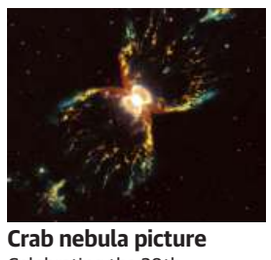
CAPSULE

Face transition
Over approximately four million years, the facial bones of humans have evolved from a form that was very different from the structure we see today. This was driven mainly by diet, says a study published in *Nature Ecology & Evolution*. While the brain and other features did change, diet played a part in shaping jaw, teeth and face.



Crab nebula picture
Celebrating the 29th anniversary of its launch, the Hubble Space Telescope imaged the Southern Crab Nebula. Resembling its namesake, the celestial structure,located in Centaurus constellation, several thousand light years away, is made of a whirling pair of stars – an ageing red giant and a burnt out white dwarf.

Trait mapping
A study published in *Nature Genetics* found more than 400 genes associated with schizophrenia. Among these, the researchers identified 67 genes that were independently associated with the disorder. They were also able to identify the regions in the brain and the developmental stages where these were expressed.

Biomarkers found for lymph node metastasis in oral cancer

Two biomarkers are heritable DNA changes while three are somatic DNA alterations

R. PRASAD

By looking out for five biomarkers, it is now possible to tell in advance if a person with oral cancer of the gum and cheek has lymph node metastasis even before surgery is undertaken, a study has found. The ability to correctly predict absence/presence of lymph node metastasis in oral cancer patients is 80-90% based on the five biomarkers, a team led by Partha Majumder from the National Institute of Biomedical Genomics, Kalyani, West Bengal, has found.

As a result, an oral cancer patient can be spared of a neck dissection to investigate if the cancer has spread to the lymph nodes in case the five biomarkers are absent. Lymph node dissection increases morbidity. However, if the patient tests positive for even one biomarker then an aggressive treatment would be required. An oral cancer patient with cancer spread to the lymph node has a 50% lower chance of survival for five years or more compared with patients in whom it has not spread to the lymph node.

Many causes: Metastasis in oral cancer is not promoted by a unique event, says Partha Majumder (right).

Hypothesis

In oral cancer patients, the cancer cells tend to commonly spread to the lymph node in the neck. But not all oral cancer patients have the tendency for the cancer to spread to other organs (metastasis). "We posited that oral cancer patients who have lymph node metastasis possess DNA alterations in specific genes that provide cancer cells the ability to spread. These DNA alterations are different from those that cause the primary cancer, and these alterations arise independent of the stage of cancer," says Prof. Majumder.

So in some patients, the cancer would have spread to the lymph node even at an early stage of oral cancer, while in some patients with advanced (T4 stage) oral cancer, the cancer would not have spread.

To find out what determines lymph node metastasis in oral cancer patients, the team studied two groups of patients – those with lymph node metastasis and those with advanced oral cancer but without lymph node metastasis. Totally, 72 patients belonging to these two groups were studied by a team led by Dr. Rajiv Sarin, Director of Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Tata Memorial Centre, Mumbai and co-author of the paper.

Five genomic biomarkers

The team found that lymph node metastasis was associated with five genomic biomarkers. The results were published in *International Journal of Cancer*.

There are five genomic features or biomarkers of lymph node metastasis in oral cancer patients. Two of these are rare, heritable DNA changes in BRCA2 and FAT1 genes. The remaining three are non-heritable (somatic) DNA alterations. The somatic DNA alterations can occur in genes belonging to three different pathways – mitotic G2/M cell-cycle pathway, homologous recombination (HR) and non-homologous end joining (NHEJ) DNA-repair pathways.

Heritable DNA changes

The protein product of FAT1 gene functions as an adhesion molecule that keeps the cells together. In the case of cancer, cellular adhesion property is sometimes lost and the cells tend to spread. "Our finding that rare alterations in FAT1 are found in metastatic oral cancer is comprehensible, but new," Prof. Majumder says. "Also, our finding that some rare alterations in BRCA2, which cause breast cancer, can also cause metastasis is novel."

A cell duplicates to produce two daughter cells. Many genes are involved in this cell-cycle pathway, called mitotic G2/M pathway. If DNA of one or more genes of this pathway is altered, then many adverse cellular events take place. Most importantly, chromosomes become unstable and abnormal chromosomal changes occur, eventually leading to metastasis. "We have mapped DNA alterations in genes that belong to the mitotic G2/M pathway in oral cancer patients and have found that altered genes of this pathway promote lymph node metastasis," says Dr. Nidhan Biswas, the first author of the paper.

Two pathways

When two major pathways – homologous recombination and non-homologous end joining – of DNA damage repair machinery gets perturbed, cells tend to behave abnormally. "We have found that DNA alterations in oral cancer patients that can be mapped to these pathways promote lymph node metastasis," Prof. Majumder says. "So, metastasis in oral cancer is not promoted by a unique event."

"The results have to be validated in a larger patient cohort," says Dr. Sarin. "We have enrolled many patients to validate the results of this study. Besides TMC, the validation has to be done at other centres in India too."

"We have found that a patient who presents without lymph node metastasis survives longer disease-free after standard treatment for oral cancer than one with metastasis of the lymph node. Therein lies the importance of our work," says Prof. Majumder.

MicroRNAs in the liver help regulate the feed-fast cycle

MicroRNA levels change in an anticipatory mechanism

SHUBASHREE DESIKAN

Researchers from Tata Institute of Fundamental Research (TIFR), Mumbai, have succeeded in identifying the mechanism that drives the feed-fast transition in the liver. They find that the oscillation in the levels of certain microRNAs in the liver drives this transition, and they study this by inhibiting the translation of the fasting responsive genes that are involved. The research has been published in *Cell Reports*.

Lifestyle disorders: Identifying such fed microRNAs in humans can aid in developing therapeutic interventions, says Ullas Kolthur

Feed-fast cycle

The feed-fast cycle is an important aspect of our body metabolism. There are four stages to it: fed state, post-absorptive state, fasting state, starvation state. Normally, we only experience the third stage and do not enter the fasting stage. Different organs in our body work to metabolise the food we consume, and they behave differently during each stage.

"The liver, for instance, is a central organ in maintaining glucose and fat metabolism both under fed and fasted conditions," explains Ullas Kolthur-Seetharam from TIFR, in whose lab this work was done. During a fasting state, liver produces glucose in a process which is critical for maintaining circulating glucose levels. An abnormality in either of these processes can lead to diabetes, obesity or other liver diseases. "There is evidence to show that aberrant molecular mechanisms that affect glucose and fat metabolism in the liver are the primary causes of several metabolic diseases and even ageing," he adds. Many of these occur due to aberrant gene expression and metabolic stress.

While fasting can last from a few hours to days, feeding (or refeeding) is a rapid process that takes from a few minutes to perhaps an hour. Therefore, when going from fasting to feeding, the liver functions must switch rapidly. "This entails stopping the mRNA translation of fasting-induced genes in a fed state. How such a tight control is exerted is being studied by researchers across the world," says Dr Kolthur-Seetharam.

Mice models

The team started by profiling microRNAs in the liver of mice models in a fed state. "We identified that these fed microRNAs could control the expression of fasting-induced genes, thus controlling liver metabolism," says Dr. Tandrika Chattopadhyay, post-doctoral fellow at the institute. They carried out several assays to check for mitochondrial functions and cellular respiration. By injecting molecular sponges that scavenged the microRNAs in the liver, they reduced the level of fed microRNAs. "This perturbed the gene expression and metabolic pathways in the liver, which, in turn, resulted in elevated glucose production and higher circulating blood glucose levels in the mice," says Babukrishna Maniyadath a PhD student and co-author of the paper.

Thus the group was able to identify the microRNAs that were responsible for the feed-fast transition and helped in maintaining liver physiology.

The study is significant in having discovered changes in microRNA levels which constitutes an anticipatory mechanism and whose abrogation leads to a diabetic like state. Most of these mechanisms are conserved between humans and mice. So identifying such fed microRNAs in humans can aid in developing therapeutic interventions for tackling lifestyle disorders and ageing-associated loss in physiological fitness.

Indoor emissions affect air-quality standards

Indoor air pollution accounted for 40% of PM 2.5 pollution in the Gangetic basin

ASWATHI PACHA

India can achieve its air quality goals if it completely eliminates emissions from household sources. A recent study has pointed out that the use of firewood, kerosene and coal in the households contributed to about 40% of the PM 2.5 pollution in the Gangetic basin districts. This number varied across the country but household emissions remained one of the major culprits behind air pollution.

The analysis was carried out by researchers from the Indian Institute of Technology (IIT), Delhi in collaboration with University of California in Berkeley, Urban Emissions, Delhi and the University of Illinois, Urbana-Champaign.

The results showed that by eliminating household emissions the average outdoor air pollution levels could be reduced and brought within the national ambient air quality standards. The paper published in the *Proceedings of the National Academy of Science* also notes that "if all households transitioned to clean fuels, about 13% of premature mortality in India could be averted." At the national scale, mitigating household emissions is also expected to bring large health benefits.

"You can't have a clean environment when about half the houses are burning dirty fuel every day. We have realised that pollution may start in the kitchen, but it doesn't stay there... it becomes part of the general outdoor air pollution," said Kirk R. Smith from UC Berkeley in a release. He is one of the corresponding authors of the paper.

Adding up: At national level, mitigating household emissions is also expected to bring large health benefits.

Satelite data

Using satellite data and chemical transport model simulations, the researchers pointed out that complete mitigation would bring down the country's average annual PM 2.5 air pollution to 38 microgram/cubic metre. Surprisingly, this is below India's national ambient air quality standard of 40 microgram/cubic metre and slightly above the World Health Organization (interim target 1) standards of 35 microgram/cubic metre.

"In many villages, they still use firewood for room heating and water heating. People prefer cheap wood fuel despite LPG being provided to many households," says Sourangsu Chowdhury, a Ph.D. scholar at IIT Delhi and the first author of the paper.

Sagnik Dey from the Centre for Atmospheric Sciences, IIT Delhi, and one of the corresponding authors, warns: "In Delhi NCR, stubble burning, industrial and power plant emission, brick kilns and vehicular emissions are the major contributors. Even after mitigating household emissions, Delhi NCR would remain out of attainment. It needs more serious and stringent measures."

Multipronged approach

"But India's pollution problem is much bigger than often perceived. Our study has demonstrated that mitigating at a household level is the easiest and more practical way out for the government to reduce not only the household pollution but also outdoor air pollution at the national scale," says Prof. Dey.

"We definitely need a multi-pronged approach to control emission from other major sectors like industries, transportation, and power plants to effectively address the air pollution issue."

Indian bullfrogs take to invasive behaviour early in Andamans

In experiments, bullfrog tadpoles ate up all tadpoles of two endemic frogs

AATHIRA PERINCHERY

Indian bullfrogs introduced in the Andaman islands are invasive, and eat native wildlife including fish and lizards. Now, experiments reveal that the frogs take to this invasive behaviour early in their lives. Even in the developmental stages, the large bullfrog tadpoles eat other native frog tadpoles, finds a study.

The Indian bullfrog *Hoplobatrachus tigerinus* (native to the Indian subcontinent) has rapidly invaded the Andaman islands after it was introduced there in the early 2000s. In human-dominated areas, it now shares space with other native (and often endemic) frog species. The bullfrogs are prolific breeders: they have short breeding seasons, and each egg clutch can contain up to 5,750 eggs. Its tadpoles are carnivorous and eat other tadpoles (including their own species).

To discern the impacts that bullfrog tadpoles have on native frog tadpoles, researchers including Nitya Prakash Mohanty (of the Andaman and Nicobar Environment Team and Stellenbosch University's Center for Invasion Biology) used a series of experiments. They first collected egg clutches (four each) of Indian bullfrogs, and native endemic frogs *Microhyla chakrapanii* and *Kaloula ghoshi*. Once the tadpoles emerged, they mixed the clutches and randomly assigned individuals to seven different 'treatments' or combinations in circular plastic pools containing 30 tadpoles each. One treatment contained equal numbers of all three species, three treatments comprised tadpoles of two species and three consisted of tadpoles of a single-species. The team replicated these experiments for up to four times for different treatments, observing 25 pools in total. They monitored the pools daily to detect metamorphosing tadpoles and recorded the survival of tadpoles in each pool every week.

Cannibal: An Indian bullfrog tadpole eating a smaller bullfrog tadpole.
▪ NITYA PRAKASH MOHANTY

Their results, published in *Biological Invasions,* reveal that Indian bullfrog tadpoles - which grew to be the largest (around 20 millimetres) - also grew the fastest. The survival of both endemic frog tadpoles reduced to zero when bullfrog tadpoles were present. In the three-species treatment too, all individuals of the endemic frog tadpoles in most pools were eaten by bullfrog tadpoles within the first week itself. The proportion of bullfrog tadpoles surviving was greater in the presence of both endemic frog tadpoles. This is worrying because other native frog species - many of which are only being described - could also be affected, said Dr. Mohanty. "Humans play a huge role in the invasive success of these frogs and urgent management actions including screening at ports of entry could help prevent their spread to other islands," he added.

In a first, IISc team directly delivers protein into cells

The team achieved nearly sixfold increase in protein uptake by cells

R. PRASAD

In a breakthrough that might have huge medical implications, researchers at Bengaluru's Indian Institute of Science (IISc) have used a novel strategy to directly deliver proteins into mammalian cells. Proteins are big molecules and so cannot enter the cells on their own. So a team led by Govindasamy Mugesh from the institute's Department of Inorganic and Physical Chemistry substituted a hydrogen atom of the protein with an iodine atom to achieve a nearly sixfold increase in protein uptake by cells.

The increased protein uptake was seen even when the molecular weight of the protein was 28,000 dalton, meaning the protein was much bigger in size than most of the therapeutic small molecules.

The researchers also tried replacing a hydrogen atom with an atom of bromine and chlorine but the uptake was way lower than when iodine was used.

In the case of bromine, the uptake of proteins increased by only about two times, while the uptake increased only marginally when chlorine was used. The results were published in the journal *Angewandte Chemie*.

Higher uptake: Iodine forms a stronger halogen bond with the receptor, say Surendar Jakka (sitting) and Govindasamy Mugesh.

Other researchers have tried tagging the protein with cell-penetrating peptides, supercharged proteins and even used virus-like particles to ferry the proteins into cells. But these approaches have severe limitations including altering the protein function inside the cell. For this reason, most of the applications involving proteins are directed to extracellular targets. Proteins inside the cells get impaired during diseased conditions such as neurodegenerative and cardiovascular disease. Supplementing the cellular protein in such cases becomes important and this is where the method used by the IISc team will come in handy.

The team had to first synthesise a green fluorescent protein with one hydrogen atom being replaced with an iodine atom. "To introduce iodine at a specific site on the protein, we had to use an iodinated amino acid. Since the iodinated amino acid used is unnatural (not genetically coded), protein synthesis machinery does not accept it. So we had to expand the genetic code of the organism to accept and incorporate the iodinated amino acid into the proteins during the biosynthesis in the cells," says Prof. Mugesh.

Since the iodinated amino acid is introduced on the surface of the protein, the secondary structure is not altered and so the protein remains functionally intact.

Strong bond

Iodine forms a halogen bond with a specific receptor (caveolin) that transports the protein from the cell membrane surface to inside the cells. "Compared with bromine and chlorine, iodine is heavier and so it forms a stronger halogen bond with the receptor. This might be responsible for more proteins getting into the cells when we substitute a hydrogen with an iodine atom," says Surendar R. Jakka from IISc's Department of Inorganic and Physical Chemistry and first author of the paper.

To be functionally useful, the proteins must enter the cytoplasm of the cell. However, the moment proteins are ferried into the cell by the receptor they are trapped inside the endosomes and transported to lysosomes, where the proteins are degraded. Significant decrease in protein concentration as measured by the fluorescence intensity was seen by the researchers after 24 hours.

To overcome the problem of protein degradation, the team treated the cells with a peptide (ppTG21). "The peptide also gets into the endosomes along with the protein and changes the pH of the endosomes. The endosome gets ruptured due to pH change leading to release of the proteins into the cytoplasm. In this case, there was no decrease in the protein concentration even after 24 hours," says Prof. Mugesh.

No toxicity

"We are substituting only one hydrogen atom with an iodine atom in the entire protein. So the toxicity is similar to native protein," says Prof. Mugesh. "We tested the cell viability by treating the cells with different concentrations of the proteins for 90 minutes. The cells were healthy after taking up the protein. The morphology of the cells that had taken up the proteins did not change even at the end of 24 hours," says Jakka.