

Estrogen and cancer in London

A Fatal Connection

Mutation is the name of the molecular game in cancer development. Recently, Svent Petersen-Mahrt and his team have shown how the sex-hormone estrogen enters this game by directly activating the cytosine-deaminase AID.

Svend Petersen-Mahrt remembers very well the most important day of his scientific career when he was working with Michael Neuberger as a postdoc at the Medical Research Council's Laboratory of Molecular Biology in Cambridge. He was particularly interested in the molecular mechanism behind white blood cells' ability to produce so many antibodies so quickly. These antibodies are, of course, needed to combat the indefinite range of antigens to which the body is exposed. After an antigen is first seen, different antibodies are generated by a series of point mutations which are restricted to the antibody's binding pocket. This process makes better and better binding antibodies. What was unknown, after forty years of intensive research, was how this variety was generated.

In 2000, an enzyme called activation-induced deaminase, or AID was shown to be always present where the mutations were generated. The mutation rate of the genome at loci which encode antibodies is staggeringly high. A DNA base is one million times more likely to change here than at most other places in the genome. It was this process, called somatic hypermutation, which really grabbed the interest of Petersen-Mahrt and his co-workers. The career-defining breakthrough came as they noticed that AID directly caused these DNA mutations by removing the amine group from cytosine at the loci of immunoglobulin genes. This, as all good biochemists know, leaves behind uracil. What it also leaves is a U:G mismatch, as the original cytosine was paired with a guanine. It is this mismatch which leads to mutations being incorporated into the genome (*Nature* 418: 99-103).

Small group, big deal

An enzyme that goes about modifying DNA is a big deal. With this discovery under his belt, Petersen-Mahrt decided to try



Estrogen can cause uracil:guanine mismatches via upregulating a DNA deaminase

to carve a niche for himself in the study of AID. He succeeded in nailing down what had been, even by most young scientists' standards, an itinerant career. Leaving Germany for six months' English practise in Texas at 16, he left the United States eleven years and two degrees later with a PhD in antibody engineering. He then headed for a postdoc at the Biomedicinska Centrum in Uppsala before arriving in Cambridge. Having completed his stint at the LMB, Petersen-Mahrt now works for Cancer Research UK at their labs at Clare Hall, north London. He runs a small group (there are no really large groups in the institute), which is funded in an exceptional way: equipment, consumables and staff are paid for solely by charitable donations.

AID has become an important target for more applied research as, when it is unregulated and accumulates in the cell, it can initiate cancer. For example, it has recently been shown that Burkitt's lymphoma, a B-

cell-specific cancer, does not develop without AID activity.

So how is the AID story developing? As might be expected, the regulation of AID expression and activity takes centre stage. Petersen-Mahrt was never convinced that AID would only be found in blood cells, and his hunch was proved correct. In collaboration with Wolf Reik at the Babraham Institute, Cambridge, UK, Petersen-Mahrt showed that AID was expressed in oocytes. "It turned out that AID is expressed in oocytes at very high levels, only ten-fold less than in the activated B-cells that made the antibody diversification, and 100-fold above what might be called a background level". Your initial reaction to this might be that an oocyte should be a cell with a very low rate of somatic mutation, so why is AID there? Well, Petersen-Mahrt and Reik thought that it was doing another interesting job. AID also works on methylated DNA (specifically 5-methyl cytosine), so it's time for some more biochemistry.

When a methylated cytosine is deaminated, it becomes a thymine, giving a T:G mismatch. This mismatch then gets repaired, but all known repair pathways introduce a cytosine and not a methylated cytosine. This becomes relevant for epigenetics as it gives a mechanism through which methylated cytosines (which can be inherited) can be reprogrammed to non-methylated cytosines. Petersen-Mahrt points out that "a recent study is corroborating this idea, in that zebra fish AID seems to do this sort of demethylation as well".

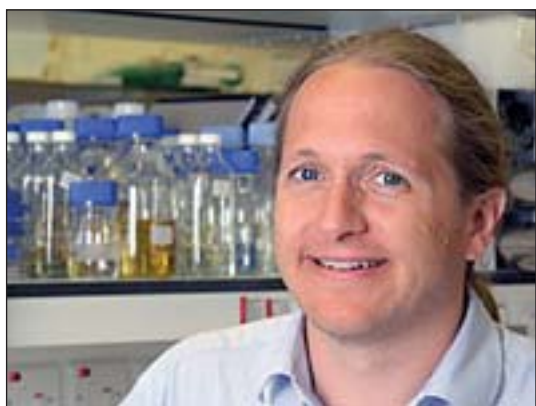
Cancer without mutation?

The regulation of AID points Petersen-Mahrt's research in another interesting direction. "Because it's expressed in the oocyte as well as B-cells, I had a hard time to think that the activation in B-cells at the transcriptional level would be through B-cell specific factors." This train of thought led Petersen-Mahrt back to one of his un-

dergraduate seminars at Texas A&M University. After his summer project presentation on cancer induction, he was asked to name an agent which does not mutate DNA but causes cancer. Unable to, he has subsequently made up for his negative answer and then some. Hormones such as estrogen, as he learned, are able to do this; a fact which has stayed with him ever since.

The first direct link

As he set out to investigate the regulation of AID he got a second push in the direction of studying the effect of hormones. A student in the lab, Siim Pauklin, arrived and immediately asked whether he could work on the link between AID and hormones. Intrigued to see whether their reasons tallied, Petersen-Mahrt naturally asked why. Siim then mentioned that nine times out of ten autoimmune disorders such as lupus are seen in women. Petersen-Mahrt explains, "B-cell autoimmunity driven through antibodies are actually almost more preva-



Itinerant career: Svent Petersen-Mahrt

lent in women. It is known that sex hormones could do something to this system, and AID, as a key factor in B-cell immunity, would make a great target."

Going back to the cancer question: estrogen causes it, but how? Is it a side effect of cell proliferation? Petersen-Mahrt thinks not, because it also causes cancer in cells which do not normally proliferate. The fundamental question of how estrogen causes a mutation, the hallmark of cancer, in the genome remains. "When I discovered in 2002 that AID was a DNA deaminase, I thought that if hormones activate the enzyme, there is a direct link between hormone and mutation." This hypothesis could only be tested in a system, which has a DNA deaminase (only vertebrates do), and so the *E. coli* assay system in which the initial discovery was made had to be shelved in favour of a mammalian system.

As it turns out, when estrogen binds its receptor, they enter the nucleus as a complex and bind to the promoters of various genes. As *AID* is a target of this complex, its transcription is activated. This provided the first direct link between the sex-hormone estrogen and a mutation process in mammalian cells (*J Exp Med.* 206(1): 99-111). But is this regulation specific to the immune system, or is it more general? To address this question, Siim Paulin and Petersen-Mahrt treated ten selected human cell lines, as well as primary tissue from mice, with estrogen. He did some real-time PCR and measured the levels of *AID* mRNA before and after the treatment and found significant differences. For example, *AID* is up-regulated 25 times in mice ovaries after exposure to estrogen. Human cell lines which express the estrogen receptor also see increases in *AID* transcript abundance. As Petersen-Mahrt explains, "This correlation has implications not only for women's immune systems, but for cancer as well.

Hormone replacement therapy is the number one concern for us, as this would mean prolonged periods of high levels of AID – unregulated." Also concerning for Petersen-Mahrt is the observation that low concentrations of Tamoxifen, an anti-estrogen drug, can activate *AID* transcription all by itself. As a treatment for breast cancer, the therapeutic high concentrations of Tamoxifen do their job by inhibiting estrogen, but what is the role of prolonged exposure to low concentrations elsewhere in the body in cells where the estrogen receptor is present? This is a question which can only be answered with correlative data, but these data suggest that it may be causing secondary cancer.

Don't stop it now

Petersen-Mahrt is at pains to stress that women should keep using estrogen therapy as it has many benefits, "Estrogen causes cancer, that's not new. Just because we know a mechanism of how it happens doesn't mean we now have to drop everything and stop." This is the area in which Petersen-Mahrt would most like make a big impact. His long-term goal is to find a way to stop estrogen-activated cells from mutating through the DNA deamination system. If successful, it would be possible to keep the benefit of estrogen therapy without causing problems elsewhere. Now that really would aid the fight against cancer.

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