Année de publication : 2022

Zucolotto Cocca L.H., Pelosi A., Sciuti L.F., M. G. Abegão L., Kamada K., Piguel S., Renato Mendonça C., De Boni L. (2022 Feb 15)

Two-photon brightness of highly fluorescent imidazopyridine derivatives: Two-photon and ultrafast transient absorption studies

Journal of Molecular Liquids : 348 : 118379 : DOI : 10.1016/j.molliq.2021.118379

Résumé

Imidazopyridine derivatives are unnatural purines isosteres and have been idealized and synthesized in the last decades, given their great applicability in several science areas. For instance, they may present potential to be used as fluorescent bioprobes on DNA or RNA spectroscopic studies. Furthermore, their synthesis can be performed to incorporate different peripheral structures in the central imidazopyridine scaffold, modeling and increasing the optical properties. Aiming applications as fluorescent probes as molecular liquids at the therapeutic window, thanks to the considerable emissive characteristics of this class of compound, optical spectroscopic studies considering nonlinear optical properties were performed on two distinct classes of N3-MEM-3H-imidazo[4,5-b]pyridine derivatives. The presented results exposed exceptionally high fluorescence quantum yields and two-photon absorption effects at the therapeutic window. The two-photon absorption spectra revealed a particular contribution of a higher excited state in some imidazopyridine derivatives containing naphthalene group. The excited state was confirmed by femtosecond time resolved spectroscopy. The obtained two-photon brightness shows substantial values for all compounds, with the potential to be used as fluorescent probes induced by two-photon excitation.

Clelia Mathieu, Quentin Chamayou, Thi Thanh Hyen Luong, Delphine Naud, Florence Mahuteau-Betzer, Mouad Alami, Elias Fattal, Samir Messaoudi, Juliette Vergnaud-Gauduchon (2022 Feb 5)

Synthesis and antiproliferative activity of 6BrCaQ-TPP conjugates for targeting the mitochondrial heat shock protein TRAP1.

European journal of medicinal chemistry : 229 : 114052 : DOI : 10.1016/j.ejmech.2021.114052

Résumé

A series of 6BrCaQ-C-TPP conjugates 3a-f and 5 was designed and synthesized as a novel class of TRAP1 inhibitors. Compound 3a displayed an excellent anti-proliferative activity with mean GI values at a nanomolar level in a diverse set of human cancer cells (GI = 0.008-0.30 μM) including MDA-MB231, HT-29, HCT-116, K562, and PC-3 cancer cell lines. Moreover, the best lead compound 6BrCaQ-C-TPP induces a significant mitochondrial membrane disturbance combined to a regulation of HSP and partner protein levels as a first evidence that his mechanism of action involves the TRAP-1 mitochondrial Hsp90 machinery.

Florence Mahuteau-Betzer, Marie Auvray, Frédéric Bolze, Delphine Naud-Martin, Matthieu Poulain, Margaux Bossuat, Gilles Clavier (2022 Jan 21)

On the road for more efficient biocompatible two-photon excitable fluorophores.

Chemistry (Weinheim an der Bergstrasse, Germany) : Accepted Article : DOI : 10.1002/chem.202104378

Résumé

Red to NIR absorption and emission wavelengths are key requirements for intravital bioimaging. One of the way to reach such excitation wavelengths is to use two-photon excitation. Unfortunately, there is still a lack of two-photon excitable fluorophores that are both efficient and biocompatible. Thus, we design a series of biocompatible quadrupolar dyes in order to study their ability to be used for live-cell imaging, and in particular for two-photon microscopy. Hence, we report the synthesis of 5 probes based on different donor cores (phenoxazine, acridane, phenazasiline and phenothiazine) and the study of their linear and non-linear photophysical properties. TD-DFT calculations were performed and were able to highlight the structure-property relationship of this series. All these studies highlight the great potential of three of these biocompatible dyes for two-photon microscopy, as they both exhibit high two-photon cross-sections (up to 3 650 GM) and emit orange to red light. This potential was confirmed through live-cell two-photon microscopy experiments, leading to images with very high brightness and contrast.

Pelosi A.G., Zucolotto Cocca L.H., Abegão L.M., Sciuti L.F., Piguel S., De Boni L., Mendonça C.R. (2022 Jan 1)

Influence of electron-withdrawing groups in two-photon absorption of imidazopyridines derivatives

Dyes and Pigments : 198 : 109972 : DOI : 10.1016/j.dyepig.2021.109972

Résumé

This work investigates the influence of different electron-withdrawing groups (Cl, Br, fluorobenzonitrile), on the two-photon absorption cross-section of six imidazo[4,5-b]pyridine derivatives. The two-photon absorption cross-section spectra were obtained by ultra-fast Z-scan technique from 470 nm up to 700 nm. The Sum-Over-States approach was applied to model the two-photon absorption spectra, using a three-level energy system. Photophysical properties, such as transition dipole moment, the difference of permanent dipole moments, and anisotropy coefficient were determined through the analysis of one-photon absorption spectra, solvatochromism, and fluorescence anisotropy, respectively. Besides, the excited state absorption spectra were measured through ultra-fast transient absorption, allowing the excited state lifetime and spectral position determination. Two-photon absorption cross-sections of about 160 GM were observed when two electron-withdrawing groups are linked to the imidazo[4,5-b]pyridine core, elucidating a path to achieve high optical nonlinearities in imidazopyridine derivatives. Furthermore, a increase in the two-photon cross-section was observed when chloride is linked at the C-6 position (90 GM) instead of the C-5 position (50 GM), which is related to the proximity of a higher excited state.

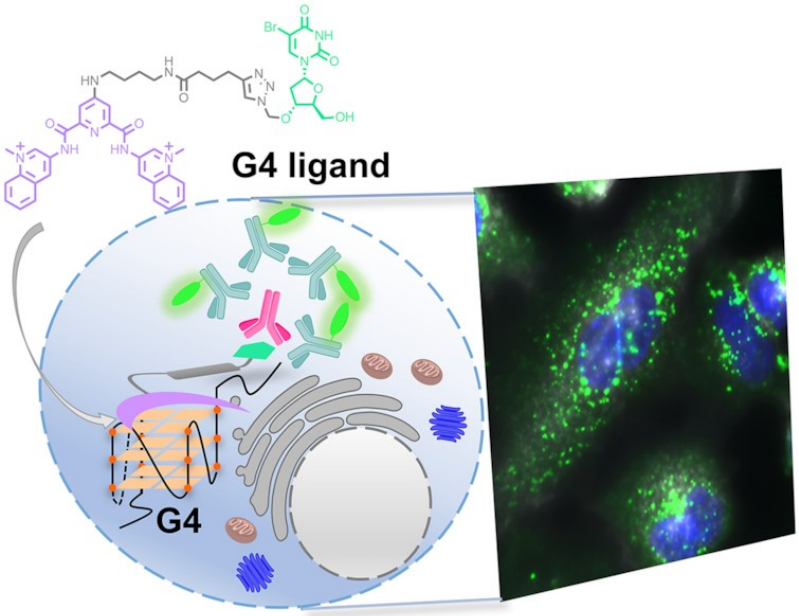
Année de publication : 2021

Masson T., Landras Guetta C., Laigre E., Cucchiarini A., Duchambon P., Teulade-Fichou M.P., Verga D. (2021 Dec 7)

BrdU immuno-tagged G-quadruplex ligands: a new ligand-guided immunofluorescence approach for tracking G-quadruplexes in cells

Nucleic Acids Research : 49 : 12644–12660 : DOI : 10.1093/nar/gkab1166

Résumé



G-quadruplexes (G4s) are secondary structures forming in G-rich nucleic acids. G4s are assumed to play critical roles in biology, nonetheless their detection in cells is still challenging. For tracking G4s, synthetic molecules (G4 ligands) can be used as reporters and have found wide application for this purpose through chemical functionalization with a fluorescent tag. However, this approach is limited by a low-labeling degree impeding precise visualization in specific subcellular regions. Herein, we present a new visualization strategy based on the immuno-recognition of 5-bromo-2′-deoxyuridine (5-BrdU) modified G4 ligands, functionalized prior- or post-G4-target binding by CuAAC. Remarkably, recognition of the tag by antibodies leads to the detection of the modified ligands exclusively when bound to a G4 target both *in vitro*, as shown by ELISA, and in cells, thereby providing a highly efficient G4-ligand Guided Immunofluorescence Staining (G4-GIS) approach. The obtained signal amplification revealed well-defined fluorescent foci located in the perinuclear space and RNase treatment revealed the preferential binding to G4-RNA. Furthermore, ligand treatment affected significantly BG4 foci formation in cells. Our work headed to the development of a new imaging approach combining the advantages of immunostaining and G4-recognition by G4 ligands leading to visualization of G4/ligands species in cells with unrivaled precision and

sensitivity.

Nils-Jørgen Knudsen Dal, Martin Speth, Kerstin Johann, Matthias Barz, Claire Beauvineau, Jens Wohlmann, Federico Fenaroli, Brigitte Gicquel, Gareth Griffiths, Noelia Alonso-Rodriguez (2021 Nov 29)

The zebrafish embryo as an in vivo model for screening nanoparticle-formulated lipophilic anti-tuberculosis compounds.

Disease models & mechanisms : Online ahead of print : DOI : 10.1242/dmm.049147

Résumé

With the increasing emergence of drug-resistant Mycobacterium tuberculosis strains, new and effective antibiotics against tuberculosis (TB) are urgently needed. However, the high frequency of poorly water-soluble compounds among hits in high-throughput drug screening (HTS) campaigns is a major obstacle in drug discovery. Moreover, in vivo testing using conventional animal TB models such as mice is time-consuming and costly, and represents a major bottleneck in lead compound discovery and development. Here, we report the use of the zebrafish embryo TB model, to evaluate the in vivo toxicity and efficacy of five poorly water-soluble nitronaphthofuran derivatives, which were recently identified to possess anti-tuberculosis activity in vitro. To aid solubilization compounds were formulated in biocompatible polymeric micelles (PM). Three of the five PM-formulated nitronaphthofuran derivatives showed low toxicity in vivo, significantly reduced bacterial burden and improved survival in infected zebrafish embryos. We propose the zebrafish embryo TB-model as a quick and sensitive tool for evaluating in vivo toxicity and efficacy of new anti-TB compounds during early stages of drug development. Thus, this model is well suited to pinpoint promising compounds for further development.

Alice J-L Zheng, Aikaterini Thermou, Pedro Guixens Gallardo, Laurence Malbert-Colas, Chrysoula Daskalogianni, Nathan Vaudiau, Petter Brohagen, Anton Granzhan, Marc Blondel, Marie-Paule Teulade-Fichou, Rodrigo Prado Martins, Robin Fahraeus (2021 Nov 17)

The different activities of RNA G-quadruplex structures are controlled by flanking sequences.

Life science alliance : 5 : e202101232 : DOI : 10.26508/lsa.202101232

Résumé

The role of G-quadruplex (G4) RNA structures is multifaceted and controversial. Here, we have used as a model the EBV-encoded EBNA1 and the Kaposi's sarcoma-associated herpesvirus (KSHV)-encoded LANA1 mRNAs. We have compared the G4s in these two messages in terms of nucleolin binding, nuclear mRNA retention, and mRNA translation inhibition and their effects on immune evasion. The G4s in the message are clustered in one repeat sequence and the G4 ligand PhenDH2 prevents all G4-associated activities. The RNA G4s in the message take part in similar multiple mRNA functions but are spread throughout

the message. The different G4 activities depend on flanking coding and non-coding sequences and, interestingly, can be separated individually. Together, the results illustrate the multifunctional, dynamic and context-dependent nature of G4 RNAs and highlight the possibility to develop ligands targeting specific RNA G4 functions. The data also suggest a common multifunctional repertoire of viral G4 RNA activities for immune evasion.

Daniel Holoch, Michel Wassef, Cecilia Lövkvist, Dina Zielinski, Setareh Aflaki, Bérangère Lombard, Tiphaine Héry, Damarys Loew, Martin Howard, Raphaël Margueron (2021 Nov 16)

A cis-acting mechanism mediates transcriptional memory at Polycomb target genes in mammals.

Nature genetics : DOI : 10.1038/s41588-021-00964-2

Résumé

Epigenetic inheritance of gene expression states enables a single genome to maintain distinct cellular identities. How histone modifications contribute to this process remains unclear. Using global chromatin perturbations and local, time-controlled modulation of transcription, we establish the existence of epigenetic memory of transcriptional activation for genes that can be silenced by the Polycomb group. This property emerges during cell differentiation and allows genes to be stably switched after a transient transcriptional stimulus. This transcriptional memory state at Polycomb targets operates in cis; however, rather than relying solely on read-and-write propagation of histone modifications, the memory is also linked to the strength of activating inputs opposing Polycomb proteins, and therefore varies with the cellular context. Our data and computational simulations suggest a model whereby transcriptional memory arises from double-negative feedback between Polycomb-mediated silencing and active transcription. Transcriptional memory at Polycomb targets thus depends not only on histone modifications but also on the gene-regulatory network and underlying identity of a cell.

Piguel S., Le Bescont J., Mouawad L., Boddaert T., Bombard S. (2021 Nov 1)

Photoactivatable small-molecule inhibitors for light-controlled TAM kinase activity

ChemPhotoChem : 5 : 989-994 : DOI : 10.1002/cptc.202100131

Résumé

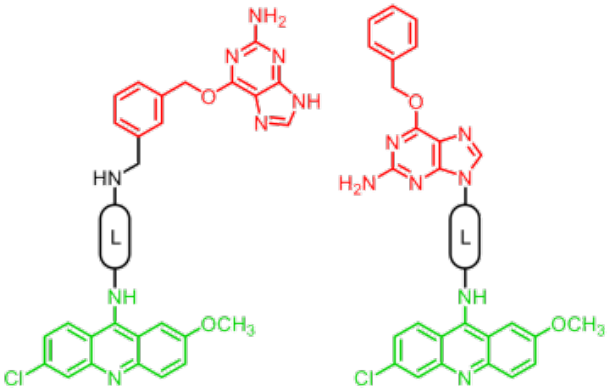
The TAM kinase family arises as a promising therapeutical target for cancer therapy, auto-immune, and viral diseases. In this study, we report the first photoactivatable caged inhibitors of Tyro3 and Mer. This strategy enables spatial and temporal control of the biological activity of the inhibitor upon irradiation with UV light. We describe the design, the synthesis, the photocleavage properties, and the inhibitory activity of four Tyro3 and Mer photoactivatable small molecules. The proof of concept on the TAM kinase family was achieved in vitro , since irradiation by UV light restored the full inhibitory activity of two prodrugs.

Jaime Franco Pinto, Alexandra Fillion, Patricia Duchambon, Sophie Bombard, Anton Granzhan
(2021 Oct 9)

Acridine–O6-benzylguanine hybrids: Synthesis, DNA binding, MGMT inhibition and antiproliferative activity

European Journal of Medicinal Chemistry : 227 : 113909 : DOI : 10.1016/j.ejmech.2021.113909

Résumé



DNA binding	☑
DNA intercalation	☒
MGMT inhibition *in vitro*	☑
MGMT inactivation in cells	☑ (**19a**)
T98G cytotoxicity (GI_{50} / 96 h)	1.1–25 μM
Synergy with TMZ	☑ (**19a**, **19c**)
DNA damage	☒ (**19a**)
Apoptosis	☑ (**19a**)

O^6-Methylguanine-DNA-methyltransferase (MGMT) is a key DNA repair enzyme involved in chemoresistance to DNA-alkylating anti-cancer drugs such as Temozolomide (TMZ) through direct repair of drug-induced *O*^{6}-methylguanine residues in DNA. MGMT substrate analogues, such as *O*^{6}-benzylguanine (BG), efficiently inactivate MGMT *in vitro* and in cells; however, these drugs failed to reach the clinic due to adverse side effects. Here, we designed hybrid drugs combining a BG residue covalently linked to a DNA-interacting moiety (6-chloro-2-methoxy-9-aminoacridine). Specifically, two series of hybrids, encompassing three compounds each, were obtained by varying the position of the attachment point of BG (*N*^{9} of guanine vs. the benzyl group) and the length and nature of the linker. UV/vis absorption and fluorescence data indicate that all six hybrids adopt an intramolecularly stacked conformation in aqueous solutions in a wide range of temperatures. All hybrids interact with double-stranded DNA, as clearly evidenced by spectrophotometric titrations, without intercalation of the acridine ring and do not induce thermal stabilization of the duplex. All hybrids, as well as the reference DNA intercalator (6-chloro-2-methoxy-9-aminoacridine **8**), irreversibly inhibit MGMT *in vitro* with variable efficiency, comparable to that of BG. In a multidrug-resistant glioblastoma cell line T98G, benzyl-linked hybrids **7a–c** and the *N*^{9}-linked hybrid **19b** are moderately cytotoxic (GI_{50} ≥ 15 μM after 96 h), while *N*^{9}-linked hybrids **19a** and **19c** are strongly cytotoxic (GI_{50} = 1–2 μM), similarly to acridine **8** (GI_{50} = 0.6 μM). Among all compounds, hybrids **19a** and **19c**, similarly to BG, display synergic cytotoxic effect upon co-treatment with subtoxic doses of TMZ, with combination index (CI) values as low as 0.2–0.3. In agreement with *in vitro* results, compound **19a** inactivates cellular MGMT but, unlike BG, does not induce significant levels of DNA damage, either alone or in combination with TMZ, as indicated by the results of γH2AX immunostaining experiments. Instead, and unlike BG, compound **19a** alone induces significant apoptosis of T98G cells, which is not further increased in a combination with TMZ. These results indicate that molecular

mechanisms underlying the cytotoxicity of **19a** and its combination with TMZ are distinct from that of BG. The strongly synergic properties of this combination represent an interesting therapeutic opportunity in treating TMZ-resistant cancers.

Paula Santabárbara-Ruiz, Pierre Léopold (2021 Oct 5)

An Oatp transporter-mediated steroid sink promotes tumor-induced cachexia in Drosophila.

Developmental cell : 2741-2751.e7 : DOI : S1534-5807(21)00727-9

Résumé

Cancer cachexia is associated with many types of tumors and is characterized by a combination of anorexia, loss of body weight, catabolic alterations, and systemic inflammation. We developed a tumor model in Drosophila larvae that causies cachexia-like syndrome, and we found that cachectic larvae show reduced levels of the circulating steroid ecdysone (Ec). Artificially importing Ec in the tumor through the use of the EcI/Oatp74D importer aggravated cachexia, whereas feeding animals with Ec rescued cachectic defects. This suggests that a steroid sink induced by the tumor promotes catabolic alterations in healthy tissues. We found that Oatp33Eb, a member of the Oatp transporter family, is specifically induced in tumors promoting cachexia. The overexpression of Oatp33Eb in noncachectic tumors induced cachexia, whereas its inhibition in cachectic tumors restored circulating Ec and reversed cachectic alterations. Oatp transporters are induced in several types of hormone-dependent tumors, and this result suggests that a similar sink effect could modify hormonal balance in cachectic cancer patients.

Nathalie Arquier, Marianne Bjordal, Philippe Hammann, Lauriane Kuhn, Pierre Léopold (2021 Sep 25)

Brain adiponectin signaling controls peripheral insulin response in Drosophila.

Nature communications : 5633 : DOI : 10.1038/s41467-021-25940-6

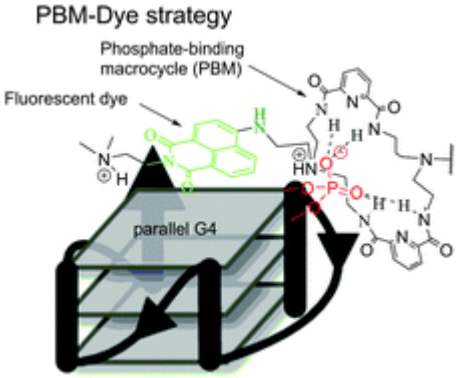
Résumé

The brain plays a key role in energy homeostasis, detecting nutrients, metabolites and circulating hormones from peripheral organs and integrating this information to control food intake and energy expenditure. Here, we show that a group of neurons in the Drosophila larval brain expresses the adiponectin receptor (AdipoR) and controls systemic growth and metabolism through insulin signaling. We identify glucose-regulated protein 78 (Grp78) as a circulating antagonist of AdipoR function produced by fat cells in response to dietary sugar. We further show that central AdipoR signaling inhibits peripheral Juvenile Hormone (JH) response, promoting insulin signaling. In conclusion, we identify a neuroendocrine axis whereby AdipoR-positive neurons control systemic insulin response.

Aleksandr S. Oshchepkov, Oksana Reznichenko, Dan Xu, Boris S. Morozov, Anton Granzhan,

Evgeny A. Kataev (2021 Sep 22)
Dye-functionalized Phosphate-binding Macrocycles: From Nucleotide to G-quadruplex Recognition and "turn-on" Fluorescence Sensing
Chemical Communications : 57 : 10632-10635 : DOI : 10.1039/D1CC04096K

Résumé



A novel strategy to design "turn-on" fluorescent receptors for G-quadruplexes of DNA is presented, which relies on the connection of phosphate binding macrocycles (PBM) with naphthalimide dyes. A new PBM-dye family was synthesized and evaluated in terms of binding and detection of nucleotides and DNA G-quadruplexes of different topologies.

Guilherme Pedreira de Freitas Nader, Sonia Agüera-Gonzalez, Fiona Routet, Matthieu Gratia, Mathieu Maurin, Valeria Cancila, Clotilde Cadart, Andrea Palamidessi, Rodrigo Nalio Ramos, Mabel San Roman, Matteo Gentili, Ayako Yamada, Alice Williart, Catalina Lodillinsky, Emilie Lagoutte, Catherine Villard, Jean-Louis Viovy, Claudio Tripodo, Jérôme Galon, Giorgio Scita, Nicolas Manel, Philippe Chavrier, Matthieu Piel (2021 Sep 22)
Compromised nuclear envelope integrity drives TREX1-dependent DNA damage and tumor cell invasion.
Cell : DOI : S0092-8674(21)01046-1

Résumé

Although mutations leading to a compromised nuclear envelope cause diseases such as muscular dystrophies or accelerated aging, the consequences of mechanically induced nuclear envelope ruptures are less known. Here, we show that nuclear envelope ruptures induce DNA damage that promotes senescence in non-transformed cells and induces an invasive phenotype in human breast cancer cells. We find that the endoplasmic reticulum (ER)-associated exonuclease TREX1 translocates into the nucleus after nuclear envelope rupture and is required to induce DNA damage. Inside the mammary duct, cellular crowding leads to nuclear envelope ruptures that generate TREX1-dependent DNA damage, thereby driving the progression of in situ carcinoma to the invasive stage. DNA damage and nuclear

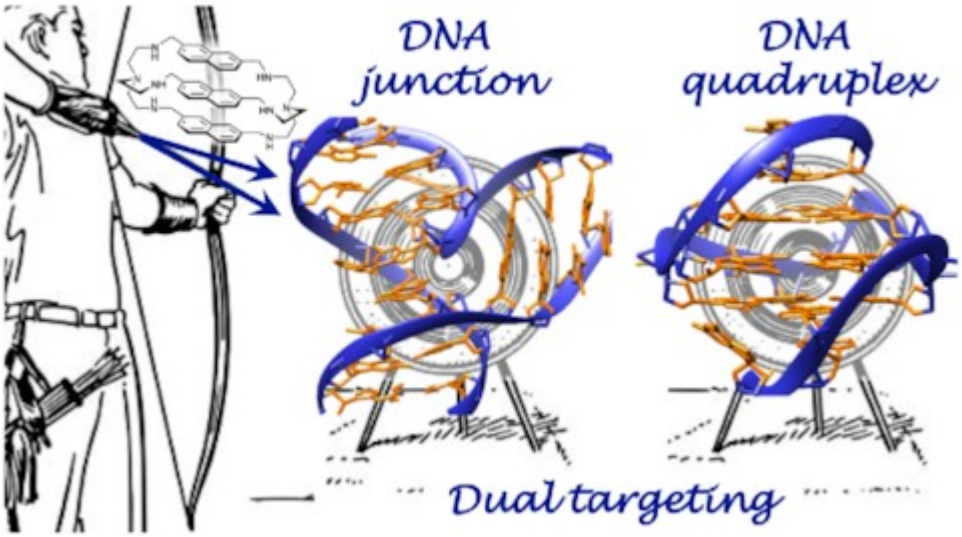
envelope rupture markers were also enriched at the invasive edge of human tumors. We propose that DNA damage in mechanically challenged nuclei could affect the pathophysiology of crowded tissues by modulating proliferation and extracellular matrix degradation of normal and transformed cells.

Joanna Zell, Katerina Duskova, Leïla Chouh, Madeleine Bossaert, Nicolas Chéron, Anton Granzhan, Sébastien Britton, David Monchaud (2021 Sep 22)

Dual targeting of higher-order DNA structures by azacryptands induces DNA junction-mediated DNA damage in cancer cells

Nucleic Acids Research : 49 : 10275–10288 : DOI : 10.1093/nar/gkab796

Résumé



DNA is intrinsically dynamic and folds transiently into alternative higher-order structures such as G-quadruplexes (G4s) and three-way DNA junctions (TWJs). G4s and TWJs can be stabilised by small molecules (ligands) that have high chemotherapeutic potential, either as standalone DNA damaging agents or combined in synthetic lethality strategies. While previous approaches have claimed to use ligands that specifically target either G4s or TWJs, we report here on a new approach in which ligands targeting both TWJs and G4s *in vitro* demonstrate cellular effects distinct from that of G4 ligands, and attributable to TWJ targeting. The DNA binding modes of these new, dual TWJ-/G4-ligands were studied by a panel of *in vitro* methods and theoretical simulations, and their cellular properties by extensive cell-based assays. We show here that cytotoxic activity of TWJ-/G4-ligands is mitigated by the DNA damage response (DDR) and DNA topoisomerase 2 (TOP2), making them different from typical G4-ligands, and implying a pivotal role of TWJs in cells. We designed and used a clickable ligand, TrisNP-α, to provide unique insights into the TWJ landscape in cells and its modulation upon co-treatments. This wealth of data was exploited to design an efficient synthetic lethality strategy combining dual ligands with clinically relevant DDR inhibitors.