Recent Advances in Lung Cancer Biology

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My charge today, originally, was to tell you some of the "exciting new things in the molecular biology of lung cancer." Lung cancer research has progressed rapidly in the last year to three or four years. As in all fields of cancer, there are two generally well-accepted types of genetic aberrations that are involved in the development of an eventual neoplasm. These are referred to as oncogenes and tumor suppressor genes, and they are the Ying Yang, or the accelerators and brakes respectively, of what makes a cell grow and not grow. It is the aberrations in these genetic processes that lead to uncontrolled growth and unfortunately often the eventual death of the individual.

Recent Advances in Lung Cancer Biology

Now, with respect to lung cancer caused by high LET radiation and radon, the primary information that is being gathered, and it is coming not as quickly as we would wish, is to study the tumors from uranium miners. That topic will be covered by Bill Bennett. I will have very little to say in my talk about the actual specifics of the miner tumors and instead will limit my talk to the generalities of what is exciting and new in lung cancer research.

One of the areas that was an old area in lung cancer research several years ago that fell in disfavor and I think is now going to come back again is the idea of an autocrine mechanism for lung cancer. Now, some of you may wonder what an autocrine is. An autocrine is typically a growth factor, but it can be a hormone, which is produced by a cell and then the cell itself has a receptor for that ligand and therefore responds. So, it is like having a perpetual motion machine where the cell makes a growth factor that causes its accelerator to move, and it just keeps going around and around and around.

Potential Autocrine Loops in Lung Cancer

A growth factor is an autocrine when it stimulates the growth of the cell that makes it. An autocrine growth factor provides a continuous stimulus; therefore, the cell may grow continuously.
In the earlier days there was some evidence that for nonsmall cell carcinoma of the lung, the growth factor involved was a factor called transforming growth factor alpha. The receptor for this factor has various names, Er-B-1 is one of them, and the cells -- the airway epithelial cells and Type II cells make this factor; this receptor is on the cells and causes cells to grow.

More recently, two growth factors that were totally unrelated to lung cancer are now coming to the fore. For nonsmall cell lung cancer there is a factor which is referred to as a hepatocyte growth factor (for reasons that are totally erroneous, it was first discovered as a mitogen for hepatocytes). This factor turns out to be rather ambiguous. It is present in the lung at high levels and the receptor for it is an oncogen referred to in the early literature at MET. This system has now been shown in at least one case to serve as an auto-stimulating system where the tumor cell makes the hepatocyte growth factor and the tumor cell has the receptor and so the cell continues to grow.

One of the very intriguing aspects of this whole system, which is not well understood yet by any means, is a second factor referred to as a hepatocyte-like growth factor. These two factors are very close to each other in their actual molecular structure but one of them, a hepatocyte-like growth factor, appears to be an antagonist of this, and they both respond to the same receptor.

There is a tumor suppressor gene at Chromosome 3p, which is a position on Chromosome 3. This factor is in that locus, so we have an exciting opportunity to try to tie all this together with a tumor suppressor gene.

In the case of small cell lung cancers, there is a stem cell factor which was originally isolated as a mitogen for leukemia cells. It is produced by the lung and the receptor kit is present in the lung, and it is now being considered as a high candidate for autocrine growth of small cell lung cancers.

As I mentioned on the first slide, there are tumor suppressor genes and one of them is located at 3p. There are several on Chromosome 3, at least three or maybe four. These losses of chromosomes are important because they give us clues as to where to look for the brakes of cell division. By losses of these brake genes then, if you're imagining the car driving, if you...
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can't brake it, it just keeps going until you run out of gas, and if we continue to feed the tumor, it will continue to grow.

We now know that there are some differences, again, between nonsmall cell and small cell cancers. These are very different kinds of cells when they finally form tumors, but there are some commonalities. A frequent loss of 9p21 contains at least two -- there are at least two tumor suppressor genes there for lung cancer. There are three to four on 3, and there are others: one on 17 and one on 3.

There is one here on 2, and these in bold are interesting because they seem to show up later in the process. I remind you that cancer is not a disease that occurs overnight; it's a disease that takes 20 to 40 years to develop, and there is a progression of genetic changes that occur during this time. These losses appear to be related to the actual transition of a cell from a tumor to a metastatic type of a disease. Again, the same sort of change occurs in small cell, but there are some other different losses.

There is a very exciting new technique that has been developed recently called comparative genomic hybridization. This allows one to try to monitor where other types of changes are occurring in a direct hybridization type of protocol. These losses come up using this assay. The gains, which are very new data and have only been so far done on small cell lung cancer and the nonsmall cell has not been published yet, show that in addition to losses there are some actual gains of chromosomal material that are quite frequent in lung cancers, and these are candidate genes for oncogene changes where you have too many go pedals, or too many accelerators in that causes the cell to go faster.

We have no idea what the genes are here. We do have an idea what the genes are here. This is a gene called retinoblastoma, and this one is called p33, and this was called APC. But other than that we don't have any idea of what a lot of these genes are.

There's another recent exciting development in cancer, now being applied to lung cancer. This is mismatch repair or microsatellite instability.

Mismatch Repair -- Microsatellite Instability

- Replication errors corrected by Mismatch repair complex (hMSHs, exonucleases, & ligases).
- Microsatellites: 2,3 or a few basepair repeats scattered throughout the genome.
- Microsatellite mutations do not provide growth advantage; however, monoclonal microsatellite mutation is an indicator of inefficient repair.
50% of SCLC and 34% of NSCLC tumors have monoclonal microsatellite mutations

Potential screening marker of premalignant disease.

What happens is that, replication errors when cells divide are corrected by a complex called a mismatch repair complex, which is a combination of many genes and many enzymes. There are things throughout the genome called microsatellites, little areas where you have two, or three, or four base pairs and they repeat themselves; we all have different lengths of these repeats called a polymorphism. You would inherit one polymorphism from your mother and one from your father and this allows us, from a genetic perspective, to trace events.

I spoke about losses and gains in the previous slide; those were in specific genes. These losses and gains are just of general markers, they don't translate to any genetic information but they do translate to a risk of something having gone wrong. So, we have now shown that about half of small cell lung cancers have these instabilities, and about a third of the nonsmall cell do. What's really exciting about this is that, these -- this loss of stability if you will, the repair process allows one a very nice potential screening marker for premalignant disease and that's an exciting area for the future. It relates not only to this mismatch repair genes, but also other DNA repair genes and other processes that control the fidelity of how well things replicate.

Another recent bit of knowledge that's come out for lung cancer is there is a very interesting relationship between two tumor suppressor genes. One of them is called Rb and another p16.

**INTERRELATIONSHIP BETWEEN Rb AND p16**

_(Lung Cancer Cell Lines)_

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<th>SCLC</th>
<th>p16&lt;sup&gt;(nu)&lt;/sup&gt; (89%)</th>
<th>p16&lt;sup&gt;(mut)&lt;/sup&gt; (11%)</th>
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<tr>
<td>Rb&lt;sup&gt;(nu)&lt;/sup&gt; (13%)</td>
<td>2%</td>
<td>11%</td>
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<tr>
<td>Rb&lt;sup&gt;(mut)&lt;/sup&gt; (87%)</td>
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<td>non-SCLC</td>
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<td>Rb&lt;sup&gt;(nu)&lt;/sup&gt; (76%)</td>
<td>11%</td>
<td>81%</td>
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<tr>
<td>Rb&lt;sup&gt;(mut)&lt;/sup&gt; (24%)</td>
<td>8%</td>
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But, what's interesting is that, p16 is mutant quite often in small cell lung cancer cell lines with a very high percentage of wild type p16 and very mutant Rb in small cell and exactly the opposite wild type and mutant in the nonsmall cell.

There is a cousin gene to p16 called p15, and they are both located on chromosome 9 in the region called 9P21.

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<th>PERCENTS OF SOMATIC MUTATIONS OF p15 &amp; p16</th>
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<tr>
<td>9p21(^{\text{DNA}})</td>
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Considering recent data from Harris' lab, a lab in Japan, and David Sidransky's lab at Hopkins, including primary tumors, metastatic tumors, and cell lines, you can see that in primaries p16 varies between zero in the United States to 30 percent in Japan for nonsmall cell, and in small cell it never occurs. For metastatic disposal and cell lines the picture is confused.

The confusion may reflect the fact that this gene can be inactivated but not mutated. It's inactivated by a chemical process called methylation, and the cytosine bases have a methyl group attached to them. There's an enzyme that does that, and what appears to be going on is that there is a fair amount of inactivation before loss of the gene actually occurs, and that this gene may be playing a much larger role in lung cancer than some have suspected in the past.
We have some very interesting findings: consider this slide. This is a cell cycle; the cells divide, and here is mitosis and two cells arising. Then a cell goes in the G1 phase, progresses along, DNA is synthesized and repackaged into metaphase chromosomes, and the cycle continues.

A number of genes are thought to be involved in lung cancer. In particular it seems that there is a lot going on in this area right here where there are some problems here; there are a lot of problems out here in a g and p53, which feeds into this. There are mutations in this gene; mutations in this gene are leading to lung cancer. Very recently in my own lab we're finding that problems in cyclin B are also involved in this area, especially in radiation-caused lung cancers in rats. We have some particular targets that we can now study and find out how frequently they're changed.

Lastly a couple of points on some other interesting areas. One is the estrogen receptor and the other is the retinoid receptor.

For reasons that we don't understand, the estrogen receptor seems to play a major role in lung cancer, even in men. It is frequently lost in nonsmall cell lung cancers and again, it seems to be due to an inactivation by methylation, not specifically a gene loss per se. In breast cancer this loss is caused by methylation and some recent evidence coming out of one of my associates' lab at the Inhalation Toxicology Research shows that this methylation correlates with radiation. In radiation-caused tumors this gene is inactivated in tumors by methylation, whereas in lung cancers caused by tobacco nitrosamines and NNK, this is not occurring.

We may have a potential signature for radiation versus spontaneous tumors but more research is needed.

With respect to the vitamin A and retinoid acid receptor, there is also interesting recent data. It's been known for a long time that vitamin A prevents squamous metaplasia. Retinoid acid beta -- receptor beta is frequently not expressed in nonsmall cell lung cancers; again, the gene is defective but without a mutation involved, implying another candidate for possible methylation inactivation; epigenetic is another term applied to this phenomenon. This is another change to assess for radiation-caused cancers.

I will now move to the possibility of taking respiratory samples to determine if people have pre-malignant disease. Now, with respect to the uranium miners that are still alive in the United States, Europe, and Asia, there are large numbers of men who have worked in the mines and received substantial doses of radiation.
Can we develop a means to obtain some of these lung cells and analyze them for genetic aberrations? Bearing on this directly is recent work from an Italian laboratory. These researchers conducted genetic and chromosomal analysis on some of the genes that I've been telling you about, and attempted to distinguish recurrences from new primaries following a first tumor.

There appears to be many people who develop a second independent tumor, and not a spread from the first tumor.

When they took samples from the uninvolved lung, other lobes, of people who had lung cancer, about half of them had genetic aberrations in "normal" cells. In other words, these were cells that had changes but not deranged to the point of producing a clinical disease. This finding suggests an opportunity and a scientific basis for taking cells from people and looking for early changes. We have done this now with a couple of miners in New Mexico and have found one individual, who now we know has a chromosomal aberration in cells in his lung, but has no evidence of cancer. Obviously, this person needs to be monitored.

In the Italian study, the people who didn't have any lung cancer had no genetic aberrations in their normal cells. However, there were only five such people so we need more information.

The most exciting, I think, in my opinion again, area of high LET radiation biology is the fantastically high level of genomic instability that is caused by these big particles. This is work that has been going on since about 1992, and is advancing rapidly.

There is early non-random chromosome instability that occurs in the progeny of cells that have been irradiated in vitro. At present, the data all come from experiments in culture. The cells are exposed to high LET radiation; in my lab we're using plutonium-238 as the source, and we're looking at 7 to 25 population doublings later, so the immediate radiation effect is long passed, the cells have grown repetitively before analysis.

A fair number of the cells show chromosomal instability, at a rate that appears higher than for any other carcinogen. The word "appears" is operative in my sentence because the comparison is to a couple of carcinogens and a few studies of X-rays. But the evidence does suggest that high LET radiation causes gene alterations that remain stable.

I will conclude with a story that I think Dr. Bennett will carry forward; that is, about two and a half years ago there was a finding of a specific mutation in the $p^{53}$ gene that was related to radon exposures. It was a mutation in Codon 249, one of the bases in the gene.
In a collection of tumors from Grand Junction, in the Colorado Plateau mining area, about half of the tumors had this mutation of the p53 gene. This finding did not agree with a previous study from New Mexico.

There was a recent paper in *LANCET* that did not find this mutation with domestic radon exposure. My lab, in collaboration with Curt Harris' lab, will expose normal human bronchial epithelial cells to radiation at high doses to learn if we can measure the actual mutation. At the moment at least, it looks like this p53 mutation may not be a particularly good signature for high LET radiation.

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