic and neuroprotective effects.

Our results indicate for the first time that two antioxidant compounds from rosemary, carnosol and carnosic acid, interfere with key events in angiogenesis. Both compounds inhibit differentiation, proliferation, migration and invasion of endothelial cells in vitro, causing a shift on the proteolytic balance of endothelial cells towards antiproteolysis. Carnosol, and carnosic acid, induce apoptosis in endothelial cells as well as in human leukemia and fibrosarcoma cell lines. Our results indicate that carnosol and carnosic acid are promising drugs for further evaluation in the treatment of angiogenesis-related pathologies.

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THE ROLE OF HOMOCYSTEINE IN ANGIOGENESIS, TUMOR INVASION AND REDOX HOMEOSTASIS.

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Homocysteine (Hcy) is a thiol-containing, nonproteinogenic amino acid, which has a high reactivity in biological systems. Hcy can be released into extracellular medium and exists largely in mixed disulfide and protein-bound forms: this is plasma total homocysteine (tHcy). An increase in plasma tHcy reflects an imbalance between homocysteine production and metabolism/excretion and elevates levels of plasma Hcy are associated with signal transduction and disease. We have investigated the roles of Hcy in cell metabolism, vascular disease, angiogenesis and tumor progression, and we have reported the anti-angiogenic effects of Hcy and its ability to inhibit tumor invasión too. Due to its reactive sulfhidryl group, Hcy is a redox active compound, which modulates the expression of endothelial cell surface molecules linked to redox homeostasis in blood and vascular tissues.

Ubiquitous plasma membrane redox systems (PMRS) are electron transporting oxidoreductases that mediate several vital functions, including proton pumping, membrane energization, ion channel regulation, iron uptake, signal transduction, growth regulation and cell proliferation, cell defense and detoxify oxidative stress damage. In this sense, PMRS are involved in the signaling of processes that modify the phenotype of vascular endothelial cells, such as atherosclerosis, thrombosis, angiogenesis and tumor progression. We have shown that Hcy is a potent modulater of PMRS activity in endothelial and tumor cells.

Currently, we are working to elucidite the role of Hcy in redox homeostasis and its involvement in angiogenesis and tumor progression.

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P19

EFFECT OF THE LACK OF SERGLYCIN ON THE PROTEOME OF BONE MARROW-DERIVED MAST CELLS

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Mast cells are known for their implication in a large number of physio-pathological processes including the innate and acquired immunity, allergy, inflammatory arthritis, asthma, angiogenesis and cancer (1). The role of mast cells in angiogenesis and cancer has been studied for long and a huge amount of information has been obtained ((2), (3)).

Although degranulation-independent actions have been described, the effect of MCs in many physio-pathological states is often a consequence of MC degranulation (1). MC secretory granules contain a wide array of preformed mediators such as histamine, cytokines and neutral proteases (chymases, tryptases and carboxypeptidase A) (4), (5). MC granules also include large amounts of proteoglycans (PGs, protein cores to which unbranched, sulfated, and thereby negatively charged polysaccharides of glycosaminoglycan type are attached). In MCs, serglycin (SG)-PGs constitutes the major type of PG (6). In SG knock-out mast cells, the storage of various proteases, histamine and serotonin were dramatically defective (7), (8). In the present work, by using proteomic approaches, we further investigate the effect of the lack of SG on the proteome of bone marrow-derived mast cells (BMMCs). 2D-gel electrophoresis followed by mass spectrometry identification of differentially expressed proteins was carried out for SG knock-out versus wild type BMMCs. Differences in protein expression are discussed in relation to the implication of mast cells in angiogenesis, cancer and other pathologies.

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P20

EXPLORING THE FRAGILITY OF ANGIOGENESIS MACHINERY

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The high number of molecular elements contributing to the angiogenic process and the complex relationships among them are far to be controlled by using reductionist approaches. Network theory provides a suitable framework for the study of interacting components as a systemic perspective.

Here we present the utilization of this systemic approach to evaluate the fragility of the angiogenic network against a single or multiple drug attack. We define the *Network of Angiogenesis* by the integration of protein-protein interaction, protein modification and transport. This information has been collected, from literature, text mining applications and databases. After filtering, it was integrated in three different networks, which we call high, medium and low confidence networks depending on their source and redundancy.

For the fragility study, we evaluate the topological properties of the graphs to determinate the "hub" nodes and the nodes with maximal betweenness. Due to the potential functional relevance of such nodes, we made an screening of those drugs that can modify the activities of these nodes. Finally, we evaluate the robustness of these graphs by the selective attack of the resultant target nodes (detaching them either one by one or at the same time) and we explore the obtained stable signaling modules.

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PERIVASCULAR GLUTAMINASES IN MAMMALIAN BRAIN: A NOVEL VASOACTIVE MECHANISM TO CONTROL CEREBRAL VASCULAR FUNCTION?

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The enzyme glutaminase (GA) is responsible for the synthesis of neurotransmitter glutamate. At least two GA isoforms, called KGA and LGA, are expressed in the brain of mammals. A strong perivascular immunoreactivity has been revealed for KGA and LGA in rat and monkey brain. Strong immunoreactivity was seen in certain blood vessels throughout the brain and in the pial surface of the cerebral cortex. Double immunofluorescence confocal laser scanning microscopy for KGA, LGA and GFAP showed that GA staining not associated with perivascular astrocyte processes.Furthermore, electron microscopy immunocytochemical studies revealed a distribution of perivascular KGA in axonal and dendritic elements, confirming the neuronal nature of KGA reactivity. On the other hand, LGA was found to colocalize in astrocytes cell processes and their perivascular endfeet with the protein GIP ("Glutaminase-Interacting Protein"), a novel PDZ protein that has been previously shown to interact with LGA. The targeting of GA's to specific population of astrocytic and neuronal processes surrounding blood vessels might be a plausible mechanism implicated in the regulation of vascular function through glutamate generation The fact that glutamate is a vasoactive compound and the existence of glutamate receptors in perivascular glia and vascular endothelial cells would implicate GA's in the regulation of the vascular tone. Furthermore, glutamate receptors, like NMDA subunit R1, was also localized surrounding the periphery of blood vessels. The findings presented here suggest a novel mechanism for neurovascular regulation.

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PROGRESSIVE AMYLOID VASCULAR PATHOLOGY IN THE SUBICULUM OF $PS1_{M146L}$/APP_{751SL} TRANSGENIC MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD), the most prevalent cause of dementia in humans, is a neurodegenerative disorder with high incidence in the older population. The major pathological hallmarks of AD are the presence of intraneuronal neurofibrillary tangles, extracellular beta-amyloid (Abeta) plaques associated with activated microglia and astrocytes, and significant neuronal loss. Cerebrovascular dysfunction such as amyloid angiopathy, cerebral microvascular pathology and deficient clearance of Abeta across the blood–brain barrier, also contributes to the cognitive decline and dementia. There is accumulating evidence that angiogenesis process is a direct contributor to AD pathology. The subiculum, a major source of hippocampal efferents to cortical and subcortical structures, is highly vulnerable in AD. The aim of the present study has been to investigate the temporal Abeta-associated vascular changes in the subiculum of 2-, 4-, 6-, 12- and 18 month-old PS1(M146L)/APP(751SL) transgenic mouse model. Using immunohistochemical and classical amyloid stainings we observed a significant increase in the number and size of extracellular amyloid deposits in the parenchyma during aging. In addition, the number of Abeta-positive blood vessels was higher in older animals, which might affect the integrity of the endothelial barrier and impairs cerebrovascular blood flow explaining the age-dependent increase in amyloid deposition. In addition, vascular pathology could be associated with a marked inflammatory response induced by Abeta in this model. Glial-derived pro-inflammatory/angiogenic cytokine TNFalpha could mediate these changes. These findings provide new insights into the pathogenic pathways for the vascular dysfunction in AD models.

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BETA-AMYLOID INDUCES VASCULAR CHANGES IN THE PERIRHINAL CORTEX OF $PS1_{M146L}/APP_{751SL}$ TRANSGENIC MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most common cause of dementia in the aging population affecting millions of people worldwide. AD is characterized by high density of extracellular beta-amyloid (Abeta) deposits, intraneuronal neurofibrillary tangles, amyloid vascular pathology and reactive gliosis leading to neuronal degeneration in discrete regions of the brain. There is increasing evidence that angiogenic changes occur in the microcirculation of the AD brain and might contribute to the course of events leading to the disease. The perirhinal cortex, a medial temporal lobe structure implicated in aspects of both perception and memory, is especially interesting for the strong reciprocal connectivity with the entorhinal cortex and hippocampus, areas first and severely affected in AD. Here we have used immunohistochemical and classical amyloid staining procedures to study the age-dependent alterations in cerebral vasculature of the perirhinal cortex of PS1(M146L)xAPP(751SL) transgenic model. These transgenic mice developed Abeta depositions at early ages (3-4 months). The number and size of amyloid plaques as well as the number of amyloid-positive blood vessels significantly increased with age. Activated microglial cells were concentrated in clusters surrounding and infiltrating plaques. In addition, there was an increase in the pro-inflammatory/angiogenic factor TNFalpha with age. Therefore, neuroinflammation induced by Abeta could be associated with perturbations in properties of vasculature including formation of new blood vessels. These findings support the idea that glial-mediated inflammatory alterations in cerebral vasculature of this AD model could contribute to the disease pathogenesis.

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