Cell Chemical Biology Call

CellPress

Principles of Chemical Biology: Iron and CSCs, Histone Acylation sans Enzymes, Eliciting Silent Gene Clusters, and 2′-deoxy-ADPR as a Second Messenger

This month: Lysosomal iron linked to cell death in cancer stem cells, non-enzymatic catalyst SynCAc for histone acylation, cytotoxins ivermectin and etoposide bring new anti-fungals out of the crypt, and 2′-deoxy-ADPR as second messenger activating TRPM2.

Ironing Out the Cancer Stem Cells

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Principles

Cancer stem cells (CSCs) exhibit self-renewal and tumor-seeding capacity. These cells are typically refractory to conventional treatments and have been linked to metastasis and relapse. Salinomycin, a natural product with pronounced ionophoric properties, has been identified as a potential drug against breast CSCs, operating through mechanisms that remained controversial. We have developed ironomycin, a more effective salinomycin surrogate, which can be subjected to click chemistry *in situ* (Mai et al., Nat. Chem., Published online May 22, 2017, 10.1038/nchem.2778). Thus, we were able to chemically label ironomycin inside cells and observe the effects it has, such as cytosolic iron depletion, production of reactive oxygen species in lysosome via Fenton chemistry, and ferroptosis. This study led to the discovery that iron homeostasis is up-regulated in CSCs, raising putative roles of iron in the maintenance of these cells and opening up unprecedented therapeutic opportunities.

> *"…iron homeostasis is up-regulated in CSCs, raising putative roles of iron in the maintenance of these cells…"*

What's Next?

It is now critical to evaluate the clinical tractability of this strategy, given that targeting iron homeostasis could potentially promote undesired side effects. To what extent these mechanisms can be exploited to treat other cancer types remains an open question and an interesting line of investigation.

Artificial Catalyst System for Histone Acylation

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Principles

Posttranslational modification of histones is crucial for epigenetic mechanisms that are involved in diverse biological processes and diseases. One of them, histone acetylation, which is physiologically catalyzed by histone acetyltransferase (HAT), constitutes a fundamental regulatory element in gene expression. In addition, several types of short-chain lysine acylations, such as malonylation, have recently been identified on histones. Mutations in HAT genes are linked to genetic disorders, such as cancer. Replacing inactivated HATs with artificial catalysts is, thus, a potential strategy for cancer treatment.

We provide a new approach to synthetically modulate histone acylation states without relying on enzymes (Ishiguro et al., Chem *2*, 840–859, 10.1016/j.chempr.2017.04.002). We have developed artificial catalyst systems ("SynCAc" for synthetic chromatin acylation), composed of nucleosome-binding catalysts and acyl donors, which preferentially acetylated or malonylated lysines on histone tails and suppressed intra- and inter-nucleosome interaction, similarly to HATs. Our study suggests a novel therapeutic strategy by complementing defects of HAT activity by artificial catalyst systems.

> *"Our study provides the starting point of "catalysis medicine," a new concept for curing diseases by substituting defective enzymes with artificial catalyst systems…"*

What's Next?

Our study provides the starting point of "catalysis medicine," a new concept for curing diseases by substituting defective enzymes with artificial catalyst systems and intervening chemical reaction networks in patients. We are developing more high-performance catalysts enabling various chemical reactions in living cells or organisms.

Opening the Antibiotic Crypt

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Principles

Bacteria are an important source of bioactive and therapeutic molecules. A given streptomycete, for example, has the potential to produce more than 25 such compounds; however, the majority of the corresponding biosynthetic gene clusters are not expressed under standard laboratory conditions. We previously established a general chemogenetic strategy for inducing these "silent" or "cryptic" gene clusters. This strategy has now been implemented in *Streptomyces albus*, a model streptomycete (Xu et al., J. Am. Chem. Soc., *139*, 9203–9212, 10.1021/jacs.7b02716). In our approach, enhanced green fluorescent protein (eGFP) is placed inside the gene cluster of interest, and high-throughput screens are carried out to identify candidate elicitors. When applied to the cryptic *sur* cluster in *S. albus*, this led to discovery that the well-known cytotoxins ivermectin and etoposide are elicitors of cryptic gene clusters. Using ivermectin/etoposide, we triggered production of numerous novel metabolites, including a cryptic anti-fungal agent. We also examined the regulatory pathways that lead to induction of *sur* and found that multiple pathways, including a new transcriptional repressor, were involved. These results highlight the ability of our approach to unveil both the products and regulation underlying silent biosynthetic gene clusters.

> *"…this led to discovery that the well-known cytotoxins ivermectin and etoposide are elicitors of cryptic gene clusters."*

What's Next?

We will apply this approach to other cryptic gene clusters in streptomycetes to find additional molecules. We will also examine the mechanisms by which known antibiotics trigger production of cryptic metabolites in bacteria.

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TRPM2 Receives a New Messenger

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Principles

Transient receptor potential melastatin type 2 (TRPM2) is a non-selective cation channel mainly expressed in cells of the immune system and the brain. TRPM2 is involved in apoptosis, inflammatory processes, insulin secretion, thermosensation, thermoadaptation, and others. Adenosine diphosphoribose (ADPR) is currently considered the main intracellular second messenger activating TRPM2. A major conceptual problem with this view is the relatively high concentration of ADPR required for activation of TRPM2. Additionally, given that NAD, a critical cellular coenzyme, is the source of cellular ADPR, an increase of ADPR in the higher mM range may well lead to a substantial loss of cellular NAD.

Upon screening diverse analogs of ADPR, we identified 2′-deoxy-ADPR as a superagonist of TRPM2 that evokes about 10-fold higher macroscopic currents as compared to ADPR, by increasing channel recruitment and open probability and delaying inactivation of TRPM2. We also detect endogenous 2′-deoxy-ADPR in T cells, further supporting its physiological relevance. Finally, oxidative stress via hydrogen peroxide evoked an increase of endogenous 2′-deoxy-ADPR, suggesting a role as second messenger activating TRPM2 (Fliegert et al., Nat. Chem. Biol., Published online June 26, 2017, 10.1038/nchembio.2415).

"…oxidative stress via hydrogen peroxide evoked an increase of endogenous 2'-deoxy-ADPR suggesting a role as second messenger activating TRPM2."

What's Next?

Quantification of endogenous concentrations of minor nucleotides such as 2′-deoxy-ADPR is very difficult given the fact that cellular extracts contain thousands of different nucleotides with very similar physicochemical properties. Thus, improved analytical methods, especially tools suitable for single-cell analysis, are required for analysis of endogenous 2′-deoxy-ADPR, and other nucleotides, in a broad range of cells and tissues.