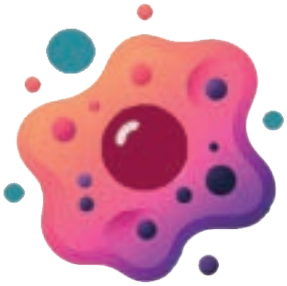
BOOKLET

KICK-OFF MEETING



CEEVEC

Centre d'Expertises & d'Expérimentations
sur les Vésicules Extracellulaires en Cancérologie

TUESDAY, MARCH 12, 2024

BEST WESTERN LA MARINA
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Life Sciences

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PROGRAM

09:30-10:00 > Registration & Welcome Coffee

10:00-10:15 > Introduction & Presentation of CEEVEC
Richard Tomasini, Project Coordinator & Sarah Tubiana, CEEVEC Manager

10:15-11:00 > Keynote Speaker - Clotilde Théry, Institut Curie, Paris
"Extracellular Vesicle heterogeneity, non-vesicular particles, & communication between cancer & the immune system"

11:00-12:00 > Short Talks

11:00-11:15 > "Extracellular vesicles: from signaling mechanistic to therapeutic engineering" Pascale Zimmermann, CRCM, Marseille & KU LEUVEN

11:15-11:30 > "Impact of EVs-mediated crosstalk in pancreatic cancer" Richard Tomasini, CRCM, Marseille

11:30-11:45 > "Role of extracellular vesicles in thrombosis" Romaric Lacroix, C2VN, Marseille

11:45-12:00 > "Presenilins as hub proteins controlling the endocytic and autophagic pathways and small extra cellular vesicle secretion" Inger Lauritzen, IPMC, Sophia Antipolis

12:00-14:00 > Lunch Break & Poster Session

14:00-15:00 > Presentation of Associated Platforms

14:00-14:10 > Flow cytometry platform, C2VN, Marseille – Stéphane Robert

14:10-14:20 > Mass spectometry platform, CRCM, Marseille – Stéphane Audebert & Luc Camoin

14:20-14:30 > Nagben, Marseille – Alain Roussel

14:30-14:40 > Flow cytometry platform, IPMC, Sophia Antipolis – Julie Cazaret

14:40-14:50 > Proteomics platform, IPMC, Sophia Antipolis – Anne-Sophie Gay

14:50-15:00 > Lipidomics platform, IPMC, Sophia Antipolis – Delphine Debayle

15:00-15:30 > Short Talks

15:00-15:15 > "Role of Ptch1 in drug efflux & chemotherapy resistance" Isabelle Mus-Veteau, IPMC, Sophia Antipolis

15:15-15:30 > "Functional analysis of the EV secreted by cancer cells following Fas death receptor activation: possible use of these EV as a biomarker of the immune response efficiency" Laurent Gagnoux, iBV, Nice

15:30-16:00 > Break & Poster Session

16:00-16:30 > Short Talks

16:00-16:15 > "In situ tumor-secreted extracellular vesicles for effective drug delivery in overcoming tumor heterogeneity and dynamic evolution" Christina Galanakou, CiNam, Marseille

16:15-16:30 > "Regulation of Cancer Associated Fibroblasts (CAF) secreting activity by SigmaR1 in pancreatic adenocarcinoma (AdKP)" Olivier Soriani, iBV, Nice

16:30-17:30 > Round Tables & Discussions
1- EVs implication in cancer / EVs in therapeutics
2 - Questions about CEEVEC organization, functioning. What is expected from the CEEVEC?

KEYNOTE SPEAKER
Clotilde Théry - Institut Curie & CurieCoreTech Extracellular Vesicles, Paris

Extracellular Vesicle heterogeneity, non-vesicular particles, and communication between cancer and the immune system

The heterogeneity of extracellular vesicles (EVs) has been increasingly recognized in the last decade, especially with demonstration of the co-existence of EVs originating from either the plasma membrane (ectosomes), or from internal multivesicular compartments (exosomes). More recently, an additional level of heterogeneity of EV preparations induced by the variable co-isolation of non-vesicular extracellular particles (NVEPs), such as exomeres, supermeres, lipoproteins, has also become recognized. I will describe how the International Society of EVs has tackled this situation, by generating community-produced guidelines, including the recently published MISEV2023. And if time allows, I will provide examples of our work, where we aim to decipher the common and specific functions of different EV subtypes and of NVEP in tumor-immune system interaction.

Pascale Zimmermann - CRCM, Marseille & KU LEUVEN

Extracellular vesicles: from signaling mechanistic to therapeutic engineering

I have spent the past 12 years uncovering molecular mechanisms that govern EV-mediated communication between cells. In particular, my team revealed the roles of syndecan-syntenin and associated partners in EV-biogenesis, -loading, -composition, -uptake and -signaling (see e.g PMIDs 37695903, 33602969, 33343836, 32108028, 29109268, 25732677, 24637612, 22660413). I recently decided investigating whether such mechanistic insight may be exploited to design potent EV-based therapeutics - by synthetic biology approaches (PCT/EP2023/050644 - WO/2023/135210). I look forward to discover the interests of the other participants and I hope my expertise will contribute (small or large) to the CEEVEC initiative and the promotion of education, research and innovation in PACA.

Richard Tomasini - DISARM Team, CRCM, Marseille

Impact of EVs-mediated crosstalk in pancreatic cancer

In the last 10 years, our team's efforts were dedicated to decipher EVs-mediated inter-cellular crosstalk between stromal and tumoral cells in pancreatic tumors. Characterized as the most complex intercellular mode of communication, EVs are a powerful tool able to bridge fundamental and translational science. In pancreatic cancers, where up to 90% of the tumor bulk is composed of non-tumoral cells, understanding these stromal/tumor cells exchanges and their consequent impact on tumor ecosystem then tumor physiopathology represents an exciting challenge.

Our studies intend to investigate EVs implication in such inter-cellular exchanges and recently determined how stromal cells-derived EVs bolster tumor cells aggressiveness. Ongoing unpublished data reveal the role of tumor cells-derived EVs on CAFs reprogramming and on fostering an immunosuppressive ecosystem.

Romaric Lacroix - C2VN / AMU, Biogenopole, Haematology Department, APHM, Marseille

Role of extracellular vesicles in thrombosis

Large extracellular vesicles (or microvesicles) have long been associated with a procoagulant activity due to the expression of anionic phospholipids and the exposure of Tissue Factor (the main initiator of the coagulation cascade). Our team has been studying the role of these EVs in hemostasis for the last 3 decades. We deciphered the role of endothelial EVs in these processes and described compensatory mechanisms through the fibrinolytic pathway. Our current view is that the role of EV in hemostasis depends on a balance between their procoagulant and fibrinolytic activities. We developed sensible and specific assays to measure these activities as prognosis biomarkers of thrombosis and survival in different clinical contexts including infectious coagulopathies and cancers. More recently, we are exploring the possibility to modify the coagulolytic balance of these EVs toward a more fibrinolytic potential as a potential vesiculotherapy in coagulopathies.

Inger Lauritzen - IPMC, Sophia Antipolis

Presenilins as hub proteins controlling the endocytic and autophagic pathways and small extracellular vesicle secretion

Emerging evidence indicates that autophagy is tightly connected to the endocytic pathway. Here, we questioned the role of Presenilins (PS1 and PS2), previously shown to be involved in autophagy, in the secretion of exosomes, which are small endocytic-originating extracellular vesicles (sEVs). Indeed, while wild-type cells responded to stimuli promoting both multivesicular endosome (MVE) formation and secretion of sEVs enriched in canonical exosomal proteins, PS-deficient cells remained unaffected to these stimuli. Moreover, in these cells, the re-expression of PS1, or of its PS1delta9 mutant, rescued most sEV secretion, while the deletion of PS1 alone phenocopied total PS invalidation. We show that in PS-deficient cells, the lack of sEV secretion was due to overactivated autophagy promoting MVE degradation. Hence, in these cells, the autophagic blocker bafilomycin A1 (BafA1) increased the intracellular levels of the MVE protein CD63, but also turned on autophagy-dependent unconventional sEV secretion, thus leading to a recovery of sEV secretion. These sEVs were, however, enriched in not only canonical exosomal proteins but also in lysosomal-autophagy-associated cargo. Altogether, we here demonstrate that PSs act as hub proteins controlling the balance between endosomal/autophagic degradation and secretion and more generally strengthens the view of a strong interconnection between the endocytic and autophagic pathways and their complementary roles in sEV secretion.

Isabelle Mus-Veteau - IPMC / UniCA, Sophia Antipolis

Role of Ptch1 in drug efflux and chemotherapy resistance

Hedgehog signaling is aberrantly activated and the Hedgehog receptor Ptch1 is overexpressed in many recurrent and metastatic cancers. We showed that Ptch1 pumps chemotherapeutic agents such as doxorubicin out of cancer cells using the proton motive force and contributes to chemotherapy resistance of several cancer cell types, and that cells overexpressing Ptch1 at their plasma membrane have persistent (or cancer stem cell) properties. We identified small molecules which inhibit the doxorubicin efflux activity of Ptch1 and enhance its cytotoxicity on adrenocortical carcinoma and melanoma cells endogenously overexpressing Ptch1, and thereby mitigates the resistance of these cancer cells to doxorubicin. We also showed that these Ptch1 drug efflux inhibitors enhance the efficacy of kinase inhibitors such as vemurafenib against melanoma cells resistant to the treatment in cellulo and in vivo on xenografts in mice. To better understand the drug efflux mechanism of Ptch1 and to strengthen the specificity of Ptch1 drug efflux inhibitors, we would be interested to overexpressing Ptch1 in extracellular vesicles and use these EV to measure the binding of inhibitors to Ptch1, to purify Ptch1 for structure studies and to produce nanobodies.

Laurent Gagnoux - iBV, Nice

Functional analysis of the EV secreted by cancer cells following Fas death receptor activation: possible use of these EV as a biomarker of the immune response efficiency

Apoptosis is a form of regulated cell death that is crucial to maintain tissue homeostasis by eliminating damaged, cancerous and infected cells. During apoptosis, the dismantling of the cells results in the formation of different types of EV. Most of these apoptotic EV (Apo-EV) arise from plasma membrane blebbing resulting in the release of large vesicles. However, smaller size Apo-EV have been identified as “apoptotic exosomes-like vesicles” (Apo-Exo). Like for EV produced by normal cells, Apo-EV have been associated with diverse physiological functions including cell proliferation during tissue regeneration and regulation of immune responses. Our team is investigating the functions of the cell death receptor Fas, a member of the TNFR superfamily. Fas is expressed on the surface of most human cells and is considered as a tumor suppressor thanks to its ability to eliminate cancer cells by engaging programmed cell death by apoptosis. Indeed, Fas ligand (FasL) is highly expressed by cytotoxic T lymphocytes (CTL) and NK that participate to apoptosis induction of cancer cells expressing the receptor Fas. However, the possible role of Apo-EV secreted following cancer cells apoptosis induced by Fas has never been studied. We show that Fas activation in human epithelial cells leads to formation of various types of extracellular vesicle (EV) including apoptotic exosomes expressing Fas (Fas+Apo-Exo). Proteomic analysis revealed that Fas+Apo-Exo are loaded with Fas, and several immune related molecules mainly involved in T cell

activation, suggesting that these EV may have an important immunomodulatory function by reinforcing T cells activation, acting as a positive feedback amplification loop which favors tumor cells elimination Our project aims now to demonstrate the immune related function of Fas Apo-EV, to decipher their mechanism of biogenesis and to test their possible use as biomarker to evaluate the immune system efficiency in cancer patients, notably upon immunotherapeutic treatment.

Christina Galanakou - Centre Interdisciplinaire de Nanoscience de Marseille (CINaM), CNRS, AMU

In situ tumor-secreted extracellular vesicles for effective drug delivery in overcoming tumor heterogeneity and dynamic evolution

Dendrimers are ideal precision materials for elaborating nanomedicine in cancer therapy by virtue of their well-defined structure, multivalent cooperativity and nanosize per se. In the past 10 years, we have been actively developing modular and adaptive self-assembling dendrimer nanosystems[1] for the delivery of anticancer drugs[2] and nucleic acid therapeutics[3] as well as imaging agents[4] in cancer detection and treatment. Recently, we have discovered that these dendrimer nanosystems are able to exploit the in-situ tumor-secreted extracellular vesicles for effective delivery and deep penetration in tumor tissue, while overcoming tumor heterogeneity and dynamic evolution.[2] This finding offer a fresh perspective for exploiting dendrimer nanomaterials to reach the ultimate goal of nanomedicine.
[1] Acc. Chem. Res. 2020, 53, 2936; Acc. Mater. Res. 2022, 3, 5, 484
[2] Proc. Natl. Acad. Sci. U.S.A. 2023, 120, e2215308120
[3] Proc. Natl. Acad. Sci. U.S.A. 2023, 120, e2220787120
[4] Adv Mater. 2023, 2308262. DOI:10.1002/adma.202308262

Olivier Soriani - iBV / UniCA, Nice

Regulation of Cancer Associated Fibroblasts (CAF) secreting activity by SigmaR1 in pancreatic adenocarcinoma (AdKP)

A significant alteration in cellular electrical patterns accompanies the onset of various diseases, including heart conditions, neurodegenerative disorders, inflammation, and cancers. Increasing evidence suggests that ion channels play a role in cancer progression by influencing calcium balance, cell morphology, and, more recently, signaling pathways via membrane potential control. However, their involvement in intercellular communication within cancerous tissues remains unclear.

The pancreatic stroma comprises immune cells, blood and lymphatic vessels, nerves, cancer-associated fibroblasts (CAF), and an extensive extracellular matrix (ECM). CAFs play a crucial role in pancreatic ductal adenocarcinoma (PDAC) by enhancing ECM deposition, thus contributing significantly to tumor aggressiveness. Through physical interaction and secretion of molecules or vesicles, CAFs enhance cancer cell invasiveness and drug resistance, leading to metastasis. Recent findings indicate that the K+ channel SK2, expressed in PDAC cells, is activated by signals from CAFs, promoting channel phosphorylation via an integrin-EGFR-AKT pathway. This activation triggers a positive feedback loop, enhancing invasiveness in vitro and metastasis in vivo. The formation of a signaling hub involving SK2 and AKT, induced by CAFs, requires the SigmaR1 chaperone. Pharmacological inhibition of SigmaR1 curtails SK2 activation by CAFs, reducing tumor progression and prolonging survival in mice.

Notably, SigmaR1 is also found in CAFs in human PDAC, suggesting its involvement in their pro-tumoral effects. Silencing SigmaR1 in CAFs inhibits proliferation and abolishes the ability of CAF-secreted factors to activate SK2, a critical event in epithelial-mesenchymal transition (EMT) and metastasis. Furthermore, SigmaR1 inhibition alters CAFs' electrical patterns and diminishes the activity of a set of receptor tyrosine kinases (RTKs).

These findings highlight SigmaR1's role in regulating intercellular communication by modulating CAFs' secretome, including soluble components, extracellular vesicles, and the matrisome. The goal is to understand how SigmaR1 and associated channels shape the three compartments of CAFs' secretome.

A live imaging screen of the Zebrafish Extracellular Vesicle/Particle Secretome

Maximilian Fürthauer, Institut de Biologie Valrose, CNRS UMR7277, INSERM1091, Université Côte d'Azur
Xavier Descombes, Equipe Morphème, INRIA/I3S Sophia Antipolis

Extracellular Vesicles (EVs) have emerged as vectors of biological information that control numerous aspects of cancer biology. In addition to EVs, non-membranous Extracellular Particles (EPs) can transport cancer-related cargo and promote tumour growth. The precise roles of EVPs remain however poorly understood, notably due to technical limitations that hamper the analysis of their functions in vivo. Our interdisciplinary consortium is taking advantage of the unique transparency of the embryonic zebrafish to establish tools for the in vivo imaging and computational analysis of EVP secretion and transport. To this aim, the Fürthauer lab has started to use high speed live imaging to characterize EVP behaviour in various organs (heart & blood vessels, brain ventricles & cerebrospinal canal…) during the first 32 hours of zebrafish development. Of particular interest, this approach provides evidence that particular organs secrete only certain types of EVPs. An interdisciplinary collaboration with the group of Xavier Descombes whose expertise lies in the computational analysis of image-based biological datasets, has moreover allowed to generate quantitative analysis pipelines to study EVP transport dynamics. Taking advantage of these approaches, we aim to perform a first imaging-based small-scale screen of the EVP secretome in an intact living vertebrate.

POSTERS

Impact of Organoid-derived Extracellular Vesicles on CAFs and Matrix in PDAC therapeutic resistance

Christopher ROVERA[1], Pierre BERTRAND[2], Thomas BREMONT[1], Sarah TUBIANA[3], Zaïnab HUSSAIN[1], Luc CAMOIN4, Stéphane AUDEBERT[4], Odile GAYET5, Julies ROQUES[5], Nelson DUSETTI[5], Richard TOMASINI[1]

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Resistance to chemotherapy in pancreatic ductal adenocarcinoma (PDAC) is due to molecular alterations in tumour cells and their microenvironment. Tumour cells modify stromal cells to create a supportive microenvironment. Cancer-Associated Fibroblasts (CAFs) produce and degrade the extracellular matrix (ECM), regulating tumour chemoresistance. There is growing evidence that extracellular vesicles (EVs) play a key role in this tumour intercellular dialogue and may be effective biomarkers of cancer progression.
However, few studies take into account the intra-tumour and inter-patient heterogeneity of tumour cells and CAFs. We are working with cultured organoids derived from primary human PDAC tumours and analysed by LC-MS/MS the proteic composition of the EVs they produce. This vesiculome correlates with the presence of locally advanced/metastatic tumours in patients, with different signatures related to the ECM.
To study the impact of these EVs on the CAF ECM, we treated CAFs with organoid-derived EVs and analysed the proteic composition of their ECM by LC-MS/MS. The ECM of CAF treated by two different patients with metastasis-derived organoid EVs is enriched in proteins associated with poor prognosis.
These results suggest that organoid-derived EVs may have predictive value for the development of metastases. These EVs may modify the ECM proteic composition towards a potentially more aggressive profile. By validating specific protein signatures in both EVs and the EV-modified ECM, this work could identify new biomarkers and therapeutic targets to prevent PDAC progression.

ADAM10 supports syndecan cleavage and the loading of pro-tumoral factors into small extracellular vesicles

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ADAM10 metalloprotease is highly expressed in metastatic cancers, mainly known to mediate the ectodomain shedding of target signaling/adhesion molecules. Also, ADAM10 was shown to be addressed to small extracellular vesicles (EV), which carry bioactive molecules that influence target cell behavior. The role of ADAM10 in the biology of tumor EVs was never addressed. Our group previously established that EV biogenesis and loading with cargo rely on syndecans. Syndecans are transmembrane proteins regulating a plethora of signaling receptors deregulated in cancer. Our previous work also suggests that syndecan needs to be cleaved, generating a C-Terminal Fragment (syndecan-CTF), to support EV biogenesis.

Here we reveal that syndecan-4 associates with ADAM10 and specific pro-tumoral factors. We show that ADAM10 loss of expression/function specifically increases the levels of syndecan-full-length into EV. Noteworthy, ADAM10

does not affect the total number of particles released but controls the presence of syndecan-CTF, syntenin and ALIX associated to the EV fraction. Finally, we show that the loss of function/expression of ADAM10 decreases EV-loading of syndecan-dependent pro-tumoral factors.

Altogether, we identify ADAM10 as a regulator of EV composition, addressing specific pro-tumoral factors into EV, most probably by regulating syndecan-4 cleavage. These findings clarify our understanding of the molecular determinants governing EV biology and function. Ultimately, syndecan/ADAM10 crosstalk might be considered as a potential target to inhibit pro-tumoral EV communication.

Dendrimer nanosystems hijack tumor-secreted extracellular vesicle for siRNA delivery

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Nucleic acid therapeutics, if delivered effectively, hold great promise for precision medicine in cancer management. However, nucleic acid delivery systems that can overcome tumor heterogeneity and evolutive nature while achieving deep tumor penetration are challenging to develop yet in high demand. We present here a delivery system based on self-assembling dendrimer nanomicelles for effective siRNA delivery via in situ tumor-secreted extracellular vehicles (EVs), an endogenous transport system that evolves with tumor microenvironment. Specifically, these dendrimers form small, spherical and uniform nanoparticles with siRNA molecules, preventing siRNA from degradation and promoting cellular uptake. Notably, following cellular uptake, these dendrimer nanoparticles have their nucleic acid payload repackaged by the cells into EVs which are further transported and internalized by other cells to propagate delivery. By exploiting the intrinsic features of tumors alongside dendrimer supramolecular chemistry, we can develop smart and effective delivery system to overcome nucleic acid innate flaws, tumor heterogeneity and dynamic evolution thereby improving cancer therapy.

Downregulation of stromal syntenin decreases the secretion of endoglin in extracellular vesicles and sustains AML development

Raphaël LEBLANC[1], Rania GHOSSOUB[1], Armelle GOUBARD[1], Rémy CASTELLANO[1], Joanna FARES[1], Marielle BALZANO[1], Luc CAMOIN[1], Stéphane AUDEBERT[1], Berna BOU-TAYEH[1], Cyril FAURIAT[1], Sylvain GARCIAZ[1,2], Norbert VEY[1,2], Jean-Paul BORG[1], Michel AURRAND-LIONS[1], Guido DAVID[1,3], Pascale ZIMMERMANN[1,3]

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The crosstalk between cancer and stromal cells plays a critical role in tumor progression. Syntenin is a small scaffold protein involved in the regulation of intercellular communication. It allows signaling factors to escape degradation by stimulating (i) their recycling at the cell surface and/or (ii) their secretion by extracellular vesicles (EVs). Syntenin is currently emerging as a target for cancer therapy. Here, we show that certain aggressive forms of acute myeloid leukemia (AML) reduce the expression of syntenin in bone marrow stromal cells (BMSC). Stromal syntenin deficiency, in turn, generates a pro-tumoral microenvironment. From serial transplantations in mice and co-culture experiments, we conclude that syntenin-deficient BMSC stimulate AML aggressiveness by promoting AML cell survival and protein synthesis. On the stromal side, we document that syntenin-deficiency causes an accumulation of endoglin (a TGFβ co-receptor) at the cell surface of the BMSC, associated with a decrease of its loading in the EVs. We establish that endoglin works in trans to support the gain of AML translational activity. In short, our study reveals a vicious signaling loop potentially at the heart of AML-stroma crosstalk and unsuspected tumor-suppressive effects of syntenin that need to be considered during systemic targeting of syntenin in cancer therapy.

Pancreatic cancer cell-derived extracellular vesicles reprogram monocytes & cancer-associated fibroblasts to shape an immunosuppressive microenvironment

Zainab HUSSAIN[1,2,3,4,5], Sarah-Simha TUBIANA[1,2,3,4,5], Eugenie LOHMANN[1,2,3,4,5], Pascal FINETTI[1,2,3,4,5], Magda RODRIGUES[1,2,4,5], Thomas BERTRAN[1,2,3,4,5], Ghislain BIDAUT[1,2,3,4,5], Daniel ISNARDON[1,2,3,4,5], François BERTUCCI[1,2,3,4,5], Stéphane AUDEBERT[1,2,3,4,5], Luc CAMOIN[1,2,3,4,5], Moacyr REGO[6], Richard TOMASINI[1,2,3,4,5]

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Cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs) are predominating cells of pancreatic cancer (PDAC) tumor microenvironment (TME), acting as a barrier against anti-tumoral immunity and therapies. However, the intricate cellular crosstalk established with tumor cells and the role of extracellular vesicles (EVs) in such communicative networks are poorly understood. We hypothesized that tumor cell-derived EVs (TC-EVs) could foster an immunosuppressive environment through simultaneous modulation of CAFs and monocytes. Using monocytes and PDAC tumor cells, we determined that direct TC-EVs treatment induced monocytes differentiation into immunosuppressive CD206+/PD-L1+/HLA-DR- macrophages. In CAFs, TC-EVs treatments induced reorganization of the extracellular matrix (ECM). Importantly, we demonstrated that monocytes cultured on TC-EVs treated CAFs derived ECM also induced differentiation into CD206+/PD-L1+/HLA-DR- macrophages, revealing the indirect action of TC-EVs to establish an immunosuppressive environment. Directly and indirectly differentiated macrophages hindered T-cell activation and anti-tumoral activity. Altogether our data highlighted novel, dual mechanisms of TC-EVs-mediated cellular crosstalk, impacting simultaneously infiltrating monocytes and CAFs to establish an immunosuppressive TME.

Fibronectin from stromal-derived extracellular vesicles drives FOLFIRINOX resistance in pancreatic cancer

Jérémy NIGRI[1,2,3,4,5], Pascal FINETTI[1,2,3,4,5], Sarah-Simha TUBIANA[1,2,3,4,5], Tristan GICQUEL[1,2,3,4,5], Dolores BAREA[1,2,3,4,5], Odile GAYET[1,2,3,4,5], Zainab HUSSAIN[1,2,3,4,5], Fabienne GUILLAUMOND[1,2,3,4,5], Sophie VASSEUR[1,2,3,4,5], Stéphane AUDEBERT[1,2,3,4,5], Nelson DUSETTI[1,2,3,4,5], Juan-Lucio IOVANNA[1,2,3,4,5], Daniel BIRNBAUM[1,2,3,4,5], Ellen VAN OBBERGHEN-SCHILLING[3,5,6,7,8], Luc CAMOIN[1,2,3,4,5], François BERTUCCI[1,2,3,4,5,9], Richard TOMASINI[1,2,3,4,5]

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Stromal to tumor cell crosstalks are mandatory for pancreatic cancer development and drug resistance, representing a growing source of therapeutic strategies. The stromal compartment represents up to 80% of the tumor mass and corroborates with tumor progression. However, our ability to assess the cellular and mechanistic basis of how stromal to tumor cell crosstalks support chemoresistance have been limited. Here, we explored the impact of FOLFIRINOX treated stromal cells-derived extracellular vesicles (EVs) on the chemoprotection of cancer cells. Through a combination of stromal cell co-cultures and cancer cell spheroids, we observed that specific EVs produced by FOLFIRINOX treated-stromal cells favor cancer cells chemoresistance. Integrated single-cell RNA-seq

and mass spectrometry studies revealed spheroid chemoresistance as associated with Fibronectin/Integrinb1 signaling and cell cycle-related clonal composition, in correlation with the patient's prognosis. Collectively, our results outline how EVs-driven stromal/tumor cell crosstalk promotes chemoresistance, paving the way for new combinatory targeted therapy.

MT1-MMP and the syntenin-syndecan exosomal pathway, partners in crime for matrix invasion?

Marie HUBER[1], Emilie CONNEN[1], Rania GHOSSOUB[1], Raphaël LEBLANC[1], Guido DAVID[2], Pascale ZIMMERMANN[1,2], Sylvie THUAULT[1]

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Matrix metalloproteinases (MMPs) control the degradation and activity of extracellular matrix components (ECM). Overexpression of MT1-MMP, a transmembrane MMP, predicts poor prognosis in triple negative breast cancer (TNBC). Cellular studies extensively document that MT1-MMP is delivered to invadopodia, cancer cell structures allowing localized ECM degradation. MT1-MMP is also present at the membrane of exosomes, small extracellular vesicles (sEVs) of endosomal origin. However, the mechanisms supporting MT1-MMP loading into exosomes and the role of exosomal MT1-MMP in invadopodia activity are obscure. The syntenin PDZ protein, in association with the syndecan heparan sulfate proteoglycans, are major players controlling exosome biogenesis. Here, we show that MT1-MMP and syntenin colocalize in MDA-MB-231 TNBC cells and that the intracellular domain of MT1-MMP directly interacts with syntenin, although independently of the PDZ binding motif of MT1-MMP. Depletion of syntenin and syndecan impairs the loading of MT1-MMP in sEVs, without affecting its levels at the plasma membrane. In addition, syntenin depletion impairs the degradative potential of MDA-MB-231 cells. These results suggest that the syntenin-syndecan exosomal pathway might regulate the invasive potential of cancer cells through sorting of MT1-MMP in sEVs, and possibly through the control of its delivery to invadopodia, as sEVs and invadopodia might function in a coordinated manner. This pathway could be targeted for TNBC treatment or used for engineering therapeutic sEVs with increased tumor penetration.

A rational and flexible genetic approach to produce customized extracellular vesicles

Lukas HYKA[1,2], Sofie MEEUSSEN[1], Guido DAVID[1,2], Patrick CHAMES[2], Zimmermann PASCALE[1,2]

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Extracellular vesicles (EVs) are nano-sized membrane-limited particles released by various cells that play a crucial role in intercellular communication. These vesicles carry proteins, lipids, and nucleic acids, influencing recipient cells and contributing to various physiological processes. Importantly, EVs can be modified e.g. to contain therapeutic cargo making them interesting vehicles for drug delivery. The field is currently exploring different strategies for efficient cargo loading inside EVs and for decorating their surface so that they can target specific cells. Here, we present a generic approach to prepare customized EVs containing, and simultaneously harboring at their surface, proteins of choice. For that, producing cells are modified by two types of expression vectors. The first encodes syndecan-1 C-terminal fragment (for surface modification) and the second syntenin (for cargo loading). The open reading frames (ORF) of the syndecan-1 C-terminal fragment and syntenin moieties are meant to be fused to ORF of polypeptide of interest to decorate the surface or load the lumen of EVs. As the syndecan-1 and syntenin moieties boost the production of EVs by non-covalent interaction, this strategy is expected to facilitate both the production of EVs and efficient cargo delivery in target cells. As a proof-of-concept we constructed vectors encoding nanobody (for surface expression) and Cas9 (for cargo loading). We patented efficient EV loading and cargo delivery as WO/2023/135210 EXTRACELLULAR VESICLES.

A rational and flexible genetic approach to produce customized extracellular vesicles

Lukas HYKA[1,2], Sofie MEEUSSEN[1], Guido DAVID[1,2], Patrick CHAMES[2], Zimmermann PASCALE[1,2]

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Extracellular vesicles (EVs) are nano-sized membrane-limited particles released by various cells that play a crucial role in intercellular communication. These vesicles carry proteins, lipids, and nucleic acids, influencing recipient cells and contributing to various physiological processes. Importantly, EVs can be modified e.g. to contain therapeutic cargo making them interesting vehicles for drug delivery. The field is currently exploring different strategies for efficient cargo loading inside EVs and for decorating their surface so that they can target specific cells. Here, we present a generic approach to prepare customized EVs containing, and simultaneously harboring at their surface, proteins of choice. For that, producing cells are modified by two types of expression vectors. The first encodes syndecan-1 C-terminal fragment (for surface modification) and the second syntenin (for cargo loading). The open reading frames (ORF) of the syndecan-1 C-terminal fragment and syntenin moieties are meant to be fused to ORF of polypeptide of interest to decorate the surface or load the lumen of EVs. As the syndecan-1 and syntenin moieties boost the production of EVs by non-covalent interaction, this strategy is expected to facilitate both the production of EVs and efficient cargo delivery in target cells. As a proof-of-concept we constructed vectors encoding nanobody (for surface expression) and Cas9 (for cargo loading). We patented efficient EV loading and cargo delivery as WO/2023/135210 EXTRACELLULAR VESICLES.

PDZ scaffolds regulate extracellular vesicle production, composition, and uptake

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Extracellular vesicles (EVs) are membrane-limited organelles mediating cell-to-cell communication in health and disease. EVs are of high medical interest, but their rational use for diagnostics or therapies is restricted by our limited understanding of the molecular mechanisms governing EV biology. Here, we tested whether PDZ proteins, molecular scaffolds that support the formation, transport, and function of signal transduction complexes and that coevolved with multicellularity, may represent important EV regulators. We reveal that the PDZ proteome (ca. 150 proteins in human) establishes a discrete number of direct interactions with the tetraspanins CD9, CD63, and CD81, well-known EV constituents. Strikingly, PDZ proteins interact more extensively with syndecans (SDCs), ubiquitous membrane proteins for which we previously demonstrated an important role in EV biogenesis, loading, and turnover. Nine PDZ proteins were tested in loss-of-function studies. We document that these PDZ proteins regulate both tetraspanins and SDCs, differentially affecting their steady-state levels, subcellular localizations, metabolism, endosomal budding, and accumulations in EVs. Importantly, we also show that PDZ proteins control the levels of heparan sulfate at the cell surface that functions in EV capture. In conclusion, our study establishes that the extensive networking of SDCs, tetraspanins, and PDZ proteins contributes to EV heterogeneity and turnover, highlighting an important piece of the molecular framework governing intracellular trafficking and intercellular communication.

NUMB inhibits invasive properties by controlling the catalytic nucleotide exchange activity of EFA6B in a breast cancer cell model

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Breast cancer is the most common cancer in women globally, but also the best described. We have observed that decreased expression of the EFA6B protein in breast tumors is associated with a poor prognostic factor. EFA6B is involved in membrane transport, actin cytoskeleton reorganization and the establishment of epithelial polarity. Part of its action derives from its ability to activate the small G protein Arf6.

Previous work carried out by the laboratory has demonstrated that a reduction in EFA6B expression levels in normal mammary cells results in the acquisition of a partially mesenchymal phenotype and collective invasion properties.
Furthermore, another study carried out by the laboratory shows that the NUMB protein, known to be a tumour suppressor but also to be involved in endocytosis processes, is a direct partner of EFA6B capable of strongly stimulating Arf6 activation. Here, we investigate the molecular mechanisms implicating these proteins in the negative control of Epithelial-Mesenchymal Transition and invasive processes. We show that overexpression of NUMB in mammary KO cells heterozygous for EFA6B inhibits the invasive process, whereas it has no effect on homozygous KO cells.
These results suggest that activation of the residual EFA6B pool is sufficient and necessary to restore a non-invasive phenotype.

Exosome-mediated paracrine signaling unveils miR-1246 as a driver of aggressiveness in fusion-negative rhabdomyosarcoma

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Rhabdomyosarcoma is a pediatric cancer associated with aggressiveness and a tendency to develop metastases. Fusion-negative rhabdomyosarcoma (FN-RMS) is the most commonly occurring subtype of RMS where metastatic disease can hinder treatment success and decrease survival rates. RMS-derived exosomes were previously demonstrated to be enriched with miRNA, including miR-1246, possibly contributing to disease aggressiveness.

We aimed to decipher the functional impact of exosomal miR-1246 on recipient cells and its role in promoting aggressiveness. Treatment of normal fibroblasts with FN-RMS-derived exosomes resulted in a significant uptake of miR-1246 paired with an increase in cell proliferation, migration and invasion. In turn, delivery of miR-1246 mimics-lipoplexes promoted fibroblast proliferation, migration and invasion in a similar manner. Conversely, when

silencing miR-1246 in FN-RMS cells, the resulting derived exosomes demonstrated reversed effects on recipient cells' phenotype. Delivery of exosomal miR-1246 targets GSK3β and promotes β-catenin nuclear accumulation, suggesting a deregulation of the Wnt pathway, known to be important in tumor progression. Finally, a pilot clinical study highlighted, for the first time, the presence of high exosomal miR-1246 levels in RMS patients' sera.

Altogether, our results demonstrate that exosomal miR-1246 has the potential to alter the tumor microenvironment of FN-RMS cells, suggesting its potential role in promoting oncogenesis.

Extracellular vesicles: a powerful tool for membrane protein studies

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Membrane proteins (MPs) expressed at the surface of living cells are involved in many biological processes such as virus entry receptors, cellular immune response antigens or cancer biomarkers. These targets are the subject of intense structural and functional studies to develop therapeutic antibodies. But the production and purification of these proteins under their native membranous forms is a difficult task because of their instability once extracted from their original membrane. Currently, most processes involve the extraction and purification of these proteins in a detergent and their stabilization in artificial environments. These costly and time-consuming methods do not always allow to preserve the natural oligomeric state and the complex architecture of these therapeutic targets. Most eukaryotic cells can produce extracellular vesicles (EVs) which constitute true intercellular communication pathways. They are made of a lipid bilayer derived from the plasma membrane and contains MPs at the vesicle surface. At CytobodX, we have developed a process to rapidly obtain and purify recombinant EVs released from mammalian cells and coated with an overexpressed MP of interest. This technology has been validated on several GPI anchored MPs (e.g. CD73), transmembrane domains type 1 MPs (e.g. ACE2 and CD16a) and transmembrane domains type 2 MPs (e.g. CD38 or GABA transporter 1). Stability, functionality, and integrity of the MPs incorporated in the recombinant EVs has been confirmed especially by using electron microscopy. EVs can be used for antibody generation and selection by immunization, phage-display, flow cytometry or biolayer interferometry. We have been able to observe the considerable advantages brought using EVs-based MPs compared to the use of soluble protein ectodomains in terms of immunization efficiency and antigen presentation. Recombinant EVs are an advantageous tool for obtaining, storing, and using MPs in an environment as close as possible to their natural state.