UPDATE ON THE FERRET GENOME PROJECT

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Because adrenal disease is so widespread in pet ferrets, a genetic basis for the large numbers of affected ferrets is being investigated, primarily by Dr. Michelle Hawkins and her team at the University of California, Davis, and Dr. Robert Wagner at the University of Pittsburgh.

The hypothesis is that if an aberrant/mutated tumor suppressor gene or genes is/are present, hyperplastic tissue with stimulation progresses to adenoma, then eventually adenocarcinoma, following models in other animals and humans. The genes may be defective, or the regulation of the genes may be defective. The hypothesis also includes the possibility of multiple tumor suppressor genes "oncogenes" being involved. A completed study looked at tumor markers in ferrets, based on the work in gonadectomized DBA/2J mice that develop adrenocortical tumors expressing transcription factor GATA-4. 86% of the ferret adrenocortical carcinomas, particularly in areas of myxoid differentiation expressed GATA-4. Normal adrenocortical cells lacked GATA-4 expression. Two other markers of adrenocortical tumors in gonadectomized mice that are co-expressed with GATA-4 are inhibin-alpha and LH receptor. These were co-expressed in some of the ferret tumors. No GATA-4 expression was observed in three cases of nodular hyperplasia; however patches of anaplastic cells expressed GATA-4 in 50% of the tumors classified as adenomas. The conclusion was that GATA-4 does function as a marker of anaplasia in ferret adrenocortical tumors. The relevance of this shows that there may be a way of tracking and marking the tumors (prognostication for the practitioner when advising the client), and pathways of cancer development in the ferrets is similar to that of other species. This also is suggestive of a genetic root to the development of the disease, as GATA-4 is a protein marker.

GENETIC RESEARCH

In humans, the appearance of benign or malignant proliferations within two or more endocrine glands is nearly always genetically determined and is termed multiple endocrine neoplasia (MEN) syndrome. There are three currently accepted human familial syndromes in which there is a progression from hyperplasia to neoplasia in endocrine tissues: MEN types 1 (MEN1), 2a (MEN2a), and 2b (MEN2b). MEN1 syndrome usually is characterized by parathyroid hyperplasia, pancreatic islet cell and/or pituitary tumors. Up to 40% of MEN1 patients also develop adrenal, thyroid or thymic tissue tumors. MEN2a and MEN2b syndromes are characterized primarily as medullary thyroid cancer (MTC) with or without pheochromocytomas and parathyroid adenomas. MEN1 and MEN2 are inherited as autosomal-dominant genetic traits. The MEN1 gene is ubiquitously expressed and is not limited to organs affected by the syndrome. A number of different mutations have been described for the MEN1 gene in humans. As there seems to be a similarity between the endocrine neoplasm patterns in the ferret and the human MEN1 syndrome, research being conducted by Dr. Hawkins is first looking for a homologous gene in the ferret to the human MEN1 gene.

DNA has been collected from buccal swabs and flash frozen tumor tissue. Polymerase chain reaction (PCR) primers from human, dog, and cow were used. Tissue culture of cancer (adrenal, thyroid and/or pancreas) cells has not been as good at yielding chromosomes for karytyping due to the clumping during tumor growth. Ferret whole blood also provided enough material for analysis. G-band karyotyping is being done using standard cytogenetic methods.

Affected ferret's chromosomal material was sequenced and both an MEN-1 and a Ret oncogene have been sequenced. The MEN-1 has a 99.40% homology with the dog MEN-1; 97.70% homology with the human gene, and 98.10% homology with bovine. The Ret has a 100% homology with dog, 97.60% with cat, 94.00% with human, but only a 75.90% with mouse. So far, no mutations have been identified. There is 100% conservation of nucleotide base pairs for both genes in all individuals, with 0% variability. This is extremely unusual as genetic material usually even among purebreds and genetically designed mice, for example, has some degree of variation.

Dr. Wagner's research has been concentrated on the p53 tumor suppressor gene and the CHEK-2 oncogene in tumor tissues. At this writing, identification of some aberrant p53s has been found.

For all of the genetic work, population genetics and an evaluation of kinship is ongoing. This looks at a panel of 30 microsatellite DNA cancer genes such as found in material from sea otters and mink, other members of the Mustelidae. A partial cancer pedigree needs to be constructed within worldwide ferret populations. Genetic material from ferrets around the world, and if possible from breeders where there are several generations of non-affected ferrets is needed. To date, material looked at from affected ferrets worldwide has virtually no genetic variation. It may be necessary to look at the genetics of the European polecat, *Mustela putorius*, that is the direct ancestor of the domestic ferret.

If genetic variation is so limited, identification of non-aberrant genes and/or regulators becomes problematic. Ferrets will need to be found without the abnormalities. Tissue typing for histocompatibility proteins should also be done to continue work on the genetic pedigree. Therefore, at present, clinicians need to continue to identify medical and surgical management of neoplasia for the individual ferret.

REFERENCES

1. Schoemaker NJ, Hawkins MG. Hyperadrenocorticism in ferrets: clinical updates. Proc Assoc Avian Vet: Assoc Exotic Mammals, Providence, RI, 2007: 79-84.