Chromosomes, Genes and Cancer

A molecular cytogenetic approach to the study of malignant histiocytosis in the Bernese Mountain Dog

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October 7, 2006
BMD Health Symposium, SIBB
Como, Italy

The Canine Genome Project was a combined effort of numerous people and was led by Dr. Kerstin Lindblad-Toh at the Broad Institute. The genome was sequenced and assembled at the Broad and was anchored by a combination of approaches including an assessment of the chromosomes, which was performed in the lab of Dr. Matthew Breen at North Carolina State University. The scientific article detailing this work was formally published in the science journal 'Nature' on Dec 8th 2005. The project cost approximately $40 million and involved 35 million reads of genetic material over seven months, followed by a detailed assembly process t put all the pieces into their correct places. The cost was met by the National Human Genetics Research Institute, in recognition of the benefits that the sequence would offer towards the development of a greater understanding of numerous human genetic diseases, including cancers.

Dr. Breen's research involves looking at genetic changes that are associated with cancers. In particular his laboratory looks at how the genome is 'shuffled' during the cancer process. The genome is divided into chromosomes (DR. Breen refers to these as nature's biological filing cabinets) and each gene has a precise location on one of these chromosomes. As cells replicate, chromosomes sometimes rearrange; this is particularly true with cancer. Chromosomes can be lost, they can move, or they can duplicate. These chromosomal aberrations are hallmarks of gene deregulation and genomic instability. As chromosomal material is lost, duplicated or relocated elsewhere in the genome, the genes within such regions are also affected accordingly. When genes move neighborhood they may not interact well with their new neighbors, if genes are lost, their function is absent from the cell, if genes are amplified their function may alter. All these features may lead to deregulation of the cell's normal function and the development a cancer.

Many chromosome changes (aberrations) are so specific to certain types of cancers that they are regarded as diagnostic of that cancer. Further, many of these changes have been shown to correlate with response to treatment and so in addition to being diagnostic these chromosome aberrations may also provide prognostic information and so ultimately assist in making treatment decisions.

Humans have 46 chromosomes, dogs have 78. Dog chromosomes all look very similar – except for the X and Y chromosomes. This is problematic for researchers in that chromosome pairs are difficult to identify unequivocally. Dr. Breen has developed a series of molecular reagents which can be used to color code chromosomes, either a single locus or gene pair, or a full chromosome pair. This technique relies on a microscopic techniques referred to fluorescence in situ hybridization, or FISH.

Some chromosomal aberrations are balanced aberrations in which there is no net gain or loss DNA in the cell- examples include inversions of gene groupings and reciprocal translocations;. Other aberrations are imbalanced, in which DNA is either lost or duplication, i.e. the gene copy number changes – examples include deletions and insertions. In order to look at these aberrations Dr. Breen needs viable cells, or fresh tumor tissue shipped quickly. In order to get this, it requires owner and veterinary awareness and willingness. Dr. Breen's lab thus requests specific samples to be sent as listed below:

The samples are used in the following manner:

Formalin fixed biopsy – this us used to obtain (usually to confirm) a pathology diagnosis, and may also be used in FISH

Blood sample (EDTA) – this is used to isolate constitutional DNA and is shared with Dr. Ostrander at the NHGRI

Sterile tumor biopsy – this is use as source of tumor cell DNA and also as source of viable cells to make chromosome preparations that are evaluated using FISH with Dr. Breen's panel of chromosome specific reagents.

Of the tumors that have been submitted to Dr. Breen's lab for Bernese Mountain Dogs (from USA dogs over approx 2 years), the breakdown is as follows:

Malignant histiocytosis/Histiocytic Sarcoma	58 (70% of total affected samples)
Hemangiosarcoma	6
Lymphoma	5
Other	15
Relatives	27

The sterile tissue is placed in a culture to grow. The cells are then fixed at metaphase. There is a very different look to the chromosomes in normal cells versus tumor cells. Dr. Breen cannot use tumor tissue that has been subjected to chemotherapy. Tumor tissue that has been preserved in wax blocks can (sometimes) be used for his research.

Dr. Breen uses a variety of molecular techniques to determine which chromosomes are involved in the cancers. Chromosome painting is an approach that literally paints the length of a chromosome. This approach is very well suited to looking for chromosome translocations but is not able to identify changes within individual chromosomes. Chromosome tiling is a technique that generates a multicolored bar code of each chromosomes and so a change in the order of the colors along the length of a chromosome identifies regions that have been changed. This process thus allows Dr. Breen to see insertions and deletions. Dr. Breen has also shown that chromosome changes seen in dog cancers may be closely related to those seen in the corresponding human cancer. This may have important consequences for the development of new therapies for both dogs and people.

In Malignant histiocytosis/Histiocytic Sarcoma there are a number of chromosomal abnormalities that can be seen. The findings so far:

- a tendency toward extremely chaotic genome reorganization
- chromosomal gains and losses are widespread
- translocation events are apparent with a reduction in total chromosome number
- some things are shared between the Flat Coat Retriever and the BMD
- others exist only within the breed
  - MH is ***NOT*** the same cancer between FCRs and BMDs at the genetic level

There are key regions of interest that are now being evaluated in more detail.

Dr. Ostrander uses blood and has received samples from 49 affected Berners, and 59 controls. Her control samples come from Berners who have reached the age of 10 years without having had any cancer. Her study has identified regions of the genome that may correlate highly with MH. These are found in normal cells that may indicate a genetic predisposition for the disease.

The studies of both Drs. Breen and Ostrander are making exciting progress. To continue making this progress requires many more samples to help refine their data and Dr. Breen urged the audience to remain very active in submitting samples for these research projects.