A new dimension to DNA and personalized medicine of the future

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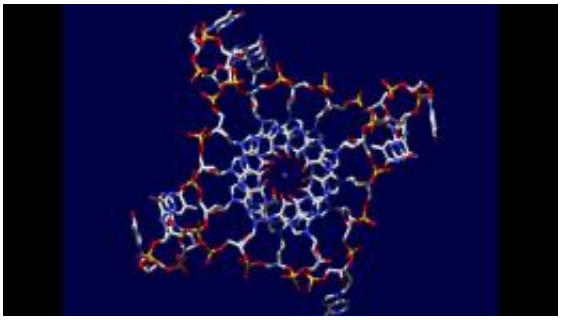

(Phys.org) -- By investigating the existence of an unusual four-stranded structure of DNA in human cells, scientists have opened the door to novel cancer therapeutics and a new era for personalised medicine.

When Watson and Crick discovered the double helix structure of DNA in 1953, they declared they had “found the secret of life”. However, as in all pursuits of science, the story did not end there. Less than 60 years later, a team led by chemist Professor Shankar Balasubramanian and cancer biologist Professor Steve Jackson has found that an unusual four-stranded configuration of DNA also forms at sites across the human genome in living cells.

Although known about by scientists for decades, the structure was considered to be something of a structural curiosity rather than a feature

found in nature. It forms in regions of DNA that are rich in one of its building blocks, guanine (G), when a single strand of the double-stranded DNA loops out and doubles back on itself, forming a four-stranded 'handle' in the genome.

G-quadruplexes have been known to occur at the ends of chromosomes in the regions known as telomeres, but it wasn't until a strong association had been noticed with genes responsible for cell proliferation that Balasubramanian and others began to suspect that G-quadruplexes might be a potential target for cancer therapy. "If you synthesize a quadruplex-binding molecule and put it into cancer cells, it can impair the growth of these cells," he said. "We've come such a long way from thinking that we understand the genome – and it appeared that this structure could tell us something new."

Dr. Raphaël Rodriguez, a Senior Research Associate who has worked with Balasubramanian for the past seven years, developed a small synthetic drug called pyridostatin to target G-quadruplexes. Two years ago the pair joined forces with Jackson, whose lab is studying the fundamental biology of cancer. In a recent article published in *Nature Chemical Biology*, they showed that not only do these structures form in cancer cells – the first time this has been demonstrated – but that pyridostatin can actually interact with G-quadruplexes to prevent proliferation of these cells. Although the function of G-quadruplexes remains a mystery, the discovery holds great potential for new, more selective approaches to treating cancer via interference with the genome, as well as offering a new dimension to personalized medicine.

Protecting the genetic code

At the heart of the new discovery is an innovative way of locating the structures in living cells and then 'capturing' them for further examination. The scientists discovered that when pyridostatin binds to G-

quadruplex DNA it causes a double-strand break in the double helix when the cell tries to replicate and copy its genes: “Pyridostatin binding to G-quadruplexes is a major impediment to copying the genome – so if a cell tries to replicate, this will generate breaks in the DNA,” said Jackson.

Over the years, Jackson’s lab has found that there are certain proteins in the cell that act as molecular policemen, patrolling the nucleus of the cell looking for damaged DNA. If they detect damage, they start making repairs, and at the same time set off alarm signals to alert the rest of the cell that there’s a problem.

When there is no DNA damage, these molecular policemen are distributed evenly throughout each cell’s nucleus. But when cells are treated with pyridostatin, they congregate in specific locations on the DNA, indicating regions of damage, and showing up as dots under the microscope. “The field really jumped on the idea that these dots represent telomeres that have G-rich sequences and in vitro have the potential to form G-quadruplexes,” said Jackson. “But we stained the dots for telomere proteins and found there was only a small amount of overlap. So clearly, this pyridostatin compound is inducing DNA damage in lots of other places, and we were faced with the issue: ‘if they are not telomeres, what are they?’”

This confirmed an earlier finding in Balasubramanian’s lab by Julian Huppert, then a PhD student, who devised a computational search algorithm to map out every spot in the entire human genome that had potential to fold into a G-quadruplex. He found there were close to 350,000 of them.

Next-generation genome sequencing

For several years, it seemed that accurately locating the specific DNA

regions that were being damaged by pyridostatin was theoretically possible by breaking up the cells, drawing out the molecular policemen that were bound to the damaged DNA, and sequencing the DNA to find its location within the genome. However, with millions of genome sequence reads required for an accurate result, finding the answers seemed a long way off.

But a new technology has changed all of this. In collaboration with colleagues in the Department of Chemistry, Balasubramanian pioneered a faster, cheaper method of decoding DNA called Solexa sequencing, which was taken up commercially a few years ago by Illumina Inc.

Using Solexa sequencing to look across the whole genome, Rodriguez together with researchers in Jackson's lab found certain hotspots of pyridostatin-induced DNA damage, indicating the locations where the drug was acting. "These hotspots were exclusively in the parts of the genome that Julian's algorithm had predicted as predisposed to folding into quadruplex structures – so it wasn't random," said Balasubramanian.

One of the key differences between cancer cells and normal cells is cancer cells are generally more vulnerable to DNA-damaging drugs. Using high-throughput sequencing, the collaboration identified that SRC, a major gene associated with breast cancer, is one of several hotspots on the genome targeted by pyridostatin. This supports the idea that certain cancer genes can be interfered with by small molecules designed to bind to specific DNA sequences. "For the very first time we're able to map structures in DNA with high resolution, in a functional context," added Balasubramanian.

"With Shankar's new technology, together with our knowledge of DNA repair, we have been able to do something that neither his nor my lab would have been able to do on its own," said Jackson. "It's a wonderful paradigm for chemical biology – we are bringing chemistry and biology

together to learn more about this unusual DNA structure and its potential as a target for cancer therapeutics."

A new approach to personalised medicine

Jackson has established two local spin-out companies, MISSION Therapeutics and KuDOS Pharmaceuticals, which have used knowledge of DNA repair to develop new anti-cancer drugs. He feels that existing and future chemicals developed by Balasubramanian and colleagues have exciting potential as anti-cancer agents, particularly if compounds can be made that hit even fewer DNA targets than pyridostatin and are thus more selective in their actions. "If drugs can be developed that only target a small number of sequences in the genome, that could be fantastic in terms of switching off a tumour-driving gene, or targeting HIV or other viral DNA," said Jackson. "It's not simple to develop these drugs, but that's the long-term goal."

For Balasubramanian and Rodriguez, this work holds potential for a new approach to testing the interaction of molecules with potentially any structure in DNA and could hail a new era for personalised genomic medicine. "There are a significant number of drugs today that work by targeting DNA," said Balasubramanian, "but we don't actually know exactly which parts of the genome they target, and how this may vary between cells or patients. Our approach could be applied to give a better understanding of why certain drugs work, or don't work, in different patients."

If the structure of the genome differs between individuals, as they believe it does, then a patient's predisposition to the effectiveness of DNA-interacting drugs might also differ. "You can imagine a day when physicians would look at the set of drugs available and decide which of these would be most suited to the patient, simply by predicting whether or not the structural hotspots for each drug exist in the patient," said

Rodriguez. “They could then choose the drug that has the best chance of working.”

“This science has not been done before so it’s potentially game changing,” added Balasubramanian. “Together, these approaches hold the potential to dramatically improve the effectiveness of future disease therapies on a patient-by-patient basis.”

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