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Nuclear fallout enables long-term experiments

Collateral Benefit

When in the 1950s and 60s, several nations conducted their nuclear weapons' tests, no one suspected that decades later, these tests would spark discoveries in regenerative medicine and forensics. *Lab Times* met "Radioactive (wo)Men", Jonas Frisen and Kirsty Spalding from the Karolinska Institute.

If you do not lose any weight during dieting, don't be upset. The reason might be your fat metabolism. Scientists around Kirsty Spalding and Peter Arner from the Karolinska Institute and Karolinska University Hospital in Stockholm, Sweden, found that obese people break down fewer and store more triglycerides in their fat cells than slim people. In the latter, fat cells renew their lipids about six times during their ten-year lifespan. Not only the lipids are exchanged but also the fat cells, approximately ten percent per year at all adult ages and levels of body mass index. In a common form of congenital blood lipid disorder, which is associated with a high risk of coronary artery disease, both lipid removal and storage rates are reduced. Under these conditions, fat is stored in alternative places and is detrimental to health (*Nature*, 453:783-7, *Nature*, 478:110–3).

The bomb peak

Both studies made use of a side-effect of above-ground nuclear weapons' tests between the late 1950s and 1963: the accidental and constant radiolabelling of humans by increased levels of radioactive carbon-14 in the atmosphere and in the food chain. In the Northern hemisphere, atmospheric isotope levels peaked in 1962. In the Southern hemisphere, they reached their maximum in 1964. Bomb carbon-14 levels in the atmosphere have been decreasing ever since and are expected to reach background levels by 2020. During duplication of the genome, cells incorporate the isotope into their DNA. "We can use measurements of carbon-14 in cells against known levels of carbon-14 in the atmosphere to date stamp a cell population," says Spalding, the developer of the method.

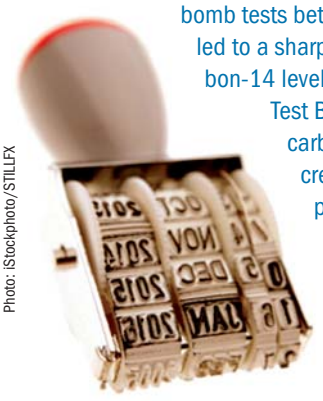
Off to the slaughter house

Spalding's and Arner's work on human fat metabolism is part of a larger endeavour to investigate the regenerative potential of the human body in health and disease and to gain insights into the dynamics of cell turnover. All began in 2001, when the Australian Spalding started to work as a postdoc in the lab of stem cell expert Jonas Frisen at the Karolinska Institute. The year before, physicists Wild and Kutschera from the University of Vienna had published how they had estimated the time of death of two elderly sisters by radiocarbon dating. They had analysed lipid and hair samples (*Nucl Instr and Meth in Phys Res B,* 172 (2000):944-50). Frisen recalls, "I suggested to Kirsty to analyse the carbon-14 content of cellular DNA to retrospectively determine the age of cells, because DNA is a very stable molecule with minimal atomic exchange after the final cell division." Spalding had to do her first experiments with rather large animals. She remembers that she had to go to the Uppsala slaughterhouse, where they put down horses every Tuesday, to collect horses' heads. "I analysed horses' brains, teeth and blood by radiocarbon dating," she recounts.

Tricky method

It took some sophistication to adapt the method for different tissues. "Brain contains a large amount of lipids. This made it impossible to obtain pure neuron preparations by density gradient centrifugation of collagenase-treated tissue," Spalding tells us. An alternative way to select a cell population of interest is fluorescence-activated cell sorting. For this purpose, cells are labelled with marker-specific antibodies, which are coupled to a fluorescent dye. "The best marker for neurons happens to be NeuN, a nuclear protein. By using nuclei, I got around the problem with the fat and could work with fresh or frozen tissue," explains Spalding. If the crucial distinguishing marker of a cell type is a cell membrane protein, it is necessary to use whole cells and to work with fresh tissue.

In the next step, the genomic DNA is isolated from the collected cells or nuclei. "The integrity and purity of the genomic DNA are incredibly important. Any external source of carbon, anything stuck to the DNA makes the analysis useless. We invest a lot of time to ensure that we have very



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Radioactive date stamp: Above-ground nuclear bomb tests between the late 1950s and 1963 led to a sharp increase of atmospheric carbon-14 levels around the globe. After the Test Ban Treaty in 1963, atmospheric carbon-14 levels started to decrease again as the radioactivity passed from the atmosphere into the oceans and the biosphere. Carbon-14 in the atmosphere reacts with oxygen to form carbon dioxide and enters the food chain through photosynthesis. Via nutrition, carbon-14 levels in the human body mirror those in the atmosphere. Because DNA is stable after a cell has gone through its final division, the carbon-14 level in DNA serves as a date mark for when a cell was born.

pure DNA," she says. The DNA is incinerated and the released carbon dioxide is transformed into graphite.

Finally, the samples are analysed in an accelerator mass spectrometer, a room-sized machine, which can separate and quantify different carbon isotopes. "In the beginning, we needed 20 million cells for an analysis. Now, we need five million. We continue to work with physicists on this aspect," Spalding tells us. Age determination is now possible with an accuracy of one to one and a half years.

New neurons in the brain

"Most analysed tissues consist of a mixture of cells. A renewing cell population should represent at least one percent of all cells to be detectable by radiocarbon dating," adds Jonas Frisen. The current price tag of a single analysis is approximately €500. In the past, the researchers collaborated with physicists in the United States, Australia and Austria for accelerator mass spectrometry. Now, they are working with the scientists Salehpour and Possnert from Uppsala University in Sweden. The latter two are currently developing a new laser-based technique of isotope analysis. "This new technique uses a much cheaper and smaller device and could increase the sensitivity of radiocarbon dating by tenfold," Frisen tells us enthusiastically.

In humans, Spalding and Frisen analysed cell turnover in different parts of the brain, in intestine, skeletal muscle, fat and heart. Their results have been published in high-ranking journals over the past seven years. Their article *Retrospective Birth Dating of Cells in Humans*, published in *Cell* in 2005 (122:133-43), even inspired the journal's cover. Currently, the scientists are interested in the details of neurogenesis in the adult human hippocampus. This part of the brain is involved in the formation of long-term memory. In a seminal study from 1998, Fred Gage and the late Peter Eriksson had described replicating cells with neuronal characteristics in the hippocampus of deceased cancer patients. These adult patients had received the nucleotide analogue bromodeoxyuridine as part of their medical treatment. The compound labels replicating cells (*Nat Med,* 4:1313-7). "There are still many open questions. We don't know the extent of neurogenesis and how it changes during aging or in pathologies," Spalding comments.


Photo: Teddy Thorlund

Dating made easy, with an accelerator mass spectrometer

A second neurogenic region in the adult human brain is the so-called ventriculo-olfactory neurogenic system. It comprises the walls of the lateral ventricles and a stream of precursor cells, which migrate to the olfactory bulb, a structure involved in the processing of odours. When Spalding, Frisen and collaborators analysed neurogenesis in the neocortex of adult humans, in general, they found no indication of neurogenesis after birth. This suggests that, with the exception of two neurogenic regions, cellular stability is favoured in the rest of the neocortex during our adult life.

Old muscles and rejuvenating hearts

In comparison with post-mitotic neurons, other cell types in our body are more short-lived. Epithelial cells in our gut, for example, have an average lifespan of about five days, whereas non-epithelial cells have an average age of nearly 16 years. The muscles between the ribs in two adults in their thirties were shown to be 15 years old.

Radiocarbon dating also revealed that the beating cells of our heart are exchanged at a very low rate. "Heart muscle cells are renewed throughout life. Young adults at age 25 exchange one percent of their cardiomyocytes per year. The rate decreases with age to half a percent per year. At the age of 75, about 40 percent of our heart muscle cells have been renewed some time after birth," Frisen tells us. Cardiomyocytes represent about 20 percent of all heart cells. To harvest cardiomyocyte nuclei from heart tissue by flow cytometry, the scientists used antibodies against cardiac troponin I and T (*Science,* 324: 98-102). Their findings suggest that it might be possible to stimulate the generation of new heart muscle cells after damage, e.g. after a heart attack.

After her daunting work with horses' heads, Spalding also plunged herself into forensics. "We collaborate with police on unsolved cases where an individual's identity has not been possible to establish. Such cases have included homicide cases and cold cases. The Canadian police asked us to determine the age of a drowned child. Our analysis helped to close the case, 30 years after the death of the boy. The family could, eventually, hold a funeral," she recalls.

She also analysed the teeth of Tsunami victims. "Teeth are usually well preserved. My work with horses showed that enamel is very stable at the molecular level and can be used for age determination by carbon-14 analysis." Even the geographical origin of a person can be narrowed down by analysis of carbon-13 in enamel (*Forensic Sci Int*, 209: 34-41). "It is nice to see your science help to make a difference," Spalding remarks.


Photo: Bettina Dupont/ Stefan Zimmerman, KI

Carbon detectives: Jonas Frisen and Kirsty Spalding

More nuts and bolts

"Fresh tissues with a short turnover time, such as lipids, have to be analysed now. Due to lower and lower atmospheric carbon-14 levels, the sensitivity of our method is decreasing for short-lived tissues," Spalding explains. "When it comes to neurological conditions, we have a long

time left to use the method because turnover in the nervous system is very slow."

However, the scientists will always be able to use frozen tissue from tissue banks. Therefore, their method will still be useful after the decline of atmospheric carbon-14 levels to pre-bomb levels by 2020. "Humans have a long lifespan. If we study individuals born in the mid-sixties, cells generated in the mid-sixties will show an even higher difference to cells generated in 2020, compared to today," Frisen adds.

A turnover map

Currently available, alternative methods to analyse cell turnover and regeneration have their drawbacks. "Most studies are done in animals and make use of cytotoxic, radioactive, mutagenic compounds, which cannot be used in humans," Spalding remarks. Scientists also look at molecular markers for cell proliferation or for immature cells, e.g. in the brain. "However, it is not possible to monitor the development or fate of a certain cell or cell population in this way," comments Frisen.

Spalding and Frisen are both members of the research network 'The Human Regenerative Map', which is funded by the Swedish Research Council until 2018. The members of the network aim to establish a map of cell turnover in the human body in health and disease. "In our centre of excellence, biologists, physicists, bioinformaticians and clinicians collaborate," says Spalding. In addition to the projects already described above, the scientists are determining the origin of different cell types in the body and are working on lineage tree reconstruction.

Frisen will continue to analyse neurogenesis in human pathologies such as stroke and psychiatric disease. "In experimental animals, stroke evokes a neurogenic response. There is also a link between depression and reduced production of nerve cells. Pharmacological treatment of depression promotes the formation of new neurons in the hippocampus in experimental animals," he explains. Frisen's department is also interested in heart pathology and the regenerative response in humans.

Focus on fat

Kirsty Spalding is developing a map of fat turnover in the human body. "We have no information on whether we make new fat cells in all parts of our body and whether there are differences between obese and lean people. I am particularly interested in visceral fat, which surrounds the organs. This is the fat most strongly linked to metabolic complications," she explains. Spalding wants to know whether there are differences in the turnover of visceral and subcutaneous fat dependent on gender, age or pathologies, like diabetes. A neurobiologist by training, she is also investigating neurogenesis in health and associated neurological disorders. We can expect upcoming publications on neurogenesis in the adult hippocampus and the olfactory bulb.

BETTINA DUPONT

Recently, the Frisen lab published their latest results on neurogenesis in the human olfactory bulb. Go to our website *www.lab-times.org* to find out what they unexpectedly discovered.