Year of publication 2019

Gaelle Boncompain, Nelly Gareil, Sarah Tessier, Aurianne Lescure, Thouis R Jones, Oliver Kepp, Guido Kroemer, Elaine Del Nery, Franck Perez (2019 Nov 5)

BML-265 and Tyrphostin AG1478 Disperse the Golgi Apparatus and Abolish Protein Transport in Human Cells.

Frontiers in cell and developmental biology : 232 : DOI : 10.3389/fcell.2019.00232

Summary

The steady-state localization of Golgi-resident glycosylation enzymes in the Golgi apparatus depends on a balance between anterograde and retrograde transport. Using the Retention Using Selective Hooks (RUSH) assay and high-content screening, we identified small molecules that perturb the localization of Mannosidase II (ManII) used as a model cargo for Golgi resident enzymes. In particular, we found that two compounds known as EGFR tyrosine kinase inhibitors, namely BML-265 and Tyrphostin AG1478 disrupt Golgi integrity and abolish secretory protein transport of diverse cargos, thus inducing brefeldin A-like effects. Interestingly, BML-265 and Tyrphostin AG1478 affect Golgi integrity and transport in human cells but not in rodent cells. The effects of BML-265 are reversible since Golgi integrity and protein transport are quickly restored upon washout of the compounds. BML-265 and Tyrphostin AG1478 do not lead to endosomal tubulation suggesting that, contrary to brefeldin A, they do not target the -Golgi ARF GEF BIG1 and BIG2. They quickly induce COPI dissociation from Golgi membranes suggesting that, in addition to EGFR kinase, the -Golgi ARF GEF GBF1 might also be a target of these molecules. Accordingly, overexpression of GBF1 prevents the effects of BML-265 and Tyrphostin AG1478 on Golgi integrity.

Gaelle Boncompain, Floriane Herit, Sarah Tessier, Aurianne Lescure, Elaine Del Nery, Pierre Gestraud, Isabelle Staropoli, Yuko Fukata, Masaki Fukata, Anne Brelot, Florence Niedergang, Franck Perez (2019 Oct 31)

Targeting CCR5 trafficking to inhibit HIV-1 infection.

Science advances : eaax0821 : DOI : 10.1126/sciadv.aax0821

Summary

Using a cell-based assay monitoring differential protein transport in the secretory pathway coupled to high-content screening, we have identified three molecules that specifically reduce the delivery of the major co-receptor for HIV-1, CCR5, to the plasma membrane. They have no effect on the closely related receptors CCR1 and CXCR4. These molecules are also potent in primary macrophages as they markedly decrease HIV entry. At the molecular level, two of these molecules inhibit the critical palmitoylation of CCR5 and thereby block CCR5 in the early secretory pathway. Our results open a clear therapeutics avenue based on trafficking control and demonstrate that preventing HIV infection can be performed at the level of its receptor delivery.

Mathilde Vinet, Samyuktha Suresh, Virginie Maire, Clarisse Monchecourt, Fariba Némati, Laetitia Lesage, Fabienne Pierre, Mengliang Ye, Auriane Lescure, Amélie Brisson, Didier Meseure, André Nicolas, Guillem Rigaill, Elisabetta Marangoni, Elaine Del Nery, Sergio Roman-Roman, Thierry Dubois (2019 Apr 9)

Protein arginine methyltransferase 5: A novel therapeutic target for triple-negative breast cancers.

Cancer medicine : 2414-2428 : DOI : 10.1002/cam4.2114

Summary

TNBC is a highly heterogeneous and aggressive breast cancer subtype associated with high relapse rates, and for which no targeted therapy yet exists. Protein arginine methyltransferase 5 (PRMT5), an enzyme which catalyzes the methylation of arginines on histone and non-histone proteins, has recently emerged as a putative target for cancer therapy. Potent and specific PRMT5 inhibitors have been developed, but the therapeutic efficacy of PRMT5 targeting in TNBC has not yet been demonstrated. Here, we examine the expression of PRMT5 in a human breast cancer cohort obtained from the Institut Curie, and evaluate the therapeutic potential of pharmacological inhibition of PRMT5 in TNBC. We find that PRMT5 mRNA and protein are expressed at comparable levels in TNBC, luminal breast tumors, and healthy mammary tissues. However, immunohistochemistry analyses reveal that PRMT5 is differentially localized in TNBC compared to other breast cancer subtypes and to normal breast tissues. PRMT5 is heterogeneously expressed in TNBC and high PRMT5 expression correlates with poor prognosis within this breast cancer subtype. Using the small-molecule inhibitor EPZ015666, we show that PRMT5 inhibition impairs cell proliferation in a subset of TNBC cell lines. PRMT5 inhibition triggers apoptosis, regulates cell cycle progression and decreases mammosphere formation. Furthermore, EPZ015666 administration to a patient-derived xenograft model of TNBC significantly deters tumor progression. Finally, we reveal potentiation between EGFR and PRMT5 targeting, suggestive of a beneficial combination therapy. Our findings highlight a distinctive subcellular localization of PRMT5 in TNBC, and uphold PRMT5 targeting, alone or in combination, as a relevant treatment strategy for a subset of TNBC.

Anahi Capmany, Azumi Yoshimura, Rachid Kerdous, Valentina Caorsi, Aurianne Lescure, Elaine Del Nery, Evelyne Coudrier, Bruno Goud, Kristine Schauer (2019 Mar 16)

MYO1C stabilizes actin and facilitates the arrival of transport carriers at the Golgi complex.

Journal of cell science : DOI : jcs225029

Summary

In this study, we aimed to identify the myosin motor proteins that control trafficking at the Golgi complex. In addition to the known Golgi-associated myosins MYO6, MYO18A and MYH9 (myosin IIA), we identified MYO1C as a novel player at the Golgi in a human cell line. We demonstrate that depletion of induces Golgi complex fragmentation and decompaction. MYO1C accumulates at dynamic structures around the Golgi complex that colocalize with

Golgi-associated actin dots. depletion leads to loss of cellular F-actin, and Golgi complex decompaction is also observed after inhibition or loss of the actin-related protein 2/3 complex, Arp2/3 (also known as ARPC). We show that the functional consequence of depletion is a delay in the arrival of incoming transport carriers, both from the anterograde and retrograde routes. We propose that MYO1C stabilizes actin at the Golgi complex, facilitating the arrival of incoming transport carriers at the Golgi.This article has an associated First Person interview with the first author of the paper.

Year of publication 2018

Marine Fanny Garrido, Nicolas Jp Martin, Matthieu Bertrand, Catherine Gaudin, Frederic Commo, Nassif El Kalaany, Nader Al Nakouzi, Ladan Fazli, Elaine Del Nery, Jacques Camonis, Franck Perez, Stéphanie Lerondel, Alain LE Pape, Martin E Gleave, Yohann Loriot, Laurent Desaubry, Stephan Vagner, Karim Fizazi, Anne Chauchereau (2018 Oct 15)

Regulation of eIF4F translation initiation complex by the peptidyl prolyl isomerase FKBP7 in taxane-resistant prostate cancer.

Clinical cancer research : an official journal of the American Association for Cancer Research : DOI : clincanres.0704.2018

Summary

Targeted therapies that use the signaling pathways involved in prostate cancer are required to overcome chemoresistance and improve treatment outcomes for men. Molecular chaperones play a key role in the regulation of protein homeostasis and are potential targets for overcoming chemoresistance.

Year of publication 2017

France Rose, Sreetama Basu, Elton Rexhepaj, Anne Chauchereau, Elaine Del Nery, Auguste Genovesio (2017 Nov 5)

Compound Functional Prediction Using Multiple Unrelated Morphological Profiling Assays.

SLAS technology : 243-251 : DOI : 10.1177/2472630317740831

Summary

Phenotypic cell-based assays have proven to be efficient at discovering first-in-class therapeutic drugs mainly because they allow for scanning a wide spectrum of possible targets at once. However, despite compelling methodological advances, posterior identification of a compound’s mechanism of action (MOA) has remained difficult and highly refractory to automated analyses. Methods such as the cell painting assay and multiplexing fluorescent dyes to reveal broadly relevant cellular components were recently suggested for MOA prediction. We demonstrated that adding fluorescent dyes to a single assay has limited

impact on MOA prediction accuracy, as monitoring only the nuclei stain could reach compelling levels of accuracy. This observation suggested that multiplexed measurements are highly correlated and nuclei stain could possibly reflect the general state of the cell. We then hypothesized that combining unrelated and possibly simple cell-based assays could bring a solution that would be biologically and technically more relevant to predict a drug target than using a single assay multiplexing dyes. We show that such a combination of past screen data could rationally be reused in screening facilities to train an ensemble classifier to predict drug targets and prioritize a possibly large list of unknown compound hits at once.

Asm Shihavuddin, Sreetama Basu, Elton Rexhepaj, Felipe Delestro, Nikita Menezes, Séverine M Sigoillot, Elaine Del Nery, Fekrije Selimi, Nathalie Spassky, Auguste Genovesio (2017 Jun 1)
Smooth 2D manifold extraction from 3D image stack.
Nature communications : 15554 : DOI : 10.1038/ncomms15554

Summary

Three-dimensional fluorescence microscopy followed by image processing is routinely used to study biological objects at various scales such as cells and tissue. However, maximum intensity projection, the most broadly used rendering tool, extracts a discontinuous layer of voxels, obliviously creating important artifacts and possibly misleading interpretation. Here we propose smooth manifold extraction, an algorithm that produces a continuous focused 2D extraction from a 3D volume, hence preserving local spatial relationships. We demonstrate the usefulness of our approach by applying it to various biological applications using confocal and wide-field microscopy 3D image stacks. We provide a parameter-free ImageJ/Fiji plugin that allows 2D visualization and interpretation of 3D image stacks with maximum accuracy.

Year of publication 2016

Peter Horvath, Nathalie Aulner, Marc Bickle, Anthony M Davies, Elaine Del Nery, Daniel Ebner, Maria C Montoya, Päivi Östling, Vilja Pietiäinen, Leo S Price, Spencer L Shorte, Gerardo Turcatti, Carina von Schantz, Neil O Carragher (2016 Sep 13)
Screening out irrelevant cell-based models of disease.
Nature reviews. Drug discovery : 751-769 : DOI : 10.1038/nrd.2016.175

Summary

The common and persistent failures to translate promising preclinical drug candidates into clinical success highlight the limited effectiveness of disease models currently used in drug discovery. An apparent reluctance to explore and adopt alternative cell- and tissue-based model systems, coupled with a detachment from clinical practice during assay validation, contributes to ineffective translational research. To help address these issues and stimulate debate, here we propose a set of principles to facilitate the definition and development of disease-relevant assays, and we discuss new opportunities for exploiting the latest advances in cell-based assay technologies in drug discovery, including induced pluripotent stem cells,

three-dimensional (3D) co-culture and organ-on-a-chip systems, complemented by advances in single-cell imaging and gene editing technologies. Funding to support precompetitive, multidisciplinary collaborations to develop novel preclinical models and cell-based screening technologies could have a key role in improving their clinical relevance, and ultimately increase clinical success rates.

Pierre Thouvenot, Barbara Ben Yamin, Lou Fourrière, Aurianne Lescure, Thomas Boudier, Elaine Del Nery, Anne Chauchereau, David E Goldgar, Claude Houdayer, Dominique Stoppa-Lyonnet, Alain Nicolas, Gaël A Millot (2016 Jun 9)

Functional Assessment of Genetic Variants with Outcomes Adapted to Clinical Decision-Making.

PLoS genetics : e1006096 : DOI : 10.1371/journal.pgen.1006096

Summary

Understanding the medical effect of an ever-growing number of human variants detected is a long term challenge in genetic counseling. Functional assays, based on in vitro or in vivo evaluations of the variant effects, provide essential information, but they require robust statistical validation, as well as adapted outputs, to be implemented in the clinical decision-making process. Here, we assessed 25 pathogenic and 15 neutral missense variants of the BRCA1 breast/ovarian cancer susceptibility gene in four BRCA1 functional assays. Next, we developed a novel approach that refines the variant ranking in these functional assays. Lastly, we developed a computational system that provides a probabilistic classification of variants, adapted to clinical interpretation. Using this system, the best functional assay exhibits a variant classification accuracy estimated at 93%. Additional theoretical simulations highlight the benefit of this ready-to-use system in the classification of variants after functional assessment, which should facilitate the consideration of functional evidences in the decision-making process after genetic testing. Finally, we demonstrate the versatility of the system with the classification of siRNAs tested for human cell growth inhibition in high throughput screening.

Year of publication 2015

Frédéric Canal, Elodie Anthony, Aurianne Lescure, Elaine Del Nery, Jacques Camonis, Franck Perez, Bruno Ragazzon, Christine Perret (2015 Dec 31)

A kinome siRNA screen identifies HGS as a potential target for liver cancers with oncogenic mutations in CTNNB1.

BMC cancer : 1020 : DOI : 10.1186/s12885-015-2037-8

Summary

Aberrant activation of the Wnt/β-catenin pathway is a major and frequent event in liver cancer, but inhibition of oncogenic β-catenin signaling has proven challenging. The identification of genes that are synthetically lethal in β-catenin-activated cancer cells would

provide new targets for therapeutic drug design.

Priscille Brodin, Elaine DelNery, Emmanuelle Soleilhac (2015 Mar 4)
[High content screening in chemical biology: overview and main challenges].
Médecine sciences : M/S : 187-96 : DOI : 10.1051/medsci/20153102016

Summary

The last two decades have seen the development of high content screening (HCS) methodology and its adaptation for the evaluation of small molecules as drug candidates or their use as chemical tools for research purpose. HCS was initially set-up for the understanding of the mechanism of action of compounds by testing them on cell based-assays for pharmacological and toxicological studies. Since the last decade, the use of HCS has been extended to academic research laboratories and this technology has become the starting point for numerous projects aiming at the identification of molecular targets and cellular pathways for a given disease on which novel type of drugs could act. This screening approach relies on image capture of fluorescently labeled cells therefore generating a large amount of data that must be handled by appropriate automated image analysis methods and storage instrumentation. These latter in addition to the integration and data sharing are current challenges that HCS must still tackle.

Year of publication 2013

Sardar Faisal Mahmood, Nadège Gruel, Elodie Chapeaublanc, Aurianne Lescure, Thouis Jones, Fabien Reyal, Anne Vincent-Salomon, Virginie Raynal, Gaëlle Pierron, Franck Perez, Jacques Camonis, Elaine Del Nery, Olivier Delattre, François Radvanyi, Isabelle Bernard-Pierrot (2013 Oct 22)
A siRNA screen identifies RAD21, EIF3H, CHRAC1 and TANC2 as driver genes within the 8q23, 8q24.3 and 17q23 amplicons in breast cancer with effects on cell growth, survival and transformation.
Carcinogenesis : 670-82 : DOI : 10.1093/carcin/bgt351

Summary

RNA interference has boosted the field of functional genomics, by making it possible to carry out ‘loss-of-function’ screens in cultured cells. Here, we performed a small interfering RNA screening, in three breast cancer cell lines, for 101 candidate driver genes overexpressed in amplified breast tumors and belonging to eight amplicons on chromosomes 8q and 17q, investigating their role in cell survival/proliferation. This screening identified eight driver genes that were amplified, overexpressed and critical for breast tumor cell proliferation or survival. They included the well-described oncogenic driver genes for the 17q12 amplicon, ERBB2 and GRB7. Four of six other candidate driver genes-RAD21 and EIF3H, both on chromosome 8q23, CHRAC1 on chromosome 8q24.3 and TANC2 on chromosome 17q23-were confirmed to be driver genes regulating the proliferation/survival of clonogenic breast cancer

cells presenting an amplification of the corresponding region. Indeed, knockdown of the expression of these genes decreased cell viability, through both cell cycle arrest and apoptosis induction, and inhibited the formation of colonies in anchorage-independent conditions, in soft agar. Strategies for inhibiting the expression of these genes or the function of the proteins they encode are therefore of potential value for the treatment of breast cancers presenting amplifications of the corresponding genomic region.

Kristine Schauer, Jean-Philippe Grossier, Tarn Duong, Violaine Chapuis, Sébastien Degot, Aurianne Lescure, Elaine Del Nery, Bruno Goud (2013 Aug 16)

A novel organelle map framework for high-content cell morphology analysis in high throughput.

Journal of biomolecular screening : 317-24 : DOI : 10.1177/1087057113497399

Summary

A screening procedure was developed that takes advantage of the cellular normalization by micropatterning and a novel quantitative organelle mapping approach that allows unbiased and automated cell morphology comparison using black-box statistical testing. Micropatterns of extracellular matrix proteins force cells to adopt a reproducible shape and distribution of intracellular compartments avoiding strong cell-to-cell variation that is a major limitation of classical culture conditions. To detect changes in cell morphology induced by compound treatment, fluorescently labeled intracellular structures from several tens of micropatterned cells were transformed into probabilistic density maps. Then, the similarity or difference between two given density maps was quantified using statistical testing that evaluates differences directly from the data without additional analysis or any subjective decision. The versatility of this organelle mapping approach for different magnifications and its performance for different cell shapes has been assessed. Density-based analysis detected changes in cell morphology due to compound treatment in a small-scale proof-of-principle screen demonstrating its compatibility with high-throughput screening. This novel tool for high-content and high-throughput cellular phenotyping can potentially be used for a wide range of applications from drug screening to careful characterization of cellular processes.

Julien Ablain, Magdalena Leiva, Laurent Peres, Julien Fonsart, Elodie Anthony, Hugues de Thé (2013 Mar 18)

Uncoupling RARA transcriptional activation and degradation clarifies the bases for APL response to therapies.

The Journal of experimental medicine : 647-53 : DOI : 10.1084/jem.20122337

Summary

In PML/RARA-driven acute promyelocytic leukemia (APL), retinoic acid (RA) induces leukemia cell differentiation and transiently clears the disease. Molecularly, RA activates PML/RARA-dependent transcription and also initiates its proteasome-mediated degradation. In contrast, arsenic, the other potent anti-APL therapy, only induces PML/RARA degradation by

specifically targeting its PML moiety. The respective contributions of RA-triggered transcriptional activation and proteolysis to clinical response remain disputed. Here, we identify synthetic retinoids that potently activate RARA- or PML/RARA-dependent transcription, but fail to down-regulate RARA or PML/RARA protein levels. Similar to RA, these uncoupled retinoids elicit terminal differentiation, but unexpectedly fail to impair leukemia-initiating activity of PML/RARA-transformed cells ex vivo or in vivo. Accordingly, the survival benefit conferred by uncoupled retinoids in APL mice is dramatically lower than the one provided by RA. Differentiated APL blasts sorted from uncoupled retinoid-treated mice retain PML/RARA expression and reinitiate APL in secondary transplants. Thus, differentiation is insufficient for APL eradication, whereas PML/RARA loss is essential. These observations unify the modes of action of RA and arsenic and shed light on the potency of their combination in mice or patients.

Year of publication 2012

Edouard Pauwels, Didier Surdez, Gautier Stoll, Aurianne Lescure, Elaine Del Nery, Olivier Delattre, Véronique Stoven (2012 Aug 29)

A probabilistic model for cell population phenotyping using HCS data.

PloS one : e42715 : DOI : 10.1371/journal.pone.0042715

Summary

High Content Screening (HCS) platforms allow screening living cells under a wide range of experimental conditions and give access to a whole panel of cellular responses to a specific treatment. The outcome is a series of cell population images. Within these images, the heterogeneity of cellular response to the same treatment leads to a whole range of observed values for the recorded cellular features. Consequently, it is difficult to compare and interpret experiments. Moreover, the definition of phenotypic classes at a cell population level remains an open question, although this would ease experiments analyses. In the present work, we tackle these two questions. The input of the method is a series of cell population images for which segmentation and cellular phenotype classification has already been performed. We propose a probabilistic model to represent and later compare cell populations. The model is able to fully exploit the HCS-specific information: “dependence structure of population descriptors” and “within-population variability”. The experiments we carried out illustrate how our model accounts for this specific information, as well as the fact that the model benefits from considering them. We underline that these features allow richer HCS data analysis than simpler methods based on single cellular feature values averaged over each well. We validate an HCS data analysis method based on control experiments. It accounts for HCS specificities that were not taken into account by previous methods but have a sound biological meaning. Biological validation of previously unknown outputs of the method constitutes a future line of work.