Molecular biological and genetic investigations of cancers in companion animals: impact on veterinary care and comparative value for (rare) cancers in the human.

Gerard R Rutteman
Utrecht University Clinic of Companion Animals
Veterinary Specialist Center de Wagenrenk
NL

Framework for comparative research
- Standardized record keeping
- Standard pathological classification (WHO)
- Storage of DNA, plasma/serum
- Storage of tissue (rapid freezing, RNA-later, FFPE-blocks)
- Collection of DNA from healthy veterans
- Development of specific cell lines

Progestins in the Dog may lead to:
Increased Growth Hormone production in mammary tissue: Potential role in mammary tumorigenesis

GH in mammary tumorigenesis
GH mRNA and protein in mammary tissue & tumors

Progestin-induced GH stimulates proliferation of stem cells in human breast epithelium
» (Lombardi ea, 2014)
Osteosarcoma in the dog

- Most appendicular
- Highly metastatic (>95%)
- Large breeds predisposed (Great Dane, Irish Wolfhound, St Bernard, Leonberger, Rottweiler, etc)

Growth / GH permissive factor?

Value of comparative research in dog/cat

- May be of use to the human
- Chance to increase financial support... ...

- For HS research in BMD, my estimate of funding: 10% veterinary resources, 90% human (at present >> 10 million Euro)

Recognition of risk for MH (HS) in BMD

- Moore & Rosin, Vet Pathol, 1984

Cancer Predisposition in Bernese Mountain Dog

- Life time risk for malignant histiocytic tumours may be 15-20% (Erich, e.a., 2013)
- Excess >500x (compared to other breeds)
Visceral HS (earlier “MH”)

* Spleen, liver, lung, BM
* Rapid multifocal development

Tumor often near joint:
Non-visceral HS
- Destructive
- Highly metastatic

HS in other organs

* Kidney,
* Stomach, intestine
* Skin (differentiate from SH)
* Head & Neck
* Bladder
* CNS
* Lymph nodes (metastatic?)

Review initial tumor diagnosis in FCR & BMD

- Selection of records from 4 labs in NL + Cambridge in UK
- Pathology (n=894) and cytology (n=396)
- Revision including IHC
  - Of cases with HS (1st diagnosis) 75% confirmed.

(Erich et al, 2018)

Follow up of Veterans (BMD / FCR)

- Data and DNA collected at 8 years or older
- 164 BMD
- 6 dogs developed HS
- 34 developed cancer (in 1/3 suspicion of HS)
- Thus: ¼ eliminated from genetic analysis
- 40 lost to follow up.

Potential association of HS risk with other cancers

* Hemangiosarcoma (concurrent in some families)
* Mal. Lymphoma (concurrent in individual dogs)
Risk of mammary cancer

“The population-based incidence rates (for all ages) of malignant mammary tumours per 1000 female dogs per year were 35.47 in boxers, 3.87 in Bernese mountain dog”

L. Moe, J Reprod Fertil, 57 (suppl) 2001

What protects BMD from mammary cancer?

Potential factors influencing mammary cancer (MC) risk

• Function endocrine system?
• Function immune system

What if factors predisposing for HS protect from MC?

Gene expression in histiocytic sarcomas

STHS: primary tumor soft tissue often near joints / tendons; metastasis in most dogs < 1 year
VHS: primary tumor visceral organs, often multiple and generalized at 1st presentation; metastasis in most dogs < 4 months

(Dobson J, 2009; Erich SA et al, 2013)

Gene expression in histiocytic sarcomas

- Fresh-frozen tissue from FCR with HS (STHS and VHS; CD18 confirmed)
- Normal canine spleens → RNA extraction
- Microarray analysis and pathway analyses
- Confirmation using quantitative real-time PCR (qPCR) analyses

Comparison of HS - Spleen

319 Probes differentially expressed (P<0.05) when 4-fold changes or larger were taking into account.

Altered expression of nine genes confirmed by qPCR.
- Down-regulated: PPBP, SpiC, VCAM1, ENPEP, ITGAD
- Up-regulated: GTSF1, Col3a1, CD90, LUM

(Boerkamp et al, 2014)

PPBP also downregulated in human myeloma
Comparing HS/ Spleen

DAVID pathway analyses revealed various pathways that were significantly involved in the development of HS in general, most of which were involved in the DNA repair and replication process.

Comparing VHS/ STHS

191 Probes were significantly differentially expressed

QPCR confirmed the significantly altered expression of three genes.
- Up-regulated: C6
- Down-regulated: CLEC12A, CCL5

CLEC12A: cell adhesion, negative regulator of granulocyte and monocyte function

(Dys)function of disease genes

Knowledge of function of genes involved in diseases such as HS, improves the chance of just intervention (prevention / treatment)

Translation of expression studies in cancer

- Immune-histochemistry / proteomics
- Functional assays
- Tumor – stroma (ECM) interactions
- Comparison with human cancers

Thanks

- To all dog owners that contributed
- And to collaborating breed societies
- And funding organizations: KWF, St DOG, EC, DRC, FRC, VBSH, NBSV, NLRV, SSV, RCS, Albert Heim Stiftung

Exposition Art Drager Meurtant

**HS in Bernese Mountain Dog**

MTAP-CDKN2A disease allele:

Cases: homozygote 65%, heterozygote 31%
Controls: homozygote 24%, heterozygote 60%

Major difference, not yet sufficient to use for selection

Also at CFA14 there is significance, but less so in USA:
European BMDs must provide the answer

---

**Histiocytic sarcomas in FCR**

Preliminary results of GWAS (NIH)

- Locus MH/HS in BMD: not relevant
- Two genomic regions highly significant variation between cases and controls: ongoing.