Counting on the virus

Math has entered the bio lab to help fight cancer, says s ananthanarayanan

WHILE genetics and molecular biology have made advances with the mechanism of life processes, scientists still cannot observe and monitor activity at the microscopic and cellular levels. Methods of statistics and analysis of numbers, hence, need to come in for an assessment of how effective an intervention has been.

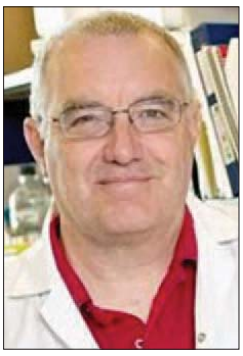
Fabrice Le Boeuf, Cory Batenchuk, Markus Vähä-Koskela, Sophie Breton, Dominic Roy, Chantal Lemay, Julie Cox, Hesham Abdelbary, Theresa Falls, Girija Waghray, Harold Atkins, David Stojdl, Jean-Simon Diallo, Mads Kaern and John Bell, a multidisciplinary team at Ontario, Canada, report in the journal, *Nature Communications*, their analyses and assessment of ways of using viruses to knock out cancer cells in the body.

Cancer cells are those that multiply without regulation. This may be due to failure of the cells' own control over growth and multiplication or the failure of the body's defence mechanisms to destroy such cells. In either case, cancer cells create tumours, invade neighbouring organs, create a blockage or ulcers and spread to other parts of the body. Curative action, apart from surgery to remove tumours, is mainly through agents that destroy cancer cells and leave healthy cells comparatively less affected. While a host of such agents has been identified, a promising line of attack is to deploy *viruses* that selectively strike at cancer cells.

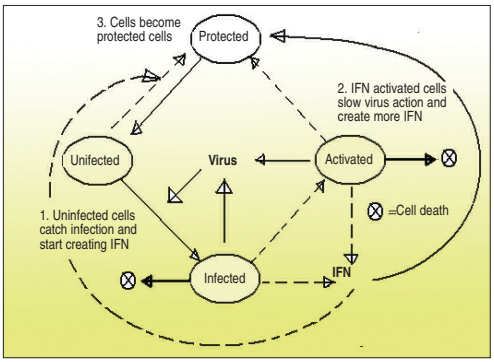
Viruses are entities, like cells, which have an envelope and are programmed with DNA, but little else, and the programme is only to reproduce. Viruses evolve the structure of the envelope to exactly fit features of the specific target cell exterior so that they are able to enter. Once within the host, they release their reproductive machinery and feed on the hosts' nutrients, since they have none of their own, to create clones of themselves. The hosts' own function is thus suspended, which causes disease, but the virus multiplies, sometimes a millionfold, within the cell. The cell wall then collapses and the generations of viruses spread out to enter other cells, and so on.

The body's defence against a virus attack is through the immune system, which kicks in when cells under stress release a signal protein called *interferon* (*IFN*). This is so called because its first role is to *interfere* with the replication of viral cells. But its other role is to communicate with other cells to slow down replication so as to impede the growth of the virus and also to convey to active agents in the immune system the features of the virus, for recognition. In this way, the immune system is often able to win the race against the virus. One encounter also leaves the body with the *template* of the virus, which helps in a faster, and generally effective, response in case of another attack.

A class of viruses known as *Oncolytic viruses* (*OV*) comprise those that get blocked by the IFN activity of normal cells but multiply in the usual way in tumour cells. The reason that normal cells can stop viruses is often that the virus is not able



Mads Kaern and John Bell.



to counter the anti-virus response that IFN sets off. In cancer cells, the IFN response is sometimes sluggish, because of changes that cause and result from malignancy. Such cells are great breeding grounds for OVs. But the extent of this IFN defect in cells is variable and this can reduce the efficacy of treatment with OVs. It is, hence, an objective of research to find ways to suppress IFN signalling in tumour cells without affecting the same function in normal cells.

The Ontario team describes the cycle of infection and protection by a schema shown in the picture. *Uninfected* cells first get the virus and start creating IFN. As their numbers increase, with the spread of infection, IFN creates an *activated* population where virus replication is controlled and more IFN is produced. And then there are *protected* cells, which have overcome the infection, and keep up the defence activity. Viruses that infect cancerous cells will have the benefit of the fast reproducing environment of the malignant cell. But at the same time, the IFN production would suppress replication and it is the balance between the two processes that would decide the efficacy of the virus in putting down the cell.

With the help of this model of the virus action and response, the team simulated the outcome of different IFN evasion strategies that were used by the OVs against three different kinds of cells — normal cells, cancer cells that did not respond to IFN and cancer cells that did. The different results would then guide the best characteristics to find in OVs, either by genetic engineering or by selection of specific OVs.

First, it was taken that the cells differed mainly in how they helped or hindered viruses in replication and in activating IFN. The reaction of these cell types to infection was then quantified, using experimental data of the response after 72 hours of infection. With these figures in place, the model was used to simulate different combinations of the rate of virus replication, IFN-mediated defence response and the destruction of cells.

Constraints in the trials were that the population was a mixture of healthy and cancerous cells and then the uncertainty of values of parameters that had been assumed. The trials, thus, had to be by thousands of simulations with random insertion of cells with different characteristics, using a technique called *Monte Carlo sampling*, to make estimates of the outcome of different strategies.

The Monte Carlo method is a statistical technique of estimating trends based on partial data. To estimate the ratio of the area of an irregular figure to that of a circle drawn within the figure, for instance, one method would be to paint the circle and then the whole figure. The quantity of paint used each time would give the ratio we need. But this would be an exact method, with full data.

Another way would be to sprinkle drops of paint randomly on the surface. A count of how many drops fall within the square, as compared to all the drops, would also give us the ratio — but approximately. Obviously, just a few drops may all land in the circle and be misleading. But the result gets very close to the correct answer as we increase the number of drops.

This method of estimation, which is useful in gambling games, was so named by its inventor after the well-known casino in Monaco.

The Ontario group, which included doctors, systems biologists and a physicist, carried out huge numbers of trials using different combinations of virus properties, both known and proposed. First, they tried the model out with a known OV, which blocks IFN production in target cells. The model correctly showed that the virus could eliminate both healthy and cancerous cells.

And also that suppressing the IFN blocking quality would make the virus ineffective against normal cells, but still effective against cells that did not respond to IFN. The group then tried out chemical manipulation, which increased the blocking action in IFN responsive cancer cells but found that the strategy seemed to affect healthy cells as well.

The third strategy they tried was where the virus was wired to create an *IFN blocking decoy* just when virus replication was initiated. This linking of the creation of the decoy with replication results in a spiralling feedback which sustains the strategy .The effect of high rate of replication, which happens in cancer cells, is to increase the rate of decoy-blocking of IFN, which would allow the virus to keep replicating and, hence, make more IFN. But as normal cells do not replicate fast, the decoy would not become active and normal cells would be able to survive the OV attack. This last strategy was also tried out in practice, successfully, with cancerous mice.

The study used sophisticated mathematical tools to simulate OV replication dynamics, including the use of differential equations, which is to make computations not of quantities but of how fast those quantities change.

"What is remarkable is how well we could actually predict the experimental outcome based on computational analysis," says Dr Bell, who, with Dr Mads Kaern, directed the study. "This work creates a useful framework for developing similar types of mathematical models in the fight against cancer."

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Courage personified

debameeta bhattacharya reports on a veritable journey of self-discovery

WHAT would you say about a girl in the Soviet Union who was petrified of heights, idolised Yuri Gagarin, the first man in space, overcame her phobia and, in 1963 at the age of 27, became the first woman to make a space flight? For Valentina Vladimirovna Tereshkova, grit was rewarded with induction into the Soviet Air Force so she could qualify for the Cosmonaut Corps.

The rest is history and to celebrate the golden jubilee of the first space flight by a woman cosmonaut, the Russian Centre of Science and Culture in Kolkata and the Birla Industrial and Technological Museum jointly organised an exhibition on 17 June.

Irina K Bashkirova (left), Consul General of the Russian Federation, Kolkata, who inaugurated the exhibition on Valentina Vladimirovna Tereshkova

Clockwise from left: Celebrations in Moscow on Yuri Gagarin becoming the first man in space; Tereshkova and BF Bykovsky return from Baikonur, 1963; commander Tereshkova of spaceship Vostok-6 and flight preparations.

Inaugurated by Irina K Bashkirova, Consul General of the Russian Federation, Kolkata, the exhibition features 54 rare photographs of Tereshkova's early life, her training period and remarkable achievements. Open to school students, faculties and the general public, it would be in the fitness of things for teachers/parents to do some research on the subject and inform their wards about Tereshkova's background to appreciate what's on view. This because some students were overheard mumbling, "Boring." And why so? "We have never heard about her and couldn't understand anything." They were busy scribbling down points from the photographs on display and when asked why, they said, "Our teachers instructed us to do so!"

Apart from the photographs, there is a 50-minute documentary featuring Tereshkova's journey from average girl to first woman cosmonaut and rare fame. That said, one wonders if Kolkata will throw up the next Tereshkova. Open till 28 June from 10 am to 5.30 pm, the exhibition is a veritable journey of self-discovery.

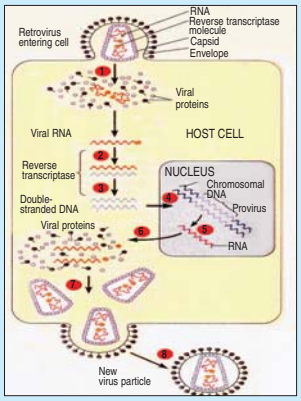
It's all in the packaging

tapan kumar maitra explains reverse transcription, retroviruses and retrotransposons

TRANSCRIPTION generally proceeds in the direction described in the central dogma, with DNA serving as a template for RNA synthesis. In certain cases, however, the process can be reversed and RNA serves as a template for DNA synthesis. This process of reverse transcription is catalysed by the enzyme reverse transcriptase, first discovered by Howard Temin and David Baltimore in certain viruses with RNA genomes. Viruses that carry out reverse transcription are called retroviruses and examples include some important pathogens such as the Human Immunodeficiency Virus, which causes Aids, and a number of viruses that cause cancers in animals.

Retroviruses: In a typical virus particle, two copies of the RNA genome are enclosed within a protein capsid that is surrounded by a membranous envelope. Each RNA copy has a molecule of reverse transcriptase attached to it. The virus first (1) binds to the surface of the host cell and its envelope fuses with the plasma membrane, releasing the capsid and its contents into the cytoplasm. Once inside the cell, the viral reverse transcriptase (2) catalyses the synthesis of a DNA strand that is complementary to the viral RNA and then (3) catalyses the formation of a second DNA strand complementary to the first. The result is a double-stranded DNA version (4) of the viral genome. This double-stranded DNA then enters the nucleus and integrates into the host cell's chromosomal DNA, much as the DNA genome of a lysogenic phage integrates into the DNA of the bacterial chromosome.

Like a prophage, the integrated viral genome, called provirus, is replicated every time the cell replicates its own DNA (5). Transcription of the proviral DNA (by cellular enzymes) produces RNA transcripts that function in two ways. First, they serve as mRNA molecules (6) that direct the synthesis of viral proteins (capsid protein, envelope protein, and reverse transcriptase). Second, some of these same RNA transcripts (7) are packaged with the viral



proteins into new virus particles (8). The new viruses then "bud" from the plasma membrane without necessarily killing the cell.

The ability of a retroviral genome to integrate into host cell DNA helps explain how some retroviruses can cause cancer. These viruses, called RNA tumor viruses, are of two types. Viruses of the first type carry a cancer-causing oncogene in their genomes, along with the genes coding for viral proteins. An oncogene is a mutated version of a normal cellular gene (a proto-oncogene) that codes for proteins of a cellular gene for a protein kinase. The protein product of the viral gene is hyperactive, and the cell cannot control it in the normal way. As a result, cells expressing this gene proliferate wildly, producing cancerous tumors called sarcomas. RNA tumor viruses of the second type do not themselves carry oncogenes, but integration of their genomes into the host chromosome alters the cellular DNA in such a way that a normal proto-oncogene is converted into an oncogene.

Retrotransposons: Reverse transcription also occurs in normal eukaryotic cells in the absence of viral infection. Much of it involves DNA elements called retrotransposons. Transposons are DNA segments that can move themselves from one site to another within the genome. Retrotransposons are a special class of transposon that use reverse transcription to carry out this movement. The transposition mechanism begins with transcription of the retrotransposon DNA followed by translation of the resulting RNA, which produces a protein exhibiting both reverse transcriptase and endonuclease activities. Next, the retrotransposon RNA and protein bind to chromosomal DNA at some other location site and the endonuclease cuts one of the DNA strands. The reverse transcriptase then uses the retrotransposon RNA as a template to make a DNA copy that is integrated into the target DNA site.

Although retrotransposons do not transpose themselves very frequently, they can attain very high copy numbers within a genome. Alu sequences are only 300 base pairs long and do not encode a reverse transcriptase, but using a reverse transcriptase encoded elsewhere in the genome, they have sent copies of themselves throughout the genomes of humans and other primates. The human genome contains about a million Alu sequences that together represent about 11 per cent of the total DNA. Another type of retrotransposon, called an LI element, is even more prevalent, accounting for roughly 17 per cent of human DNA. The LI retrotransposon is larger than Alu and encodes its own reverse transcriptase and endonuclease. The reason why genomes retain so many copies of retrotransposon sequences such as LI and Alu is not well understood, but they are thought to contribute to evolutionary flexibility and variability.

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