

Gatekeepers are discovered in the human cell 'shredder' - a new target for cancer drugs?

Human cells make use of a 'shredder', the proteasome, to degrade proteins that are old, misfolded or no longer needed. For example, insulin, a hormone released in large quantities when food is consumed, is reduced by 50% only three to five minutes later. However, if the cell's internal waste disposal system malfunctions and the misfolded proteins are not destroyed, illnesses such as Alzheimer's or Parkinson's disease may occur. To prevent this from happening, the complex process of protein degradation first needs to be fully understood at an atomic level so that appropriate drugs can be developed. Biochemists at Frankfurt University, collaborating with an international team of scientists have just taken an important step towards unravelling the workings of this mechanism. In the current edition of the scientific journal "Nature" they report finding the long-awaited receptor for ubiquitin on the proteasome. This receptor may well turn out to have a key role in fighting tumours.

"A discovery of this kind happens only once in a researcher's lifetime" comments Professor Ivan Dikic, in whose group at the Institute for Biochemistry the significant finding was made. The editors of "Nature" agree and have accepted two manuscripts describing this discovery: an article (leading manuscript in the issue) and a letter (regular publication). The Institute's director Professor Werner Müller-Esterl is delighted by his colleague's success: "This sort of recognition is only achieved by one in a thousand scientists".

However, things were looking very different only a year ago when it appeared that the research groups involved in this project - in Frankfurt, Munich, Minnesota and Harvard - were treading water. The scientists were hoping to solve structure of the portal protein from yeast using protein crystallography but the protein refused to crystallize. However, Koraljka Husnjak, a postdoctoral researcher found a way to isolate the ubiquitin binding domain in the mammalian protein, that was amenable for rapid crystallization and subsequent determination of its structure.

Already some 30 years ago, the basic mechanism of cellular waste disposal was elucidated by three scientists, Aaron Ciechanover, Avram Hershko, and Irwin Rose, for which they won Nobel Prize in Chemistry in 2004. Since then it has been known that proteins due for disposal are marked with ubiquitin molecules, which are present throughout the cell. They reach the barrel-like proteasome complex via 'shuttle' molecules or through diffusion. On the upper side of the proteasome there is a kind of gatekeeper's lodge with a narrow entrance leading to an inner chamber, where aggressive enzymes cleave the protein. But first the protein is subjected to a strict control procedure to ensure that it is indeed destined for the shredder. If the gatekeeper - a receptor - recognizes that the protein is tagged with ubiquitin, the tagged protein is unfolded and can then pass through the narrow opening. While this takes place the ubiquitin separates from the protein, ready to be re-used. Until now, only one such gatekeeper, a proteasomal receptor called Rpn 10, was known. The scientists then conducted experiments to genetically remove Rpn 10 from the cell and were surprised to discover that the

proteasome continued to function normally. This led them to suspect that there must be an additional protein in the cell, which compensates in the absence of Rpn 10 and serves a similar purpose. This has now been discovered: protein Rpn 13.

According to Koraljka Husnjak the first breakthrough occurred about four years ago, when they found out that ubiquitin binds to a subunit in the gatekeeper's lodge. "So it became clear to us that the proteasome subunit might act as ubiquitin receptor on the proteasome. But first of all we had to clarify this binding site's function and understand the details of the binding process at an atomic level". Ivan Dikic then asked other leading international groups for their expertise in helping to solve this complex research problem. The X-ray structural analysis was carried out by Prof. Michael Groll and his group at the Technical University in Munich, and a group led by Prof. Kylie Walters at the University of Minnesota, Minneapolis undertook the NMR structure work. As soon as the binding mechanism had been understood at an atomic level, Professor Finley and his group at Harvard Medical School conducted experiments with various yeast strains in which they were able to prove that in living cells the process was indeed identical to that already suggested by the structural model.

The discovery of this second receptor on the proteasome is of particular significance in cancer research since it has the potential to be blocked by specific drugs. This would then prevent the proteins in the cell from being broken down. Since developing cancer cells depend on the breakdown of specific proteins in signalling cascades, which appear critical for tumour cell survival and proliferation, the cancer cells would no longer be able to multiply. It is likely that both these receptors react selectively to certain groups of proteins. So even if one is blocked, the other continues to ensure that the proteins that are no longer needed nevertheless still gain access to the proteasome.