Année de publication : 2016

Laetitia Saint-Paul, Chi-Hung Nguyen, Anne Buffière, Jean-Paul Pais de Barros, Arlette Hammann, Corinne Landras-Guetta, Rodolphe Filomenko, Marie-Lorraine Chrétien, Pauline Johnson, Jean-Noël Bastie, Laurent Delva, Ronan Quéré (2016 Sep 1)

CD45 phosphatase is crucial for human and murine acute myeloid leukemia maintenance through its localization in lipid rafts.

Oncotarget : 7 : 64785-64797 : DOI : 10.18632/oncotarget.11622

Résumé

CD45 is a pan-leukocyte protein with tyrosine phosphatase activity involved in the regulation of signal transduction in hematopoiesis. Exploiting CD45 KO mice and lentiviral shRNA, we prove the crucial role that CD45 plays in acute myeloid leukemia (AML) development and maintenance. We discovered that CD45 does not colocalize with lipid rafts on murine and human non-transformed hematopoietic cells. Using a mouse model, we proved that CD45 positioning within lipid rafts is modified during their oncogenic transformation to AML. CD45 colocalized with lipid rafts on AML cells, which contributes to elevated GM-CSF signal intensity involved in proliferation of leukemic cells. We furthermore proved that the GM-CSF/Lyn/Stat3 pathway that contributes to growth of leukemic cells could be profoundly affected, by using a new plasma membrane disrupting agent, which rapidly delocalized CD45 away from lipid rafts. We provide evidence that this mechanism is also effective on human primary AML samples and xenograft transplantation. In conclusion, this study highlights the emerging evidence of the involvement of lipid rafts in oncogenic development of AML and the targeting of CD45 positioning among lipid rafts as a new strategy in the treatment of AML.

Clément Grandin, Marianne-LucasHourani, Yves L Janin, Daniel Dauzonne, Hélène Munier-Lehmann, Adeline Paturet, Fabrice Taborik, Astrid Vabret, Hugues Contamin, Frédéric Tangy, Pierre-Olivier Vidalain (2015 Nov 25)

Respiratory syncytial virus infection in macaques is not suppressed by intranasal sprays of pyrimidine biosynthesis inhibitors.

Antiviral research : 58-62 : DOI : 10.1016/j.antiviral.2015.11.006

Résumé

There is imperious need for efficient therapies against ubiquitous and life-threatening respiratory viruses, foremost among them being the human respiratory syncytial virus (hRSV). Several research groups who performed functional screens for broad-spectrum antivirals identified compounds targeting the de novo pyrimidine biosynthesis pathway. Despite their strong antiviral activity in vitro, whether such antimetabolites are effective in vivo remains highly controversial. Here, we evaluated two potent pyrimidine biosynthesis inhibitors developed in our laboratory, IPPA17-A04 and GAC50, in a model of mild hRSV-infection in cynomolgus macaques. In this model, hRSV replication is restricted to the epithelium of the upper respiratory tract, and is compatible with a topical treatment by

intranasal sprays. The local administration of palivizumab, a neutralizing anti-hRSV antibody used in clinics, significantly reduced virus replication. In contrast, pyrimidine biosynthesis inhibitors did not show any inhibitory effect on hRSV growth when delivered topically as experimented in our model. Our results should help to better define the potential applications of this class of antimetabolites in the treatment of viral infections.

Année de publication : 2015

Ly-Thuy-Tram Le, Morgane Couvet, Bertrand Favier, Jean-Luc Coll, Chi-Hung Nguyen, Annie Molla (2015 Sep 8)

Discovery of benzo[e]pyridoindolones as kinase inhibitors that disrupt mitosis exit while erasing AMPK-Thr172 phosphorylation on the spindle.

Oncotarget : 6 : 22152-22166 : DOI : 10.18632/oncotarget.4158

Résumé

Aurora kinases play an essential role in mitotic progression and are attractive targets in cancer therapy. The first generation of benzo[e]pyridoindole exhibited powerful aurora kinase inhibition but their low solubility limited further development. Grafting a pyperidine-ethoxy group gives rise to a hydrosoluble inhibitor: compound C5M.C5M could efficiently inhibit the proliferation of cells from different origins. C5M prevented cell cycling, induced a strong mitotic arrest then, cells became polyploid and finally died. C5M did not impair the spindle checkpoint, the separation of the sister chromatids and the transfer of aurora B on the mid-zone. C5M prevented histone H3 phosphorylation at mitotic entry and erased AMPK-Thr172 phosphorylation in late mitosis. With this unique profile of inhibition, C5M could be useful for understanding the role of phospho-Thr172-AMPK in abscission and the relationship between the chromosomal complex and the energy sensing machinery.C5M is a multikinase inhibitor with interesting preclinical characteristics: high hydro-solubility and a good stability in plasma. A single dose prevents the expansion of multicellular spheroids. C5M can safely be injected to mice and reduces significantly the development of xenograft. The next step will be to define the protocol of treatment and the cancer therapeutic field of this new anti-proliferative drug.

Florence Mahuteau-Betzer (2015 May 12)

The French National Compound Library: advances and future prospects.

Médecine sciences : M/S : 31 : 417-422 : DOI : 10.1051/medsci/20153104016

Résumé

The French National Compound Library (Chimiothèque Nationale) has been created in 2003 and is the federation of local collections. It contains more than 56 000 small molecules and natural compounds synthesised or isolated in different laboratories over the past years. This explains the diversity of the collection. The strength of this initiative is the ability to connect chemists and biologists for the development of hits. This development involves the synthesis

of analogues or/and chemical tools to find new targets. These collaborations lead to the identification of new chemical probes. These probes able to modulate a biological function are essential to study biological pathways. They can also be useful for therapeutic applications. This article will describe the major achievements and perspectives of the French Chemical Library.

Année de publication : 2013

Marianne Lucas-Hourani, Daniel Dauzonne, Pierre Jorda, Gaëlle Cousin, Alexandru Lupan, Olivier Helynck, Grégory Caignard, Geneviève Janvier, Gwénaëlle André-Leroux, Samira Khiar, Nicolas Escriou, Philippe Desprès, Yves Jacob, Hélène Munier-Lehmann, Frédéric Tangy, Pierre-Olivier Vidalain (2013 Oct 8)

Inhibition of pyrimidine biosynthesis pathway suppresses viral growth through innate immunity.

PLoS pathogens : e1003678 : DOI : 10.1371/journal.ppat.1003678

Résumé

Searching for stimulators of the innate antiviral response is an appealing approach to develop novel therapeutics against viral infections. Here, we established a cell-based reporter assay to identify compounds stimulating expression of interferon-inducible antiviral genes. DD264 was selected out of 41,353 compounds for both its immuno-stimulatory and antiviral properties. While searching for its mode of action, we identified DD264 as an inhibitor of pyrimidine biosynthesis pathway. This metabolic pathway was recently identified as a prime target of broad-spectrum antiviral molecules, but our data unraveled a yet unsuspected link with innate immunity. Indeed, we showed that DD264 or brequinar, a well-known inhibitor of pyrimidine biosynthesis pathway, both enhanced the expression of antiviral genes in human cells. Furthermore, antiviral activity of DD264 or brequinar was found strictly dependent on cellular gene transcription, nuclear export machinery, and required IRF1 transcription factor. In conclusion, the antiviral property of pyrimidine biosynthesis inhibitors is not a direct consequence of pyrimidine deprivation on the virus machinery, but rather involves the induction of cellular immune response.

Ly-Thuy-Tram Le, Hong-Lien Vu, Delphine Naud-Martin, Marianne Bombled, Chi-Hung Nguyen, Annie Molla (2013 Jan 3)

Hydrosoluble benzo[e]pyridoindolones as potent inhibitors of aurora kinases.

ChemMedChem : 8 : 289-96 : DOI : 10.1002/cmdc.201200479

Résumé

Aurora kinases play an essential role in mitotic progression and are potentially druggable targets in cancer therapy. We identified benzo[e]pyridoindoles (BePI) as powerful aurora kinase inhibitors. Their efficiency was demonstrated both in enzymatic inhibition studies and

in cell culture assays. New BePI molecules were synthesized, and a structure-activity relationship study was conducted with the aim of improving the activity and solubility of the lead compound. Tetracyclic BePI derivatives are characterized by a particular curved shape, and the presence of an oxo group on the pyridine ring was found to be required for aurora kinase B inhibition. New hydrosoluble benzo[e]pyridoindolones were subsequently designed, and their efficacy was tested by a combination of cell-cycle analysis and time-lapse experiments in live cells. The most active BePI derivative, 13 b, inhibited the cell cycle, drove cells to polyploidy, and eventually induced apoptosis. It exhibited high antiproliferative activity in HeLa cells with an IC(50) value of 63 nM. Relative to compounds tested in clinical trials, this antiproliferative potency places 13 b among the top 10 aurora kinase inhibitors. Our results justify further in vivo evaluation in preclinical animal models of cancer.

Année de publication : 2012

Ada Collura, Laetitia Marisa, Diletta Trojan, Olivier Buhard, Anaïs Lagrange, Arnaud Saget, Marianne Bombled, Patricia Méchighel, Mira Ayadi, Martine Muleris, Aurélien de Reynies, Magali Svrcek, Jean-François Fléjou, Jean-Claude Florent, Florence Mahuteau-Betzer, Anne-Marie Faussat, Alex Duval (2012 Sep 26)

Extensive characterization of sphere models established from colorectal cancer cell lines.

Cellular and molecular life sciences : CMLS : 2 : 729-42 : DOI : 10.1007/s00018-012-1160-9

Résumé

Links between cancer and stem cells have been proposed for many years. As the cancer stem cell (CSC) theory became widely studied, new methods were developed to culture and expand cancer cells with conserved determinants of « stemness ». These cells show increased ability to grow in suspension as spheres in serum-free medium supplemented with growth factors and chemicals. The physiological relevance of this phenomenon in established cancer cell lines remains unclear. Cell lines have traditionally been used to explore tumor biology and serve as preclinical models for the screening of potential therapeutic agents. Here, we grew cell-forming spheres (CFS) from 25 established colorectal cancer cell lines. The molecular and cellular characteristics of CFS were compared to the bulk of tumor cells. CFS could be isolated from 72 % of the cell lines. Both CFS and their parental CRC cell lines were highly tumorigenic. Compared to their parental cells, they showed similar expression of putative CSC markers. The ability of CRC cells to grow as CFS was greatly enhanced by prior treatment with 5-fluorouracil. At the molecular level, CFS and parental CRC cells showed identical gene mutations and very similar genomic profiles, although microarray analysis revealed changes in CFS gene expression that were independent of DNA copy-number. We identified a CFS gene expression signature common to CFS from all CRC cell lines, which was predictive of disease relapse in CRC patients. In conclusion, CFS models derived from CRC cell lines possess interesting phenotypic features that may have clinical relevance for drug resistance and disease relapse.

Renaud Prudent, Émilie Vassal-Stermann, Chi-Hung Nguyen, Marjorie Mollaret, Jean Viallet, Agnès Desroches-Castan, Anne Martinez, Caroline Barette, Catherine Pillet, Glaucio Valdameri, Emmanuelle Soleilhac, Attilio Di Pietro, Jean-Jacques Feige, Marc Billaud, Jean-Claude Florent, Laurence Lafanechère (2012 Sep 26)

Azaindole derivatives are inhibitors of microtubule dynamics, with anti-cancer and anti-angiogenic activities.

British journal of pharmacology : 673-85 : DOI : 10.1111/j.1476-5381.2012.02230.x

Résumé

Drugs targeting microtubules are commonly used for cancer treatment. However, the potency of microtubule inhibitors used clinically is limited by the emergence of resistance. We thus designed a strategy to find new cell-permeable microtubule-targeting agents.

Renaud Prudent, Emilie Vassal-Stermann, Chi-Hung Nguyen, Catherine Pillet, Anne Martinez, Chloé Prunier, Caroline Barette, Emmanuelle Soleilhac, Odile Filhol, Anne Beghin, Glaucio Valdameri, Stéphane Honoré, Samia Aci-Sèche, David Grierson, Juliana Antonipillai, Rong Li, Attilio Di Pietro, Charles Dumontet, Diane Braguer, Jean-Claude Florent, Stefan Knapp, Ora Bernard, Laurence Lafanechère (2012 Jul 5)

Pharmacological inhibition of LIM kinase stabilizes microtubules and inhibits neoplastic growth.

Cancer research : 4429-39

Résumé

The emergence of tumor resistance to conventional microtubule-targeting drugs restricts their clinical use. Using a cell-based assay that recognizes microtubule polymerization status to screen for chemicals that interact with regulators of microtubule dynamics, we identified Pyr1, a cell permeable inhibitor of LIM kinase, which is the enzyme that phosphorylates and inactivates the actin-depolymerizing factor cofilin. Pyr1 reversibly stabilized microtubules, blocked actin microfilament dynamics, inhibited cell motility in vitro and showed anticancer properties in vivo, in the absence of major side effects. Pyr1 inhibition of LIM kinase caused a microtubule-stabilizing effect, which was independent of any direct effects on the actin cytoskeleton. In addition, Pyr1 retained its activity in multidrug-resistant cancer cells that were resistant to conventional microtubule-targeting agents. Our findings suggest that LIM kinase functions as a signaling node that controls both actin and microtubule dynamics. LIM kinase may therefore represent a targetable enzyme for cancer treatment.

Année de publication : 2011

Alexandre Ceccaldi, Arumugam Rajavelu, Christine Champion, Christine Rampon, Renata Jurkowska, Gytis Jankevicius, Catherine Sénamaud-Beaufort, Loïc Ponger, Nathalie Gagey, Hana

Dali Ali, Jörg Tost, Sophie Vriz, Sindu Ros, Daniel Dauzonne, Albert Jeltsch, Dominique Guianvarc'h, Paola B Arimondo (2011 Jun 3)

C5-DNA methyltransferase inhibitors: from screening to effects on zebrafish embryo development.

Chembiochem : a European journal of chemical biology : 12 : 1337-45 : DOI : 10.1002/cbic.201100130

Résumé

DNA methylation is involved in the regulation of gene expression and plays an important role in normal developmental processes and diseases, such as cancer. DNA methyltransferases are the enzymes responsible for DNA methylation on the position 5 of cytidine in a CpG context. In order to identify and characterize novel inhibitors of these enzymes, we developed a fluorescence-based throughput screening by using a short DNA duplex immobilized on 96-well plates. We have screened 114 flavones and flavanones for the inhibition of the murine catalytic Dnmt3a/3L complex and found 36 hits with IC(50) values in the lower micromolar and high nanomolar ranges. The assay, together with inhibition tests on two other methyltransferases, structure-activity relationships and docking studies, gave insights on the mechanism of inhibition. Finally, two derivatives effected zebrafish embryo development, and induced a global demethylation of the genome, at doses lower than the control drug, 5-azacytidine.

Année de publication : 2010

Renaud Prudent, Virginie Moucadel, Chi-Hung Nguyen, Caroline Barette, Frédéric Schmidt, Jean-Claude Florent, Laurence Lafanechère, Céline F Sautel, Eve Duchemin-Pelletier, Elodie Spreux, Odile Filhol, Jean-Baptiste Reiser, Claude Cochet (2010 Dec 2)

Antitumor activity of pyridocarbazole and benzopyridoindole derivatives that inhibit protein kinase CK2.

Cancer research : 9865-74 : DOI : 10.1158/0008-5472.CAN-10-0917

Résumé

The alkyloid compound ellipticine derived from the berrywood tree is a topoisomerase II poison that is used in ovarian and breast cancer treatment. In this study, we report the identification of ellipticine derivatives and their tetracyclic angular benzopyridoindole analogues as novel ATP-competitive inhibitors of the protein kinase CK2. In vitro and in vivo assays showed that these compounds have a good pharmacologic profile, causing a marked inhibition of CK2 activity associated with cell cycle arrest and apoptosis in human cancer cells. Further, in vivo assays demonstrate antitumor activity in a mouse xenograft model of human glioblastoma. Finally, crystal structures of CK2-inhibitor complex provide structural insights on the molecular basis of CK2 inhibition. Our work lays the foundation for development of clinically useful CK2 inhibitors derived from a well-studied scaffold with suitable pharmacokinetics parameters.

Miriam López-Ramos, Renaud Prudent, Virginie Moucadel, Céline F Sautel, Caroline Barette, Laurence Lafanechère, Liliane Mouawad, David Grierson, Frédéric Schmidt, Jean-Claude Florent, Panagis Filippakopoulos, Alex N Bullock, Stefan Knapp, Jean-Baptiste Reiser, Claude Cochet (2010 Apr 20)

New potent dual inhibitors of CK2 and Pim kinases: discovery and structural insights.

FASEB journal : official publication of the Federation of American Societies for Experimental Biology : 3171-85 : DOI : 10.1096/fj.09-143743

Résumé

Protein kinase casein kinase 2 (CK2) is a serine/threonine kinase with evidence of implication in growth dysregulation and apoptosis resistance, making it a relevant target for cancer therapy. Several CK2 inhibitors have been developed showing variable efficiency, emphasizing the need to expand the chemical diversity of those inhibitors. We report the identification and characterization of 2,8-difurandicarboxylic acid derivatives as a new class of nanomolar ATP-competitive inhibitors. Selectivity profiling pointed out proviral insertion Moloney virus kinases (Pim kinases) as the only other kinases that are significantly inhibited. By combining structure-activity relationship analysis with structural determination, we were able to determine the binding mode of these inhibitors for both kinases and to explain their strong inhibitory potency. Essential chemical features necessary for activity on both kinases were then identified. The described compounds are not cell permeable: however, they could provide a lead for developing novel inhibitors usable also in vivo. Given the similar but not redundant pathophysiological functions of CK2 and Pim family members, such inhibitors would provide new attractive leads for targeted cancer therapy. This work highlights that 2 functionally related kinases from different kinome branches display exquisite sensitivity to a common inhibitor.

Thérèse David-Pfeuty, Michel Legraverend, Odile Ludwig, David S Grierson (2010 Mar 4)

Targeting the cell cycle and the PI3K pathway: a possible universal strategy to reactivate innate tumor suppressor programmes in cancer cells.

International journal of oncology : 873-81

Résumé

Corruption of the Rb and p53 pathways occurs in virtually all human cancers. This could be because it lends oncogene-bearing cells a surfeit of Cdk activity and growth, enabling them to elaborate strategies to evade tumor-suppressive mechanisms and divide inappropriately. Targeting both Cdk activities and the PI3K pathway might be therefore a potentially universal means to palliate their deficiency in cancer cells. We showed that the killing efficacy of roscovitine and 16 other purines and potentiation of roscovitine-induced apoptosis by the PI3K inhibitor, LY294002, decreased with increasing corruption of the Rb and p53 pathways. Further, we showed that purines differing by a single substitution, which exerted little lethal effect on distant cell types in rich medium, could display widely-differing cytotoxicity profiles toward the same cell types in poor medium. Thus, closely-related compounds targeting

similar Cdks may interact with different targets that could compete for their interaction with therapeutically-relevant Cdk targets. In the perspective of clinical development in association with the PI3K pathway inhibitors, it might thus be advisable to select tumor cell type-specific Cdk inhibitors on the basis of their toxicity in cell-culture-based assays performed at a limiting serum concentration sufficient to suppress their interaction with undesirable crossreacting targets whose range and concentration would depend on the cell genotype.

Année de publication : 2009

Anne Keriel, Florence Mahuteau-Betzer, Chantal Jacquet, Marc Plays, David Grierson, Marc Sitbon, Jamal Tazi (2009 Feb 20)

Protection against retrovirus pathogenesis by SR protein inhibitors.

PloS one : e4533 : DOI : 10.1371/journal.pone.0004533

Résumé

Indole derivatives compounds (IDC) are a new class of splicing inhibitors that have a selective action on exonic splicing enhancers (ESE)-dependent activity of individual serine-arginine-rich (SR) proteins. Some of these molecules have been shown to compromise assembly of HIV infectious particles in cell cultures by interfering with the activity of the SR protein SF2/ASF and by subsequently suppressing production of splicing-dependent retroviral accessory proteins. For all replication-competent retroviruses, a limiting requirement for infection and pathogenesis is the expression of the envelope glycoprotein which strictly depends on the host splicing machinery. Here, we have evaluated the efficiency of IDC on an animal model of retroviral pathogenesis using a fully replication-competent retrovirus. In this model, all newborn mice infected with a fully replicative murine leukemia virus (MLV) develop erythroleukemia within 6 to 8 weeks of age. We tested several IDC for their ability to interfere ex vivo with MLV splicing and virus spreading as well as for their protective effect in vivo. We show here that two of these IDC, IDC13 and IDC78, selectively altered splicing-dependent production of the retroviral envelope gene, thus inhibiting early viral replication in vivo, sufficiently to protect mice from MLV-induced pathogenesis. The apparent specificity and clinical safety observed here for both IDC13 and IDC78 strongly support further assessment of inhibitors of SR protein splicing factors as a new class of antiretroviral therapeutic agents.

Thi My-Nhung Hoang, Bertrand Favier, Annie Valette, Caroline Barette, Chi Hung Nguyen, Laurence Lafanechère, David S Grierson, Stéfan Dimitrov, Annie Molla (2009 Feb 18)

Benzo[e]pyridoindoles, novel inhibitors of the aurora kinases.

Cell cycle (Georgetown, Tex.) : 765-72

Résumé

Aurora kinases are serine/threonine protein kinases that are involved in cancer development and are important targets for cancer therapy. By high throughput screening of a chemical

library we found that benzo[e]pyridoindole derivatives inhibited Aurora kinase. The most potent compound (compound 1) was found to be an ATP competitive inhibitor, which inhibited in vitro Aurora kinases at the nanomolar range. It prevented, ex vivo, the phosphorylation of Histone H3, induced mitosis exit without chromosome segregation, known phenomena observed upon Aurora B inactivation. This compound was also shown to affect the localization of Aurora B, since in the presence of the inhibitor the enzyme was delocalized on the whole chromosomes and remained associated with the chromatin of newly formed nuclei. In addition, compound 1 inhibited the growth of different cell lines derived from different carcinoma. Its IC(50) for H358 NSCLC (Non Small Cancer Lung Cells), the most sensitive cell line, was 145 nM. Furthermore compound 1 was found to be efficient towards multicellular tumor spheroid growth. It exhibited minimal toxicity in mice while it had some potency towards aggressive NSCLC tumors. Benzo[e]pyridoindoles represent thus a potential new lead for the development of Aurora kinase inhibitors.