

# IHV Researchers Hunt for an Infectious Cause of Some Human Lung Cancers

Lung cancer kills more people than any other type of cancer. Although smoking is known to be the major cause of bronchial lung cancer, there are other forms of lung cancer. Epidemiological studies have found that HIV infection is associated with their increased risk, independent of smoking. This finding has led researchers at the Institute of Human Virology (IHV) of the University of Maryland School of Medicine to hypothesize that some lung cancers may have an infectious cause, and they are attempting to find such a causative agent.

"When HIV influences the incidence of cancer, most of the time it's turned out to be a viral induced cancer or at least a virus playing a role in that cancer," says Dr. Robert Gallo, Director of the IHV. "We became interested because of our long interest in viral oncology and because we are involved in a viral oncology at the University's Greenebaum Cancer Center, particularly with Dr. Martin Edelman, Director of Medical and Thoracic Oncology," he says. "It's not long ago that people thought no cancer had an infectious cause," but now it's estimated that between 20% and 25% of all cancers have a viral cause or a role with other factors, he adds.

HIV has previously been associated with certain cancers like Kaposi sarcoma and non-Hodgkin lymphoma. In both cases, the cancers are associated with the expression of tumor-inducing viral oncogenes, and their occurrence is dependent on immune system suppression that may increase the expression of latent viruses. However, the advent of highly-active antiretroviral therapy and longer life spans caused a shift from the occurrence of these AIDS-related malignancies to other types of cancer, including lung cancer. The increased incidence of these cancers has led researchers to search for their cause.

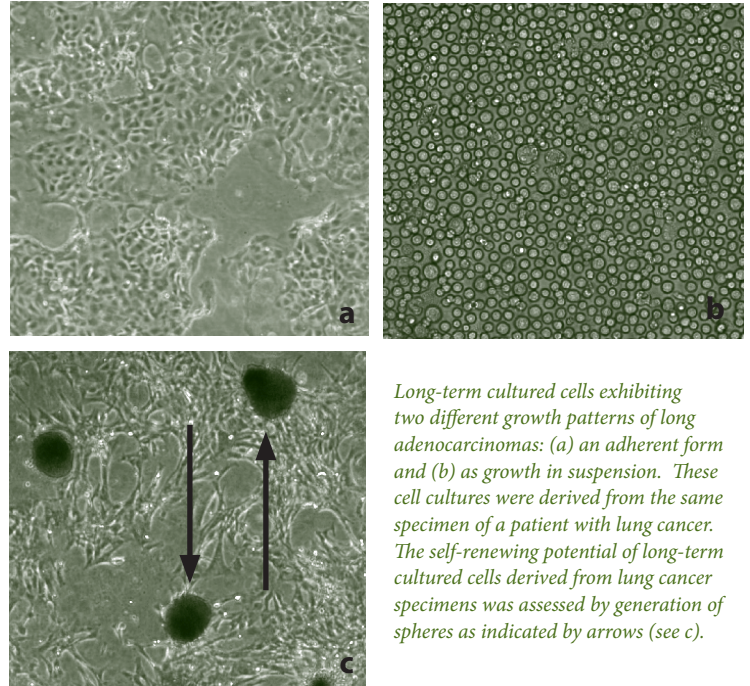
Apart from the association with HIV, another indication that there might be a microbial cause for human lung cancers came from a naturally occurring lung cancer in sheep, says IHV senior researcher Dr. Mika Popovic, who is conducting the study along with IHV's Director of Animal Models, Dr. Joseph Bryant, Gallo and other IHV scientists. It was discovered that this lung cancer is caused by Jaagsiekte sheep retrovirus (JSRV). Interestingly, JSRV-induced sheep tumors bear strong similarities to human lung cancer adenocarcinomas, leading IHV researchers to look for a similar infectious cause of human lung cancers.

To achieve their research goals, Popovic is extracting and culturing tumor cells from patients with lung cancer. "Virus infection frequently induces changes in the cells," he says. For example, viral infection can create giant, multinucleated cells, and as such Popovic identifies and cultures these cells before analyzing them for any infectious agents.

Apart from potential virus-modified cells, "Popovic is trying to pull out stem cell-like cells of the cancer," that are thought today to be the driving cells of the disease, says Gallo. These 'cancer stem cells' are thought to constitute just a small portion of tumor cells, but have stem cell-like characteristics that allow them to regenerate the whole tumor.

Once he has these cells, "the ultimate marker of a cancer stem cell is that you inoculate it into immunodeficient mice and see whether it generates a tumor," says Popovic. And so far, the cells he extracted have successfully generated human lung cancer tumors in mice.

Popovic has currently extracted cells from 5 patients with lung cancer,



*Long-term cultured cells exhibiting two different growth patterns of long adenocarcinomas: (a) an adherent form and (b) as growth in suspension. These cell cultures were derived from the same specimen of a patient with lung cancer. The self-renewing potential of long-term cultured cells derived from lung cancer specimens was assessed by generation of spheres as indicated by arrows (see c).*

and has established 3 cell culture lines. "We have short and long term cultures, as well as primary cultures for characterization, and that's where we are," he says. In the past "people have not had much success in culturing cells from lung cancer," says Gallo, but "Popovic has plenty of experience, talent, and the necessary patience to make advances." Now that Popovic has managed to grow them, "he's looking for different types of viruses," says Gallo.

Because of the link between JSRV and sheep lung cancer, the researchers definitely wanted to include retroviruses in their search, says Gallo. To identify retroviruses, Popovic looks for the expression of the viral enzyme reverse transcriptase, whose expression increases dramatically during retroviral infection. Popovic also uses polymerase chain reaction (PCR) to screen peripheral blood cells, mononuclear cells, or cells from pleural effusions extracted from patients. PCR is used to try to amplify regions of DNA specific for different viruses, including human papilloma viruses, Herpes viruses, Cytomegalovirus, and JSRV. According to Gallo, in the future the researchers could also sequence patients' genomes to look for any information in the DNA that might indicate a virus.

Once the researchers find a virus, they will need to characterize it and find out how it is associated with lung cancer. This would involve a combination of serological assays and molecular biology techniques. Among other things, to be associated with the disease a virus should be present in the lesions caused by the disease, says Popovic.

Finding a viral or other infectious cause for lung cancer would have a major impact on the treatment and prevention of lung cancer. "If we discover a virus that's novel, or we find an old virus and can link it positively to that kind of tumor, the implications would be obvious—anti-viral therapy, methods for early diagnosis, or prevention by vaccine," says Gallo.