Rolf Breinbauer

Controlling Protein Function by Small Molecules

A major goal in modern biology is to understand how a cell and its various components are functioning. Various genetic tools (e.g. RNA interference, CRISPR/Cas9) have been developed, which have revolutionized this research. However, these tools have their limitations since they only interfere with the flow of information in a cell. In contrast, the use of small molecules which modulate protein function are a more direct way of controlling cell function, which offers the additional advantage that it can be carried out it in a time- and concentration-dependent way.

According to the "central dogma of molecular biology" the genetic information of a human cell is encoded in the DNA of the nucleus. A small region (gene) of the long DNA molecule will be transcribed into mRNA (messenger RNA), which ultimately gets translated into a certain protein (Figure 1). Proteins are the essential components of the cell, which are responsible for transport, communication ("signal transduction"), catalytic function (enzymes), and mechanical structure.

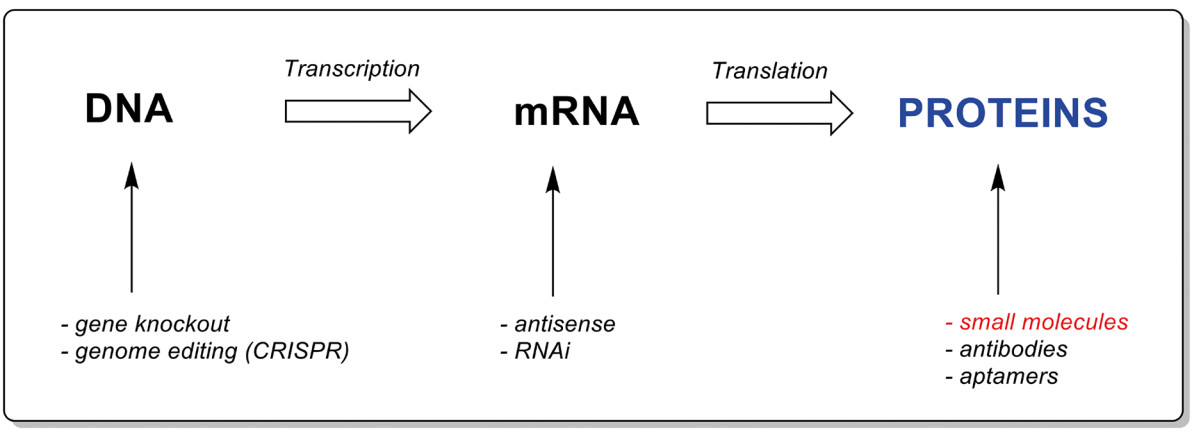
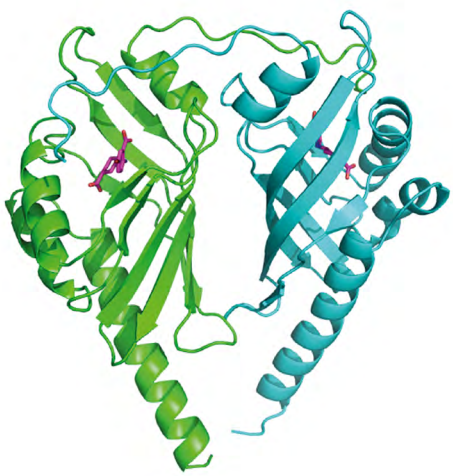


Figure 1: Organization of a cell and methods used to control the flow of information or the function of proteins in a cell.

SMALL MOLECULE INHIBITORS

In order to understand how the different components in a cell work, several tools have been developed which either delete a certain gene or prevent the production of the corresponding protein (Figure 1). Genetic methods such as gene knock out, CRISPR/Cas9 and RNA interference have proven to be powerful tools which are easy to design and show high specificity. However, they have certain limitations as they control only the flow of information in a cell (and not proteins directly), cause complete "on" or complete "off" events, or lead to problems regarding the delivery of the genetic tools into the cells. >

Figure 2: A small molecule inhibitor (depicted in magenta) inhibits protein function by binding to the active site of a protein (depicted in green and blue) according to the lock-key principle.



In contrast, small organic molecules can function as a molecular key (inhibitor) which binds directly to the protein in question. Additional advantages of small molecules are that they can easily reach the interior of a cell and control protein function in a dose- and time-dependent manner, which allow new levels of precision over genetic methods. However, there is one fundamental challenge: How can you find a specific key – a small molecule which binds selectively to just one out of the tens of thousands different proteins of a human cell?

Over the past 17 years my organic chemistry laboratory at TU Graz has collaborated with biochemists, structural biologists and cell biologists to develop the first small molecule inhibitors for several enzymes (Figure 3).

CONTROLLING LIPID METABOLISM

In 2004 the groups of Rudi Zechner and Robert Zimmermann at the Institute of Molecular Biosciences of the University of Graz published a study in the journal *Science* that demonstrated that a protein called ATGL (Adipose Triglyceride Lipase) is the rate-determining enzyme in the degradation of triglycerides in adipocytes (fat cells) of human fat tissue. Genetic studies performed in their lab revealed that a mouse which does not express the ATGL protein shows interesting features: it does not develop

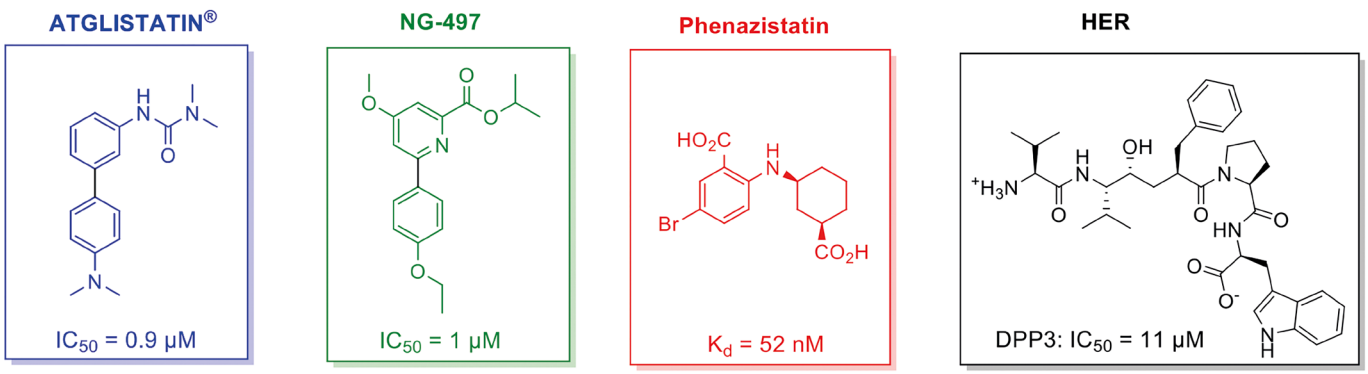


Figure 3: Small molecules inhibitors against adipose triglyceride lipase (ATGL), phenazine biosynthesis enzyme PhzA, dipeptidylpeptidase 3 (DPP3).

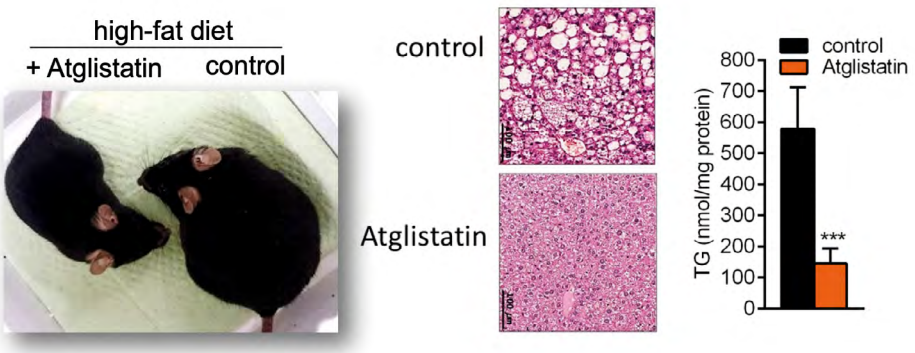


Figure 4: Atglistatin prevents metabolic-associated fatty liver disease (MAFLD) in mice as can be shown by the loss in body weight (left), the lack of white lipid droplets in liver tissue (middle) and the reduced content of triglycerides in liver tissue (right).

diabetes even on a high-fat diet, and it is resistant against tumor-induced cachexia. This motivated us to find a molecular key controlling ATGL function.

In a collaborative effort we have developed small molecule inhibitors of ATGL. It was necessary to synthesize and test almost 1,000 compounds before we could identify the first inhibitor of murine ATGL[1] with Atglistatin® and – more recently – the first inhibitor of human ATGL[2] with NG-497. Atglistatin has stimulated significant research activities worldwide, thus increasing our understanding of the physiological role of ATGL. The Zimmermann/Zechner lab could show that Atglistatin prevents metabolic-associated fatty liver disease (MAFLD) in mice (Figure 4). MAFLD affects ca. 25% of the Austrian population and in severe cases can lead to liver cirrhosis. With cardiologists in Edmonton (Canada) and at the Charité in Berlin (Germany) we could show that Atglistatin also prevents and cures heart failure in mice. These data have validated ATGL as an attractive drug target. We are currently investigating the preclinical development of ATGL inhibitors in collaboration with the wings4innovation-initiative. In addition, we are pursuing several other research programmes identifying small molecule inhibitors of various other proteins, especially transcription factors.

Rolf Breinbauer

has been head of the Institute of Organic Chemistry at TU Graz since 2007. His research interests encompass the development of tool compounds for chemical biology and the development of new methods for organic synthesis using biocatalysis, transition metal catalysis or electrochemistry.

Source: Christian Lembacher-Fadum – TU Graz

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