Année de publication : 2019

Hélène Lazareth, Carole Henique, Olivia Lenoir, Victor G Puelles, Martin Flamant, Guillaume Bollée, Cécile Fligny, Marine Camus, Lea Guyonnet, Corinne Millien, François Gaillard, Anna Chipont, Blaise Robin, Sylvie Fabrega, Neeraj Dhaun, Eric Camerer, Oliver Kretz, Florian Grahammer, Fabian Braun, Tobias B Huber, Dominique Nochy, Chantal Mandet, Patrick Bruneval, Laurent Mesnard, Eric Thervet, Alexandre Karras, François Le Naour, Eric Rubinstein, Claude Boucheix, Antigoni Alexandrou, Marcus J Moeller, Cédric Bouzigues, Pierre-Louis Tharaux (2019 Jul 26)

The tetraspanin CD9 controls migration and proliferation of parietal epithelial cells and glomerular disease progression.

Nature communications : 3303 : DOI : 10.1038/s41467-019-11013-2

Résumé

The mechanisms driving the development of extracapillary lesions in focal segmental glomerulosclerosis (FSGS) and crescentic glomerulonephritis (CGN) remain poorly understood. A key question is how parietal epithelial cells (PECs) invade glomerular capillaries, thereby promoting injury and kidney failure. Here we show that expression of the tetraspanin CD9 increases markedly in PECs in mouse models of CGN and FSGS, and in kidneys from individuals diagnosed with these diseases. Cd9 gene targeting in PECs prevents glomerular damage in CGN and FSGS mouse models. Mechanistically, CD9 deficiency prevents the oriented migration of PECs into the glomerular tuft and their acquisition of CD44 and β1 integrin expression. These findings highlight a critical role for de novo expression of CD9 as a common pathogenic switch driving the PEC phenotype in CGN and FSGS, while offering a potential therapeutic avenue to treat these conditions.

André Görgens, Michel Bremer, Rita Ferrer-Tur, Florian Murke, Tobias Tertel, Peter A Horn, Sebastian Thalmann, Joshua A Welsh, Christine Probst, Coralié Guerin, Chantal M Boulanger, Jennifer C Jones, Helmut Hanenberg, Uta Erdbrügger, Joanne Lannigan, Franz L Ricklefs, Samir El-Andaloussi, Bernd Giebel (2019 Apr 6)

Optimisation of imaging flow cytometry for the analysis of single extracellular vesicles by using fluorescence-tagged vesicles as biological reference material.

Journal of extracellular vesicles : 1587567 : DOI : 10.1080/20013078.2019.1587567

Résumé

Extracellular vesicles (EVs) mediate targeted cellular interactions in normal and pathophysiological conditions and are increasingly recognised as potential biomarkers, therapeutic agents and drug delivery vehicles. Based on their size and biogenesis, EVs are classified as exosomes, microvesicles and apoptotic bodies. Due to overlapping size ranges and the lack of specific markers, these classes cannot yet be distinguished experimentally. Currently, it is a major challenge in the field to define robust and sensitive technological

platforms being suitable to resolve EV heterogeneity, especially for small EVs (sEVs) with diameters below 200 nm, i.e. smaller microvesicles and exosomes. Most conventional flow cytometers are not suitable for the detection of particles being smaller than 300 nm, and the poor availability of defined reference materials hampers the validation of sEV analysis protocols. Following initial reports that imaging flow cytometry (IFCM) can be used for the characterisation of larger EVs, we aimed to investigate its usability for the characterisation of sEVs. This study set out to identify optimal sample preparation and instrument settings that would demonstrate the utility of this technology for the detection of single sEVs. By using CD63eGFP-labelled sEVs as a biological reference material, we were able to define and optimise IFCM acquisition and analysis parameters on an Amnis ImageStreamX MkII instrument for the detection of single sEVs. In addition, using antibody-labelling approaches, we show that IFCM facilitates robust detection of different EV and sEV subpopulations in isolated EVs, as well as unprocessed EV-containing samples. Our results indicate that fluorescently labelled sEVs as biological reference material are highly useful for the optimisation of fluorescence-based methods for sEV analysis. Finally, we propose that IFCM will help to significantly increase our ability to assess EV heterogeneity in a rigorous and reproducible manner, and facilitate the identification of specific subsets of sEVs as useful biomarkers in various diseases.

Elisa Rossi, Sonia Poirault-Chassac, Ivan Bieche, Richard Chocron, Anne Schnitzler, Anna Lokajczyk, Pierre Bourdoncle, Blandine Dizier, Nour C Bacha, Nicolas Gendron, Adeline Blandinieres, Coralie L Guerin, Pascale Gaussem, David M Smadja (2019 Mar 18)

Human Endothelial Colony Forming Cells Express Intracellular CD133 that Modulates their Vasculogenic Properties.

Stem cell reviews and reports : 590-600 : DOI : 10.1007/s12015-019-09881-8

Résumé

Stem cells at the origin of endothelial progenitor cells and in particular endothelial colony forming cells (ECFCs) subtype have been largely supposed to be positive for the CD133 antigen, even though no clear correlation has been established between its expression and function in ECFCs. We postulated that CD133 in ECFCs might be expressed intracellularly, and could participate to vasculogenic properties. ECFCs extracted from cord blood were used either fresh ($n = 4$) or frozen ($n = 4$), at culture days <30, to investigate the intracellular presence of CD133 by flow cytometry and confocal analysis. Comparison with HUVEC and HAEC mature endothelial cells was carried out. Then, CD133 was silenced in ECFCs using specific siRNA (siCD133-ECFCs) or scramble siRNA (siCtrl-ECFCs). siCD133-ECFCs ($n = 12$), siCtrl-ECFCs ($n = 12$) or PBS ($n = 12$) were injected in a hind-limb ischemia nude mouse model and vascularization was quantified at day 14 with H&E staining and immunohistochemistry for CD31. Results of flow cytometry and confocal microscopy evidenced the positivity of CD133 in ECFCs after permeabilization compared with not permeabilized ECFCs ($p < 0.001$) and mature endothelial cells ($p < 0.03$). In the model of mouse hind-limb ischemia, silencing of CD133 in ECFCs significantly abolished post-ischemic revascularization induced by siCtrl-ECFCs; indeed, a significant reduction in cutaneous blood flows ($p = 0.03$), capillary density (CD31) ($p = 0.01$) and myofiber regeneration ($p = 0.04$) was observed. Also, a significant necrosis ($p = 0.02$) was observed in mice receiving

siCD133-ECFCs compared to those treated with siCtrl-ECFCs. In conclusion, our work describes for the first time the intracellular expression of the stemness marker CD133 in ECFCs. This feature could resume the discrepancies found in the literature concerning CD133 positivity and ontogeny in endothelial progenitors.

Alicja Czopek, Rebecca Moorhouse, Léa Guyonnet, Tariq Farrah, Olivia Lenoir, Elizabeth Owen, Job van Bragt, Hannah M Costello, Filippo Menolascina, Véronique Baudrie, David J Webb, David C Kluth, Matthew A Bailey, Pierre-Louis Tharaux, Neeraj Dhaun (2019 Jan 19)
A novel role for myeloid endothelin-B receptors in hypertension.
European heart journal : 768-784 : DOI : 10.1093/eurheartj/ehy881

Résumé

Hypertension is common. Recent data suggest that macrophages (Mφ) contribute to, and protect from, hypertension. Endothelin-1 (ET-1) is the most potent endogenous vasoconstrictor with additional pro-inflammatory properties. We investigated the role of the ET system in experimental and clinical hypertension by modifying Mφ number and phenotype.

Année de publication : 2018

Nour C Bacha, Marilyne Levy, Coralie L Guerin, Bernard Le Bonniec, Annie Harroche, Isabelle Szezepanski, Jean M Renard, Pascale Gaussem, Dominique Israel-Biet, Chantal M Boulanger, David M Smadja (2018 Nov 29)
Treprostinil treatment decreases circulating platelet microvesicles and their procoagulant activity in pediatric pulmonary hypertension.
Pediatric pulmonology : 66-72 : DOI : 10.1002/ppul.24190

Résumé

Pulmonary arterial hypertension (PAH) results from pulmonary vascular disease and may eventually lead to right heart failure and death. Vasodilator therapy has greatly improved PAH prognosis. Circulating microvesicles are considered as surrogate markers of endothelial and hematopoietic cell activation.

Marina Wierz, Sandrine Pierson, Ernesto Gargiulo, Coralie Guerin, Etienne Moussay, Jerome Paggetti (2018 Nov 23)
Purification of Leukemia-Derived Exosomes to Study Microenvironment Modulation.
Methods in molecular biology (Clifton, N.J.) : 231-245 : DOI : 10.1007/978-1-4939-8885-3_16

Résumé

Exosomes are membrane-enclosed vesicles released by different cell types into the extracellular space. As mediators of intercellular communication, they are involved in multiple physiological processes, but they are also associated with the pathogenesis of human malignancies including leukemia. Isolation of exosomes enables the characterization of their role in microenvironment modulation as well as their participation in disease pathology. A variety of strategies and techniques exists to purify exosomes from many biological fluids (e.g., blood, urine, and saliva). Here, we describe the efficient production of large quantities of exosomes from leukemic cell lines by using CELLine bioreactors based on two-compartment technology, as well as their isolation and purification by combining differential centrifugation and ultracentrifugation through a density gradient (17% OptiPrep cushion). Thus, exosomes are appropriately prepared for characterization by western blotting to detect exosome markers or imaging flow cytometry (ImageStream), and for downstream analyses such as the internalization in microenvironmental cells by confocal imaging or flow cytometry, methods which are also described in this chapter.

Antoun Al Absi, Hannah Wurzer, Coralie Guerin, Celine Hoffmann, Flora Moreau, Xianqing Mao, Joshua Brown-Clay, Rémi Petrolli, Carla Pou Casellas, Monika Dieterle, Jean-Paul Thiery, Salem Chouaib, Guy Berchem, Bassam Janji, Clément Thomas (2018 Aug 15)

Actin Cytoskeleton Remodeling Drives Breast Cancer Cell Escape from Natural Killer-Mediated Cytotoxicity.

Cancer research : 5631-5643 : DOI : 10.1158/0008-5472.CAN-18-0441

Résumé

Elucidation of the underlying molecular mechanisms of immune evasion in cancer is critical for the development of immunotherapies aimed to restore and stimulate effective antitumor immunity. Here, we evaluate the role of the actin cytoskeleton in breast cancer cell resistance to cytotoxic natural killer (NK) cells. A significant fraction of breast cancer cells responded to NK-cell attack via a surprisingly rapid and massive accumulation of F-actin near the immunologic synapse, a process we termed « actin response. » Live-cell imaging provided direct evidence that the actin response is associated with tumor cell resistance to NK-cell-mediated cell death. High-throughput imaging flow cytometry analyses showed that breast cancer cell lines highly resistant to NK cells were significantly enriched in actin response-competent cells as compared with susceptible cell lines. The actin response was not associated with a defect in NK-cell activation but correlated with reduced intracellular levels of the cytotoxic protease granzyme B and a lower rate of apoptosis in target cells. Inhibition of the actin response by knocking down CDC42 or N-WASP led to a significant increase in granzyme B levels in target cells and was sufficient to convert resistant breast cancer cell lines into a highly susceptible phenotype. The actin response and its protective effects were fully recapitulated using donor-derived primary NK cells as effector cells. Together, these findings establish the pivotal role of actin remodeling in breast cancer cell resistance to NK-cell-mediated killing. These findings establish the pivotal role of the actin cytoskeleton in driving breast cancer cell resistance to natural killer cells, a subset of cytotoxic lymphocytes with important roles in innate antitumor immunity. .

Xavier Loyer, Ivana Zlatanova, Cecile Devue, Min Yin, Kiave-Yune Howangyin, Phatchanat Klaihmon, Coralie L Guerin, Marouane Kheloufi, Jose Vilar, Konstantinos Zannis, Bernd K Fleischmann, Do Won Hwang, Jongmin Park, Hakho Lee, Philippe Menasché, Jean-Sébastien Silvestre, Chantal M Boulanger (2018 Mar 30)

Intra-Cardiac Release of Extracellular Vesicles Shapes Inflammation Following Myocardial Infarction.

Circulation research : 100-106 : DOI : 10.1161/CIRCRESAHA.117.311326

Résumé

A rapid and massive influx of inflammatory cells occurs into ischemic area after myocardial infarction (MI), resulting in local release of cytokines and growth factors. Yet, the mechanisms regulating their production are not fully explored. The release of extracellular vesicles (EVs) in the interstitial space curbs important biological functions, including inflammation, and influences the development of cardiovascular diseases. To date, there is no evidence for in situ release of cardiac EVs after MI.

Marina Wierz, Sandrine Pierson, Léa Guyonnet, Elodie Viry, Audrey Lequeux, Anaïs Oudin, Simone P Niclou, Markus Ollert, Guy Berchem, Bassam Janji, Coralie Guérin, Jerome Paggetti, Etienne Moussay (2018 Feb 15)

Dual PD1/LAG3 immune checkpoint blockade limits tumor development in a murine model of chronic lymphocytic leukemia.

Blood : 1617-1621 : DOI : 10.1182/blood-2017-06-792267

Résumé

Lynda Zeboudj, Mikael Maître, Lea Guyonnet, Ludivine Laurans, Jeremie Joffre, Jeremie Lemarie, Simon Bourcier, Wared Nour-Eldine, Coralie Guérin, Jonas Friard, Abdelilah Wakkach, Elizabeth Fabre, Alain Tedgui, Ziad Mallat, Pierre-Louis Tharaux, Hafid Ait-Oufella (2018 Jan 13)

Selective EGF-Receptor Inhibition in CD4 T Cells Induces Anergy and Limits Atherosclerosis.

Journal of the American College of Cardiology : 160-172 : DOI : S0735-1097(17)41588-9

Résumé

Several epidermal growth factor receptor (EGFR) inhibitors have been successfully developed for the treatment of cancer, limiting tumor growth and metastasis. EGFR is also expressed by leukocytes, but little is known about its role in the modulation of the immune response.

Année de publication : 2017

Lynda Zeboudj, Andréas Giraud, Lea Guyonnet, Yujiao Zhang, Ludivine Laurans, Bruno Esposito, Jose Vilar, Anna Chipont, Nikolina Papac-Milicevic, Christoph J Binder, Alain Tedgui, Ziad Mallat, Pierre-Louis Tharaux, Hafid Ait-Oufella (2017 Dec 2)

Selective EGFR (Epidermal Growth Factor Receptor) Deletion in Myeloid Cells Limits Atherosclerosis-Brief Report.

Arteriosclerosis, thrombosis, and vascular biology : 114-119 : DOI : 10.1161/ATVBAHA.117.309927

Résumé

To determine the consequences of specific inhibition of EGFR (epidermal growth factor receptor) in myeloid cells in atherosclerosis development.

Nour C Bacha, Adeline Blandinieres, Elisa Rossi, Nicolas Gendron, Nathalie Nevo, Séverine Lecourt, Coralie L Guerin, Jean Marie Renard, Pascale Gaussem, Eduardo Angles-Cano, Chantal M Boulanger, Dominique Israel-Biet, David M Smadja (2017 Nov 5)

Endothelial Microparticles are Associated to Pathogenesis of Idiopathic Pulmonary Fibrosis.

Stem cell reviews and reports : 223-235 : DOI : 10.1007/s12015-017-9778-5

Résumé

Idiopathic pulmonary fibrosis (IPF) is a devastating disease characterized by obliteration of alveolar architecture, resulting in declining lung function and ultimately death. Pathogenic mechanisms remain unclear but involve a concomitant accumulation of scar tissue together with myofibroblasts activation. Microparticles (MPs) have been investigated in several human lung diseases as possible pathogenic elements, prognosis markers and therapeutic targets. We postulated that levels and cellular origins of circulating MPs might serve as biomarkers in IPF patients and/or as active players of fibrogenesis. Flow cytometry analysis showed a higher level of Annexin-V positive endothelial and platelet MPs in 41 IPF patients compared to 22 healthy volunteers. Moreover, in IPF patients with a low diffusing capacity of the lung for carbon monoxide (DL<40%), endothelial MPs (EMPs) were found significantly higher compared to those with DL>40% ($p = 0.02$). We then used EMPs isolated from endothelial progenitor cells (ECFCs) extracted from IPF patients or controls to modulate normal human lung fibroblast (NHLF) properties. We showed that EMPs did not modify proliferation, collagen deposition and myofibroblast transdifferentiation. However, EMPs from IPF patients stimulated migration capacity of NHLF. We hypothesized that this effect could result from EMPs fibrinolytic properties and found indeed higher plasminogen activation potential in total circulating MPs and ECFCs derived MPs issued from IPF patients compared to those isolated from healthy controls MPs. Our study showed that IPF is associated with an increased level of EMPs in the most severe patients, highlighting an active process of endothelial activation in the latter. Endothelial microparticles might contribute to the lung fibroblast invasion

mediated, at least in part, by a fibrinolytic activity.

Takouhie Mgrditchian, Tsolere Arakelian, Jérôme Paggetti, Muhammad Zaeem Noman, Elodie Viry, Etienne Moussay, Kris Van Moer, Stephanie Kreis, Coralie Guerin, Stephanie Buart, Caroline Robert, Christophe Borg, Philippe Vielh, Salem Chouaib, Guy Berchem, Bassam Janji (2017 Oct 29)

Targeting autophagy inhibits melanoma growth by enhancing NK cells infiltration in a CCL5-dependent manner.

Proceedings of the National Academy of Sciences of the United States of America : E9271-E9279 : DOI : 10.1073/pnas.1703921114

Résumé

While blocking tumor growth by targeting autophagy is well established, its role on the infiltration of natural killer (NK) cells into tumors remains unknown. Here, we investigate the impact of targeting autophagy gene Beclin1 () on the infiltration of NK cells into melanomas. We show that, in addition to inhibiting tumor growth, targeting increased the infiltration of functional NK cells into melanoma tumors. We provide evidence that driving NK cells to the tumor bed relied on the ability of autophagy-defective tumors to transcriptionally overexpress the chemokine gene Such infiltration and tumor regression were abrogated by silencing CCL5 in BECN1-defective tumors. Mechanistically, we show that the up-regulated expression of CCL5 occurred through the activation of its transcription factor c-Jun by a mechanism involving the impairment of phosphatase PP2A catalytic activity and the subsequent activation of JNK. Similar to , targeting other autophagy genes, such as , /, or inhibiting autophagy pharmacologically by chloroquine, also induced the expression of in melanoma cells. Clinically, a positive correlation between CCL5 and NK cell marker NKp46 expression was found in melanoma patients, and a high expression level of CCL5 was correlated with a significant improvement of melanoma patients' survival. We believe that this study highlights the impact of targeting autophagy on the tumor infiltration by NK cells and its benefit as a novel therapeutic approach to improve NK-based immunotherapy.

Clément d'Audigier, Sophie Susen, Adeline Blandinieres, Virginie Mattot, Bruno Saubamea, Elisa Rossi, Nathalie Nevo, Séverine Lecourt, Coralie L Guerin, Blandine Dizier, Nicolas Gendron, Bertrand Caetano, Pascale Gaussem, Fabrice Soncin, David M Smadja (2017 Oct 6)

Egfl7 Represses the Vasculogenic Potential of Human Endothelial Progenitor Cells.

Stem cell reviews and reports : 82-91 : DOI : 10.1007/s12015-017-9775-8

Résumé

Egfl7 (VE-statin) is a secreted protein mostly specific to the endothelial lineage during development and in the adult and which expression is enhanced during angiogenesis. Egfl7 involvement in human postnatal vasculogenesis remains unresolved yet. Our aim was to

assess Egfl7 expression in several angiogenic cell types originating from human bone marrow, peripheral blood, or cord blood. We found that only endothelial colony forming cells (ECFC), which are currently considered as the genuine endothelial precursor cells, expressed large amounts of Egfl7. In order to assess its potential roles in ECFC, Egfl7 was repressed in ECFC by RNA interference and ECFC angiogenic capacities were tested in vitro and in vivo. Cell proliferation, differentiation, and migration were significantly improved when Egfl7 was repressed in ECFC in vitro, whereas miR-126-3p levels remained unchanged. In vivo, repression of Egfl7 in ECFC significantly improved post-ischemic revascularization in a model of mouse hind-limb ischemia. In conclusion, ECFC are the sole postnatal angiogenic cells which express large amounts of Egfl7 and whose angiogenic properties are repressed by this factor. Thus, Egfl7 inhibition may be considered as a therapeutic option to improve ECFC-mediated postnatal vasculogenesis and to optimize in vitro ECFC expansion in order to develop an optimized cell therapy approach.

Elisa Rossi, David Smadja, Celine Goyard, Audrey Cras, Blandine Dizier, Nour Bacha, Anna Lokajczyk, Coralie L Guerin, Nicolas Gendron, Benjamin Planquette, Virginie Mignon, Carmelo Bernabéu, Olivier Sanchez, David M Smadja (2017 Aug 4)

Co-injection of mesenchymal stem cells with endothelial progenitor cells accelerates muscle recovery in hind limb ischemia through an endoglin-dependent mechanism.

Thrombosis and haemostasis : 1908-1918 : DOI : 10.1160/TH17-01-0007

Résumé

Endothelial colony-forming cells (ECFCs) are progenitor cells committed to endothelial lineages and have robust vasculogenic properties. Mesenchymal stem cells (MSCs) have been described to support ECFC-mediated angiogenic processes in various matrices. However, MSC-ECFC interactions in hind limb ischemia (HLI) are largely unknown. Here we examined whether co-administration of ECFCs and MSCs bolsters vasculogenic activity in nude mice with HLI. In addition, as we have previously shown that endoglin is a key adhesion molecule, we evaluated its involvement in ECFC/MSC interaction. Foot perfusion increased on day 7 after ECFC injection and was even better at 14 days. Co-administration of MSCs significantly increased vessel density and foot perfusion on day 7 but the differences were no longer significant at day 14. Analysis of mouse and human CD31, and in situ hybridization of the human ALU sequence, showed enhanced capillary density in ECFC+MSC mice. When ECFCs were silenced for endoglin, coinjection with MSCs led to lower vessel density and foot perfusion at both 7 and 14 days ($p<0.001$). Endoglin silencing in ECFCs did not affect MSC differentiation into perivascular cells or other mesenchymal lineages. Endoglin silencing markedly inhibited ECFC adhesion to MSCs. Thus, MSCs, when combined with ECFCs, accelerate muscle recovery in a mouse model of hind limb ischemia, through an endoglin-dependent mechanism.