Ulm researchers are writing a new chapter of the thalidomide story

Thalidomide, which was sold in Germany in the late 1950s under the trade name Contergan, is mainly known for having caused one of the biggest pharmaceutical scandals in Germany. However, what was once a sleeping pill is increasingly being used as an immunomodulatory drug for treating tumours of the haematopoietic system, something that is not yet widely known. Dr. Jan Krönke is the head of a junior research group at Ulm University Hospital studying the mechanism of action of the thalidomide analogue lenalidomide.

Uncovering the secret with proteomics

The anti-tumour properties of the molecule and its analogues were only finally explained four years after its discovery. In 2014, three research groups, including Krönke's (Science 2014), simultaneously managed to uncover the secrets of the anti-cancer drugs thalidomide, lenalidomide and pomalidomide using different, though complementary proteomics methods. Krönke's group found that lenalidomide causes the selective degradation of IKZF1 and IKFZ3, which are essential transcription factors in multiple myeloma. These results reveal a previously unknown mechanism of action for lenalidomide.

Lenalidomide binds to the substrate receptor cereblon, which belongs to an E3 ubiquitin ligase complex that is also associated with other proteins (DDB1, CUL4A and Roc1). Binding to cereblon activates E3 ubiquitin ligase and triggers a signalling cascade that causes ubiquitin to attach to IKZF1 and IKFZ3 (ubiquitination), thus signalling their degradation via the proteasome. Krönke and his colleagues were able to show that Ikarrs (IKZF1) and Aiolos (IKFZ3) bind selectively to cereblon.

Thanks to a DFG grant, Jan Krönke spent 2011 to 2014 at the Brigham and Women's Hospital at the Harvard Medical School where he met and learnt some important lessons from Benjamin Ebert. Ebert is an expert in myelodysplastic syndrome (MDS) and lenalidomide. Back in 2008, Ebert discovered that the loss of the long arm of chromosome 5 (del(5q)) plays a key part in MDS pathogenesis. One in five MDS patients lack the long arm of chromosome 5, and 70 to 80
percent of these patients respond to treatment with lenalidomide.

Why is lenalidomide so specific?

During his post-doctoral stay in Boston, Krönke found out why lenalidomide, but not thalidomide and pomalidomide, has an effect on (del(5q)) MDS. Using proteomics, molecular investigations and a mouse model, the German-American group of researchers showed that only lenalidomide can degrade casein kinase 1A (CK1A). The group of researchers led by Ebert has recently discovered (Cancer Cell. 2014 Oct 13;26(4):509-20.doi: 10.1016/j.ccr.2014.08.001) that this enzyme plays a key role in the biology of (del (5q)) MDS. Further research is needed to identify all the biological effects of CK1A degradation.

"CK1\(\alpha\) is encoded by a gene located on the deleted chromosome region (del(5q)). del(5q) MDS cells therefore only have small amounts of this protein and they are particularly sensitive to lenalidomide, which exploits the loss of the gene in the cancer cells in order to destroy them," says Krönke. The mechanism of action is based on the haploinsufficient expression of CK1\(\alpha\), which, as mentioned above, sensitises cells to lenalidomide therapy. Haploinsufficiency has been known as a driving force in cancer for around 20 years (Nature 2015, p. 183, 187).

A single amino acid alters effect

Krönke and his (former) colleagues also discovered that a single amino acid in the murine cereblon protein is enough to make it resistant to thalidomide and its analogues. Genetic modifications of the protein cereblon will one day make it possible to reverse this resistance and carry out investigations with lenalidomide and other potential drugs in mouse models.

Krönke’s experiments show that tiny chemical modifications seem to suffice to change the effect of the molecule group. Krönke comments: "This enables us to adjust this mechanism precisely and control it with minor chemical modifications. This may well be important for new drugs with similar effects such as the ones Celgene develops, and in particular drugs that can degrade disease-relevant proteins.

Can transcription factors now be attacked?

Experts (e.g. Stewart, Ito, Licht) believe these studies have huge implications. Researchers are aware of the immense regulatory power of transcription factors and have been looking for them and ways to suppress them for around 30 years now. "It is difficult to develop drugs to counteract the action of transcription factors as, unlike enzymes, they are hard to attack," explains Krönke. A group of researchers (Winter, Science 2015) provided evidence that further chemical modifications of thalidomide analogues also make other proteins (BET or FKBP12) vulnerable to attack and delay the progression of cancer in the mouse model. This has fuelled speculations in the research community about a potential new drug class. However, the journey from animal experiments to clinical studies is a rocky road. Jan Krönke knows this from personal experience as head of an Emmy Noether research group (http://www.uniklinik-ulm.de/struktur/kliniken/innere-medizin/klinik-fuer-innere-medizin-iii/home/forschung/forschungsbereiche/emmy-noether-research-group.html).

MDS is a cancer characterised by the loss of blood-forming cells and platelets in the bone marrow. This often leads to severe anaemia, infections and bleeding. Around 50 percent of all MDS patients carry some type of cytogenetic or chromosomal abnormality, and current research shows that 30 percent of patients lack the long arm of chromosome 5 (5q deletion, del(5q)). MDS has a bad prognosis and can develop into acute myeloid leukaemia, which is often fatal (EMA/31457/2015).

In addition to lenalidomide, 5-azacytidine, a chemical analogue of cytidine that removes methyl groups from DNA, has also been shown to be suitable for treating MDS. MDS develops slowly. However, therapeutic options are rather limited. Allogeneic stem cell transplantation is probably the only available cure, but can only be used in very fit patients under 70.

References:

Krönke, Jan et al.: Lenalidomide Causes Selective Degradation of IKZF1 and IKZF3 in Multiple Myeloma Cells, Science 343, (17.1.2014), 301-305; DOI: 10.1126/science.1244851.

