Année de publication : 2019

Emeline Bonsergent, Gregory Lavieu (2019 Jun 9)

Content release of extracellular vesicles in a cell-free extract.

FEBS letters : DOI : 10.1002/1873-3468.13472

Résumé

Extracellular Vesicles (EVs) transfer molecules from donor to acceptor cells. The EV-content delivery process within the acceptor cell is poorly characterized. We developed a new cell-free assay to assess EV-content release in vitro. We found that EV-cytosolic cargoes are released from EVs when isolated vesicles are incubated with purified plasma membrane sheets at acidic pH, a characteristic of the endo/lysosomal environment. This process is protein dependent. Our results suggest that EV-content delivery occurs within the endo/lysosomes of acceptor cells and is triggered by acidification. This process resembles virus content delivery and may require membrane fusion. The assay presented here will facilitate investigations into the core machinery and mechanisms underlying EV content delivery. This article is protected by copyright. All rights reserved.

Ouahiba Sihali-Beloui, Djamila Aroune, Fella Benazouz, Adile Hadji, Salima El-Aoufi, Sergio Marco (2019 Jun 2)

A hypercaloric diet induces hepatic oxidative stress, infiltration of lymphocytes, and mitochondrial reshuffle in Psammomys obesus, a murine model of insulin resistance.

Comptes rendus biologies : DOI : S1631-0691(19)30041-1

Résumé

The aim of this study was to show, for the first time, the effect of a hypercaloric diet on the mitochondrial reshuffle of hepatocytes during the progression from steatosis to steatohepatitis to cirrhosis in Psammomys obesus, a typical animal model of the metabolic syndrome. Metabolic and oxidative stresses were induced by feeding the animal through a standard laboratory diet (SD) for nine months. Metabolic parameters, liver malondialdehyde (MDA) and glutathione (GSH), were evaluated. The pathological evolution was examined by histopathology and immunohistochemistry, using CD3 and CD20 antibodies. The dynamics of the mitochondrial structure was followed by transmission electron microscopy. SD induced a steatosis in this animal that evolved under the effect of oxidative and metabolic stress by the appearance of adaptive inflammation and fibrosis leading the animal to the cirrhosis stage with serious hepatocyte damage by the triggering, at first the mitochondrial fusion-fission cycles, which attempted to maintain the mitochondria intact and functional, but the hepatocellular oxidative damage was increased inducing a vicious circle of mitochondrial alteration and dysfunction and their elimination by mitophagy. P. obesus is an excellent animal model of therapeutic research that targets mitochondrial dysfunction in the progression of steatosis.

Juan José Sáez, Ana-María Lennon-Duménil, María-Isabel Yuseff (2019 Jun 1)
Studying MHC Class II Presentation of Immobilized Antigen by B Lymphocytes.
Methods in molecular biology (Clifton, N.J.) : 419-437 : DOI : 10.1007/978-1-4939-9450-2_29

Résumé

The ability of B lymphocytes to capture external antigens (Ag) and present them as peptide fragments, loaded on major histocompatibility complex (MHC) class II molecules, to CD4 T cells is a crucial part of the adaptive immune response. This allows for T-B cooperation, a cellular communication that is required for B cells to develop into germinal centers (GC) and form mature high affinity antibody producing cells and to further develop B cell memory. MHC class II antigen presentation by B lymphocytes is a multistep process involving (1) Recognition and capture of external Ag by B lymphocytes through their B cell receptor (BCR), (2) Ag processing, which comprises the degradation of Ag in internal compartments within the B cell and loading of the corresponding peptide fragments on MHC class II molecules, and (3) Presentation of MHCII-peptide complexes to CD4 T cells. Here, we describe how to study the biochemical and morphological changes that occur in B lymphocytes at these three major levels.

Lou Fourriere, Amal Kasri, Nelly Gareil, Sabine Bardin, Hugo Bousquet, David Pereira, Franck Perez, Bruno Goud, Gaelle Boncompain, Stéphanie Miserey-Lenkei (2019 May 31)
RAB6 and microtubules restrict protein secretion to focal adhesions.
The Journal of cell biology : DOI : 10.1083/jcb.201805002

Résumé

To ensure their homeostasis and sustain differentiated functions, cells continuously transport diverse cargos to various cell compartments and in particular to the cell surface. Secreted proteins are transported along intracellular routes from the endoplasmic reticulum through the Golgi complex before reaching the plasma membrane along microtubule tracks. Using a synchronized secretion assay, we report here that exocytosis does not occur randomly at the cell surface but on localized hotspots juxtaposed to focal adhesions. Although microtubules are involved, the RAB6-dependent machinery plays an essential role. We observed that, irrespective of the transported cargos, most post-Golgi carriers are positive for RAB6 and that its inactivation leads to a broad reduction of protein secretion. RAB6 may thus be a general regulator of post-Golgi secretion.

Kevin Grosselin, Adeline Durand, Justine Marsolier, Adeline Poitou, Elisabetta Marangoni, Fariba Nemati, Ahmed Dahmani, Sonia Lameiras, Fabien Reyal, Olivia Frenoy, Yannick Pousse, Marcel Reichen, Adam Woolfe, Colin Brenan, Andrew D. Griffiths*, Céline Vallot* & Annabelle Gérard* (2019 May 31)
High-throughput single-cell ChIP-seq identifies heterogeneity of chromatin

states in breast cancer
Nature Genetics : 1060–1066 : DOI : 10.1038/s41588-019-0424-9

Résumé

Dapeng Zhang, Yujiao Fan, Hui Chen, Sylvain Trépout, Min-Hui Li (2019 May 31)
CO_2-activated reversible transition between polymersomes and micelles with AIE fluorescence.
Angewandte Chemie (International ed. in English) : Accepted Author Manuscript : DOI : 10.1002/anie.201905089

Résumé

Fluorescent polymersomes with both aggregation-induced emission (AIE) and CO_2-responsive properties were developed from amphiphilic block copolymer PEG-b-P(DEAEMA-co-TPEMA) in which the hydrophobic block was a copolymer made of tetraphenylethene functionalized methacrylate (TPEMA) and 2-(diethylamino)ethyl methacrylate (DEAEMA) with unspecified sequence arrangement. Four block copolymers with different DEAEMA/TPEMA and hydrophilic/hydrophobic ratios were synthesized and bright AIE polymersomes were prepared by nanoprecipitation in THF/water and dioxane/water systems. Polymersomes of PEG45-b-P(DEAEMA36-co-TPEMA6) were chosen to study the CO_2-responsive property. Upon CO_2 bubbling vesicles transformed to small spherical micelles, and upon Ar bubbling micelles returned to vesicles with the presence of a few intermediate morphologies. These polymersomes might have promising applications as sensors, nanoreactors or controlled release systems.

Aude Burlion, Rodrigo N Ramos, Pukar Kc, Kélhia Sendeyo, Aurélien Corneau, Christine Ménétrier-Caux, Eliane Piaggio, Daniel Olive, Christophe Caux, Gilles Marodon (2019 May 31)
A novel combination of chemotherapy and immunotherapy controls tumor growth in mice with a human immune system.
Oncoimmunology : 1596005 : DOI : 10.1080/2162402X.2019.1596005

Résumé

Mice reconstituted with a human immune system and bearing human tumors represent a promising model for developing novel cancer immunotherapies. Here, we used mass cytometry and multi-parametric flow cytometry to characterize human leukocytes infiltrating a human breast cancer tumor model in immunocompromised NOD.SCID.γc-null mice reconstituted with a human immune system and compared it to samples of breast cancer patients. We observed highly activated human CD4 and CD8 T cells in the tumor, as well as minor subsets of innate immune cells in both settings. We also report that ICOS CD4 regulatory T cells (Treg) were enriched in the tumor relative to the periphery in humanized mice and patients, providing a target to affect Treg and tumor growth. Indeed,

administration of a neutralizing mAb to human ICOS reduced Treg proportions and numbers and improved CD4 + T cell proliferation in humanized mice. Moreover, a combination of the anti-ICOS mAb with cyclophosphamide reduced tumor growth, and that was associated with an improved CD8 to Treg ratio. Depletion of human CD8 T cells or of murine myeloid cells marginally affected the effect of the combination therapy. Altogether, our results indicate that a combination of anti-ICOS mAb and chemotherapy controls tumor growth in humanized mice, opening new perspectives for the treatment of breast cancer. One sentence summary: Targeting ICOS in combination with chemotherapy is a promising strategy to improve tumor immunity in humans.

Olivier Lantz, François Legoux (2019 May 30)
MAIT cells: programmed in the thymus to mediate immunity within tissues.
Current opinion in immunology : 75-82 : DOI : S0952-7915(18)30079-7

Résumé

MAIT cells are an evolutionarily conserved T cell subset recognizing ubiquitous microbial metabolites. Herein, we review recent literature showing that MAIT cells can be divided into type 1 and type 17 subsets, which acquire a tissue resident differentiation program in the thymus and localize in specific tissues. We also discuss the nature and in vivo availability of the different agonist and antagonist MAIT ligands with potential consequences for MAIT cell biology.

Emilia Puig Lombardi, Allyson Holmes, Daniela Verga, Marie-Paule Teulade-Fichou, Alain Nicolas, Arturo Londoño-Vallejo (2019 May 23)
Thermodynamically stable and genetically unstable G-quadruplexes are depleted in genomes across species.
Nucleic acids research : gkz463 : 2019-2020 : DOI : 10.1093/nar/gkz463

Résumé

G-quadruplexes play various roles in multiple biological processes, which can be positive when a G4 is involved in the regulation of gene expression or detrimental when the folding of a stable G4 impairs DNA replication promoting genome instability. This duality interrogates the significance of their presence within genomes. To address the potential biased evolution of G4 motifs, we analyzed their occurrence, features and polymorphisms in a large spectrum of species. We found extreme bias of the short-looped G4 motifs, which are the most thermodynamically stable in vitro and thus carry the highest folding potential in vivo. In the human genome, there is an over-representation of single-nucleotide-loop G4 motifs (G4-L1), which are highly conserved among humans and show a striking excess of the thermodynamically least stable G4-L1A (G3AG3AG3AG3) sequences. Functional assays in yeast showed that G4-L1A caused the lowest levels of both spontaneous and G4-ligand-induced instability. Analyses across 600 species revealed the depletion of the most stable G4-L1C/T quadruplexes in most genomes in favor of G4-L1A in vertebrates or G4-L1G in

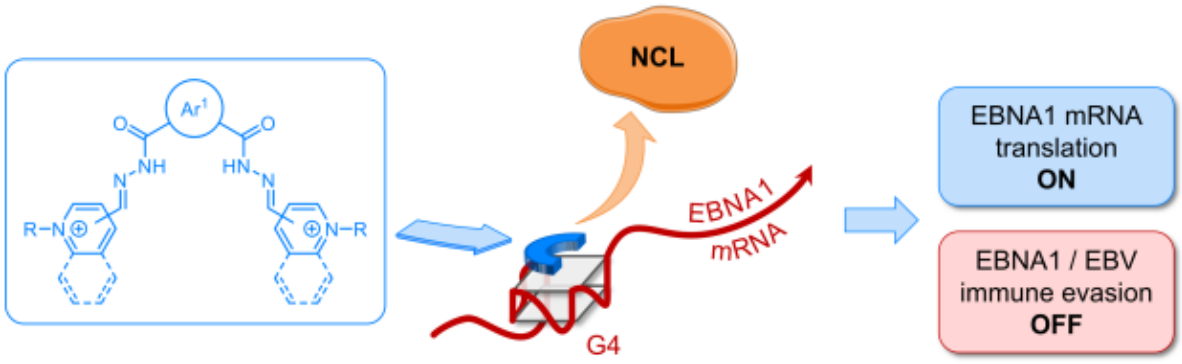
other eukaryotes. We discuss how these trends might be the result of species-specific mutagenic processes associated to a negative selection against the most stable motifs, thus neutralizing their detrimental effects on genome stability while preserving positive G4-associated biological roles.

Oksana Reznichenko, Alicia Quillévéré, Rodrigo Prado Martins, Nadège Loaëc, Hang Kang, María José Lista, Claire Beauvineau, Jorge González-García, Régis Guillot, Cécile Voisset, Chrysoula Daskalogianni, Robin Fåhraeus, Marie-Paule Teulade-Fichou, Marc Blondel, Anton Granzhan (2019 May 23)

Novel cationic bis(acylhydrazones) as modulators of Epstein–Barr virus immune evasion acting through disruption of interaction between nucleolin and G-quadruplexes of EBNA1 mRNA

European Journal of Medicinal Chemistry : 178 : 13-29 : DOI : 10.1016/j.ejmech.2019.05.042

Résumé



The oncogenic Epstein-Barr virus (EBV) evades the immune system through limiting the expression of its highly antigenic and essential genome maintenance protein, EBNA1, to the minimal level to ensure viral genome replication, thereby also minimizing the production of EBNA1-derived antigenic peptides. This regulation is based on inhibition of translation of the virally-encoded EBNA1 mRNA, and involves the interaction of host protein nucleolin (NCL) with G-quadruplex (G4) structures that form in the glycine–alanine repeat (GAr)-encoding sequence of the EBNA1 mRNA. Ligands that bind to these G4-RNA can prevent their interaction with NCL, leading to disinhibition of EBNA1 expression and antigen presentation, thereby interfering with the immune evasion of EBNA1 and therefore of EBV (M.J. Lista et al., *Nature Commun.*, **2017**, *8*, 16043). In this work, we synthesized and studied a series of 20 cationic bis(acylhydrazone) derivatives designed as G4 ligands. The *in vitro* evaluation showed that most derivatives based on central pyridine (Py), naphthyridine (Naph) or phenanthroline (Phen) units were efficient G4 binders, in contrast to their pyrimidine (Pym) counterparts, which were poor G4 binders due to a significantly different molecular geometry. The influence of lateral heterocyclic units (*N*-substituted pyridinium or quinolinium residues) on G4-binding properties was also investigated. Two novel compounds, namely **PyDH2** and **PhenDH2**, used at a 5 µM concentration, were able to significantly enhance EBNA1 expression in H1299 cells in a GAr-dependent manner, while being significantly less

toxic than the prototype drug **PhenDC3** ($GI_{50} > 50\,\mu M$). Antigen presentation, RNA pull-down and proximity ligation assays confirmed that the effect of both drugs was related to the disruption of NCL–EBNA1 mRNA interaction and the subsequent promotion of GAr-restricted antigen presentation. Our work provides a novel modular scaffold for the development of G-quadruplex-targeting drugs acting through interference with G4–protein interaction.

Gervais L, van den Beek M, Josserand M, Sallé J, Stefanutti M, Perdigoto CN, Skorski P, Mazouni K, Marshall OJ, Brand AH, Schweisguth F, Bardin AJ (2019 May 20)
Stem Cell Proliferation Is Kept in Check by the Chromatin Regulators Kismet/CHD7/CHD8 and Trr/MLL3/4
Developmental Cell : DOI : https://doi.org/10.1016/j.devcel.2019.04.033

Résumé

Chromatin remodeling accompanies differentiation, however, its role in self-renewal is less well understood. We report that in Drosophila, the chromatin remodeler Kismet/CHD7/CHD8 limits intestinal stem cell (ISC) number and proliferation without affecting differentiation. Stem-cell-specific whole-genome profiling of Kismet revealed its enrichment at transcriptionally active regions bound by RNA polymerase II and Brahma, its recruitment to the transcription start site of activated genes and developmental enhancers and its depletion from regions bound by Polycomb, Histone H1, and heterochromatin Protein 1. We demonstrate that the Trithorax-related/MLL3/4 chromatin modifier regulates ISC proliferation, colocalizes extensively with Kismet throughout the ISC genome, and co-regulates genes in ISCs, including Cbl, a negative regulator of Epidermal Growth Factor Receptor (EGFR). Loss of kismet or trr leads to elevated levels of EGFR protein and signaling, thereby promoting ISC self-renewal. We propose that Kismet with Trr establishes a chromatin state that limits EGFR proliferative signaling, preventing tumor-like stem cell overgrowths.

Mélanie Durand, Thomas Walter, Tiphène Pirnay, Thomas Naessens, Paul Gueguen, Christel Goudot, Sonia Lameiras, Qing Chang, Nafiseh Talaei, Olga Ornatsky, Tatiana Vassilevskaia, Sylvain Baulande, Sebastian Amigorena, Elodie Segura (2019 May 11)
Human lymphoid organ cDC2 and macrophages play complementary roles in T follicular helper responses.
The Journal of experimental medicine : DOI : jem.20181994

Résumé

CD4 T follicular helper (Tfh) cells are essential for inducing efficient humoral responses. T helper polarization is classically orientated by dendritic cells (DCs), which are composed of several subpopulations with distinct functions. Whether human DC subsets display functional specialization for Tfh polarization remains unclear. Here we find that tonsil cDC2 and CD14 macrophages are the best inducers of Tfh polarization. This ability is intrinsic to the cDC2 lineage but tissue dependent for macrophages. We further show that human Tfh cells comprise two effector states producing either IL-21 or CXCL13. Distinct mechanisms drive

the production of Tfh effector molecules, involving IL-12p70 for IL-21 and activin A and TGFβ for CXCL13. Finally, using imaging mass cytometry, we find that tonsil CD14 macrophages localize in situ in the B cell follicles, where they can interact with Tfh cells. Our results indicate that human lymphoid organ cDC2 and macrophages play complementary roles in the induction of Tfh responses.

Edith Borcoman, Philippe De La Rochere, Wilfrid Richer, Sophie Vacher, Walid Chemlali, Clémentine Krucker, Nanour Sirab, Francois Radvanyi, Yves Allory, Géraldine Pignot, Nicolas Barry de Longchamps, Diane Damotte, Didier Meseure, Christine Sedlik, Ivan Bieche, Eliane Piaggio (2019 May 10)

Inhibition of PI3K pathway increases immune infiltrate in muscle-invasive bladder cancer.

Oncoimmunology : e1581556 : DOI : 10.1080/2162402X.2019.1581556

Résumé

Although immune checkpoint inhibitors have shown improvement in survival in comparison to chemotherapy in urothelial bladder cancer, many patients still fail to respond to these treatments and actual efforts are made to identify predictive factors of response to immunotherapy. Understanding the tumor-intrinsic molecular basis, like oncogenic pathways conditioning the presence or absence of tumor-infiltrating T cells (TILs), should provide a new rationale for improved anti-tumor immune therapies. In this study, we found that urothelial bladder cancer from human samples bearing gene mutations was significantly associated with lower expression of a defined immune gene signature, compared to unmutated ones. We identified a reduced 10-gene immune gene signature that discriminates muscle-invasive bladder cancer (MIBC) samples according to immune infiltration and mutation. Using a humanized mouse model, we observed that BKM120, a pan-PI3K inhibitor, significantly inhibited the growth of a human bladder cancer cell line bearing a mutation, associated to increased immune cell infiltration (hCD45+). Using qRT-PCR, we also found an increase in the expression of chemokines and immune genes in mutated tumors from mice treated with BKM120, reflecting an active immune infiltrate in comparison to untreated ones. Moreover, the addition of BKM120 rendered -mutated tumors sensitive to PD-1 blockade. Our results provide a relevant rationale for combination strategies of PI3K inhibitors with immune checkpoint inhibitors to overcome resistance to immune checkpoint inhibitors.

Perrine Lavalou, Helene Eckert, Louise Damy, Florian Constanty, Sara Majello, Angelo Bitetti, Antoine Graindorge, Alena Shkumatava (2019 May 3)

Strategies for Genetic Inactivation of Long Noncoding RNAs in Zebrafish.

RNA (New York, N.Y.) : DOI : rna.069484.118

Résumé

The number of annotated long noncoding RNAs (lncRNAs) continues to grow, however their functional characterization in model organisms has been hampered by the lack of reliable

genetic inactivation strategies. While partial or full deletions of lncRNA loci disrupt lncRNA expression, they do not permit the formal association of a phenotype with the encoded transcript. Here, we examined several alternative strategies for generating lncRNA null alleles in zebrafish and found that they often resulted in unpredicted changes to lncRNA expression. Removal of the transcriptional start sites (TSSs) of lncRNA genes resulted in hypomorphic mutants due to the usage of either constitutive or tissue-specific alternative TSSs. Deletions of short, deeply conserved lncRNA regions can also lead to overexpression of truncated transcripts. By contrast, a knock-in of a polyadenylation signal enabled complete inactivation of malat1, the most abundant vertebrate lncRNA. In summary, lncRNA null alleles require extensive in vivo validation and we propose insertion of transcription termination sequences as the most reliable approach to generate lncRNA-deficient zebrafish.

El Hassen Mokrani, Abderrahmane Bensegueni, Ludovic Chaput, Claire Beauvineau, Hanane Djeghim, Liliane Mouawad (2019 May 1)

Identification of New Potent Acetylcholinesterase Inhibitors Using Virtual Screening and *In Vitro* Approaches.

Molecular informatics : 38 : 1800118 : DOI : 10.1002/minf.201800118

Résumé

Acetylcholinesterase (AChE) is currently the most favorable target for the symptomatic treatment and reduction of Alzheimer's disease (AD). In order to identify new potent inhibitors of this enzyme, we describe herein a new structure-based virtual screening (SBVS) using the Institut Curie-CNRS chemical library (ICCL), which contained at the screening date 14307 compounds. The strategy undertaken in this work consisted of the use of several docking programs in SBVS calculations followed by the application of a consensus method (vSDC) and a scrupulous visual analysis. It allowed us to obtain a high degree of success, with a yield of almost 86 %, since 12 hits were identified among only 14 molecules tested *in vitro*. Still more remarkably, 6 of these hits were more active than galantamine, the reference inhibitor. These hits were predicted to have good ADMET properties. The two most promising compounds can serve as leads for AD treatment.