Current Science Reports

Cotton Flower and Fibre
Role of microRNA

The 167th microRNA identified in cotton plants seems to regulate auxin hormone signalling. Higher amounts of microRNA167 appear to reduce flower and fibre size. But how exactly does that happen?

Researchers at Delhi University and the Gautam Buddha University, Uttar Pradesh decided to explore the question. They selected a strain of *Gossypium hirsutum* amenable to *in vitro* regeneration to simplify their investigations. Using *Agrobacterium tumefaciens*, a bacteria found to be useful for gene transfer, they introduced a DNA construct with sequences that mimic the target of the microRNA167. This reduces the action of the microRNA in cellular processes.

The DNA construct contained a marker to help distinguish the genetically transformed plantlets.

To compare morphological differences between the transformed and non-transformed plants, the team grew both types in the greenhouse at the Gautam Buddha University.

In the transgenic plants, the arrangement and development of floral organs were deformed. Flower buds, flowers and floral organs were smaller. The number of anthers per flower had also decreased. The lower the action of the microRNA167, the greater were these effects. In some cases, even the formation of the cotton boll was inhibited.

Is this a direct consequence of the reduced action of the microRNA167 or is it due to other microRNAs and their gene targets?

To find out, the researchers analysed the gene expression of two of microRNA167's target genes in the young leaves of transgenic cotton using real-time polymerase chain reaction. They found elevated activity of both the target auxin responsive genes in the floral, leaf and fibre tissues of the transgenic plants. They also identified the significantly biassed activity of six other microRNAs in the tissues of transgenic cotton.

The decrease in the activity of miR167 directly affected the other flowering-associated regulatory miRNAs in the plants. This, in turn, led to differences in the synthesis of another hormone, gibberellin, and that of flavonoids and anthocyanins.

This understanding of the crucial role of microRNA167 can now be leveraged upon to improve cotton production.

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Disease-free Potatoes
Selection using molecular markers

Pathogenic microbes and pests destroy 17% of potatoes globally. To reduce the loss, we need to breed potato varieties that are resistant to multiple diseases. Conventional breeding consumes time.

To identify disease-resistant potato lines in a quick and cost-effective manner, researchers from the ICAR-Central Potato Research Institute, Himachal Pradesh used a molecular marker-assisted selection of potato germplasm.

They screened 216 potato accessions available at the National Germplasm Repository, Shimla for late blight, potato virus and potato cyst nematodes.

The researchers infected the potatoes with viruses, nematodes and water mould, *Phytophthora infestans*, a causative agent for late blight disease. They observed changes in the plants and assessed the presence of 15 known molecular markers for disease resistance.

The molecular markers were then correlated with the phenotypical or observable characteristics of the infected plants.

'The idea was to choose the resistant molecular markers that show maximum concordance with the phenotypic data,' says Vikas Mangal, ICAR-Central Potato Research Institute, Shimla.

For late blight, one of the resistance gene markers, *Rpi-abpt*, showed a maximum of 83% concordance with the phenotype. However, using two resistance genetic markers, the concordance was cent percent. Similarly, for the potato cyst nematode *Globodera pallida*, a combination of three resistance molecular markers showed complete concordance with phenotypic data.

Unfortunately, none of the molecular markers for viruses showed significant concordance with the phenotypic data.

Though the researchers could not find potato accessions with resistant genes for all diseases or pest traits, they identified 54 potato accessions with at least two genes for resistance to late blight, viruses or nematodes.

'The most promising potato strain for high-throughput breeding was CP 2049. It has two resistant genes for late blight, two for potato virus X, and four for nematodes,' says Devendra Kumar, ICAR-Central Potato Research Institute, Shimla.

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Manual Small Incision Surgery
In cataracts with corneal opacity

In some cases of cataract, besides the clouding of the lens, the cornea of the eye may also become less transparent. In such circumstances, a surgical removal of the opaque lens may not be enough to restore clear vision. Manual small incision surgery is then preferred for managing cataracts.

What are the outcomes of manual small incision cataract surgery in eyes with corneal opacity?

R. Sankaranathan and associates from the Aravind Eye Hospital, Madurai examined manual small incision cataract surgeries conducted in the hospital between 2020 and 2022.

There were around 285 individuals, who had corneal opacity along with cataract. The researchers reviewed all clinical data prior to the surgery and found that most of the individuals had lower grade cataracts.

Most patients showed opacity of the superficial stroma, a collagenous multi-layered structure between the corneal epithelium and the endothelium. The common causes of corneal opacity were trauma, infection and inflammation.

During the manual small incision cataract surgeries, complications such as posterior capsular rent and absence

of lens had occurred, but had been resolved with appropriate management strategies. Complications like corneal edema and blood clots in the anterior chamber had also been adequately handled.

The team reviewed the status of the patients on the first day and one month after surgery and assessed surgical outcomes such as visual acuity and complications, and graded corneal opacity and cataract. The vision of all the individuals was better after surgery.

Treating cataracts with concomitant corneal opacity using manual small incision surgery may be useful to provide improved vision. Yet, it is critical to compare the results of the surgery with those of regular procedures before suggesting a small incision cataract surgery.

Hepatitis C Infection
Repurposing drugs

Hepatitis C is a blood-borne virus that causes inflammation of the liver. In most cases, the symptoms are mild. And the symptoms are difficult to distinguish from those of various other illnesses. So an estimated 58 million people live with chronic hepatitis C infection.

Direct-acting antivirals are effective against hepatitis C infections. But high costs and low availability remain challenges.

Developing new drugs is time consuming. To save time and money, can we repurpose existing drugs?

Kalyan Giri and team from Presidency University, Kolkata came up with a strategy: identify the structural parameters of the virus and the receptor that facilitates its entry into cells and find molecules that can block the interaction.

From a protein data bank, the group took the crystal structure of the envelope protein, E2, of the virus. This is the protein that interacts with the liver cell receptor, CD81, to gain entry to the cell. The team observed that the binding region of the envelope 2 protein was rather disordered.

Which parts were most crucial in binding with CD81?

The team ran molecular dynamics simulations to identify 36 potential clusters. The clusters were then compared for structural diversity using the Dali server, a network service for comparing protein structures in 3D.

The structures were all different from each other. So the team decided to use all of them for drug binding studies.

Using the Computed Atlas of Surface Topography of Proteins and the Pocket Druggability Prediction server, they identified two sites that overlap with the binding sites of the CD81 receptor.

To identify the binding capacities of the residues in these druggable sites, they used FTMap, a computational mapping server that recognizes binding hot spots of macromolecules.

Upon ligand binding, proteins tend to change their conformations, which, in turn, changes the dynamics of binding between the protein and the CD81 receptor. So, to confirm binding, the team used site-specific protein perturbation responses.

Then, using IgemDock, a graphical environment for recognizing pharmacological interactions, the group screened a drug dataset of 9213 molecules. They selected 17 molecules which showed a binding energy of less than a hundred kilocalories per mole for final screening.

To negate any bias or false positives, they used two different docking algorithms, AutoDock Vina and LeDock, on the selected molecules. The molecular dynamics of selected molecules in both the druggable sites was simulated for 50 nanoseconds each. The criteria for this round of selection was that the binding energy should be negative, by a few kilocalories per mole.

Five compounds made it to final list: acarbose, a drug for Type 2 diabetes mellitus, candesartan cilexetil and azilsartan medoxomil for lowering high blood pressure, cefiderocol, for urinary tract infections and hesperidin, for various medical conditions.

Since none of these drugs has shown side effects even in people without the indications prescribed for their use, it will not be difficult to repurpose them for hepatitis C infections, say the researchers.

All five may inhibit the entry of the virus into cells, a very different mode of action than that of existing antivirals used for hepatitis C infection. Some of them are more easily available and less costly too. So now, only testing their potential to treat hepatitis C infection remains.

Hesperetin-loaded Nanofibres
Boosting neuroprotection

Hesperetin is a flavonoid found in citrus fruits. It is a good antioxidant, can cross the blood brain barrier, and can be used for treating brain disorders such as Parkinson's and Alzheimer's diseases. But hesperetin has poor solubility in water and, hence, it has low bioavailability, which limits its therapeutic potential. How can we improve its bioavailability?

Researchers from the UP University of Medical Sciences and the Talla Padmavathi College of Pharmacy, Warangal thought of loading nanofibres with hesperetin. They used a hydrophilic polymer, polyvinylpyrrolidone, as a matrix to load hesperetin. This matrix has anti-nucleating properties, which prevent the drug from crystallizing, thus improving its aqueous solubility.

The researchers loaded hesperetin nanofibres into a hard gelatin capsule and subjected it to dissolution studies to compare the release with the release of hesperetin powder from a gelatin capsule. There was a significant improvement in hesperetin release from the nanofibre sample than from the one with the raw hesperetin crystalline powder.

The team investigated the *in vivo* performance of hesperetin nanofibres in albino rats. One group of rats received hesperetin powder and another group received hesperetin nanofibres orally. The serum level of hesperetin was much higher when hesperetin nanofibres were used. But can hesperetin nanofibres address cognitive decline?

The researchers treated one group of rats with scopolamine, known to increase acetylcholinesterase activity. The degradation of acetylcholine impairs cholinergic transmission and degrades cognitive function, inducing amnesia.

Another group was pretreated with hesperetin powder and later given scopolamine to induce amnesia. A third group was pretreated with hesperetin

nanofibres and later given scopolamine.

The rats were then evaluated for long-term memory and spatial learning. The results indicated that hesperetin-loaded nanofibres improved long term memory in scopolamine-treated rats, reducing amnesia. In fact, hesperetin nanofibre-treated rats demonstrated better memory than rats pretreated with hesperetin powder.

Hesperetin nanofibres decreased acetylcholinesterase activity in the rat hippocampus and accelerated cholinergic transmission, improving cognitive function. Scopolamine-treated rats showed oxidative stress in hippocampus brain cells: lower glutathione levels and higher lipid peroxidation. But hesperetin nanofibre therapy improved antioxidant activity, enhanced glutathione content and decreased lipid peroxidation.

Investigations of the brain revealed degenerative changes in the hippocampus of scopolamine-treated rats. However, in hesperetin nanofibre-pretreated rats, the hippocampus was normal.

Hesperetin-loaded polymeric nanofibres show potential for the treatment of neurodegenerative diseases. However, further studies to establish safety and efficacy in human subjects are needed before the formulation becomes available in the market.

Photo-chemo Therapy
For cancer treatment

Materials which absorb light and release it as heat can be used to kill cancer cells. To reach target cells, the materials are injected into the bloodstream. These chemicals, when irradiated with near infrared light, get hot and kill tumour cells. This is a remotely controlled treatment with no known side effects.

But the uneven distribution of tumour cells makes it difficult to penetrate deep into them. This reduces the efficiency of the therapy. Can we tweak photothermal therapy to make it more effective for cancer treatment?

Researchers from Tamil Nadu now propose a combination of photothermal therapy and chemotherapy.

Since molybdenum disulphide is biocompatible, they chose it as material for photothermal therapy. Flower-like nanostructures can be easily synthesized using molybdenum disulphide. Such structures have larger surface area.

To improve photothermal stability, the researchers coated the molybdenum disulphide nanoflowers with polydopamine, a biopolymer obtained by oxidating dopamine.

To this structure, they conjugated folic acid molecules. Since tumour cells have a greater number of folate receptors, the technique is a useful to target tumour cells. The team loaded the nanostructures with gemcitabine, commonly used for cancer treatment.

Would the modified nanoflowers efficiently convert light to heat?

The team compared the conversion efficiency of bare and modified nanoflowers by irradiating them with a laser. Modified molybdenum disulphide nanoflowers showed about 33% conversion efficiency.

The team then treated breast cancer cell lines with molybdenum disulphide with polydopamine, folic acid and gemcitabine. For comparison, they also used only molybdenum disulphide, molybdenum disulphide with polydopamine, molybdenum disulphide with polydopamine and folic acid. The results were best when the cells were treated with molybdenum disulphide with polydopamine, folic acid and gemcitabine.

Labelling the nanoflowers with a fluorescent dye, the researchers compared the cellular uptake. It was maximum for molybdenum disulphide with polydopamine, folic acid and gemcitabine, when exposed to near infrared.

This combination of photothermal and chemotherapy is an effective strategy for better cancer treatment, claim the researchers.

Monitoring Drug in Blood
Using magnetic nanosystem

Gemcitabine is used to treat pancreatic cancer. However, the oral dose of the drug is modified when it enters the bloodstream and the serum concentrations achieved may not be adequate for the intended action. How can we measure gemcitabine in blood to adjust the dosage?

Researchers from the National Institute of Technology, Warangal and Syngene International Ltd, Bengaluru joined hands to design a material to adsorb gemcitabine from patient blood samples.

Their strategy was to use magnetic nanomaterials modified with molecules having a large surface area and properties to attract and hold the hydrophobic drug.

Adding ferric chloride solution at high temperature and pressure to graphene oxide, they made magnetic oxide graphene sheets, easy to separate from serum using the magnetic field.

The researchers functionalized the magnetic graphene oxide sheets with round calixarene, macrocyclic molecules with hydrophobic interior and calix-like hydrophilic exterior. They selected a calixarene with six petal-like hydrophilic parts. From the structure of gemcitabine, the hydrophobic central part would act like a bucket to hold gemcitabine, they reasoned.

The researchers tested their material. After the blood samples were deproteinized and centrifuged, they used the magnetic graphene oxide-calixarene adsorbent nanosystem to trap gemcitabine molecules. They validated this method of sample preparation using high performance liquid chromatography analysis.

This method was capable of detecting gemcitabine at concentrations as low as 2 nanograms per millilitre. Accurate quantification was possible for concentrations of up to 13 nanograms per millilitre.

Moreover, the material is reusable, say the researchers. They used ethyl acetate as an effluent to remove gemcitabine from the adsorbent. More than 97% of the adsorbent was recoverable for reuse. The same material could be reused at least six times.

The method cuts down the costs of pharmacokinetic studies of gemcitabine besides being convenient, quick, reusable and eco-friendly.

Tale of Two Tapeworms
Measuring prevalence in India

Humans are the primary hosts for the tapeworms, *Taenia solium* and *Taenia saginata*. Tapeworm eggs in the stool of infected people pass into the soil where they are consumed by the main secondary host. In the case of *T. solium*,

these are pigs and, for *T. saginata*, cattle. Eating undercooked pork or beef brings the infection back to us.

Most people with taeniasis show no symptoms. In some cases, there may be stomach pain and weight loss. In some, however, taeniasis can lead to intestinal blockage, and other more serious conditions like epileptic seizures and retinal edema. However, we lack reliable estimates from large scale epidemiological studies to understand the extent of the problem in India.

So, Nasir Salam, Central University of Punjab, collaborated with researchers at Jamia Millia Islamia, New Delhi to do a systematic review on the problem. From the Web of Science and PubMed databases, they extracted more than 1200 scientific papers published between 2009 and 2019.

Based on the title, abstract, prevalence data and inclusion of various epidemiological parameters, they selected 234 studies reported from India. The researchers tabulated data about attributes like location, gender, age, methods and diagnosis. They found that the infection was prevalent in all age groups.

Most of the reports were from Tamil Nadu and Kashmir. There were more reports of *T. saginata* infections from Kashmir and *T. solium* from Goa.

To detect publication bias caused by the failure to publish clinical results, the researchers clubbed the papers with positive results and used a funnel plot. They statistically analysed the data using pooled prevalence, pooling together results from multiple epidemiological studies. The results suggest the prevalence of *T. solium* cysts in 11% of the population. Four per cent of occurrences of taeniasis were caused by *T. saginata*.

'To break the cycle of these infections, it is important to create awareness about the need for better sanitation and livestock vaccination. But we lack adequate research to identify areas at high risk for focused intervention,' says Nasir Salam.

Artificial Synapses for Computing
Cobalt ferrite transistors

The brain with its neurons and synapses is a more versatile, adaptable and energy-efficient information processor than computers with their electronic parts and digitally stored memory.

Synapses communicate electrical impulses from one neuron to another using chemical signals and are responsible for processing information and memory formation. Can we develop artificial synapses that can process and send data as signals?

Researchers from IISc Bengaluru collaborated with colleagues in Singapore to fabricate an artificial synapse.

In a biological synapse, calcium channels are activated only at particular ion concentrations. The signal is passed on to the next neuron via neurotransmitters. Similarly, artificial synapses are dependent on input voltage to transfer electrical signals.

The researchers designed a three-terminal transistor controlled by voltage drop through an ionic liquid. They used cobalt ferrite, a ferroelectric material with both magnetic and electrical properties. Cobalt ferrite has been used in memory storage devices and operates on voltage fluctuations.

To make the system sensitive to low voltages, the researchers doped it with electron-rich ruthenium and reduced the resistivity of the material. Using pulses of laser on ruthenium-doped cobalt ferrite to dislodge the material and using electrostatic forces to deposit it on magnesium oxide, they created thin films of about 40 nanometres thickness. Using argon ions, they etched out a three terminal gated electrode with gold or chromium deposited on top via thermal evaporation. The researchers used an ionic liquid as gate. They chose an ionic liquid with small ionic radius. This allows high capacitance in low volumes.

When tested under near vacuum conditions, the device exhibited spike duration-dependent plasticity, where the duration of the input pulse influences the magnitude and direction of the conductance change. And the amplitude of the input voltage determines the magnitude and direction of the conductance change.

In other words, the electrolyte-gated transistor emulates spike-amplitude-dependent plasticity. The device also showed long-term potentiation on increasing conductance and, on decreasing, long-term depression. These characteristics are similar to those of biological neurons.

They applied a series of positive gate pulses of different voltage amplitudes. The system was brought back to the original state by a series of negative gate pulses. The researchers say that the device will be useful for analog computing with ultra-high-density memory applications.

'The synaptic device can store information in conductance states programmed by applying voltage,' says Monalisha Peda, IISc Bengaluru.

'Such artificial synapses have great potential in neuromorphic computing and artificial intelligence,' adds P. S. Anil Kumar, her mentor.

There are some limitations, admit the researchers. For instance, compared to the biological synapse, the time taken by the artificial synapse is greater. But such issues can be tackled by tweaking the system, they say.

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