THE GENETICS OF CANCER

David Malkin, MD

DR. LOWDEN: Our next speaker, Dr. David Malkin, will be discussing the genetics of cancer. Dr. Malkin is an Associate Staff Member, Division of Oncology and a Project Director of the Research Institute, The Hospital for Sick Children, Toronto. He received his medical degree from the University of Toronto. After completing his residency in pediatrics, he served a two year fellowship in Clinical Hematology/Oncology at the Hospital for Sick Children. After completing post-doctoral training in Boston, Dr. Malkin returned to the Hospital for Sick Children in the capacity of Assistant Professor in the Division of Oncology, Dept. of Pediatrics. With both institutional and external support, he has set up a laboratory to screen high risk populations for germ line mutations of P53, as well as pursuing his interest in the role of this and other genes in familial and hereditary cancer. He also pursues an active clinical practice in the field of pediatric oncology. Please join me in giving a warm welcome to Dr. Malkin. (applause)

DR. MALKIN: Thank you very much for the very kind introduction and thank you again for inviting me to speak to you today.

I would like to talk to you about the role of genetics in cancer. It is an impossible task to address this whole issue in one session. I will concentrate my discussion on the role of genetics in familial cancer and, as you will see shortly, on one specific gene which appears to be of great importance.

The lifetime risk in North America to develop cancer, if one does not include skin cancers, is about 1 in 3. Between 1 in 4 and 1 in 5 individuals will die from cancer. Three approaches exist to decrease the morbidity, mortality and incidence of this group of diseases: the prevention of cancer altogether, early detection and the presumed benefits of early detection, and, of course, improved treatments. We will concentrate for the most part on the first two of these.

Twenty years ago, Dr. Alfred Knudson, a geneticist and one of the forefathers of modern cancer genetics, presented the concept of "oncodemes." This term refers to the influence and interactions of both environment and genetics on cancer risk. On the one hand are the background level of cancer risk, which may include factors such as cosmic rays, nuclear disasters, and other things that are beyond our control. On the other extreme, is inherent genetic predisposition to cancer inherited from generation to generation, and perhaps, in many ways independent of any environmental influences. We all are aware of the environmental causes of cancer, such as cigarette smoking, diet, and sun exposure. Many of these carcinogens may be modulated by genetic susceptibility.

The familial nature of cancer is demonstrated in a study from Switzerland (Fig. 1). Of over 450 patients with cancer, primarily adults, in which the frequency of first-degree relatives with cancer was ascertained, twenty-nine percent had at least one first-degree relative with cancer, 11% had two affected first-degree relatives and 6% had more than three affected first-degree relatives. In addition, almost 10% of the children had at least one first-degree relative with cancer. In our practice in Toronto, where we see between 250 and 300 new cancer patients at the Hospital for Sick Children each year, one would predict that 25 or 30 will have a first-degree relative with cancer. In the past year, we have analyzed this by rudimentary family history-taking, and have determined, in fact, that the rate is probably a bit higher than 10%.

The significance of those numbers is borne out by a study that was conducted by Drs. Frederick Li and Joseph Fraumeni, then at the National Cancer Institute, in 1969. They were interested in identifying children
with soft tissue sarcomas, who had family histories of cancer. After examining the medical history and hospital records of over 600 childhood sarcoma patients, they were able to identify four families in which at least one individual had a sarcoma at an early age. Multiple other primary malignancies occurred in the family and there were always two first-degree relatives or one first-degree and one second-degree relative with cancer. Frequently, breast cancer was apparent, but other malignancies were also found. The Li-Fraumeni syndrome is a relatively rare syndrome, and until 1990 only some 150 such pedigrees had been described in the world's literature. Since 1990 there have been many more described, and they are being more readily identified as oncologists become more aware of the constellation of tumors.

What is this syndrome and why is it important? The clinical criteria include the presence of a sarcoma under the age of 45, cancer in two first degree relatives under the age of 45 in the kindred, and multiple primary neoplasms frequently occurring in affected individuals.

Dr. Louise Strong at the M.D. Anderson Cancer Center has demonstrated the syndrome to be an autosomal dominantly inherited disorder. The gene penetrance is high, with a 50% chance of cancer occurring in a gene carrier by the age of 30, as opposed to 1% in the general population, and a 90% or more chance by the age of 70.

As shown in Figure 3, one can consider that normal cell growth and proliferation are directed by two pathways. The first is driven by a set of genes, termed proto-oncogenes, which are normal cellular genes. At least one or two hundred of these exist in the human genome. They are thought to control cell growth and proliferation in a regulated manner. On the other hand, the so-called tumor suppressor genes, or growth suppressor genes, or anti-oncogenes, which, as the name implies, suppress abnormal growth of cells, and thus regulate cell growth in a "negative" manner. The interaction of both of these sets of genes allows for normal growth and proliferation.

Cancer might develop in the following manner. First, under the control of normal tumor suppressor genes, one might have a cell in which a proto-oncogene has been activated in some manner, such as a point mutation, translocation, or amplification, perhaps by an environmental influence, to become an oncogene. This directly affects cell growth and proliferation and the cell may eventually go on to transform in a malignant fashion.

On the other hand, one might have normal proto-oncogenes but one or more of the tumor suppressor genes may become altered, inactivated by mutations, deleted or lost. Negative control is lost, and one ends up again with malignant transformation. Obviously, one can also...
have interactions of both of these, which presumably would accelerate the events. This is an overly simplistic outline of malignant transformation, yet it identifies the significant steps.

Cancer-related genes are scattered throughout the cell. A number have been cloned to date. Many more have been localized to particular chromosomes, but are not yet characterized. Genes may encode proteins which are ligands outside the cellular membrane, within the membrane, in the cytoplasm or in the nucleus. Both the tumor suppressor genes and the proto-oncogenes encode proteins that can be found in different regions of the cell.

When one has identified a familial cancer syndrome, how does one go about finding its associated gene(s)? Although one could identify with some degree of certainty who may be at risk by family history, linkage analysis will not absolutely identify all those at risk and all those who are not. Identifying a gene can be done in four major ways. These have been described by Dr. Rotter, but I’ll simply identify these four methods as they apply to cancer.

**Figure 4**

**DIFFERENT STRATEGIES FOR DNA LINKAGE ANALYSIS**

1) RANDOM MARKER
2) TUMOR DELETION
3) CANDIDATE CHROMOSOME
4) CANDIDATE GENE

The random marker approach is one that was used for the identification of the retinoblastoma tumor suppressor gene, which was the first such gene identified. Retinoblastoma is an extremely rare ocular tumor of childhood. It actually is one of a few tumors that are potentially curable by surgery alone. In children who had retinoblastoma, markers on the long arm of chromosome 13 near the putative retinoblastoma gene had previously been identified and were used to localize the precise location of the gene.

The tumor deletion approach simply implies that if one is born with two normal copies of the gene, and then for some reason one of those copies is lost in a particular cell, then one would be able to detect the deletion or the loss of that gene by performing a Southern blot analysis or polymorphism analysis, where you lose one of the two bands you would expect to see on the gel. And by doing that, you can then suggest that perhaps the gene of interest is one that was lost in the tumor.

The candidate chromosome approach is useful where there is an actual deletion, alteration, or translocation of large or small fragments of the chromosomes, indicating the presence of a gene within those altered sites.

Finally, the candidate gene approach is used when a gene has already been cloned and some of its function is known. The functions have something to do with the type of disease you’re looking at, and you can then decide to look at that gene to see whether it’s associated specifically with that disease.

The problems in isolating genes associated with the familial cancer syndromes, including Li-Fraumeni, are, first, that the syndromes are very rare. Secondly, the definition of a syndrome is complicated and often not clear. Thirdly, the tumors are lethal in the vast majority of affected members, which means that large families with abundant material to examine are simply not available, and genetic linkage is difficult to perform.

Sporadic tumors can occur in non-gene carriers. The term for these individuals is "phenocopies." For example, if a member of the family develops sporadic breast cancer, a component tumor of the syndrome, this would not be distinguishable from breast cancer in a gene carrier. There are no obvious phenotypic changes in Li-Fraumeni patients. They look completely normal and have no specific chromosomal alterations in their blood, or for that matter, in the tumors that they develop.

For all these reasons, linkage analysis in the standard ways is really quite difficult, and one is left using what has been called the candidate gene approach. In this case, those are the tumor suppressor genes.

In 1971 Knudson proposed a genetic model of cancer formation using retinoblastoma and Wilm's tumor, an uncommon tumor of the kidney in children. Both of these occur in both hereditary and sporadic forms. He proposed a model of genetic inheritance that assumed the presence of genes that, when lost, would produce cancer.
In the sporadic model, the individual is born with two normal copies of the gene, one from the mother and one from the father. Two somatic, acquired mutations are required, one in each copy of the gene, to go on and develop the malignancy. This usually occur singly, unilaterally and of later onset. The likelihood of both events occurring in more than one cell is extremely low (Fig. 5).

On the other hand, for hereditary malignancies to occur, one is born with one abnormal copy of the gene, and therefore, requires only one acquired mutation in the normal allele to develop multiple, bilateral tumors of early onset. This is the classic model for tumor suppressor genes.

A number of potential tumor suppressor genes exist, and about 60 have been chromosomally localized. As of 1990, however, only two had been cloned: the retinoblastoma gene (RB1) and one called TP53. Retinoblastoma is not found in the Li-Fraumeni Syndrome, nor is it found in any other familial cancer syndrome except for hereditary retinoblastoma. Neither the Wilms' tumor gene nor the neurofibromatosis gene, NF-1, had been cloned and we were left with only one other gene that could be a candidate.

This gene, TP53, so called because it produces a 53 kilodalton protein, is located on the short arm of human chromosome 17. It is a nuclear phosphoprotein that undergoes phosphorylation at distinct stages of the cell cycle. It is thought that the state the protein is in at a given time in the cell cycle defines whether it has an inhibitory or a non-inhibitory effect on driving the cell cycle. TP53 is a protein that binds to strands of DNA to activate transcription and eventual translation of other genes downstream to it. It cooperates with dominant oncogenes to transform cells and behaves as a tumor suppressor gene. Deletions of a large part of the gene yield incomplete binding to the TP53 binding site and one gets reduced transcription. When a missense mutation within the TP53 gene yields a mutant protein, one again does not get complete binding. Nonsense mutations, meaning mutations in the gene that eventually yield a truncated or shortened protein, also lead to reduced transcription.

The other mechanism of TP53 inactivation is based on the property of TP53 to bind to a variety of constitutional proteins within a cell, or exogenous proteins such as the HPV virus particles. When they are bound, TP53 can no longer bind to DNA and no longer activate transcription. In other words, when TP53 is inactivated, DNA transcription no longer occurs. Bert Vogelstein and his group, in 1989, and others, have shown that TP53 may be abnormal in almost all human malignancies, making it the most frequently altered gene in sporadic cancers.

The structural features of the P53 gene are outlined in Figure 6. The gene encodes 393 amino acids. There are five regions (I to V), which are so-called conserved domains of the gene. The amino acid sequence in these regions is identical or close to identical from humans down to trout and probably to some of the lower invertebrates as well.

It is thought that conserved regions of a gene confer functionally important properties to the gene and its protein. Therefore, mutations in these regions would presumably lead to major gene dysfunction. Interestingly, of the hundreds of mutations identified in TP53
ingly, of the hundreds of mutations identified in TP53 in sporadic tumors, >95% of them occur in these "hot spot" regions which correspond very closely to the four conserved domains.

Because sporadic TP53 mutations are so frequently identified in the conserved regions of the gene, these domains were felt to be the best candidates to search for germline mutations in the Li-Fraumeni Syndrome. Other evidence to support TP53's candidacy arose from observations in transgenic mice that carried altered TP53 who were shown to develop multiple tumors similar to those in Li-Fraumeni families.

The methodology involved initial extraction of DNA from lymphocytes or skin fibroblasts. The region spanning exons 5 to 8 were amplified using the polymerase chain reaction. This fragment was then subcloned into a vector, and then sequenced to look for mutations.

In one of the families initially examined all members were alive. The proband had a soft tissue sarcoma at age 1, and developed osteosarcoma at 8. Her sister had a brain tumor at age 5. Their mother had breast cancer at age 30 and her sister had bilateral breast cancer at age 28. All four affected members carried two TP53 alleles, one was wild type or normal and the other had a mutation at codon 248 in the gene, which is a highly conserved amino acid.

There were several unaffected members who carried only normal TP53 and there were two members, the grandfather and a cousin of the proband, who were unaffected but were carriers. The grandfather was 57 at the time of study, and could potentially still develop a malignancy. The other carrier was only 4 years old.

After several families were analysed, it could be demonstrated that germline TP53 mutations did predispose families to malignancy, at least in those members who were carrying the abnormal gene. The Li-Fraumeni syndrome is rare; therefore, the question arises whether this sort of information can be applied to more common clinical situations. The answer is probably, "yes." One might look at other individuals who have components of the Li-Fraumeni syndrome, but whose kindreds cannot be clearly identified as such. For example, we know that multiple malignancies occur in affected family members. Perhaps those individuals who develop more than one tumor after surviving their first cancer, even without a family history, may fit this sort of syndrome, or at least may carry abnormal TP53. Early presentations of cancer are a hallmark of the syndrome, and, of course, they can occur outside of these unusual families.

To perform any of these studies, one must develop efficient large scale screening procedures. A variety of techniques have been developed over the last two to three years, to screen large numbers of patients rapidly for mutations in any gene.

![Figure 7](image) One of the techniques most commonly used is called Single Strand Conformational Polymorphism, SSCP. (Fig. 7) A PCR amplified fragment of double stranded DNA is heat denatured to yield two single strands that will have a distinctive conformation based on inherent biochemical interactions of one base with the other. If a mutation exists, then that conformation will be different.

If the wild type DNA is run on an acrylamide gel, which acts as a matrix for the DNA to run within an electrical current, one ends up with two single strands that will have a distinctive conformation based on inherent biochemical interactions of one base with the other. If a mutation exists, then that conformation will be different.

The advantage of this technique is that if you run fifty samples and you only have five that are mutant, you only have to sequence five. Previously, one would have had to sequence all fifty, which is much too laborious.

For the last few minutes, I will discuss one of the high risk groups in which one might look for germline TP53
mutations, namely children who develop second malignancies.

By 25 years after the first tumor develops, there is a 10 to 15 percent risk of developing a second malignancy, irrespective of whether the patient has been irradiated for the first. We studied 59 children and young adults who had survived their first malignancy and went on to develop a second and who did not have a strong family history of cancer. In almost half of these patients, both their first and second tumors were components of Li-Fraumeni Syndrome, even though none of these patients had family histories of cancer. Four of the 59 had germline mutations in the TP53 gene. Three could be shown to be inherited. In one, the earlier generations were not available. More importantly, it was found that there were a variety of malignancies that were not component tumors of Li-Fraumeni Syndrome: colon cancer, non-Hodgkin’s lymphoma, gastric CA, neuroblastoma. Although these are not component tumors of the syndrome, yet they were very evident as first or second malignancies in patients carrying germ line mutations.

It had thus been demonstrated that this very rare Li-Fraumeni Syndrome was only the tip of an iceberg, suggesting that there are many subpopulations, cancer populations that carry mutations in TP53 gene, and probably other genes as well, that predispose them to cancer.

However, the problems only start there. Even though some families have the classic syndrome, they have a normal genotype at this particular locus.

Simply identifying that an individual harbors a DNA mutation does not necessarily mean it’s a functional mutation. It does not necessarily mean they’ll develop disease. Mutations at some codons of TP53 produce altered function, but not complete dysfunction. The spectrum of germline mutations in TP53 is not unlike those in sporadic tumors. They can occur anywhere throughout the gene. Although there have been several hundred sporadic mutations found less than 50 germ line mutations have been identified, and that’s really only because this work has just been getting underway in a number of labs.

Clear advantages exist in screening for TP53 mutations. It will improve the technology of mutation analysis generally and may help clarify whether specific mutations are associated with specific tumors. One can reassure non-carriers and determine the frequency of gene carriers. As well, one may identify other at-risk populations and develop long-term clinical follow-up programs for those patients shown to be carriers of the abnormal gene. But those perceived advantages have to be balanced with the problems.

Screening is currently an error-prone, laborious, expensive technique. It is not appropriate to screen the general population, let alone the general cancer population. There are inadequate clinical screening methods and, of course, other psychological effects and risks of discrimination that have yet to be evaluated.
And so, we have several issues of predictive testing in cancer exist which will, of course, be discussed in the course of this meeting. Thank you very much.

DR. A.C. FAVORS, General American Life Insurance Company: A year ago we felt that it was not appropriate to use PSA as a screening test, even though now there is an ongoing trial. Is there pressure, outside pressure from women, other groups, to test for P53 even before it’s ready? Could you comment on that, please?

DR. MALKIN: That’s absolutely right. There’s pressure to do something to the point that any time an article comes out on this subject we get several hundred phone calls from interested patients or families. What it leaves us with is a dilemma in terms of approaching these patients, because we can do the test. That’s no problem. We don’t know how to transmit back the information. In a pediatric setting we are doing the test on a child based on the consent given by the parent, but who’s to say that the child, if he or she was able to understand and consent, may themselves not want to be tested. At present the pressure is such that testing is only being done in a research setting. There are specific protocols and specific studies being done and those include testing families that resemble Li-Fraumeni, but maybe miss one or two of criteria, and testing children with component tumors of this syndrome to see whether those cancer populations carry the gene, and one may identify other families that are out there. Probably 90 percent or so of the patients who are tested come to us for testing rather than our going out looking for them.

I should just mention there was an earlier question related to cost of the TP53 test and I scribbled down a few numbers. From the time the patient walks into the clinic and blood is drawn, he or she undergoes some sort of genetic, psychological, and oncologic counseling before the testing is done. The actual test is performed in a lab with extractions, the SSCP, and so on. That’s usually at least duplicated, if not triplicated. There’s the labor cost involved in that, as well as the reagent cost. Before transmitting the information back to the patient, there’s another round of counseling done to see what their reaction to the test will be, whether it’s positive or negative. Crude calculation of that came up to between $2,000 per sample.

DR. JOHN PHILLIPS, Vanderbilt University: Can you clarify? I was confused by what portion of Li-Fraumeni cases have a germ line TP53 mutation.

DR. MALKIN: We’re still working on that actually, but, initially, we thought it was 100 percent with very small numbers. Then it dropped off to about 60 percent and it turned out that the screening procedures that we were doing were not effective. For a variety of reasons, technically, a lot of mutations were being missed. It now appears that probably about 85 percent of the Li-Fraumeni families carry these mutations. Probably about 50 percent of "almost-Li-Fraumeni" families carry the mutations.

DR. PHILLIPS: What about the 15 percent where you don’t find the mutation? What do you tell them?

DR. MALKIN: The 15 percent that don’t find them, we say it’s not there, that presumably there may be other genes or other things, environmental or otherwise, which are predisposing them to develop this similar phenotype to Li-Fraumeni Syndrome.

DR. MICHAEL KABACK, University of California at San Diego: Regarding the point about reassuring those who are not carriers, the last pedigree shows a woman who had both bone and breast cancer and was negative on both alleles for a mutation. So, in fact, for research purposes, the person is negative?

DR. MALKIN: No, if you identify the mutation in one individual, but you do the functional assay on that mutation and it’s not functionally active, all you can say is TP53 is not what’s giving you this pedigree. We can’t reassure them that they’re not going to get cancer, but we can reassure them that it’s not related to TP53. So you identify the mutation first, but, as I mentioned, identifying the mutation itself is not enough. You have to identify that it is functionally inactivating the protein.

DR. KABACK: But in a family with a history of cancer, what the people that are not affected are most worried about is whether or not they’re going to get cancer. They don’t care about the numbers. They’re anxious about their risk for cancer and if you tell them that you’ve done the test and their results are negative, their insurance companies will feel wonderful until they get breast cancer and colon cancer the following year.

DR. MALKIN: The pedigree shows we are doing one test for one gene of the several dozens, perhaps hundreds that predispose to cancer, and all we tell these patients is that they have a normal TP53 gene. We don’t tell them that they’re not going to get cancer. They already know they’re at high risk for cancer. We’re just providing them one gene that is not causing it.

DR. LOWDEN: I’d like to thank Dr. Malkin again.
Bibliography


