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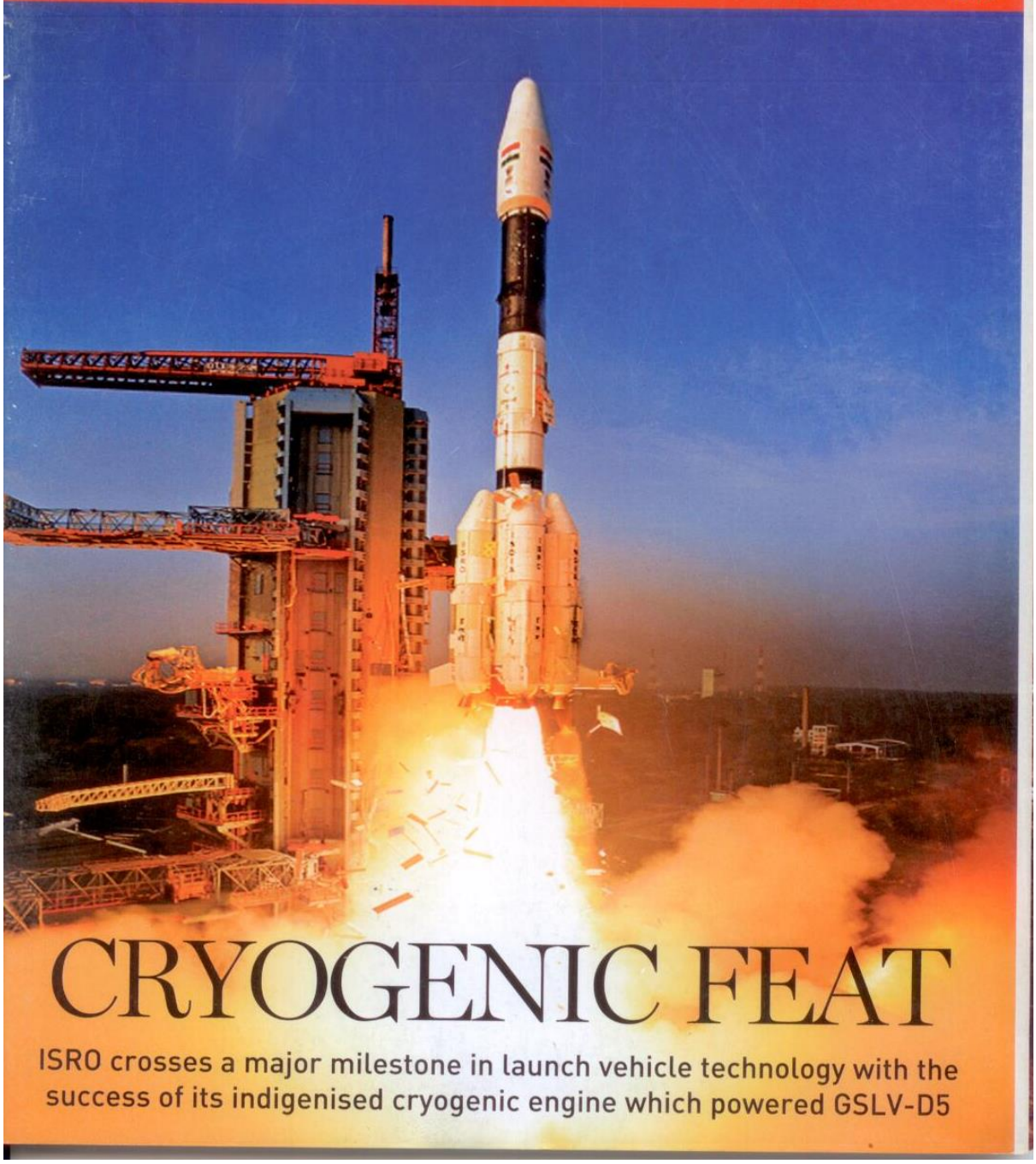

CRYOGENIC FEAT

ISRO crosses a major milestone in launch vehicle technology with the success of its indigenised cryogenic engine which powered GSLV-D5

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The ICGC-India team's study of oral cancer, the leading cancer among males in India, has revealed that alterations in several genes and biological pathways are specific to driving the "Indian variety" of oral cancer—the gingivo-buccal oral cancer. By PARTHA P. MAJUMDER and R. RAMACHANDRAN

CANCER is a genetic disease. For any type of cancer—lung, oral, cervical, and so on—only rarely does one encounter families in which multiple family members are affected with the disease. In other words, familial cancers are rare. Cancer is mostly sporadic. This is not what one expects of a genetic disease. A genetic disease, like thalassaemia, usually runs in families because the genetic alterations that are responsible for causing it are transmitted from parents to their children, to some but usually not to all.

Even though cancer generally does not run in families, one invariably observes alterations in the deoxyribonucleic acid (DNA) contained in the cells of the tumour tissue compared with that contained in the cells of the normal tissue of the same patient. Thus, alterations in the genetic

material are responsible for causing cancer. Yet, these genetic changes are not passed on to offspring because these changes rarely take place in the cells through which genetic material is passed on to children from parents. These DNA alterations arise because of lifestyle or environmental factors, such as exposure to tobacco, ultraviolet radiation, certain types of chemicals, and infection by specific viruses, mostly in body cells (somatic cells), such as cells of the lung and the mouth.

The DNA alterations in body cells are called somatic alterations. In the minority of instances in which cancer is found to occur in multiple family members, DNA alterations are present at birth in patients. These alterations, called germ-line alterations, are inherited by the patient from his or her parents and are transmitted from one generation to the next. All cases of cancer are therefore the result of DNA alterations, mostly somatic, but sometimes germ-line or a mixture of germ-line and somatic.

The hallmark of cancer is the uncontrolled growth of cells. Normal cells have very regulated growth and die at a certain period of time after they are born. Tumour cells, on the other hand, have uncontrolled growth and mostly refuse to even die. One or more DNA alterations in a normal cell provide a growth advantage to the cell, which then uncontrollably and abnormally makes duplicate copies of itself to form a "clone" of cells, which forms a tumour. A tumour often contains multiple clones. These clones usually differ in their sets of DNA alterations. These DNA alterations are mostly somatic but can be germ-line, that is, inherited, as well.

Many germ-line alterations, and the genes in which they occur to provide growth advantage to a cell that eventually causes cancer, have been known for several decades. However, genes which acquire somatic alterations that provide the cell with a growth advantage, called driver mutations, have not been systematically identified. It is important to do so to understand the process of the development of cancer, thereby paving the way for the development of drugs to combat the disease. Most somatic alterations, however, do not provide any growth advantage to a cell and are called passenger mutations. Passenger mutations are therefore not responsible for causing cancer.

Because a tumour contains multiple clones, the clones often carry distinct sets of DNA alterations. To identify the "drivers", it is essential to search for all DNA alterations in every one of the clones. The ideal way to accomplish this is to determine the sequence of the DNA contained in a large representative set of single cells sampled from the tumour tissue.

However, at this time, sequencing the DNA contained in a single cell is technically difficult and highly expensive. The only advanced technology of DNA sequencing that existed until about 10 years ago, called capillary sequencing, or Sanger sequencing, (named after Fred Sanger, who invented the technology and was awarded the Nobel Prize twice, and who passed away recently), was incapable of even providing a reasonable picture of the diversity of DNA alterations present in a tumour tissue.

About a decade ago, a new technology, nicknamed next-generation sequencing technology—which obtained DNA from a pool of cells, fragmented it and sequenced every fragment a large number of times using the massively parallel sequencing method—became accessible and

popular. This technology, even if it is still not capable of capturing DNA alterations in every single cell of a tissue, is a good approximation to capturing the complete landscape of DNA alterations in the tissue. Alterations in tumour cells comprise both somatic and germ-line alterations. DNA derived from the blood of a patient provides the landscape of genomic alterations that an individual was born with, namely germ-line alterations. Most of these alterations are possibly harmless to the individual. The totality of DNA alterations in the cells of the tumour tissue is the sum of germ-line alterations and those DNA alterations that the individual acquired during his lifespan, that is, somatic alterations. Hidden in the set of somatic alterations are one or more driver alterations. To capture them, it is necessary to first "subtract out" the germ-line alterations found in the DNA of the blood from the totality of all alterations found in the tumour cells.

When massively parallel DNA sequencing technology became widely available, cancer geneticists from various parts of the world got together and decided to identify those DNA alterations that drive cells to produce various types of cancer and to understand the biological actions of these alterations. The International Cancer Genome Consortium (ICGC) was thus formed in 2009. India is a founding member of the ICGC.

After a series of national consultations, the Department of Biotechnology, Government of India, decided that India would participate in the ICGC to conduct research on the genomics and biology of oral cancer. The India Project of the ICGC is being conducted collaboratively by the National Institute of Biomedical Genomics (NIBMG) in Kalyani, West Bengal, and the Advanced Centre for Treatment, Research and Education in Cancer (ACTREC) of the Tata Memorial Centre, Mumbai. The first set of results from the work carried out by the India Project Team was published in the renowned international journal *Nature Communications* on December 2, 2013.

Many scientists are critical of this approach of trying to understand genomic underpinnings of cancer by what they call a "fishing expedition" strategy of sequencing all genes in cancer patients. They argue that one must first propose some hypotheses regarding which genes are likely to cause cancer, and then carry out sequencing and other types of research on these "candidate" genes. The response to the critics was provided over a quarter century ago, in an article published in the journal *Science* in 1986, by the Nobel laureate Renato Dulbecco: "If we wish to learn more about cancer, we must concentrate on the cellular genome.… We have two options: either to try to discover the genes important in malignancy by a piecemeal approach, or to sequence the whole genome… it will be far more useful to begin by sequencing the cellular genome."

India and oral cancer

Why did the Indian study focus on oral cancer? Oral cancer is the eighth most common cancer worldwide and is the leading cancer among males in India. Therefore, this type of cancer is of great societal importance in India. Annually, over 260,000 new cases arise and about 128,000 deaths occur. Tobacco smokers have a 27-times higher rate of oral cancer than non-smokers. Chewing of areca nut and tobacco with slaked lime causes over half of the oral cancers in India. Tobacco-chewing is widespread in India, even among the youth. It is important to understand the

interplay between genetic reasons and exposure to the chemicals present in tobacco. The regions of the oral cavity frequently affected in cancer patients in India are different from those observed among patients in the West. Oral cancer predominantly presents itself as tongue cancer (~65 per cent) in the West, while in India it predominantly (~60 per cent) affects the lining of the mouth, lower gum and other regions of the oral cavity (called the gingivo-buccal region). Are different sets of driver genes involved in tongue cancer and cancers of other regions of the oral cavity?

In the Indian study, 110 patients suffering from oral cancer were profiled. Most patients were middle-aged and predominantly male. All patients, except two, were tobacco users; most had used tobacco for over 10 years. The blood and tumour DNA of each patient was screened, using massively parallel sequencing, for all genes— about 20,000—in the human genome.

In the study, the scientists looked for DNA alterations in the first 50 oral cancer patients and identified the genes that frequently harboured alterations in these patients. Then, these findings were tested on the remaining 60 patients to validate the discoveries. This kind of checking on a separate group of subjects is done to avoid the vagaries of chance in the findings from the former group.

A large number of DNA alterations were observed in each patient. Many of these were innocuous, without any impact on the protein coded by the gene. On an average, each patient harboured 85 somatic alterations in various genes that likely had an adverse impact on the proteins coded by the genes. Some of these alterations are potential drivers. From past studies it is known that different types of environmental factors that cause DNA alterations leave different types of marks on the genome.

The DNA molecule is essentially a double stranded helix consisting of long chains of four types of molecules called nucleotides—A, C, G and T, with the A nucleotide on one strand always pairing with the T nucleotide on the other and, similarly, the C nucleotide on the one always pairing with the G nucleotide on the other. DNA alterations are usually random. However, DNA alterations induced by chemicals in tobacco preferentially alter C nucleotides to A nucleotides (which is the same as a G to T change). This phenomenon was observed in the genomes of oral cancer patients and was not surprising as most patients were heavy users of tobacco. This also indicates that tobacco has a specificity of impact on DNA, which provides a useful link between chemicals in tobacco, our DNA and the propensity for oral cancer.

The Indian study has identified five new genes that frequently (10-22 per cent) harbour driver DNA alterations in oral cancer patients. These genes were not found to be frequently altered in past studies on oral cancer conducted in the United States, predominantly on tongue cancer patients. This implies that site differences in cancer within the oral cavity are associated with different sets of genes.

Genes are responsible for producing proteins that are essential for carrying out various functions that are normally required. One of the genes that control the growth of a cell is designated as epidermal growth factor receptor (EGFR). When this gene functions normally, cells grow, perform their normal functions and then die. When the EGFR gene does not function normally and produces more protein than it normally should, it sends signals to some other genes which

then start to behave abnormally, because of which cells forget to die and go on an uncontrolled growth spree, resulting in cancer. The normal function of the EGFR is disrupted when it acquires some specific alterations. EGFR gene alterations are observed in many, though not all, lung cancer patients.

Similarly, many human genes produce proteins that normally suppress the formation of tumours. One set of such genes produces proteins that regulate the growth of cells by "asking" a cell to die after it has reproduced and lived a happy life for a certain period of time. Thus, unregulated cell growth is prevented. However, if the DNA sequences of these genes are altered, then they produce defective proteins which cannot carry out their normal function of tumour suppression. The new genes found associated with oral cancer in India predominantly function as tumour suppressors. (There is another class of genes that usually carries out useful normal functions but if altered becomes virulent and promotes cancer. These genes are called oncogenes.)

In addition to simple DNA alterations, many cancer patients are found to harbour large-scale disruptions in their DNA. These disruptions are usually insertions of large chunks of DNA from elsewhere or amplifications of whole genes or parts of genes, or deletions of large chunks of DNA or duplications of parts of chromosomes or even whole chromosomes, and so on.

The Indian study found several genes to be amplified or deleted, either partially or fully, in multiple oral cancer patients. These genes have not been reported to be disrupted in oral cancer patients from Western countries. One of these genes, GSTT1, was found to be fully deleted on both chromosomes in many oral cancer patients. GSTT1 plays a key role in removing from the body many toxic substances that are known to cause cancer, perhaps including various cancer-causing chemicals found in tobacco. The deletion of this gene obviously results in the inability of the body to remove these harmful chemicals and leads to cancer.

Biological pathways

Evolution by natural selection has provided resilience to humans. Genes act in concert. The proteins coded by genes are often members of a biological pathway. An alteration in a gene may not have a major adverse impact if other proteins in the pathway function normally. The ICGC-India team identified several pathways that are altered in oral cancer. Many of these are known to be altered in other cancers, but some were unknown. One such pathway is associated with neurotransmission. It is possible that early changes in this pathway may contribute to tobacco addiction, which in turn, enhances the risk of oral cancer.

The Indian team's efforts have yielded results that are relevant to the management of oral cancer patients. The team has identified the existence of subgroups among oral cancer patients. Patients belonging to a subgroup possessed DNA alterations in specific genes that are not possessed by patients of other subgroups. These subgroups of patients showed differences in periods of survival after receiving cancer treatment.

The most notable was the finding that oral cancer patients who possessed alterations in a gene (MLL4) that works in concert with a "famous" tumour suppressor gene (TP53) had a significantly longer disease-free survival period than those who did not. The Indian study has

thus revealed that alterations in several genes and biological pathways are specific to driving the "Indian variety" of oral cancer—gingivo-buccal oral cancer. Further, detailed study of the biology of these genes will also likely result in the discovery of therapies for gingivo-buccal oral cancer.

In cancer chemotherapy, the chemicals that are toxic to cells are used to kill cells, usually the fast-growing cells. Since cancer cells are fast growing, they get killed by chemotherapeutic agents. However, cancer cells are not the only cells that are fast-growing in a patient's body. Cells in the outer layers of various organs, such as the lining of the oral cavity, also divide fast. So these cells also get killed during chemotherapy. This is why patients undergoing chemotherapy often lose their ability to taste food and refuse to eat. For the same reason, they also lose hair during chemotherapy. These are the horrible side effects of using cellular poisons. Also, while chemotherapy is effective, there is no guarantee of a complete cure because many cancer cells often escape the firepower of the chemotherapeutic agents.

Research has shown that the overproduction of protein caused by alterations in the EGFR gene and the consequent unregulated cellular growth are restricted to the cells comprising the tumour. If this overproduction can be inhibited by a drug, then it may be possible to control cell growth and treat the cancer. Afatinib is such a drug, and it can be orally administered.

This is a better alternative to injectable chemotherapy since the drug (which is also a chemical and hence a chemotherapeutic agent, but more specific than the older agents) will have an impact on only the cells in the tumour tissue and not on other cells of the body. It has recently been shown that the response of patients to Afatinib oral therapy is far greater (more than double) than that to the "standard" chemotherapy, provided that they harbour changes in the EGFR gene. Moreover, patients on Afatinib also survive longer without progression of their cancer.

However, alterations in the EGFR gene are mainly found in lung and breast cancers. Therefore, unlike the older chemotherapeutic agents that are usually applicable to a wide variety of cancers, drugs such as Afatinib, which are specific to EGFR gene alterations, are not expected to be effective against all types of cancer. Now, just as in the case of EFGR alterations and the inhibitor drug Afatinib, identifying alterations in the driver genes associated with oral cancer could lead to finding drugs that would be milder than the standard hospital-based chemotherapy and can be given orally at home.

The Indian study has also unambiguously revealed the advantage of large-scale genomic studies. Many of the new genes discovered and validated could certainly not have been identified as "candidate genes" for oral cancer but for the methodology used, which drew inspiration from the remarks of Renato Dulbecco.

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