Healthcare industry BW

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.

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University of Ulm researchers led by Jan Krönke, along with American colleagues, have discovered a new mechanism of action of the thalidomide analogue lenalidomide (Krönke, Nature 9, July 2015). Their research suggests that thalidomide analogues have the potential to make hitherto unreachable cancer proteins (transcription factors) vulnerable.

A story full of dramatic turns and twists

The story of thalidomide is full of dramatic turns and twists. The compound was developed in the 1950s by a research director at the German drug company Chemie Grünenthal and sold under the trade name Contergan. The sedative was advertised as harmless and was taken by many expectant mothers as it also helped alleviate morning sickness.

Thalidomide's high teratogenic potential, which was not known at the time, led to an increased number of births of deformed children with stunted arms and legs. Some children were born without any limbs at all, and exhibited damage to multiple organs or loss of entire organs. In Germany, thalidomide was sold as an over-the-counter drug and led to the largest number of victims, causing, in the early 1960s, the biggest drug scandal in the country's history. In 1968, the research director and other senior company personnel were put on trial. The case ended in December 1970 and the drug disappeared from the market.

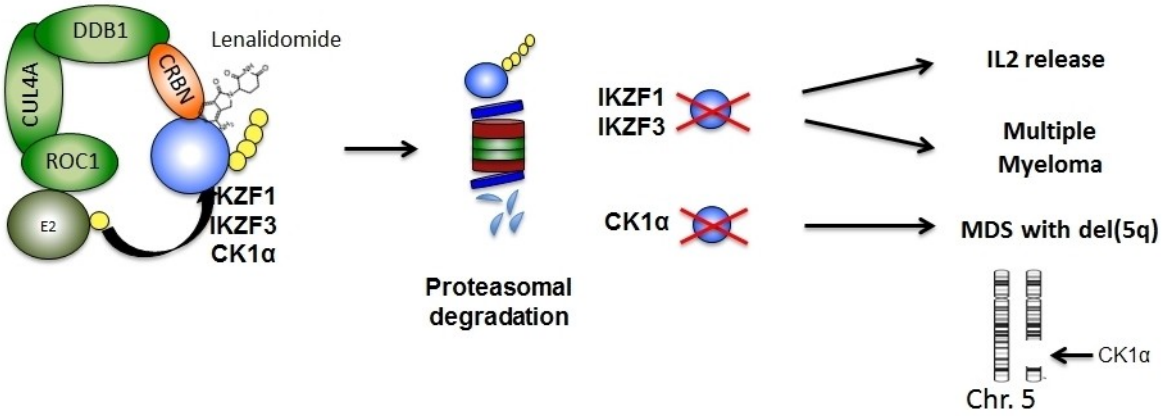
In the early 1990s, the thalidomide patents were acquired by the American pharmaceutical company Celgene, which then started to produce and market immunomodulatory drugs based on thalidomide and thalidomide derivatives (http://www.celgene.com/research-development/medical-innovation/imids/). In 1999, thalidomide was shown to prevent the development of new blood vessels, and thus stop tumour growth: researchers discovered that thalidomide unfolds and has a strong effect in patients with multiple myeloma (MM), and that its derivatives, lenalidomide and pomalidomide (Stewart, 257), which have received marketing authorisation for the treatment of MM and other blood cancers (Stewart, 256), are even more potent. However, due to their teratogenic effect, these drugs can only be administered in the EU (see link to BfArM publication) and the US under strict safety conditions. The mechanism of action of this molecule group has long remained elusive. Many experiments have revealed the compounds' anti-tumour effect. They prevent the formation of blood vessels and strengthen the immune system by augmenting natural killer cell cytotoxicity and increasing the number of cytotoxic immunomodulators such as interleukin-2. In 2010, the Japanese scientist Ito found that the protein cereblon (CRBN) was inhibited by thalidomide. Inactivation of cereblon was identified as the reason for thalidomide's teratogenic effect (Licht, 11).

Uncovering the secret with proteomics

The anti-tumour properties of the molecule and its analogues were only finally explained four years after Ito's 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 polamidomide 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 Ikaros (IKZF1) and Aiolos (IKZF3) 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.



Lenalidomide binds to E3 ubiquitine ligase, and activates the binding of IKFZ1 and IKFZ3 as well as casein kinase 1α. These substrate proteins are labelled with the small protein ubiquitin and subsequently degraded in the proteasome. The degradation of IKFZ1 and IKFZ3 is responsible for the clinical effects of lenalidomide, thalidomide and pomalidomide in MM and the release of interleukin-2 of T cells. CK1α is only ubiquitinated by 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.

"CK1α 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 CK1α, which, as mentioned above, sensitises cells to lenalidomide therapy. Haploinsufficy 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. Allogenic stem cell transplantation is probably the only available cure, but can only be used in very fit patients under 70.

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