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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 level. Methods of statistics and analysis of numbers hence need to come in for 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 body cells 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 defense mechanisms to destroy such cells. In either case, cancer cells create tumours, invade neighbouring organs, create 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 have been identified, a promising line of attack is to deploy ***viruses*** which selectively strike at cancer cells.

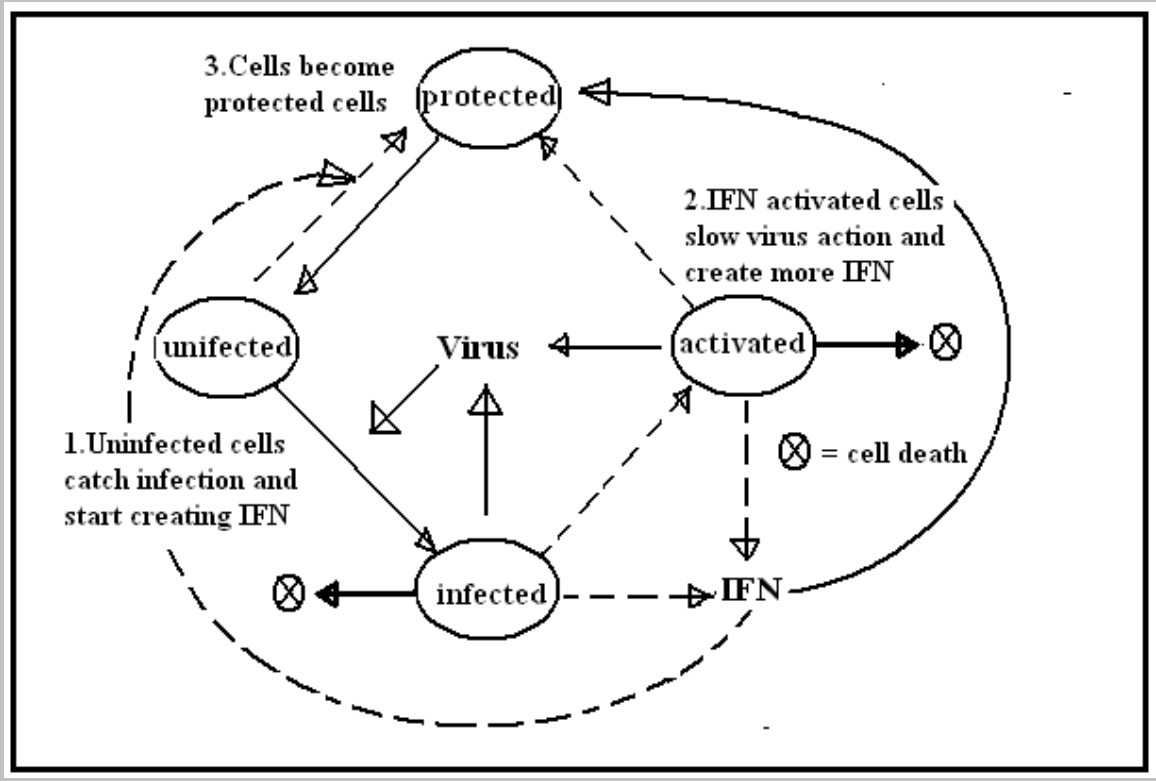
The virus

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 million-fold, 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 defense against a virus attack is through the immune system, which kicks in when cells under stress release a signal protein called ***interferon*** (***IFN***). IFN 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 faster, and generally effective, response in case of another attack.

A class of viruses, known as ***Oncolytic viruses*** (***OV***) are viruses that get blocked by 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 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 OV. It is hence an objective of research to find ways to suppress IFN signaling 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, which 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 defense 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 defense 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 smpling***, 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 combination 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 is initiated. This linking of the creation of the decoy with replication results in a spiraling 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, s 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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