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Technology Offer

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New KDAC assay and selection system

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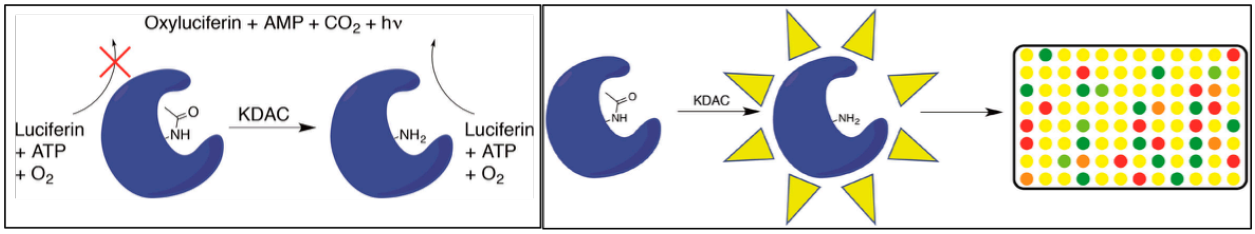
Background

Acetylation of the N(ε)-amino group of lysine residues has shown to bear a large variety of functional roles in almost every physiological process. These modifications can be reversed by lysine deacetylases (KDACs), which themselves play a prominent role in many physiological and cellular processes, e.g. by acting as transcriptional repressors. Defects in KDACs are associated with a variety of diseases such as diabetes, cancer and ageing. However, the exact mechanisms of how KDAC misregulation contributes to the disease etiology is hard to asses, as there is only a limited specificity of the enzymes for particular protein substrates and types of acylation. Inhibition of KDACs by either genetic ablation or use of chemical inhibitors has already enlightened their function and generated active leads in pharmaceutical design. For their identification and characterization, the availability of reliable KDAC assays is key to success. However, the interpretation of results has often been challenging, due to the low-selectivity for specific KDACs. To fully elucidate the role of different lysine acylations, KDAC variants selective for particular types of lysine modifications will be necessary.

Technology

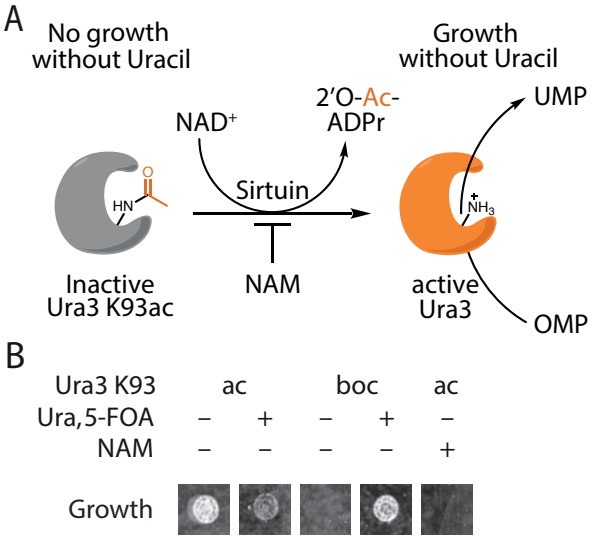
Our scientists have developed a new KDAC assay based on Firefly luciferase harboring an acetylation on an essential active site lysine incorporated by genetic code expansion. Several KDACs can reverse this modification and hence activate luciferase. This new assay is extremely sensitive, reliable, and fast and can be performed in a continuous format. In a recently performed high-throughput screen testing >200,000 compounds, about 20 novel specific inhibitors of SirT1 (IC_{50} 30 – 3000 nM) have been identified.
Furthermore, our scientists developed a bacterial selection system for KDACs with altered substrate specificity or reactivity against bioorthogonal chemical protecting groups. The selection system uses a reporter enzyme inactivated by lysine modifications at an essential active site residue, thereby linking deacetylase activity to a selectable output. They used the selection system to identify acyl-type specific KDACs from libraries of >30 million different variants. The new enzymes can be used for partial complementation of KDAC deletion strains to reveal the physiological role of particular lysine acylations.
In the same way they engineered KDAC variants capable of removing chemical protecting groups that may be used in prodrug strategies of cancer therapy. Inactive precursor molecules of toxic substances, modified at their essential lysine residues are often used in cancer therapies. KDAC variants derived from human progenitors are being developed and will be tested in human cancer cell lines for their ability to remove the modification and thus activate the pro-toxins. The present technology can be therefore an important and highly specific tool to study and treat cancer.

KDAC assay



Left panel: Acetylated Firefly luciferase is activated upon deacetylation by a KDAC. Right panel: Schematic of KDAC screen: Several KDACs can deacetylase and thereby activate the luciferase, creating a specific and selectable output.

KDAC selection system



Design of KDAC selection system. A) Deacetylation of OMP decarboxylase K93ac (Ura3 K93ac) by Sirtuins enables growth of E. coli in the absence of uracil. B) E. coli producing Ura3 K93ac as the sole source of OMP decarboxylase depend on KDAC activity. Cell growth of E. coli DB6566 (ΔpyrF) expressing plasmids to encode Ura3 K93ac or K93boc (not cleavable, tert-butyloxycarbonyl-lysine) on agar plates with or without uracil and 5-FOA. Nicotinamide (NAM), which inhibits endogenous CobB, prevents growth.

We are now looking for either a licensing partner, or a collaboration partner to further develop this project. Licensing and collaborations can also be established regarding partial aspects of the patent.

Publication

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Patent Information

A PCT application was filed on 21.09.2018 EP3460057A1.