Publications
Mass Spectrometry and Proteomics Facility

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Anabelle Planques, Vanessa Oliveira Moreira, David Benacom, Clémence Bernard, Laurent Jourdren, Corinne Blugeon, Florent Dingli, Vanessa Masson, Damarys Loew, Alain Prochiantz, Ariel A. Di Nardo (2021 Aug 19)
OTX2 Homeoprotein Functions in Adult Choroid Plexus
International Journal of Molecular Sciences : DOI : 10.3390/ijms22168951

Summary

The choroid plexus is an important blood barrier that secretes cerebrospinal fluid, which essential for embryonic brain development and adult brain homeostasis. The OTX2 homeoprotein is a transcription factor that is critical for choroid plexus development and remains highly expressed in adult choroid plexus. Through RNA sequencing analyses of constitutive and conditional knockdown adult mouse models, we reveal putative functional roles for OTX2 in adult choroid plexus function, including cell signaling and adhesion, and show that OTX2 regulates the expression of factors that are secreted into the cerebrospinal fluid, notably transthyretin. We also show that Otx2 expression impacts choroid plexus immune and stress responses, and affects splicing, leading to changes in the mRNA isoforms of proteins that are implicated in the oxidative stress response and DNA repair. Through mass spectrometry analysis of OTX2 protein partners in the choroid plexus, and in known non-cell-autonomous target regions, such as the visual cortex and subventricular zone, we identify putative targets that are involved in cell adhesion, chromatin structure, and RNA processing. Thus, OTX2 retains important roles for regulating choroid plexus function and brain homeostasis throughout life.

Virginia Sanchez-Quiles, Ming-Jun Shi, Florent Dingli, Clémentine Krucker, Damarys Loew, Isabelle Bernard-Pierrot, François Radvanyi (2021 Aug 8)
Triple extraction method enables high quality mass spectrometry-based proteomics and phospho-proteomics for eventual multi-omics integration studies
Proteomics : DOI : 10.1002/pmic.202000303

Summary

Large-scale multi-omic analysis allows a thorough understanding of different physiological or pathological conditions, particularly cancer. Here, an extraction method simultaneously yielding DNA, RNA and protein (thereby referred to as “triple extraction”, TEx) was tested for its suitability to unbiased, system-wide proteomic investigation. Largely proven efficient for transcriptomic and genomic studies, we aimed at exploring TEx compatibility with mass spectrometry-based proteomics and phospho-proteomics, as compared to a standard urea extraction. TEx is suitable for the shotgun investigation of proteomes, providing similar results as urea-based protocol both at the qualitative and quantitative levels. TEx is likewise compatible with the exploration of phosphorylation events, actually providing a higher number of correctly localized sites than urea, although the nature of extracted modifications
appears somewhat distinct between both techniques. These results highlight that the presented protocol is well suited for the examination of the proteome and modified proteome of this bladder cancer cell model, as efficiently as other more widely used workflows for mass spectrometry-based analysis. Potentially applicable to other mammalian cell types and tissues, TEx represents an advantageous strategy for multi-omics on scarce and/or heterogenous samples.

Matthieu Meryet-Figuiere, Mégane Vernon, Mamy Andrianteranagna, Bernard Lambert, Célia Brochen, Jean-Paul Issartel, Audrey Guttin, Pascal Gauduchon, Emilie Brotin, Florent Dingli, Damarys Loew, Nicolas Vigneron, Anaïs Wambecke, Edwige Abeilard, Emmanuel Barillot, Laurent Poulain, Loredana Martignetti, Christophe Denoyelle (2021 Aug 5)

Network-Based Integration of Multi-Omics Data Identifies the Determinants of miR-491-5p Effects

Cancers : DOI : 10.3390/cancers13163970

Summary

The identification of miRNAs’ targets and associated regulatory networks might allow the definition of new strategies using drugs whose association mimics a given miRNA’s effects. Based on this assumption we devised a multi-omics approach to precisely characterize miRNAs’ effects. We combined miR-491-5p target affinity purification, RNA microarray, and mass spectrometry to perform an integrated analysis in ovarian cancer cell lines. We thus constructed an interaction network that highlighted highly connected hubs being either direct or indirect targets of miR-491-5p effects: the already known EGFR and BCL2L1 but also EP300, CTNNB1 and several small-GTPases. By using different combinations of specific inhibitors of these hubs, we could greatly enhance their respective cytotoxicity and mimic the miR-491-5p-induced phenotype. Our methodology thus constitutes an interesting strategy to comprehensively study the effects of a given miRNA. Moreover, we identified targets for which pharmacological inhibitors are already available for a clinical use or in clinical trials. This study might thus enable innovative therapeutic options for ovarian cancer, which remains the leading cause of death from gynecological malignancies in developed countries.

Mathilde Mathieu, Nathalie Névo, Mabel Jouve, José Ignacio Valenzuela, Mathieu Maurin, Frederik J. Verweij, Roberta Palmulli, Danielle Lankar, Florent Dingli, Damarys Loew, Eric Rubinstein, Gaëlle Boncompain, Franck Perez, Clotilde Théry (2021 Jul 19)

Specificities of exosome versus small ectosome secretion revealed by live intracellular tracking of CD63 and CD9

Nature Communications : DOI : 10.1038/s41467-021-24384-2

Summary

Despite their roles in intercellular communications, the different populations of extracellular
vesicles (EVs) and their secretion mechanisms are not fully characterized: how and to what extent EVs form as intraluminal vesicles of endocytic compartments (exosomes), or at the plasma membrane (PM) (ectosomes) remains unclear. Here we follow intracellular trafficking of the EV markers CD9 and CD63 from the endoplasmic reticulum to their residency compartment, respectively PM and late endosomes. We observe transient co-localization at both places, before they finally segregate. CD9 and a mutant CD63 stabilized at the PM are more abundantly released in EVs than CD63. Thus, in HeLa cells, ectosomes are more prominent than exosomes. By comparative proteomic analysis and differential response to neutralization of endosomal pH, we identify a few surface proteins likely specific of either exosomes (LAMP1) or ectosomes (BSG, SLC3A2). Our work sets the path for molecular and functional discrimination of exosomes and small ectosomes in any cell type.

Erwan Dumontet, Céline Pangault, David Roulois, Matthais Desoteux, Simon Léonard, Tony Marchand, Maelle Latour, Patricia Legoix, Damarys Loew, Florent Dingli, Joelle Dulong, Erwan Flecher, Cédric Coulouarn, Guillaume Cartron, Thierry Fest, Karin Tarte (2021 Jul 8)

**Extracellular vesicles shed by follicular lymphoma B cells promote polarization of the bone marrow stromal cell niche**
*Blood* : [DOI : 10.1182/blood.2020008791](https://doi.org/10.1182/blood.2020008791)

**Summary**

Follicular lymphoma (FL) originates in the lymph nodes (LNs) and infiltrates bone marrow (BM) early in the course of the disease. BM FL B cells are characterized by a lower cytological grade, decreased proliferation, and a specific phenotypic and subclonal profile. Mesenchymal stromal cells (MSCs) obtained from FL BM display a specific gene expression profile (GEP), including enrichment for a lymphoid stromal cell signature, and an increased capacity to sustain FL B-cell growth. However, the mechanisms triggering the formation of the medullar FL permissive stromal niche have not been identified. In the current work, we demonstrate that FL B cells produce extracellular vesicles (EVs) that can be internalized by BM-MSCs, making them more efficient to support FL B-cell survival and quiescence. Accordingly, EVs purified from FL BM plasma activate transforming growth factor β-dependent and independent pathways in BM-MSCs and modify their GEP, triggering an upregulation of factors classically associated with hematopoietic stem cell niche, including CXCL12 and angiopoietin-1. Moreover, we provide the first characterization of BM FL B-cell GEP, allowing the definition of the landscape of molecular interactions they could engage with EV-primed BM-MSCs. This work identifies FL-derived EVs as putative mediators of BM stroma polarization and supports further investigation of their clinical interest for targeting the crosstalk between BM-MSCs and malignant B cells.

Jérôme Ribot, Rachel Breton, Charles-Félix Calvo, Julien Moulard, Pascal Ezan, Jonathan Zapata, Kevin Samama, Matthieu Moreau, Alexis-Pierre Bemelmans, Valentin Sabatet, Florent Dingli, Damarys Loew, Chantal Milleret, Pierre Billuart, Glenn Dalléram, Nathalie Rouach (2021 Jul 2)

**Astrocytes close the mouse critical period for visual plasticity**
Brain postnatal development is characterized by critical periods of experience-dependent remodeling of neuronal circuits. Failure to end these periods results in neurodevelopmental disorders. The cellular processes defining critical-period timing remain unclear. Here, we show that in the mouse visual cortex, astrocytes control critical-period closure. We uncover the underlying pathway, which involves astrocytic regulation of the extracellular matrix, allowing interneuron maturation. Unconventional astrocyte connexin signaling hinders expression of extracellular matrix-degrading enzyme matrix metalloproteinase 9 (MMP9) through RhoA-guanosine triphosphatase activation. Thus, astrocytes not only influence the activity of single synapses but are also key elements in the experience-dependent wiring of brain circuits.

Meetali Singh, Eric Cornes, Blaise Li, Piergiuseppe Quarato, Loan Bourdon, Florent Dingli, Damarys Loew, Simone Proccacia, Germano Cecere (2021 Jun 9)

Translation and codon usage regulate Argonaute slicer activity to trigger small RNA biogenesis

In the Caenorhabditis elegans germline, thousands of mRNAs are concomitantly expressed with antisense 22G-RNAs, which are loaded into the Argonaute CSR-1. Despite their essential functions for animal fertility and embryonic development, how CSR-1 22G-RNAs are produced remains unknown. Here, we show that CSR-1 slicer activity is primarily involved in triggering the synthesis of small RNAs on the coding sequences of germline mRNAs and post-transcriptionally regulates a fraction of targets. CSR-1-cleaved mRNAs prime the RNA-dependent RNA polymerase, EGO-1, to synthesize 22G-RNAs in phase with translating ribosomes, in contrast to other 22G-RNAs mostly synthesized in germ granules. Moreover, codon optimality and efficient translation antagonize CSR-1 slicing and 22G-RNAs biogenesis. We propose that codon usage differences encoded into mRNA sequences might be a conserved strategy in eukaryotes to regulate small RNA biogenesis and Argonaute targeting.

Cristina Chiva, Teresa Mendes Maia, Christian Panse, Karel Stejskal, Thibaut Douché, Mariette Matondo, Damarys Loew, Dominic Helm, Mandy Rettel, Karl Mechtler, Francis Impens, Paolo Nanni, Anna Shevchenko, Eduard Sabidó (2021 Jun 4)

Quality standards in proteomics research facilities

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Core facilities and research infrastructures have become an essential part of the scientific ecosystem. In the field of proteomics, national and international networks and research platforms have been established during the past decade that are supposed to set standards for high-quality services, promote an exchange of professional information, and enable access to cutting-edge, specialized proteomics technologies. Either centralized or distributed, these national and international proteomics infrastructures and technology platforms are generating massive amounts of data for the research community, and support a broad range of translational, computational and multi-omics initiatives and basic research projects.

By delegating part of their work to these services, researchers expect that the core facility adjusts their analytical protocols appropriately for their project to acquire data conforming best research practice of the scientific community. The implementation of quality assessment measures and commonly accepted quality controls in data generation is therefore crucially important for proteomics research infrastructures and the scientists who rely on them.

However, current quality control and quality assessment procedures in proteomics core facilities and research infrastructures are a motley collection of protocols, standards, reference compounds and software tools. Proteomics relies on a customized multi-step workflow typically consisting of sample preparation, data acquisition and data processing, and the implementation of each step differs among facilities. For example, sample preparation involves enzymatic digestion of the proteins, which can be performed in-solution, in-gel, or on-beads, with often different proteolytic enzymes, chemicals, and conditions among laboratories. Data acquisition protocols are often customized to the particular instrument set up, and the acquired spectra and chromatograms are processed by different software tools provided by equipment vendors, third parties or developed in-house.

Moreover, core facilities implement their own guidelines to monitor the performance and quality of the entire workflow, typically utilizing different commercially available standards such as pre-digested cell lysates, recombinant proteins, protein mixtures, or isotopically labeled peptides. Currently, there is no clear consensus on if, when and how to perform quality control checks. There is even less quality control in walk-in facilities, where the staff is only responsible for correct usage of the instruments and users select and execute the analytical workflow themselves. It is not surprising therefore that instrument stability and robustness of the applied analytical approach are often unclear, which compromises analytical rigor.

Dipti Vinayak Vernekar, Giordano Reginato, Céline Adam, Lepakshi Ranjha, Florent Dingli, Marie-Claude Marsolier, Damarys Loew, Raphaël Guérois, Bertrand Llorente, Petr Cejka, Valérie Borde (2021 May 7)

The Pif1 helicase is actively inhibited during meiotic recombination which restrains gene conversion tract length

Nucleic Acids Research : DOI: 10.1093/nar/gkab232
Summary

Meiotic recombination ensures proper chromosome segregation to form viable gametes and results in gene conversions events between homologs. Conversion tracts are shorter in meiosis than in mitotically dividing cells. This results at least in part from the binding of a complex, containing the Mer3 helicase and the MutLβ heterodimer, to meiotic recombination intermediates. The molecular actors inhibited by this complex are elusive. The Pif1 DNA helicase is known to stimulate DNA polymerase delta (Pol δ) -mediated DNA synthesis from D-loops, allowing long synthesis required for break-induced replication. We show that Pif1 is also recruited genome wide to meiotic DNA double-strand break (DSB) sites. We further show that Pif1, through its interaction with PCNA, is required for the long gene conversions observed in the absence of MutLβ recruitment to recombination sites. In vivo, Mer3 interacts with the PCNA clamp loader RFC, and in vitro, Mer3-MutLβ ensemble inhibits Pif1-stimulated D-loop extension by Pol δ and RFC-PCNA. Mechanistically, our results suggest that Mer3-MutLβ may compete with Pif1 for binding to RFC-PCNA. Taken together, our data show that Pif1’s activity that promotes meiotic DNA repair synthesis is restrained by the Mer3-MutLβ ensemble which in turn prevents long gene conversion tracts and possibly associated mutagenesis.

Lorena Martin-Jaular, Nathalie Nevo, Julia P Schessner, Mercedes Tkach, Mabel Jouve, Florent Dingli, Damarys Loew, Kenneth W Witwer, Matias Ostrowski, Georg H H Borner, Clotilde Théry (2021 Apr 15)

Unbiased proteomic profiling of host cell extracellular vesicle composition and dynamics upon HIV-1 infection
The EMBO Journal : DOI : 10.15252/embj.2020105492

Summary

Cells release diverse types of extracellular vesicles (EVs), which transfer complex signals to surrounding cells. Specific markers to distinguish different EVs (e.g. exosomes, ectosomes, enveloped viruses like HIV) are still lacking. We have developed a proteomic profiling approach for characterizing EV subtype composition and applied it to human Jurkat T cells. We generated an interactive database to define groups of proteins with similar profiles, suggesting release in similar EVs. Biochemical validation confirmed the presence of preferred partners of commonly used exosome markers in EVs: CD81/ADAM10/ITGB1, and CD63/syntenin. We then compared EVs from control and HIV-1-infected cells. HIV infection altered EV profiles of several cellular proteins, including MOV10 and SPN, which became incorporated into HIV virions, and SERINC3, which was re-routed to non-viral EVs in a Nef-dependent manner. Furthermore, we found that SERINC3 controls the surface composition of EVs. Our workflow provides an unbiased approach for identifying candidate markers and potential regulators of EV subtypes. It can be widely applied to in vitro experimental systems for investigating physiological or pathological modifications of EV release.

Jeremy Bigot, Ana I. Lalanne, Francesca Lucibello, Paul Gueguen, Alexandre Houy, Stephane
Dayot, Olivier Ganier, Jules Gilet, Jimena Tosello, Fariba Nemati, Gaëlle Pierron, Joshua J. Waterfall, Raymond Barnhill, Sophie Gardrat, Sophie Piperno-Neumann, Tatiana Popova, Vanessa Masson, Damarys Loew, Pascale Mariani, Nathalie Cassoux, Sebastian Amigorena, Manuel Rodrigues, Samar Alsafadi, Marc-Henri Stern, Olivier Lantz (2021 Apr 2)

**Splicing Patterns in SF3B1-Mutated Uveal Melanoma Generate Shared Immunogenic Tumor-Specific Neoepitopes**

*Cancer Discovery*: [DOI: 10.1158/2159-8290.CD-20-0555](https://doi.org/10.1158/2159-8290.CD-20-0555)

**Summary**

Disruption of splicing patterns due to mutations of genes coding splicing factors in tumors represents a potential source of tumor neoantigens, which would be both public (shared between patients) and tumor-specific (not expressed in normal tissues). In this study, we show that mutations of the splicing factor SF3B1 in uveal melanoma generate such immunogenic neoantigens. Memory CD8+ T cells specific for these neoantigens are preferentially found in 20% of patients with uveal melanoma bearing SF3B1-mutated tumors. Single-cell analyses of neoepitope-specific T cells from the blood identified large clonal T-cell expansions, with distinct effector transcription patterns. Some of these expanded T-cell receptors are also present in the corresponding tumors. CD8+ T-cell clones specific for the neoepitopes specifically recognize and kill SF3B1-mutated tumor cells, supporting the use of this new family of neoantigens as therapeutic targets.

**Significance:** Mutations of the splicing factor SF3B1 in uveal melanoma generate shared neoantigens that are uniquely expressed by tumor cells, leading to recognition and killing by specific CD8 T cells. Mutations in splicing factors can be sources of new therapeutic strategies applicable to diverse tumors.

Gaetana Sessa, Belén Gómez-González, Sonia Silva, Carmen Pérez-Calero, Romane Beaurepere, Sonia Barroso, Sylvain Martineau, Charlotte Martin, Åsa Ehlén, Juan S Martínez, Bérangère Lombard, Damarys Loew, Stephan Vagner, Andrés Aguilera, Aura Carreira (2021 Apr 1)

**BRCA2 promotes DNA-RNA hybrid resolution by DDX5 helicase at DNA breaks to facilitate their repair**

*The EMBO Journal*: [DOI: 10.15252/embj.2020106018](https://doi.org/10.15252/embj.2020106018)

**Summary**

The BRCA2 tumor suppressor is a DNA double-strand break (DSB) repair factor essential for maintaining genome integrity. BRCA2-deficient cells spontaneously accumulate DNA-RNA hybrids, a known source of genome instability. However, the specific role of BRCA2 on these structures remains poorly understood. Here we identified the DEAD-box RNA helicase DDX5 as a BRCA2-interacting protein. DDX5 associates with DNA-RNA hybrids that form in the vicinity of DSBs, and this association is enhanced by BRCA2. Notably, BRCA2 stimulates the DNA-RNA hybrid-unwinding activity of DDX5 helicase. An impaired BRCA2-DDX5 interaction, as observed in cells expressing the breast cancer variant BRCA2-T207A, reduces the
association of DDX5 with DNA-RNA hybrids, decreases the number of RPA foci, and alters the kinetics of appearance of RAD51 foci upon irradiation. Our findings are consistent with DNA-RNA hybrids constituting an impediment for the repair of DSBs by homologous recombination and reveal BRCA2 and DDX5 as active players in their removal.

Alena Ivashenka, Christian Wunder, Valerie Chambon, Roger Sandhoff, Richard Jennemann, Estelle Dransart, Katrina Podsypanina, Bérangère Lombard, Damarys Loew, Christophe Lamaze, Francoise Poirier, Hermann-Josef Gröne, Ludger Johannes, Massiullah Shafaq-Zadah (2021 Feb 10)

Glycolipid-dependent and lectin-driven transcytosis in mouse enterocytes.

Communications biology : 173 : DOI : 10.1038/s42003-021-01693-2

Summary

Glycoproteins and glycolipids at the plasma membrane contribute to a range of functions from growth factor signaling to cell adhesion and migration. Glycoconjugates undergo endocytic trafficking. According to the glycolipid-lectin (GL-Lect) hypothesis, the construction of tubular endocytic pits is driven in a glycosphingolipid-dependent manner by sugar-binding proteins of the galectin family. Here, we provide evidence for a function of the GL-Lect mechanism in transcytosis across enterocytes in the mouse intestine. We show that galectin-3 (Gal3) and its newly identified binding partner lactotransferrin are transported in a glycosphingolipid-dependent manner from the apical to the basolateral membrane. Transcytosis of lactotransferrin is perturbed in Gal3 knockout mice and can be rescued by exogenous Gal3. Inside enterocytes, Gal3 is localized to hallmark structures of the GL-Lect mechanism, termed clathrin-independent carriers. These data pioneer the existence of GL-Lect endocytosis in vivo and strongly suggest that polarized trafficking across the intestinal barrier relies on this mechanism.

Xue Zhao, Achal Rastogi, Anne Flore Deton Cabanillas, Ouardia Ait Mohamed, Catherine Cantrel, Bérangère Lombard, Omer Murik, Auguste Genovesio, Chris Bowler, Daniel Bouyer, Damarys Loew, Xin Lin, Alaguraj Veluchamy, Fabio Rocha Jimenez Vieira, Leila Tirichine (2021 Feb 3)

Genome wide natural variation of H3K27me3 selectively marks genes predicted to be important for cell differentiation in Phaeodactylum tricornutum.

The New phytologist : DOI : 10.1111/nph.17129

Summary

In multicellular organisms, Polycomb Repressive Complex2 (PRC2) is known to deposit trimethylation of lysine 27 of histone H3 (H3K27me3) to establish and maintain gene silencing, critical for developmentally regulated processes. The PRC2 complex is absent in both widely studied model yeasts, which initially suggested that PRC2 arose with the emergence of multicellularity. However, its discovery in several unicellular species including microalgae questions its role in unicellular eukaryotes. Here, we use Phaeodactylum tricornutum
enhancer of zeste E(z) knockouts and show that *P. tricornutum* E(z) is responsible for di- and tri-methylation of lysine 27 of histone H3. H3K27me3 depletion abolishes cell morphology in *P. tricornutum* providing evidence for its role in cell differentiation. Genome-wide profiling of H3K27me3 in fusiform and triradiate cells further revealed genes that may specify cell identity. These results suggest a role for PRC2 and its associated mark in cell differentiation in unicellular species, and highlight their ancestral function in a broader evolutionary context than currently is appreciated.

Simon Bourdareau, Leila Tirichine, Bérangère Lombard, Damarys Loew, Delphine Scornet, Yue Wu, Susana M Coelho, J Mark Cock (2021 Jan 5)

**Histone modifications during the life cycle of the brown alga *Ectocarpus*.**

*Genome biology*: 12 : DOI: [10.1186/s13059-020-02216-8](https://doi.org/10.1186/s13059-020-02216-8)

**Summary**

Brown algae evolved complex multicellularity independently of the animal and land plant lineages and are the third most developmentally complex phylogenetic group on the planet. An understanding of developmental processes in this group is expected to provide important insights into the evolutionary events necessary for the emergence of complex multicellularity. Here, we focus on mechanisms of epigenetic regulation involving post-translational modifications of histone proteins.