IISc: Natural shield protects certain DNA regions from radiation damage

When exposed to radiation, there were fewer breaks in G-quadruplexes present in the telomeres

R. PRASAD

It is well known that ionizing radiation can break the double-stranded DNA in one or both the strands. Now a study by researchers at the Indian Institute of Science (IISc) Bengaluru have shown that regions of the genome rich in four-stranded DNA made of guanine nucleotide base – G-quadruplexes (G4-DNA) – are more resistant to irradiation. As a result, there are fewer DNA breaks seen in G-quadruplexes when exposed to radiation. The lower sensitivity to radiation was seen in studies carried out *in vitro* and inside cells.

The team led by Sathees Raghavan from the Department of Biochemistry at IISc found that contrary to the general notion that radiation-induced DNA breaks are random in nature and can occur throughout the genome, the breaks are sequence-dependent. Certain regions of the genome were found to be resistant to radiation with fewer strand breaks in the DNA, and these regions are rich in G-quadruplexes.

G-quadruplexes typically consist of three-guanine nucleotide base found together and repeated four times. When a guanine nucleotide gets repeated it tends to fold itself into a four-stranded DNA. There are 3.5 to 7 lakh G-quadruplexes in the human genome, and these are found in certain regions of the genome such as the telomeres that act as caps on either end of the chromosomes.

Natural protection: When we exposed double-stranded DNA to radiation, regions containing G-quadruplexes had fewer breaks and so were protected from radiation, says Sathees Raghavan (second from left).

"When we exposed double-stranded DNA to radiation, the DNA was getting cut randomly. But those regions of the DNA containing G-quadruplexes had fewer breaks and so were protected from radiation," says Prof. Raghavan. The results were published in the journal *iScience*.

Resilience of guanine

To test the resistance of guanine to radiation, the researchers started with single DNA strands. When single DNA strands made entirely of one of the four nucleotides – adenine, cytosine, guanine, or thymine – were exposed to gamma radiation, all except the strand made of guanine were sensitive to radiation.

In the case of a single DNA strand containing only thymine in one half and guanine in the other half, the guanine half alone showed better resistance to radiation.

But guanine loses its resistance when paired into double-strands and exposed to radiation. "We found guanine was resistant to radiation when present in a single strand but becoming sensitive to radiation when present in a double-strand form," he says.

When the team made three double-stranded DNAs containing AT-rich, GC-rich and scrambled sequence and exposed to gamma radiation, all the three were equally susceptible to radiation. "When DNA is in normal double-helical form then guanine is equally susceptible to radiation unlike when it is in the G-quadruplex structure," says Prof. Raghavan.

While dimethyl sulphate is able to cause DNA breaks when guanine is present in a double-strand, its ability to break DNA strands is compromised when guanine is present as G-quadruplex. "The position required for methylation [addition of methyl groups to the DNA] is occupied due to bonding, and so the G-quadruplex is resistant to dimethyl sulphate and no breaks are seen," he says.

"Potassium chloride is essential for G-quadruplex formation. And in the presence of potassium chloride, the ability of dimethyl sulphate to induce cleavage in guanine is less, suggesting that guanine forms a G-quadruplex structure," says Nitu Kumari from IISc and one of the first authors of the paper.

Inside the cells

The researchers tested the radiation resistance of G-quadruplex inside cells. The cells were exposed to 10 Gray gamma radiation and then stained with a fluorescent-labelled antibody to study if the telomere remains protected.

"There were fewer DNA breaks in the G-quadruplex present in telomeres compared with centromere [another part of the chromosome]. This suggests that G-quadruplex offers radioprotection inside the cell," says Sumedha Dahal from IISc and another first author of the paper.

To reconfirm the protection offered by G-quadruplex, the researchers used an antibody that binds to the G-quadruplex structure and then irradiated the cell using 5, 10 and 20 Gray. "We examined the entire genome and found wherever G-quadruplex was present there was less DNA damage. Even at 20 gray gamma radiation no breaks were seen in the G-quadruplex structure unlike the rest of the genome," says Susmita Kumari from IISc and another first author of the paper.

Public database

Data available in public database of whole genome sequencing of two cell lines post-irradiation were analysed for radioprotection offered by G-quadruplex by collaborators from Institute of Bioinformatics and Applied Biotechnology (IBAB). "There were fewer DNA breaks in the regions where G-quadruplex is present unlike the other regions of the genome," says Bibha Choudhary.

To confirm the analysis of publicly available data, the researchers irradiated the genome and amplified the DNA sequences using a PCR. "All the genes that contained G-quadruplex structure showed less DNA breaks unlike other genes," says Nitu Kumari.

Several other studies carried out too confirmed that G-quadruplex structure offered better protection to the DNA against radiation.

"Our study provides a new dimension to the role of altered DNA structures within the human genome, and helps study potential evolution of these structures. We also anticipate that our study will aid in exploring differential radiosensitivity across living organisms in correlation with the GC content of the genome," says Prof. Raghavan.

Deserving protection: Meghalayan rainforests, northernmost in the world, had a higher proportion of rare species

Meghalayan rainforests similar to equatorial ones

ASWATHI PACHA

The northeastern State of Meghalaya known for its wettest districts and living root bridges is also home to a lowland tropical rainforest north of the Tropic of Cancer. A new study discovers that this rainforest, the northernmost in the world, is similar in structure and diversity to the other rainforests found near the Equator.

Rainforests usually occur near the Equator and about five degrees North and South latitudes from the Equator are considered the real home of the lowland tropical rainforest. The extreme spread of tropical rainforests in northern limits in the world has been found in northeastern region of India where high rainfall-receiving habitats with hot and humid climate, especially in Meghalaya and Namdapha in Arunachal Pradesh are now known to boast species-rich rainforests.

Stretched distributions

Uma Shankar from the Department of Botany, Northeastern Hill University set out to study the Namdapha rainforests in Arunachal Pradesh in late 1990s and in Meghalaya a few years ago. "We wanted to note how far north these rainforests extend and how different they are from the ones found near the Equator," he adds. "Decoding what were the precursors for their stretched distribution in this region was also an interesting task."

The team found that the climatic conditions in the region – high rainfall and humidity, and perfect annual mean temperature – were conducive for the survival of the rainforests. Since rainforests have a complicated structure, the team looked at over two hectares of forest area, studying nearly 2,500 individuals including trees, shrubs and herbs. Over 180 different taxa were identified of the total, and it was noted that tropical Asian species made up 95% of the abundance. Although these rainforests had fewer species and individuals of liana or woody climbing plants, the levels of beta diversity were high. Also compared to Equatorial rainforests, they had a higher proportion of rare species and good representation of the members of families of *Fagaceae* and *Theaceae* in the Meghalayan rainforests.

Shorter trees

The results published recently in *Plant Diversity* note that though the species diversity was similar to the other rainforests, the Meghalayan rainforest trees showed short stature. While the trees in the Equatorial region are known to grow from 45 to 60 m in height, the highest ones in Meghalaya could reach only up to about 30 m. Dr. Shankar adds that in order to survive at this higher latitude the trees would have to make some compromises.

The region had a high density of 467 trees per hectare. Though this is lower compared with equatorial rainforests, it fell in the intermediate category for rainforests around the Tropic of Cancer. Also, the richness of species per hectare was the highest among all lowland rainforests near the Tropic of Cancer.

Ignored in maps

Though it has so many special aspects, the team writes that this region has been virtually ignored on the world maps of tropical rainforests.

But recent developmental and tourist activities have started to degrade patches of these rainforests.

Superbug crisis: Are doctors to blame?

Doctors are responsible for 10% or less of global misuse of antibiotics

ABDUL GHAFUR

Antimicrobial resistance (AMR) is considered one of the most significant challenges the world faces today. To keep it simple, let us use the term superbug crisis instead of AMR. Globally, thousands succumb to untreatable superbug infections on a daily basis. Irrational antibiotic usage is a major reason behind this.

Major causes

Are doctors to blame for the crisis? Excessive usage of antibiotics creates resistance, and doctors are notorious for this. But it may surprise many that over two-thirds of the antibiotics manufactured by the pharmaceutical industry are used as growth promoters for poultry and cattle. An article published this year in *Science* points out that globally 73% of all antimicrobials sold are used in animals raised for food.

The remaining one-third is used to treat human ailments. Of this, the common public purchases more than half without a doctor's prescription, according to WHO. That leaves us just 15% of the global antibiotic production for the doctors to use and misuse. Let us say doctors utilise half of this for rational indications. Hence, doctors have to bear the cross for 10% or less for global antibiotic misuse.

Antibiotic stewardship is considered to be the most important intervention to tackle super bug crisis. A remarkable, though unachievable, 100% success of antibiotic stewardship among doctors to rationalise antibiotic use can correct only one-tenth of the global antibiotic misuse. Should we not invest our limited resources and efforts in other more fruitful components?

Over the last 10 years, antibiotic stewardship efforts by various medical societies in our country and other stakeholders including the Chennai Declaration have significantly raised awareness of the super bug problem among the medical community. It is doubtful whether this awareness translated into rational antibiotic usage. Lack of infrastructure and inadequate diagnostic facilities in our health-care sector is one of the major triggers of the irrational antibiotic use by doctors and the public. Unless we correct the root causes, it is very unlikely that in a country like India with a million doctors and half a million pharmacies, rational antibiotic usage can ever be implemented.

I do not dare question the relevance of antibiotic stewardship as a patient safety measure. Rational antibiotic use is choosing the right drug at the right dose at the right time. No doubt this is one of the cornerstones of modern medicine practice. Successful antibiotic stewardship programmes may make some impact in countries with good sanitation standards. However, it is doubtful whether this component will make any real difference in superbug rate in developing countries.

Priority factors

Improving cleanliness in hospitals and sanitation in the community is much more important than antibiotic stewardship. In countries with high existing superbug rate and sanitation issues, rational antibiotic use, unless it is comprehensive, may not help reverse the rate or halt its progression. There are many scientific publications that underscore this argument. There is no conclusive evidence to support antibiotic stewardship as an effective measure to reduce the Gram-negative superbug bacteria, such as *E. coli* and *Klebsiella*, the most prevalent group in South Asia.

Mistaken notion

Unfortunately, the medical community is under the erroneous impression that doctors created the superbug crisis. It is high time doctors come out of this. The medical community must emerge from the never-ending antibiotic stewardship rituals, especially those backed or orchestrated by the pharmaceutical industry. Doctors are the most enlightened ones on superbug crisis. If they don't realise their own folly, future generations will indeed blame the medical community for the superbug crisis, but for an entire different reason – self-deception!

(The writer is the coordinator of the Chennai Declaration. drghafur@hotmail.com)

Seaweed extract shows anti-retroviral activity

Bioactive compound from two macroalgae shows anti-HIV-1 effect in lab tests

SHUBASHREE DESIKAN

The medicinal properties of compounds extracted from seaweed have been used for medicinal purposes. These compounds, as a class called sulphated polysaccharides, have been shown to have anti-inflammatory and antiviral properties. Of interest here is their anti-retroviral activity which makes them potential drugs against HIV.

Bioactive compound

A team from Chennai has extracted one such sulphated polysaccharide known as fucoidan from two seaweed species collected from the Mandapam, Thondi and Rameswaram regions along the coast of Tamil Nadu. The team further shows that, *in vitro,* this compound inhibits the functioning of the HIV-1 strain of the human immunodeficiency virus to a degree that is comparable to the drug tenofovir that is presently in vogue for anti-retroviral action. The research was published in *Scientific Reports*.

Brown algae: *Turbinaria decurrence* found along the Tamil Nadu coastline was rich in fucoidan.

Two species of marine brown algae, also known as macroalgae (seaweed), *Dictiyota bartaysiana* and *Turbinaria decurrence* were chosen by the team for extraction of the bioactive compound. "We studied many algae species along with these macroalgae and found that these two have more sulphated polysaccharide (fucoidan) than others," says Sanniyasi Elumalai Professor and Head, Department of Biotechnology, University of Madras.

Two species

The idea that sulphated polysaccharides can inhibit the activity of viral strains and arrest their growth has been known for some time now. The Chennai group's effort has been in identifying and extracting such a compound in two species of algae mentioned here that are common and available at low cost.

HIV comes in two strains, HIV-1 and HIV-2, and of these the former strain is more widespread and this is the strain that the group studied. The proliferation of HIV in cells is related to the expression of a protein called gagP24. The bioactive compound extracted from the seaweed and purified was used to treat cell lines *(in vitro)* and these were compared with two types of control cells. One set of controls were not treated with any chemical, and the second set was treated with tenofovir. The HIV cell lines treated with fucoidan did show a high percentage of inhibition (close to 90%) of the expression of the protein as compared with untreated controls. The drug tenofovir had a high degree of inhibition effect also.

"We are planning to test this macroalgal sulphated polyacrylamide in animal models," says Prof. Elumalai.

Managing change: It is high time to change track and take to proven methods like drip irrigation and aerobic cultivation. ▪ G.N. RAO

India's efforts towards mitigating climate change

SPEAKING OF SCIENCE

D. BALASUBRAMANIAN

At the recently held meeting on climate change at the U.N. in New York, the Swedish school student Greta Thunberg directed two scathing statements towards the attending representatives of over a hundred nations. One was, "You have stolen my childhood with empty words," and the other: "You all come to us young people for hope (in mitigating the damage…). How dare you?" As Krishna Kumar's perceptive analysis of her statements (November 1, issue of *The Hindu*) showed, the audience did not own up that their industries were responsible for the climate change; instead they agreed upon comfortable targets of decades for the reduction of carbon emission. As he points out, not only the richer nations but also the richer people in every nation continue to believe that they can buy relief and escape from the consequence of climate change for their progeny.

It is because of the carbon-rich fossil fuel-burning which started during the Industrial Revolution of the 1750s till today that the globe has warmed by about 2 degrees, affecting the lives of humans, animals, plants, and microbes. Oceans are warmer, icebergs are melting, and hence Greta's *J'accuse*.

India's challenge

It was in 2015 when nations across the globe met in Paris, and 197 signatory countries have promised to own up and to limit the increase to no more than 1.5 degrees over the pre-industrial levels by 2030. India is one of them. Vishnu Padmanabhan, in his blog, points out the four big climate challenges for India. India has promised to cut its emission intensity by 33-35% by the year 2030, as compared to 2015 levels. It looks like this is desirable and achievable. First challenge: Most of India's emissions come from energy (largely coal-based) production (68%), industry (20%), agriculture, food and land use (10%). It becomes vital that we use other means of energy, produced by, for instance, hydroelectric power, windmills, solar power, nuclear power and others. India hopes to produce 40% of its energy from such non-coal sources.

Next, turning to agriculture, land use and water resources, these too contribute to climate change. How? The minimum support price, subsidies, free 24-hour electric power supply, and water-intensive crops are some. It is high time we restrict these and take to proven methods, and work on innovative ones. Some of these are drip irrigation (as Israel has done), aerobic cultivation (a water- saving agronomic practice, and researching on improving specific traits that lead to better roots that go down to deeper levels in the ground, as initiated by the University of Agricultural Sciences, Bengaluru), better and more nutritious grains. Doing these on rice – a major water-guzzling plant of India – will go far in water conservation. More nutritious varieties such as the new Samba Masoori (developed at CCMB and NIPGR, which is incidentally lower in carbohydrate, hence good for diabetics) should be promoted among farmers. Stubble burning must stop; we need to find better ways. This is no 'rocket science'; Indian scientists and technologists can and should find ways that are better and safer.

The third is to bring down atmospheric CO_2 levels through natural means. Forestation and planting of local varieties of trees must increase. Here, it is worth following the steps taken by the Philippines government. Each student there must plant and nurture 10 locally-suited trees before he/she gets a school certificate/ college degree. Note that local trees absorb water and send it down to earth. India has planned to create additional 'carbon sinks' through forestation and tree plantation, so as to bring down 2.5-3 billion tonnes of CO_2.

Health issues

Several publications have focused on how climate change and global warming has gradually become injurious to health. The paper "Global climate change and infectious diseases" by Shuman (*NEJM* 2010, 362:1061-63; doi:10.1056/NEJM/09129310) points out, as we burn more fossil fuel, the temperature rise, associated heat wave and heavier rain make perfect conditions for insects (and the germs/viruses they host) to thrive. Thanks to the warmer climate, waterborne diseases such as cholera, diarrhoea, as well as malaria, dengue and chikungunya have increased in numbers and in geographical spread across hilly, cold as well as warm deserts and sea coasts. Another important paper by V Ramana Dhara et al. (Climate change and infectious diseases in India: implications for health care providers, *Ind. J. Med. Res.* 2013: 138(6):847.852) points out how rising sea surface temperatures increase tropical cyclones and storm surges, leading to polluted water, insanitary conditions, population displacement, toxic exposures, hunger and malnutrition across the Bay of Bengal and Arabian Sea coast. Some are transmitted from animals to humans and of course human-to-human. The latest example is Nipah virus, transmitted by bats to humans. It is here that we should appreciate the prompt action taken by Kerala government in arranging to isolate people, work with biological labs in India and abroad to identify more such initiatives from State and Central governments.

Happily enough, many of our labs and drug companies are involved both in-house and collaborative research in order to design drugs from India's natural plant sources, biosimilars, repurposing known drugs for other ailments and vaccines. We will be able to rise to the occasion and can even be world- leaders in this field. Note how our drug and biotechnology companies have provided drugs to the needy across the word at affordable costs, how just a handful of vaccine companies in India supply almost 40% of the world's childhood vaccines and how some of them are already working on vaccines for other current epidemics.

dbala@lvpei.org