Année de publication : 2018

Athanasia Stoupa, Frédéric Adam, Dulanjalee Kariyawasam, Catherine Strassel, Sanjay Gawade, Gabor Szinnai, Alexandre Kauskot, Dominique Lasne, Carsten Janke, Kathiresan Natarajan, Alain Schmitt, Christine Bole-Feysot, Patrick Nitschke, Juliane Léger, Fabienne Jabot-Hanin, Frédéric Tores, Anita Michel, Arnold Munnich, Claude Besmond, Raphaël Scharfmann, François Lanza, Delphine Borgel, Michel Polak, Aurore Carré (2018 Nov 18)

TUBB1 mutations cause thyroid dysgenesis associated with abnormal platelet physiology.

EMBO molecular medicine : DOI : e9569

Résumé

The genetic causes of congenital hypothyroidism due to thyroid dysgenesis (TD) remain largely unknown. We identified three novel gene mutations that co-segregated with TD in three distinct families leading to 1.1% of mutations in TD study cohort. (Tubulin, Beta 1 Class VI) encodes for a member of the β-tubulin protein family. gene is expressed in the developing and adult thyroid in humans and mice. All three mutations lead to non-functional α/β-tubulin dimers that cannot be incorporated into microtubules. In mice, knock-out disrupted microtubule integrity by preventing β1-tubulin incorporation and impaired thyroid migration and thyroid hormone secretion. In addition, mutations caused the formation of macroplatelets and hyperaggregation of human platelets after stimulation by low doses of agonists. Our data highlight unexpected roles for β1-tubulin in thyroid development and in platelet physiology. Finally, these findings expand the spectrum of the rare paediatric diseases related to mutations in tubulin-coding genes and provide new insights into the genetic background and mechanisms involved in congenital hypothyroidism and thyroid dysgenesis.

Cáceres R, Bojanala N, Kelley LC, Dreier J, Manzi J, Di Federico F, Chi Q, Risler T, Testa I, Sherwood DR, Plastino J (2018 Nov 6)

Forces drive basement membrane invasion in Caenorhabditis elegans

Proceedings of the National Academy of Sciences USA : 115 : 11537-11542 : DOI : 10.1073/pnas.1808760115

Résumé

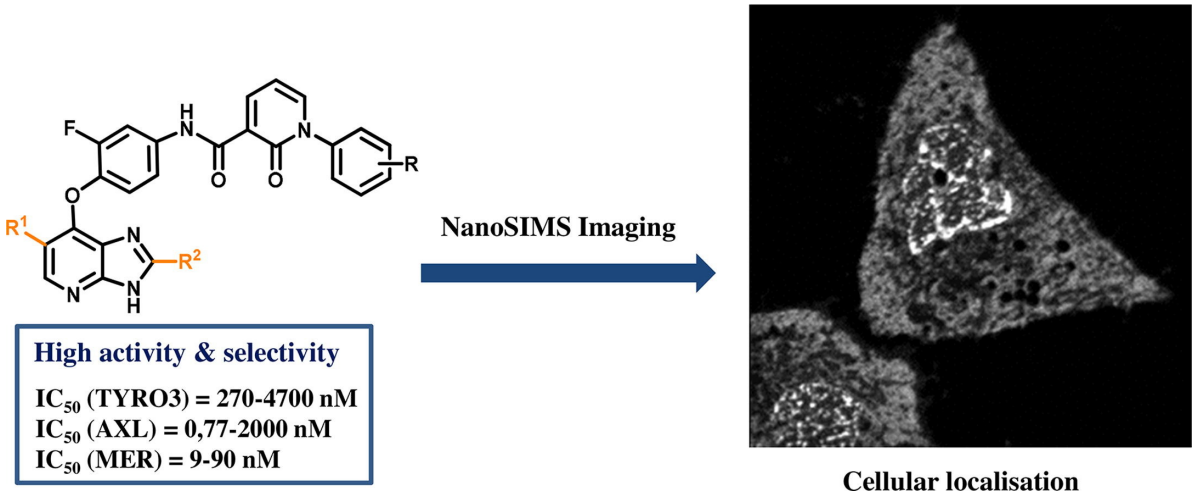
Tom Baladi, Jessy Aziz, Florent Dufour, Valentina Abet, Véronique Stoven, François Radvanyi, Florent Poyer, Ting-Di Wu, Jean-Luc Guerquin-Kern, Isabelle Bernard-Pierrot, Sergio Marco Garrido, Sandrine Piguel (2018 Nov 1)

Design, synthesis, biological evaluation and cellular imaging of imidazo[4,5-b]pyridine derivatives as potent and selective TAM inhibitors.

Bioorganic & medicinal chemistry : 26 : 5510-5530 : DOI : 10.1016/j.bmc.2018.09.031

Résumé

The TAM kinase family arises as a new effective and attractive therapeutic target for cancer therapy, autoimmune and viral diseases. A series of 2,6-disubstituted imidazo[4,5-b]pyridines were designed, synthesized and identified as highly potent TAM inhibitors. Despite remarkable structural similarities within the TAM family, compounds 28 and 25 demonstrated high activity and selectivity in vitro against AXL and MER, with IC value of 0.77 nM and 9 nM respectively and a 120- to 900-fold selectivity. We also observed an unexpected nuclear localization for compound 10Bb, thanks to nanoSIMS technology, which could be correlated to the absence of cytotoxicity on three different cancer cell lines being sensitive to TAM inhibition.



Hee-Sheung Lee, Mar Carmena, Mikhail Liskovykh, Emma Peat, Jung-Hyun Kim, Mitsuo Oshimura, Hiroshi Masumoto, Marie-Paule Teulade-Fichou, Yves Pommier, William C Earnshaw, Vladimir Larionov, Natalay Kouprina (2018 Nov 1)

Systematic Analysis of Compounds Specifically Targeting Telomeres and Telomerase for Clinical Implications in Cancer Therapy.

Cancer research : 78 : 6282-6296 : DOI : 10.1158/0008-5472.CAN-18-0894

Résumé

The targeting of telomerase and telomere maintenance mechanisms represents a promising therapeutic approach for various types of cancer. In this work, we designed a new protocol to screen for and rank the efficacy of compounds specifically targeting telomeres and telomerase. This approach used two isogenic cell lines containing a circular human artificial chromosome (HAC, lacking telomeres) and a linear HAC (containing telomeres) marked with the EGFP transgene: compounds that target telomerase or telomeres should preferentially induce loss of the linear HAC but not the circular HAC. Our assay allowed quantification of chromosome loss by routine flow cytometry. We applied this dual-HAC assay to rank a set of known and newly developed compounds, including G-quadruplex (G4) ligands. Among the

latter group, two compounds -Cu-ttpy and Pt-ttpy- induced a high rate of linear HAC loss with no significant effect on the mitotic stability of a circular HAC. Analysis of the mitotic phenotypes induced by these drugs revealed an elevated rate of chromatin bridges in late mitosis and cytokinesis as well as UFB (Ultrafine Bridges). Chromosome loss after Pt-ttpy or Cu-ttpy treatment correlated with the induction of telomere-associated DNA damage. Overall, this platform enables identification and ranking of compounds that greatly increase chromosome mis-segregation rates as a result of telomere dysfunction and may expedite the development of new therapeutic strategies for cancer treatment.

M Lupu, Ph Maillard, J Mispelter, F Poyer, C D Thomas (2018 Nov 1)

A glycoporphyrin story: from chemistry to PDT treatment of cancer mouse models.

Photochemical & photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology : 17 : 1599-1611 : DOI : 10.1039/c8pp00123e

Résumé

Photodynamic therapy (PDT) represents a non-toxic and non-mutagenic antitumor therapy. The photosensitizer's (PS) chemo-physical properties are essential for the therapy, being responsible for the biological effects induced in the targeted tissues. In this study, we present the synthesis and development of some glycoconjugated porphyrins based on lectin-type receptor interaction. They were tested *in vitro* for finally choosing the most effective chemical structure for an optimum antitumor outcome. The most effective photosensitizer is substituted by three diethylene glycol α-D-mannosyl groups. *In vivo* studies allow firstly the determination of some characteristics of the biological processes triggered by the initial photochemical activation. Secondly, they make it possible to improve the therapeutic protocol in the function of the structural architecture of the targeted tumor tissue.

Annalisa Patriarca, Charles Fouillade, Michel Auger, Frédéric Martin, Frédéric Pouzoulet, Catherine Nauraye, Sophie Heinrich, Vincent Favaudon, Samuel Meyroneinc, Rémi Dendale, Alejandro Mazal, Philip Poortmans, Pierre Verrelle, Ludovic De Marzi (2018 Nov 1)

Experimental set-up for FLASH proton irradiation of small animals using a clinical system

International Journal of Radiation Oncology • Biology • Physics : 102 : 619-626 : DOI : 10.1016/j.ijrobp.2018.06.403

Résumé

Purpose

Recent *in vivo* investigations have shown that short pulses (FLASH) of electrons are less harmful to healthy tissues, but just as efficient as conventional dose-rate radiation to inhibit

tumor growth. In view of the potential clinical value of FLASH and the availability of modern proton therapy infrastructures to achieve this goal, we herein describe a series of technological developments required to investigate the biology of FLASH irradiation, using a commercially available clinical proton therapy system.

Methods and materials

Numerical simulations and experimental dosimetric characterization of a modified clinical proton beamline, upstream from the isocenter were performed with Monte Carlo toolkit and different detectors. A single scattering system was optimized together with a ridge filter and a high current monitoring system. In addition, a submillimetric set-up protocol based on image-guidance using a digital camera and an animal positioning system was also developed.

Results

The dosimetric properties of the resulting beam and monitoring system were characterized: linearity with dose rate and homogeneity for a 12×12 mm^2 field size were assessed. Dose rates exceeding 40 Gy/s at energies between 138 and 198 MeV were obtained, enabling uniform irradiation for radiobiology investigations on small animals in a modified clinical proton beam line.

Conclusion

This approach will enable us to conduct FLASH proton therapy experiments on small animals, specifically for mouse lung irradiation. Dose rates exceeding 40 Gy/s were achieved, which was not possible with the conventional clinical mode of the existing beamline.

Veronica Rodilla, Silvia Fre (2018 Nov 1)

Cellular Plasticity of Mammary Epithelial Cells Underlies Heterogeneity of Breast Cancer.

Biomedicines : DOI : 10.3390/biomedicines6040103

Résumé

The hierarchical relationships between stem cells, lineage-committed progenitors, and differentiated cells remain unclear in several tissues, due to a high degree of cell plasticity, allowing cells to switch between different cell states. The mouse mammary gland, similarly to other tissues such as the prostate, the sweat gland, and the respiratory tract airways, consists of an epithelium exclusively maintained by unipotent progenitors throughout adulthood. Such unipotent progenitors, however, retain a remarkable cellular plasticity, as they can revert to multipotency during epithelial regeneration as well as upon oncogene activation. Here, we revise the current knowledge on mammary cell hierarchies in light of the most recent lineage tracing studies performed in the mammary gland and highlight how stem cell differentiation or reversion to multipotency are at the base of tumor development and progression. In addition, we will discuss the current knowledge about the interplay

between tumor cells of origin and defined genetic mutations, leading to different tumor types, and its implications in choosing specific therapeutic protocols for breast cancer patients.

Tanguy Lucas, Huy Tran, Carmina Angelica Perez Romero, Aurélien Guillou, Cécile Fradin, Mathieu Coppey, Aleksandra M Walczak, Nathalie Dostatni (2018 Oct 27)
3 minutes to precisely measure morphogen concentration.
PLoS genetics : e1007676 : DOI : 10.1371/journal.pgen.1007676

Résumé

Morphogen gradients provide concentration-dependent positional information along polarity axes. Although the dynamics of the establishment of these gradients is well described, precision and noise in the downstream activation processes remain elusive. A simple paradigm to address these questions is the Bicoid morphogen gradient that elicits a rapid step-like transcriptional response in young fruit fly embryos. Focusing on the expression of the major Bicoid target, hunchback (hb), at the onset of zygotic transcription, we used the MS2-MCP approach which combines fluorescent labeling of nascent mRNA with live imaging at high spatial and temporal resolution. Removing 36 putative Zelda binding sites unexpectedly present in the original MS2 reporter, we show that the 750 bp of the hb promoter are sufficient to recapitulate endogenous expression at the onset of zygotic transcription. After each mitosis, in the anterior, expression is turned on to rapidly reach a plateau with all nuclei expressing the reporter. Consistent with a Bicoid dose-dependent activation process, the time period required to reach the plateau increases with the distance to the anterior pole. Despite the challenge imposed by frequent mitoses and high nuclei-to-nuclei variability in transcription kinetics, it only takes 3 minutes at each interphase for the MS2 reporter loci to distinguish subtle differences in Bicoid concentration and establish a steadily positioned and steep (Hill coefficient ~ 7) expression boundary. Modeling based on the cooperativity between the 6 known Bicoid binding sites in the hb promoter region, assuming rate limiting concentrations of the Bicoid transcription factor at the boundary, is able to capture the observed dynamics of pattern establishment but not the steepness of the boundary. This suggests that a simple model based only on the cooperative binding of Bicoid is not sufficient to describe the spatiotemporal dynamics of early hb expression.

Abhijit Saha, Sophie Bombard, Anton Granzhan, Marie-Paule Teulade-Fichou (2018 Oct 27)
Probing of G-Quadruplex Structures via Ligand-Sensitized Photochemical Reactions in ^{Br}U-Substituted DNA.
Scientific Reports : 8 : 15814 : DOI : 10.1038/s41598-018-34141-z

Résumé

We studied photochemical reactions of ^{Br}U-substituted G-quadruplex (G4) DNA substrates with two pyrene-substituted polyazamacrocyclic ligands, M-1PY and M-2PY. Both ligands bind to and stabilize G4-DNA structures without altering their folding topology, as demonstrated

by FRET-melting experiments, fluorimetric titrations and CD spectroscopy. Notably, the bis-pyrene derivative (M-2PY) behaves as a significantly more affine and selective G4 ligand, compared with its mono-pyrene counterpart (M-1PY) and control compounds. Upon short UVA irradiation (365 nm) both ligands, in particular M-2PY, efficiently sensitize photoreactions at ^{Br}U residues incorporated in G4 structures and give rise to two kinds of photoproducts, namely DNA strand cleavage and covalent ligand-DNA photoadducts. Remarkably, the photoinduced strand cleavage is observed exclusively with G4 structures presenting ^{Br}U residues in lateral or diagonal loops, but not with parallel G4-DNA structures presenting only propeller loops. In contrast, the formation of fluorescent photoadducts is observed with all ^{Br}U-substituted G4-DNA substrates, with M-2PY giving significantly higher yields (up to 27%) than M-1PY. Both ligand-sensitized photoreactions are specific to ^{Br}U-modified G4-DNA structures with respect to double-stranded or stem-loop substrates. Thus, ligand-sensitized photoreactions with ^{Br}U-substituted G4-DNA may be exploited (i) as a photochemical probe, allowing « photofootprinting » of G4 folding topologies *in vitro* and (ii) for covalent trapping of G4 structures as photoadducts with pyrene-substituted ligands.

Arthur Charles-Orszag, Feng-Ching Tsai, Daria Bonazzi, Valeria Manriquez, Martin Sachse, Adeline Mallet, Audrey Salles, Keira Melican, Ralitza Staneva, Aurélie Bertin, Corinne Millien, Sylvie Goussard, Pierre Lafaye, Spencer Shorte, Matthieu Piel, Jacomine Krijnse-Locker, Françoise Brochard-Wyart, Patricia Bassereau, Guillaume Duménil (2018 Oct 27)

Adhesion to nanofibers drives cell membrane remodeling through one-dimensional wetting.

Nature communications : 4450 : DOI : 10.1038/s41467-018-06948-x

Résumé

The shape of cellular membranes is highly regulated by a set of conserved mechanisms that can be manipulated by bacterial pathogens to infect cells. Remodeling of the plasma membrane of endothelial cells by the bacterium Neisseria meningitidis is thought to be essential during the blood phase of meningococcal infection, but the underlying mechanisms are unclear. Here we show that plasma membrane remodeling occurs independently of F-actin, along meningococcal type IV pili fibers, by a physical mechanism that we term 'one-dimensional' membrane wetting. We provide a theoretical model that describes the physical basis of one-dimensional wetting and show that this mechanism occurs in model membranes interacting with nanofibers, and in human cells interacting with extracellular matrix meshworks. We propose one-dimensional wetting as a new general principle driving the interaction of cells with their environment at the nanoscale that is diverted by meningococci during infection.

Antoine Hocher, Myriam Ruault, Petra Kaferle, Marc Descrimes, Mickaël Garnier, Antonin Morillon, Angela Taddei (2018 Oct 26)

Expanding heterochromatin reveals discrete subtelomeric domains delimited by

chromatin landscape transitions.
Genome research : DOI : gr.236554.118

Résumé

The eukaryotic genome is divided into chromosomal domains of heterochromatin and euchromatin. Transcriptionally silent heterochromatin is found at subtelomeric regions, leading to the telomeric position effect (TPE) in yeast fly and human. Heterochromatin generally initiates and spreads from defined loci, and diverse mechanisms prevent the ectopic spread of heterochromatin into euchromatin. Here, we overexpressed the silencing factor Sir3 at varying levels in yeast and found that Sir3 spreads into Extended Silent Domains (ESDs), eventually reaching saturation at subtelomeres. We observed the spread of Sir3 into subtelomeric domains associated with specific histone marks in wild-type cells and stopping at zones of histone mark transitions including H3K79 tri-methylation levels. Our study shows that the conserved H3K79 methyltransferase Dot1 is essential in restricting Sir3 spread beyond ESDs, thus ensuring viability upon overexpression of Sir3. Lastly, our analyses of published data demonstrate how ESDs unveil uncharacterized discrete domains isolating structural and functional subtelomeric features from the rest of the genome. Our work offers a new approach on how to separate subtelomeres from the core chromosome.

Stéphanie Torrino, Wei-Wei Shen, Cédric M Blouin, Satish Kailasam Mani, Christine Viaris de Lesegno, Pierre Bost, Alexandre Grassart, Darius Köster, Cesar Augusto Valades-Cruz, Valérie Chambon, Ludger Johannes, Paolo Pierobon, Vassili Soumelis, Catherine Coirault, Stéphane Vassilopoulos, Christophe Lamaze (2018 Oct 24)

EHD2 is a mechanotransducer connecting caveolae dynamics with gene transcription.
The Journal of cell biology : 4092-4105 : DOI : 10.1083/jcb.201801122

Résumé

Caveolae are small invaginated pits that function as dynamic mechanosensors to buffer tension variations at the plasma membrane. Here we show that under mechanical stress, the EHD2 ATPase is rapidly released from caveolae, SUMOylated, and translocated to the nucleus, where it regulates the transcription of several genes including those coding for caveolae constituents. We also found that EHD2 is required to maintain the caveolae reservoir at the plasma membrane during the variations of membrane tension induced by mechanical stress. Metal-replica electron microscopy of breast cancer cells lacking EHD2 revealed a complete absence of caveolae and a lack of gene regulation under mechanical stress. Expressing EHD2 was sufficient to restore both functions in these cells. Our findings therefore define EHD2 as a central player in mechanotransduction connecting the disassembly of the caveolae reservoir with the regulation of gene transcription under mechanical stress.

Anne C Ferguson-Smith, Deborah Bourc'his (2018 Oct 23)

The discovery and importance of genomic imprinting.
eLife : DOI : 10.7554/eLife.42368

Résumé

The discovery of genomic imprinting by Davor Solter, Azim Surani and co-workers in the mid-1980s has provided a foundation for the study of epigenetic inheritance and the epigenetic control of gene activity and repression, especially during development. It also has shed light on a range of diseases, including both rare genetic disorders and common diseases. This article is being published to celebrate Solter and Surani receiving a 2018 Canada Gairdner International Award « for the discovery of mammalian genomic imprinting that causes parent-of-origin specific gene expression and its consequences for development and disease ».

Huy Tran, Carmina Angelica Perez-Romero, Teresa Ferraro, Cécile Fradin, Nathalie Dostatni, Mathieu Coppey, Aleksandra M Walczak (2018 Oct 17)
LiveFly: A Toolbox for the Analysis of Transcription Dynamics in Live Drosophila Embryos.
Methods in molecular biology (Clifton, N.J.) : 183-195 : DOI : 10.1007/978-1-4939-8772-6_11

Résumé

We present the LiveFly toolbox for quantitative analysis of transcription dynamics in live Drosophila embryos. The toolbox allows users to process two-color 3D confocal movies acquired using nuclei-labeling and the fluorescent RNA-tagging system described in the previous chapter and export the nuclei's position as a function of time, their lineages and the intensity traces of the active loci. The toolbox, which is tailored for the context of Drosophila early development, is semiautomatic, and requires minimal user intervention. It also includes a tool to combine data from multiple movies and visualize several features of the intensity traces and the expression pattern.

Carmina Angelica Perez-Romero, Huy Tran, Mathieu Coppey, Aleksandra M Walczak, Cécile Fradin, Nathalie Dostatni (2018 Oct 17)
Live Imaging of mRNA Transcription in Drosophila Embryos.
Methods in molecular biology (Clifton, N.J.) : 165-182 : DOI : 10.1007/978-1-4939-8772-6_10

Résumé

Live imaging has been used in recent years for the understanding of dynamic processes in biology, such as embryo development. This was made possible by a combination of advancements in microscopy, leading to improved signal-to-noise ratios and better spatial and temporal resolutions, and by the development of new fluorescence markers, allowing for the quantification of protein expression and transcriptional dynamics in vivo. Here we describe a general protocol, which can be used in standard confocal microscopes to image

early Drosophila melanogaster embryos, in order to learn about the transcriptional dynamics of a fluorescently labeled RNA.