**Year of publication 2019**


**PML-Regulated Mitochondrial Metabolism Enhances Chemosensitivity in Human Ovarian Cancers**

*Cell Metabolism*

**Summary**

High-grade serous ovarian cancer (HGSOC) remains an unmet medical challenge. Here, we unravel an unanticipated metabolic heterogeneity in HGSOC. By combining proteomic, metabolomic, and bioenergetic analyses, we identify two molecular subgroups, low- and high-OXPHOS. While low-OXPHOS exhibit a glycolytic metabolism, high-OXPHOS HGSOCs rely on oxidative phosphorylation, supported by glutamine and fatty acid oxidation, and show chronic oxidative stress. We identify an important role for the PML-PGC-1α axis in the metabolic features of high-OXPHOS HGSOC. In high-OXPHOS tumors, chronic oxidative stress promotes aggregation of PML-nuclear bodies, resulting in activation of the transcriptional co-activator PGC-1α. Active PGC-1α increases synthesis of electron transport chain complexes, thereby promoting mitochondrial respiration. Importantly, high-OXPHOS HGSOCs exhibit increased response to conventional chemotherapies, in which increased oxidative stress, PML, and potentially ferroptosis play key functions. Collectively, our data establish a stress-mediated PML-PGC-1α-dependent mechanism that promotes OXPHOS metabolism and chemosensitivity in ovarian cancer.

**Year of publication 2018**

Tatiana Cañéque, Sebastian Müller, Raphaël Rodriguez (2018 Aug 15)

**Visualizing biologically active small molecules in cells using click chemistry**

*Nature Rev. Chem.*

**Summary**

Natural products and synthetic small molecules can be used to perturb, dissect and manipulate biological processes, thereby providing the basis for drug development. Over the past decades, the evolution of molecular biology protocols and microscopy techniques has made it possible to visually detect proteins in living systems with valuable spatiotemporal resolution, in which dynamic topological information has proved to be insightful. By contrast, although small molecules have become essential for biological studies, general methods to track them in cells remain underexplored. In this Review, we discuss how bioorthogonal chemistry, and click chemistry in particular, can be exploited to label and visualize almost any biologically active small molecule in cells and tissues. We review recent developments, highlighting cases in which visualizing small molecules has provided crucial mechanistic insights. This methodology is facile to implement, is versatile and is illuminating.
Antoine Versini, Lou Saier, Fabien Sindikubwabo, Sebastian Müller, Tatiana Cañeque, Raphaël Rodriguez (2018 Aug 1)

**Chemical biology of salinomycin**

**Summary**

Cancer stem cells (CSC) have been shown to be refractory to conventional therapeutic agents, can promote metastasis, and have been linked to cancer relapse. The natural product Salinomycin has been identified by means of high throughput phenotypic screening as a selective killer of CSC in vitro and in vivo. In this article we comprehensively review the chemistry of Salinomycin, documenting early total syntheses, along with strategies that have been developed over the years to effectively modify this natural product at key positions with the view to establish a robust structure-activity-relationship and to delineate the complex mechanism of action of this fascinating molecule in the context of cancer research. Then, we document the biology of Salinomycin, putting forward phenotypic alterations that have been observed in the relevant biological models and highlighting how chemistry has been instrumental in discovering unprecedented physiological features of cancer stem cells that can be exploited for therapeutic benefits.

Tatiana Cañeque, Sebastian Müller, Anne Lafon, Fabien Sindikubwabo, Antoine Versini, Lou Saier, Manon Barutaut, Christine Gailet, Raphaël Rodriguez (2018 May 1)

**Reprogramming the reactivity of iron in cancer stem cells**

**Summary**

Cancer stem cells (CSCs) have been shown to be refractory to conventional therapeutic agents, can promote metastasis, and have been linked to cancer relapse. Salinomycin can selectively kill CSCs. We have shown that salinomycin derivatives accumulate in lysosomes and sequester iron in this organelle. As a result, accumulation of iron leads to the production of reactive oxygen species and lysosomal membrane permeabilization, which in turn promotes cell death by ferroptosis. These findings have revealed the prevalence of iron homeostasis in CSCs and paved the way toward the development of next-generation therapeutics.


**Targeting NAT10 enhances healthspan and lifespan in a mouse model of human accelerated aging syndrome**
*Nature Communications* : 9 : [DOI: 10.1038/s41467-018-03770-3]
Summary

Hutchinson-Gilford Progeria Syndrome (HGPS) is a rare, but devastating genetic disease characterized by segmental premature aging, with cardiovascular disease being the main cause of death. Cells from HGPS patients accumulate progerin, a permanently farnesylated, toxic form of Lamin A, disrupting the nuclear shape and chromatin organization, leading to DNA-damage accumulation and senescence. Therapeutic approaches targeting farnesylation or aiming to reduce progerin levels have provided only partial health improvements. Recently, we identified Remodelin, a small-molecule agent that leads to amelioration of HGPS cellular defects through inhibition of the enzyme N-acetyltransferase 10 (NAT10). Here, we show the preclinical data demonstrating that targeting NAT10 in vivo, either via chemical inhibition or genetic depletion, significantly enhances the healthspan in a Lmna<sup>G609G</sup> HGPS mouse model. Collectively, the data provided here highlights NAT10 as a potential therapeutic target for HGPS.

Year of publication 2017

Sarah Benabdi, François Peurois, Agata Nawrotek, Jahnavi Chekireddy, Tatiana Cañeque, Takao Yamori, Isamu Shiina, Yoshimi Ohashi, Shingo Dan, Raphaël Rodriguez, Jacqueline Cherfils, Mahel Zeghouf (2017 Sep 1)

Family-wide analysis of the inhibition of Arf guanine nucleotide exchange factors with small molecules: evidence for unique inhibitory profiles.

Biochemistry : 56 : 5125–5133 : DOI : 10.1021/acs.biochem.7b00706

Summary

Arf GTPases and their guanine nucleotide exchange factors (ArfGEFs) are major regulators of membrane traffic and organelle structure in cells. They are associated with a variety of diseases and are thus attractive therapeutic targets for inhibition by small molecules. Several inhibitors of unrelated chemical structures have been discovered, which have shown their potential in dissecting molecular pathways and blocking disease-related functions. However, their specificity across the ArfGEF family has remained elusive. Importantly, inhibitory responses in the context of membranes, which are critical determinants of Arf and ArfGEF cellular functions, have not been investigated. Here, we compare the efficiency and specificity of four structurally distinct ArfGEF inhibitors, Brefeldin A, SecinH3, M-COPA, and NAV-2729, toward six ArfGEFs (human ARNO, EFA6, BIG1, and BRAG2 and Legionella and Rickettsia RaLF). Inhibition was assessed by fluorescence kinetics using pure proteins, and its modulation by membranes was determined with lipidated GTPases in the presence of liposomes. Our analysis shows that despite the intra-ArfGEF family resemblance, each inhibitor has a specific inhibitory profile. Notably, M-COPA is a potent pan-ArfGEF inhibitor, and NAV-2729 inhibits all GEFs, the strongest effects being against BRAG2 and Arf1. Furthermore, the presence of the membrane-binding domain in Legionella RaLF reveals a strong inhibitory effect of BFA that is not measured on its GEF domain alone. This study demonstrates the value of family-wide assays with incorporation of membranes, and it should enable accurate dissection of Arf pathways by these inhibitors to best guide their use and development as therapeutic agents.

**An iron hand over cancer stem cells**


**Summary**

The paradigm of cancer stem cells (CSCs) defines the existence of cells exhibiting self-renewal and tumor-seeding capacity. These cells have been associated with tumor relapse and are typically resistant to conventional chemotherapeutic agents. Over the past decade, chemical biology studies have revealed a significant number of small molecules able to alter the proliferation of these cells in various settings. The natural product salinomycin has emerged as the most promising anti-CSC agent. However, an explicit mechanism of action has not yet been characterized, in particular due to the pleiotropic responses salinomycin is known for. In this punctum, we describe our recent discovery that salinomycin and the more potent synthetic derivative we named ironomycin sequester lysosomal iron. We found that these compounds, by blocking iron translocation, induce an iron-depletion response leading to a lysosomal degradation of ferritin followed by an iron-mediated lysosomal production of reactive oxygen species (ROS) and a cell death pathway that resembles ferroptosis. These unprecedented findings identified iron homeostasis and iron-mediated processes as potentially druggable in the context of CSCs.


**Click chemistry enables preclinical evaluation of targeted epigenetic therapies**

*Science*: [DOI: 10.1126/science.aal2066]

**Summary**

The success of new therapies hinges on our ability to understand their molecular and cellular mechanisms of action. Here we modify BET bromodomain inhibitors, an epigenetic-based therapy, to create functionally conserved compounds that are amenable to click-chemistry and can be used as molecular probes in vitro and in vivo. Using click-proteomics and click-sequencing we explore the gene regulatory function of bromodomain containing 4 protein (BRD4) and the transcriptional changes induced by BET inhibitors. Studying mouse models of acute leukemia, we use high-resolution microscopy and flow cytometry to highlight the heterogeneity of drug activity within tumor cells located in different tissue compartments. We also demonstrate the differential distribution and effects of BET inhibitors in normal and malignant cells in vivo. This study provides a potential framework for the pre-clinical assessment of a wide range of drugs.
**Salinomycin kills cancer stem cells by sequestering iron in lysosomes**

*Nature Chemistry*: DOI:10.1038/nchem.2778

**Summary**

Cancer stem cells (CSCs) represent a subset of cells within tumours that exhibit self-renewal properties and the capacity to seed tumours. CSCs are typically refractory to conventional treatments and have been associated to metastasis and relapse. Salinomycin operates as a selective agent against CSCs through mechanisms that remain elusive. Here, we provide evidence that a synthetic derivative of salinomycin, which we named ironomycin (AM5), exhibits a more potent and selective activity against breast CSCs *in vitro* and *in vivo*, by accumulating and sequestering iron in lysosomes. In response to the ensuing cytoplasmic depletion of iron, cells triggered the degradation of ferritin in lysosomes, leading to further iron loading in this organelle. Iron-mediated production of reactive oxygen species promoted lysosomal membrane permeabilization, activating a cell death pathway consistent with ferroptosis. These findings reveal the prevalence of iron homeostasis in breast CSCs, pointing towards iron and iron-mediated processes as potential targets against these cells.

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**Chromatin Regulates Genome Targeting with Cisplatin**

*Angewandte Chemie*: DOI:10.1002/anie.201701144

**Summary**

Cisplatin derivatives can form various types of DNA lesions (DNA-Pt) and trigger pleiotropic DNA damage responses. Here, we report a strategy to visualize DNA-Pt with high resolution, taking advantage of a novel azide-containing derivative of cisplatin we named APPA, a cellular pre-extraction protocol and the labeling of DNA-Pt by means of click chemistry in cells. Our investigation revealed that pretreating cells with the histone deacetylase (HDAC) inhibitor SAHA led to detectable clusters of DNA-Pt that colocalized with the ubiquitin ligase RAD18 and the replication protein PCNA. Consistent with activation of translesion synthesis (TLS) under these conditions, SAHA and cisplatin cotreatment promoted focal accumulation of the low-fidelity polymerase Polη that also colocalized with PCNA. Remarkably, these cotreatments synergistically triggered mono-ubiquitination of PCNA and apoptosis in a RAD18-dependent manner. Our data provide evidence for a role of chromatin in regulating genome targeting with cisplatin derivatives and associated cellular responses.
Targeting cancer stem cells with small molecules

Summary
Cancers arise as a result of physiological imbalances and subsequent uncontrolled cell division. Cancer initiation requires a set of biochemical alterations, including some occurring at the genetic and epigenetic levels. Thus, tumors are heterogeneous in nature making it challenging to selectively target different cancer cells by means of small molecule intervention. The paradigm of cancer stem cells (CSCs) describes subpopulations of cells with high self-renewal and tumor-seeding capacity. These cells, typically refractory to conventional therapies, can give rise to relapse after treatment. Combinatorial strategies, including drugs that selectively target this population of cells, have emerged in recent years. Here, we review how discovery-based – unbiased – screening approaches[1] have helped identify small molecules that specifically target CSCs. We also highlight biological pathways characteristic of CSCs that can potentially be selectively targeted in a hypothesis-driven manner by small molecules. We describe molecules that effectively target CSCs and emphasize what is known about their biological modes of action. The diversity and complexity of biochemical processes that CSCs may be addicted to, raises the question of how selective targeting of these pathways can be achieved. This challenge may be addressed by the continuing production of structurally complex and diverse small molecules using target and diversity-oriented synthesis approaches.[2]

Year of publication 2016
Emmanouil Zacharioudakis, Tatiana Cañéque, Raúl Custodio, Sebastian Müller, Ana M Cuadro, Juan J Vaquero, Raphaël Rodriguez (2016 Dec 7)
Quinolizinium as a new fluorescent lysosomotropic probe.
Bioorganic & medicinal chemistry letters : DOI : S0960-894X(16)31239-2

Summary
We have synthesized a collection of quinolizinium fluorescent dyes for the purpose of cell imaging. Preliminary biological studies in human U2OS osteosarcoma cancer cells have shown that different functional groups appended to the cationic quinolizinium scaffold efficiently modulate photophysical properties but also cellular distribution. While quinolizinium probes are known nuclear staining reagents, we have identified a particular quinolizinium derivative salt that targets the lysosomal compartment. This finding raises the question of predictability of specific organelle targeting from structural features of small molecules.

Year of publication 2014
Sebastian Müller, Katta Laxmi-Reddy, Prakrit V. Jena, Benoit Baptiste, Zeyuan Dong, Frédéric
Godde, Taekjip Ha, Raphaël Rodriguez, Shankar Balasubramanian, Ivan Huc (2014 Nov 24)
**Targeting DNA G-quadruplexes with helical small molecules**
*Chembiochem* : 15 : DOI : 10.1002/cbic.201402439

**Summary**

We previously identified quinoline-based oligoamide helical foldamers and a trimeric macrocycle as selective ligands of DNA quadruplexes. Their helical structures might permit targeting of the backbone loops and grooves of G-quadruplexes instead of the G-tetrads. Given the vast array of morphologies G-quadruplex structures can adopt, this might be a way to achieve sequence selective binding. Here, we describe the design and synthesis of molecules based on macrocyclic and helically folded oligoamides. We tested their ability to interact with the human telomeric G-quadruplex and an array of promoter G-quadruplexes by using FRET melting assay and single-molecule FRET. Our results show that they constitute very potent ligands—comparable to the best so far reported. Their modes of interaction differ from those of traditional tetrad binders, thus opening avenues for the development of molecules specific for certain G-quadruplex conformations.

Delphine Larrieu, Raphaël Rodriguez, Sébastien Britton (2014 Nov 2)
**Chemical inhibition of NAT10 corrects defects of laminopathic cells**
*Science* : 344 : 527 : DOI : 10.1126/science.1252651

**Summary**

Down-regulation and mutations of the nuclear-architecture proteins lamin A and C cause misshapen nuclei and altered chromatin organization associated with cancer and laminopathies, including the premature-aging disease Hutchinson-Gilford progeria syndrome (HGPS). Here, we identified the small molecule “Remodelin” that improved nuclear architecture, chromatin organization, and fitness of both human lamin A/C-depleted cells and HGPS-derived patient cells and decreased markers of DNA damage in these cells. Using a combination of chemical, cellular, and genetic approaches, we identified the acetyltransferase protein NAT10 as the target of Remodelin that mediated nuclear shape rescue in laminopathic cells via microtubule reorganization. These findings provide insights into how NAT10 affects nuclear architecture and suggest alternative strategies for treating laminopathies and aging.

Ján Cvengroš, Raphaël Rodriguez (2014 Oct 1)
**A Ray of Light Piercing through the Clouds The 49th EUCHEMS Conference on Stereochemistry Bürgenstock Conference 2014**
*Chimia (Aarau)* : 68 -10 : 742-745

**Summary**
Publications

Chemical Biology of Cancer