Photo: Fotolia/Schliemer

Toxin research in Oslo, Norway

Friends or Foes?

Endocytosis and intracellular transport of toxins have been the passion of Kirsten Sandvig and her colleagues at the Norwegian Radium Hospital in Oslo for years. Her persistence in clarifying the underlying basic cellular processes may soon pay off, as toxins face promising applications in cancer treatment and molecular imaging.

It was first suggested that toxins may be useful in combating cancer a short time before Kirsten Sandvig started her research career at the Institute for Cancer Research at the Norwegian Radium Hospital in Oslo in 1974. Since then, apart from two research visits to the United States, she has remained loyal to her work place and her subject. We look out over the town – Oslo is covered in a thick layer of fresh snow – as we talk in her office in the new research building annexed to the hospital. Her research group is part of the Centre of Cancer Biomedicine, a Centre of Excellence in biomedicine in Norway. In addition, Kirsten Sandvig is a professor at the University of Oslo.

Changing beliefs

"They tell us a lot about basic mechanisms in the cell," Sandvig explains her fascination with toxins. Her first work on ricin, a plant toxin shown to be more toxic to cancer cells than to healthy cells, addressed the compound's interaction with cell surface receptors and its internalisation by endocytosis. At the time, the term 'endocytosis' stood for 'clathrin-mediated endocytosis' (by means of clathrin-coated pits). However, using ricin she showed that clathrin-independent endocytosis also existed. "When I presented this for the first time at a meeting, people at the back got up from their seats and said that they did not believe it," she remembers. Now, clathrin-independent endocytosis is well established, whilst more and more different endocytic mechanisms are being identified.

By the end of the 1980s, the dicision to take on another toxin, Shiga toxin, was motivated by the discovery that its receptor, the glycosphingolipid globotriaosylceramide (Gb3) aka CD77, is much more abundant in cancer cells than in healthy cells. What's more, Shiga and Shiga-like toxins are important in connection with infectious diseases caused by the bacterium *Shigella dysenteriae* and specific strains of *Escherichia coli* present in contaminated food. Infection with these bacteria can cause diarrhoea, abnormal lysis of red blood cells and kidney failure, especially in children.

Outside the mammalian cell, the toxin is an inactive complex of a binding subunit B and an enzymatically active A subunit. The B subunit binds the cell surface and assists the toxin in entering the cell. During transit to the cytosol, the A subunit is cleaved into two fragments by the protease furin. One of the fragments, A_1, reaches the cytoplasm, where it modifies and thus inactivates the ribosomal 28S RNA, part of the 60S subunit of the ribosome. Protein synthesis comes to a halt.

How the toxin's A_1 subunit reaches the cytosol was still unknown at the time, as were many other aspects of its mode of action. Few would have guessed that the toxin followed the entire secretory pathway in reverse direction. But this was exactly what Sandvig and her colleagues discovered back in 1992 (*Nature,* 358:510-512). From endosomes, the toxin enters the *trans*-Golgi network and travels through the Golgi apparatus to the endoplasmic reticulum (ER), from where it enters the cytosol. It even reaches the nuclear envelope.

Not that toxic at all, Kirsten Sandvig (left) and her group

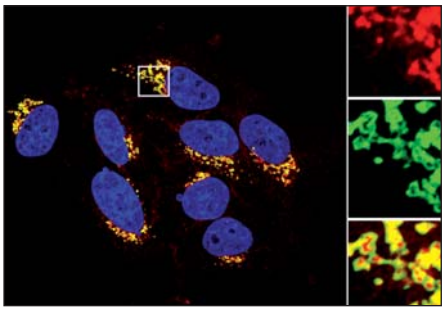
The internalisation of Shiga toxin occurs via both clathrin-coated pits, as well as by clathrin-independent mechanisms, although these latter mechanisms are not very well characterised. "The problem with endocytic pathways is that they are very difficult to study. When you hit one molecule or pathway, others may be affected as well, and it is difficult to assess what is the normal state of the cell," explains Sandvig.

Some ligands manipulate cellular processes to boost their own uptake into the cell. Shiga toxin turned out to be one of those: it triggers the phosphorylation of a clathrin subunit (Clathrin Heavy Chain, CHC) by activating the tyrosine kinase Syk, which is also implicated in signal transduction events in B and T-cells. When Syk is depleted by small interfering RNA or inhibited using piceatannol, clathrin is no longer phosphorylated and the uptake of Shiga toxin is reduced *(Mol Biol Cell,* 17:1096–1109). To study endocytosis, the Sandvig lab uses a biochemical assay, which measures the amount of chemiluminescent and biotinylated Shiga toxin taken up by the cell. However, to have a closer look at how the Syk kinase modulates endocytosis, Audrun Utskarpen, a PhD student in Sandvig's lab at the time, spent a few months in Thomas Kirchhausen's lab at Harvard Medical School. "This group uses a lot of advanced microscopy and has been studying clathrin-coated pits for a long time, so it was the ideal collaborator on this project," says Sandvig. The technique Utskarpen used at Harvard was live-cell spinning disk confocal imaging. Using this technique, she was able to record the appearance and disappearance of clathrin-coated pits (lifetimes between 30 and 90 s) in real time. These time series showed very neatly that cells, in their case HeLa cells, respond to Shiga toxin with the formation of increased numbers of clathrin-coated pits and that the response is dependent on the Syk kinase (*PloS One,* 5(7):e10944).

Toxin therapy

Many different types of tumours and cancer cells express the Shiga toxin receptor, Gb3, at high levels, while in healthy tissue Gb3 expression is very limited (the highest amounts are found in renal epithelial tissue and the endothelium). This is an excellent starting point for making use of

Shiga toxin in targeted cancer therapy. Shiga toxin is an extremely efficient killer and only very low doses are needed to kill cancer cells. Another advantage is that it kills



Confocal laser scanning microscopy image showing cultured HEp-2 cells, where the B-subunit of Shiga toxin (red colour) has been internalised and transported to the Golgi apparatus (green colour). Cell nuclei are stained in blue. Scale bar: 10 µm.

by a distinct mechanism to most cancer drugs, and will, therefore, avoid problems with drug resistance. The proof of concept came from experiments by a number of groups, in which Shiga toxin was injected into tumours in mice. The mice became tumour-free within a few days and did not show any side-effects. However, the road to establishing Shiga toxin as an approved drug is still long, as more work needs to be done on the short and long-term effects of the toxin in humans. An example of an approved anti-cancer drug based on a bacterial toxin is 'Denileukin diftitox', a conjugate between diphtheria toxin and interleukin 2, which targets leukaemia cells.

An alternative way to exploit Shiga toxin for cancer research is by using only the non-toxic binding subunit (Shiga toxin B) for treatment or cancer imaging. The binding subunit is a very effective vehicle to get substances into cells. By binding it to fluorescent labels and isotopes, successful visualisation of mice tumours was achieved, using confocal laser endoscopy and positron emission tomography (PET), respectively.

Nanoparticle movement

Kirsten Sandvig is, however, not directly involved in these trials. "Insight into the fundamental mechanisms responsible for toxin transport and uptake is our group's main contribution," she explains. Nevertheless, she describes the "short distance between basic research and the applications in the clinic" as one of the aspects of her work that inspire her most.

This inspiration can also be recognised in another of her many lines of research: uptake and intracellular transport of nanoparticles. Certain nanoparticles, especially quantum dots, which are very bright and photostable nanoparticles, are used widely in tracking molecular dynamics in cellular imaging and in tumour targeting. However, "There is a clear lack of knowledge on how cells take up and transport these particles," explains Tore-Geir Iversen, who works with the nanoparticles in Sandvig's group. "Uptake experiments reported in the literature are often of poor quality, and we saw immediately that we could use our extensive knowledge on endocytosis and transport to make important contributions to this field."

His original intention was to use quantum dots to visualise the retrograde pathways from endosomes to cytoplasm that had been described for Shiga toxin and ricin. To this end, he conjugated the toxins' binding domains to quantum dots. However, he discovered that the toxin-quantum dot conjugates accumulated in endosomes and did not travel to the Golgi, ER or cytoplasm. Caught in endosomal compartments, they also perturbed normal cellular transport (*Nano Lett,* 8(7):1858-65).

This has implication for the clinical use of quantum dots: "It is clear that, once inside the cell, the particles stay there and can even be harmful," he clarifies. Elaborating, he describes that there is a lot of interest from pharmaceutical companies to produce novel nanoparticles for use in combination with different imaging modalities, such as optical imaging, positron emission tomography (PET) and single photon emission computed tomography (SPECT). Important for these types of particles is that they are rapidly cleared from the body in order to minimise background signals and obtain better imaging results. "Sound cell biological research is crucial to assess the impact of the size, surface properties and chemical composition of these particles on their interaction with, and behaviour inside or outside, cells," he explains.

In the years to come, both Iversen and Sandvig will continue using the toxins to study endocytic and other intracellular transport pathways in healthy cells. They will also apply their knowledge to develop methods facilitating the identification of cancerous cells using tissue arrays. "The toxins really contributed to our current view on endocytosis," concludes Sandvig. Her publication record with over 200 papers thus far is mind-blowing. This must be taken as a testimony of how important that contribution is.

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