Session 6: Moonlighting enzymes

Lectures

L6.1

Targeting glycolytic aldolase: an Achilles heel in cancer metabolism

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Increased nutrient uptake is a major hallmark of cancer and correlates with a poor survival rate. Many of the oncogenic events that drive cancer development influence metabolism by increasing glucose and glutamine uptake while other genetic events and processes also influence metabolism without increased nutrient uptake. Together these events reprogram metabolism away from catabolic metabolism and promote the anabolic pathways necessary for cell growth and proliferation. Glycolysis/gluconeogenesis is the key metabolic pathway that provides intermediates for cell growth and proliferation. We have targeted the central glycolytic enzyme aldolase which reversibly cleaves fructose-bisphosphate into triose-Ps by screening a 135 000 compound library for inhibitors of aldolase activity. One compound, UM0112176, allosterically inhibits aldolase activity at micromolar levels (IC50 ≈ 5 μM) and is cytotoxic to cancer cells (EC50 ≈ 5 μM) grown in glucose only, in glutamine only, and in complete cell culture media. The compound has good selectivity when tested against normal tissue culture cells exhibiting a 10–20-fold therapeutic window. Intracellular targeting of aldolase by UM0112176 is consistent with aldolase substrate/product accumulation depending on the direction of the glycolytic flux. The mechanism of action by UM0112176 is surprising as the compound not only targets the important metabolic role of aldolase but also impacts its moonlighting activities in F-actin polymerization, and novel activities related to ROS production and DNA repair. The elucidation of the essential role played by aldolase in metabolism and associated moonlighting activities, as revealed through the mechanism of action by UM0112176, will be the topic of the conference.

L6.2

The role of SWI/SNF ATP-dependent chromatin remodeling complex in triple negative breast cancer (TNBC) development

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TNBC is a subtype of breast cancers that are associated with early recurrence and an aggressive metastatic progression and mostly is chemotherapy-resistant. In this type of cancer metabolic switch to glycolysis is observed. Furthermore, the epigenetic silencing of gene encoding fructose-1,6-biphosphatase (FPB1) enzyme by the transcriptional repressor Snail protein is obligatory and characteristic for the development of TNBC. Snail together with Slug proteins belong to SNAI family transcription factors. Snail and Slug are the epithelial to mesenhymal trasition (EMT) driving factors. EMT is a process leading to the loss of epithelial cell characteristics and gain migratory and invasive properties manifested by the mesenchymal phenotype featured by reduced level of typical epithelial markers and up-regulation of vimentin and N-cadherin. In cancer cells, EMT is associated with increased aggressiveness, as well as invasive and metastatic potential. EMT markers expression is associated with poor prognosis.
Our data indicated that SWI/SNF chromatin remodeling complex directly controls genes coding for glycolysis enzymes. We have also shown that Snail and Slug proteins co-precipitate from HeLa cells with BAF155 and other core subunit of SWI/SNF complex. Therefore, our study provided new evidences for an important regulatory function of SWI/SNF complexes in control of main metabolic genes expression and likely EMT process. Furthermore, we analyzed more than 100 TNBC clinical samples and found the aberration of main SWI/SNF subunits. We confirmed those data by hightroughput analysis from 106 clinical samples obtained from GEO database.

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Oral presentations

O6.1

Structural studies of cold adaptation mechanism of GH2 β-D-galactosidases from *Arthrobacter* sp. 32cB and *Paracoccus* 32d

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Glycoside Hydrolase Family 2, β-D-galactosidases (βDGs) (EC 3.2.1.23) are large, usually five-domain molecules of molecular mass around 110 kDa. The biologically active forms of these enzymes are mostly tetramers and they act upon $\beta(1\rightarrow4)$-glycosidic bond in β-D-galactosides, starting from the non-reducing β-D-galactose residue. Multiple studies have shown that some of them are able to catalyze synthesis of glycosidic bonds, resulting in creation of oligosaccharide chains.

The most abundant substrate for βDGs is lactose, a disaccharide found in large quantities in milk. Hence, these enzymes are useful in production of low-lactose milk, which is associated with multiple benefits. Such milk is not only perfect for people with lactose intolerance, but it is also used to accelerate production of dairy products. Removal of lactose increases natural sweetness of these products as lactose is hydrolyzed into galactose and glucose, thus no addition of sugar is needed to improve the taste of the final product. Moreover, the transglycosylation activity makes βDGs useful, mainly in food industry, for production of galactooligosaccharides, carbohydrate-based functional food ingredients with prebiotic properties.

The implementation of cold-adapted βDGs would be beneficial for the industry. By lowering the temperature of a technological process, one may decrease production costs, minimize the risk of product contamination, and shift the production toward more environmentally-friendly parameters. Moreover, this enables quick reaction termination by a relatively small temperature increase, which additionally prevents unwanted thermal conversions of the product.

The structures of two cold-adapted β-D-galactosidases (from *Arthrobacter* sp. 32 cB and *Paracoccus* 32d) were determined and used for the analysis of structural features of cold adaptation mechanism. Apart from, abundantly reported in case of such enzymes, presence of flexible loops, features like: formation of hinge regions by glycine residues and higher arginine content and their presence close to the protein surface were discovered. The conserved catalytic site is indeed no surprise, since it is responsible for the enzyme's selectivity. Thus it seems reasonable to associate the mechanism of cold adaptation with structural features present close to the protein surface. Both cold-adapted βDGs form functional dimers, which is atypical, as other enzymes classified as GH2, forming either functional tetramers or hexamers.

Posters

P6.1

Chaperone activity of the mutated forms of *Helicobacter pylori* DsbK (HP0231) protein

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Background: The mechanism of disulfide bond formation in microorganisms is extremely diverse. The *H. pylori* Dsb systemseems to be novel and different from that functioning the model microorganism – *E. coli*. It possesses only two extracytoplasmic Dsb proteins named HP0377 and HP0231. HP0377 is a reductase involved in the process of cytochrome c maturation. Additionally, it also possesses disulfide isomerase activity. HP0231 was the first periplasmic dimeric oxidoreductase involved in disulfide generation to be described.

Objectives: HP0231 was characterized as a protein functioning parallel as a molecular chaperone and in an oxidizing pathway in *H. pylori* cells. HP0231 acts as periplasmic oxidase, as EcDsbA, despite its structural resemblance to EcDsbG. The characteristic motifs of HP0231 are identical to that of EcDsbA (CPHC/VcP) but different from that of EcDsbG (CPYC/TcP). To assess relations between HP0231 structure and function we tested *in vitro* properties of mutated forms of HP0231 containing motifs characteristic for EcDsbG or EcDsbC. Thus, we also examined whether the HP0231-mutated versions function as molecular chaperones by checking their impact on the thermal aggregation of citrate synthase. Moreover, to distinguish between chaperone and redox activities of HP0231, mutated form with its second cysteine replaced by serine in the CXXC motif (CPHS), was used.

Methods: Mutated forms of HP0231 were constructed by site-directed mutagenesis and overexpressed in *E. coli* Rosetta strain. Proteins were purified using NGC™ Medium-Pressure Chromatography and used to evaluate their chaperone activity.

Results: All tested mutated forms of HP0231 possess chaperone activity slightly higher than that of native protein. The HP0231 variant (CPHC/TcP), a protein defective in all the *in vivo* tests, functioned similarly to native HP0231. The two variants having the CXXC motifs of DsbC or DsbG paired with VcP were slightly more active in this assay than their equivalents with TcP. HP0231, with its CPHS motif was disfunctional in disulfide bond formation *in vivo* but remained active as chaperone, which indicates that chaperone activity is independent of the CXXC motif.

Conclusions: The high chaperone activity of DsbK is independent of the CXXC motif and it is rather conditioned by dimeric HP0231 structure.

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P6.2

Slight difference in the structure makes huge difference in activity – PR peptides as activators and inhibitors of the 20S proteasome

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The proteasome is a main protease in the ubiquitin-proteasome pathway responsible for degradation of majority of intracellular proteins, such as damaged, mutated, oxidized and short-lived regulatory proteins. This system controls critical biological processes, like cell cycle progression, apoptosis, transcription, antigen presentation and oncogenesis. Dysfunction of the proteasome could lead to development of diverse diseases including cancer, autoimmune, neurodegenerative and rheumatoid disorders, and cardiac dysfunctions [1]. Due to its broad implication in physiological and pathological processes the proteasome becomes an attractive target in drug development. It has been found that malignant cells are more susceptible for the proteasome inhibition than normal cells, thus inhibitors of the proteasome are applied mostly in anticancer therapy. To date, three inhibitors of the proteasome have been approved for the treatment of hematological cancers [2]. On the other hand, activation of the proteasome could be one of the strategies preventing accumulation of damaged or oxidized proteins – the condition characteristic for neurodegenerative disorders. But so far a very few proteasome activators have been developed.
In search for allosteric modulators of the proteasome we focused on the *N*-terminal fragment of PR39. It is a naturally occurring, proline-and arginine-rich peptide. In the contrast to the conventional proteasomal inhibitors, PR39 inhibits the proteasome activity in a noncompetitive manner. The 11-residue-long *N*-terminal fragment of PR39 is responsible for its interaction with the enzyme [3]. We obtained a set of analogs of peptide PR11 with quite good inhibitory capacity (IC50 in nanomolar range). What is interesting, in some cases alteration of the original sequence has led to change in the mode of action of PR analogs: from inhibition to quite strong stimulation of the proteasome activity. The influence of PR analogs on the isolated proteasome and cultured cell lines will be presented and the structure-activity correlation will be discussed.

Reference:

1. Jung T *et al* (2009) *Mol Aspects Med* **30**: 191-296.
2. Kisselev A F *et al* (2012) *Chem Biol* **19**: 99-115.
3. Gaczynska M *et al* (2003) *Biochemistry* **42**: 8663-8670.

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P6.3

Searching for modulators of proteasome activity among phenolic acids

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The degradation of proteins plays a key role in maintaining normal homeostasis of the intracellular protein. The proteasome is a multimeric and multicatalytic complex responsible for nonlysosomal degradation of incorrectly folded, damaged by free radicals and aging proteins [1]. The 20S proteasome is a cylinder which is formed by four heptameric rings named α and β. Two β rings carry the catalytic sites having three different activities: caspase-like, trypsin-like and chymotrypsin-like [2]. Catalytic activity of the 20S can be regulated by both – activators and inhibitors. Currently, the best known synthetic regulators are the proteasome inhibitors. However few compounds that can act as activators have been described in the literature [3].
Polyphenolic compounds of plant origin are known for their antioxidant properties [4, 5]. A small part of them can also exhibit the properties of modulating the activity of the proteasome. These compounds increase the elimination from the cells of the oxidized proteins formed by oxidative stress thereby preventing the aging processes.
The aim of the present study was to look for potential new compounds stimulating the proteasome activity among extracts rich in phenolic acids. The idea of our studies emerged from the results described by Katsiki *et al.* [6] who showed that extract from the olive *Olea europaea* can affect – the activity of the proteasome. The main component found in this extract – oleuropein, significantly increases chymotrypsin-like, trypsin-like and caspase-like activity of the studied enzyme. Within this project we have tested if the extract of plants isolated by us lead to a similar effect. The results of our biological tests will be presented and discussed.

References:

1. Marques A *et al* (2009) *Chem Rev* **109:** 1509-1536.
2. Dal Vechio F *et al* (2014) *Free Rad Biol Med* **67:** 3014-313.
3. Bonfili L *et al* (2008) *FEBS* **275:** 5512-5526
4. Argyropoulou A *et al* (2013) *Nat Prod Rep* **30:** 1412-1437.
5. Brewer M (2011) *Comp Rev Food Sci F* **10:** 221-247.
6. Katsiki M *et al* (2007) *Rejuvenation Res* **10:** 157-172.

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P6.4

The role of ATP-dependent chromatin remodeling complexes in control of metabolic processes

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Several types of cancer i.e. breast and kidney cancers are featured by the loss of FBP1 (fructose-1,6-bisphosphatase) – the enzyme acting in glycolysis and gluconeogenesis in cytoplasm. This enzyme is also modulator of HIF1α transcription factor in the nucleus. Given its different cytoplasmic and nuclear function, FBP1 belongs to the family of moonlighting enzymes. The loss of FBP1 is thought as one of the main reasons of metabolic switch observed in these types of cancer.

The study on *Saccharomyces cerevisiae* provided some evidences that the expression of *FBP1* gene may be directly controlled by the SWI/SNF-type ATP-dependent chromatin remodeling complexes (CRCs).

The SWI/SNF class of ATP-dependent chromatin remodeling complexes, a prototype of which was first described in *Saccharomyces cerevisiae*, is conserved from fungi to mammals and plants. SWI/SNF CRCs regulate the structure, activity and organization of chromatin and play key roles in maintenance, transmission and expression of eukaryotic genome. The central SNF2 ATPase of SWI/SNF CRCs is associated with a small set of highly conserved „core" subunits including homologues of yeast SWI3 and SNF5-type proteins, which when reconstituted *in vitro*, have remodeling activity. In multicellular eukaryotes, the lack or aberrant stoichiometry of individual core subunits causes embryolethality or severe defects in development, and in animals carcinogenesis. Depending on subunit composition, distinct mammalian SWI/SNF complexes can act as either activators or repressors of transcription, and may thus exert opposite effects on cellular activities and serve as the interface for integration of various processes. Genetic and molecular analyses confirm that different classes of plant SWI/SNF complexes also are involved in regulation of specific processes.

Here we show by comparative functional analysis of plant and human SWI/SNF CRCs their conserved involvement in control of evolutionary most ancient regulatory processes, including regulation of metabolic status and energy homeostasis.

Our findings also suggest that impairment of SWI/SNF CRCs activity may be the real cause of metabolic switch observed in some types of cancer. Furthermore, our study justify using *Arabidopsis thaliana* as attractive model for investigation of molecular mechanisms controlling highly conserved processes in Eukaryotes.

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P6.5

C-terminal mobile catalyst of aldolase A can be positioned at subunit interface

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Aldolase is an important enzyme catalysing the conversion of fructose-1,6-bisphosphate to 3-phosphoglyceraldehyde and dihydroxyacetone phosphate in glycolysis or the reverse reaction in gluconeogenesis. Human muscle isoenzyme of aldolase – aldolase A – is a homotetramer of 363aa subunits. Its C-terminal fragment is very mobile and is missing in many crystal structures of aldolase. In others, particularly those with bound substrates, it can be found in vicinity of the active site. Tyr-363 is known to be crucial for fructose-1,6-bisphosphate aldolase activity – its loss essentially converts aldolase to transaldolase. Here we report a crystal structure of human aldolase A with 1.94Å resolution and tetramer present in the asymmetric unit. Our model demonstrates that the C-terminal fragment can also be located at the subunit interface and such location is stabilised by a salt bridge between carboxyl of C-terminus and ε-amino group of Lys-12 of adjacent subunit. This suggests that aldolase A can be an allosteric enzyme.