Linking Bone to Breast

Fourteen years after its identification, RANKL still has many surprises in store – not only for Josef Penninger and his group in Vienna. From a protein involved in bone remodelling it has now turned into a major player in the development of breast cancer.

Without bones you’d be just a puddle of skin and guts on the floor. Therefore it’s nice to have bone-forming cells called osteoblasts. Their counterparts, the osteoclasts, dissolve bone tissue – both work together to guarantee a constant reshaping of the tissue. On a more pathological note, hyperactivation of osteoclasts results in weaker bones, as is the case in osteoporosis. Inactivation, in contrast, hardens the skeleton, a condition called osteoporosis or marble bone disease. One important molecule in osteoclast differentiation and activation is RANKL or Receptor Activator of Nuclear Factor Kappa-B Ligand (just one of its many aliases).

The legacy of the small mice

Four different groups (three from the US and one group from Japan) cloned RANKL at almost the same time, between 1997 and 1998. Two of the groups (based in Seattle and New York) discovered the protein also in T-cells, while the two other groups (in California and Japan) had established a link with osteoclasts. At that time, Josef Penninger, of Austrian origin, was leading a group at the Amgen Institute in Toronto, Canada. He saw that RANKL could yield something worthwhile. However, when he suggested to his then postdoc Young-Yun Kong, to create a knock-out mouse, the researcher was wary at first. Then, an unexpected phone call from his colleague Bill Boyle, who dropped the same idea, encouraged him. The result was a publication in Nature (397 (6717):315-23), two years later.

Originally trained as a medical doctor at the University of Innsbruck, Penninger got hooked onto immunology during his PhD. A postdoctoral position in Canada turned him into a geneticist: he learned gene-targeting and to generate knock-out mice. Then, when the US biotech company Amgen created a new research institute, he was offered a position as a group leader. “A fantastic opportunity to work at a great institute with plenty of money to do good research in freedom”, is how Penninger describes the start of his career as an independent researcher instead of a hospital doctor. In 2003, Penninger moved his group to Vienna, to become the founding director of the Institute of Molecular Biotechnology (IMBA), the largest institute of the Austrian Academy of Sciences.

Bones and immunity

So what about the knock-out mice Young-Yun Kong was asked to produce? The poor fellows turned out to be small, have deformed heads, and suffer from severe osteoporosis, providing the genetic demonstration of the function of RANKL in osteoclast differentiation and bone remodelling. A second phenotype of the RANKL-deficient mice, the complete absence of lymph nodes, hinted at the possibility that RANKL and its receptor RANK connect bone physiology and immunology. Such a direct relation between immune cells and bone cells was still difficult to grasp for many at that time.

That is why Penninger calls the experiment his group performed, to establish how the immune system and bone metabolism fit together, ‘heretic’. “The idea that white blood cells control how much bone we have was not well-received by some colleagues”, he explains. In the experiment, he activated T-cells in mice, which led to increased osteoclastogenesis and bone loss as a result (Nature, 402(6759):304-9).

The finding had huge implications. It explained why patients with chronic activation of the immune system, like leukaemia, HIV, or inflammatory bowel disease, suffer from bone loss. It also explained why periodontitis, inflammation of the gums, leads to bone loss in the jaws. It even provided the key to the mechanism behind auto-immune diseases, such as rheumatic arthritis, which are characterised by inflammation of the joints, and which lead to severe degradation of bone tissue. Every T-cell-activating agent increases the level of RANKL, and, hence, of the number of osteoclasts. “It felt like a light switch: so many pieces suddenly fell into place”, Penninger remembers.

Sex hormones

What’s more, all the pups of RANKL knock-out mice died soon after birth. In hindsight, this was a clue to an even more surprising role for RANKL but it was long left unexplored. “At first, we did not think that the fact that they did not survive was very interesting. These mice lack teeth because osteoclasts are needed for teeth eruption and the mice are generally in poor health. We thought they were just unable to feed their babies.” Then, one day, they noticed that the mother mice did not produce any milk. The knock-out mice have normal mammary glands at birth and the development of these glands continues as normal during puberty. However, the expansion of the mammary epithelium during pregnancy to form a lactating mammary gland, a process controlled by the sex hormone progesterone, was impaired, hence the failure to produce milk. It turned out that the mammary glands in RANKL-deficient mice failed to respond to progesterone. Apparently, progesterone triggers epithelial cells to produce RANKL, which, in turn, drives the cells into cell division to form a lactating gland (Cell, 103(1):41-50).
Sex hormones controlling bone mass!
It seemed as if something had gone awry in evolution. Yet, Penninger was slowly starting to make sense of it all; calcium is the most important building block for the expanding skeleton in a newborn. To guarantee that the levels of calcium in the blood and in the milk are sufficient for the developing embryo and newborn, progesterone, through RANKL, prompts osteoclasts to release it from the mother's bones. Thus, RANKL plays an essential role in mammalian reproduction.

Ten years of preparation
Once RANKL was established as a downstream target of the sex hormone progesterone, the link with breast cancer was no longer a big step. Two large studies published in 2002 and 2003 demonstrated that synthetic progesterone (progestin), used in hormone replacement therapy for postmenopausal women and in various contraceptives, increases the risk of developing breast cancer.

Penninger and his group took ten years to prepare for the experiments to answer the obvious question whether RANKL and its receptor RANK are the missing link between progestin and mammary cancer.

They created mice in which RANK is specifically knocked-out in the mammary epithelium. Daniel Schrameck spent large amounts of his PhD time getting ready for the experiments. “At first, we were looking in the wrong direction. Once we settled on the hormone treatment, the result was very convincing.” He joined forces with Verena Sigl, an undergraduate student in the lab, dropped all his other projects temporarily, and finished the experiments as soon as possible. They published the results last year (Nature, 469(7320):98-102).

Smoke and hormones
To induce breast cancer in the mice, they treated them with MPA, a synthetic progesterone, and with a carcinogen. The carcinogen induces DNA damage, while MPA, through a surge in RANKL, triggers proliferation of mammary epithelial cells. “Compare it to a woman who smokes like a chimney and who is on the pill or on hormone replacement therapy”, Daniel Schrameck explains. The smoking triggers the DNA damage and the hormone pushes the damaged cell to grow out and proliferate. The treatment resulted in breast tumours in all the normal mice. Of the RANK-deficient mice, on the other hand, 70% remained tumour-free. They further showed that, apart from inducing proliferation in mammary epithelial cells, RANKL protects DNA-damaged cells from cell death after treatment with γ-irradiation. “Together, these two effects create an environment in which tumours thrive”, Penninger points out.

Penninger’s group collaborated closely with Bill Dougell’s group in Seattle, USA. “They had the same findings around RANKL and breast cancer and published them in the same issue of Nature. That meant that we had immediate confirmation that our results are real.”

A human anti-RANKL antibody called denosumab is already approved for treatment of osteoporosis and skeletal-related events in various forms of cancer. It lowers the levels of RANKL by directly binding to it. Penninger does not hide his excitement: “I cannot wait to see the first sound clinical trial with this antibody for breast cancer prevention in women who use hormone replacement therapy.”

And more functions
This will not be the end of the RANKL story. Verena Sigl, now a PhD student in Penninger’s lab, is trying to unravel the molecular pathways between RANKL and breast tumour development. There are indications that hormones play a role in hereditary breast cancer in carriers of mutations in one of the brca genes. Verena is exploring possible links between RANKL and these hereditary forms of cancer.

Yet more unexpected functions for RANKL emerged in the last two years: when you pull out the hairs of a RANKL knock-out mouse, the mouse remains bald. Apparently, RANKL does not only affect proliferation of mammary epithelium, it also regulates the epithelial cells involved in hair regrowth (PNAS, 108(13): 5342-7). So far, in this story, there has not been any mention of RANKL in the brain. But also this organ cannot stand at the side line: RANKL expression influences fever and body temperature control in the brain. Together with Japanese colleagues, Penninger and his group identified this novel function and they are currently delving into the brain looking for additional roles.

“Although I have never treated a patient in my life, in my heart I am still a medical doctor. It is fantastic that my research has taken me so close to possible clinical applications that can really make a difference to patients’ lives. That is what drives me in my every-day work”, Penninger concludes.

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