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Genomic and proteomic profiling for cancer diagnosis in dogs

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Abstract

Global gene expression, whereby tumours are classified according to similar gene expression patterns or ‘signatures’ regardless of cell morphology or tissue characteristics, is being increasingly used in both human and veterinary fields to assist in cancer diagnosis and prognosis. Many studies on canine tumours have focussed on RNA expression using techniques such as microarrays or next generation sequencing, however, proteomic studies combining two-dimensional polyacrylamide gel electrophoresis or two-dimensional differential gel electrophoresis with mass spectrometry have also provided a wealth of data on gene expression in tumour tissues. In addition, proteomics has been instrumental in the search for tumour biomarkers in blood and other body fluids.

Keywords: Biomarkers; Gene expression signatures; Mass spectrometry; Microarray; Next generation sequencing

Introduction

A diagnosis of ‘cancer’ can be challenging in both dogs and man. Historically, it has relied on histopathological interpretation of biopsy samples with an emphasis on cell morphology and tissue architecture, using immunohistochemical (IHC) stains to confirm the precise cell of origin or specify subtype, to help with treatment decisions and estimates of prognosis.

Examination of single gene product expression by IHC can be useful diagnostically and practical to assess in the clinical laboratory setting, but more recently global gene expression is being used in both human and veterinary fields. This classifies tumours according to similar gene expression patterns or ‘signatures’ consisting of several tens or hundreds of genes, regardless of cell morphology or tissue characteristics. Ultimately, gene expression patterns from individual tumours may direct personal patient treatment plans, using therapy targeted to specific pathways of gene expression (Chantrill et al., 2015). The feasibility of prospective molecular profiling of canine cancers for personalised medicine (PMed) has already been investigated successfully, making it a practical option for clinical use (Monks et al., 2013; Paoloni et al., 2014). In the shorter term, genomic profiling can usefully identify a few key genes for which IHC or RT-PCR assays can be developed for clinical diagnostic use.

Global gene expression is most easily measured using cellular RNA (Nambiar et al., 2005), however protein expression gives a more dynamic view, providing information on protein interactions, alternative splicing and post-translational modifications, as well as protein abundance (Maes et al., 2015). Global protein expression may therefore be of more use clinically than genomics, since it is proteins that perform most biological processes.

Most genomic and proteomic profiling studies focus on tumour tissue, however, since biopsy samples can be time consuming, stressful and expensive to obtain, the use of tumour biomarkers in blood or other body fluids (urine, cerebrospinal fluid) for diagnosis and monitoring of treatment response is an attractive tool for clinicians (McCaw et al., 2007). In addition, circulating tumour DNA released from tumour cells in the blood can reflect the genomic changes in the tumour itself and is an exciting prospect for determining tumour gene expression from the blood (Schaefer et al., 2007; Forshew et al., 2012; Bidard et al., 2013).

This review will summarise recent developments in genomic and proteomic profiling of canine tumour tissue and blood, which could be of value in diagnosis and therapeutic decisions. It includes the most relevant publications, but is not an exhaustive list of all veterinary studies using genomic and proteomic profiling.

Genomic Profiling - techniques

Sequencing of the canine genome (Lindblad-Toh et al., 2005) and more recent improvements in genome detail (Hoeppner et al., 2014) have facilitated high throughput genomic or gene expression profiling (GEP) of canine tumour tissues.

Initial global gene expression studies in dogs used microarray techniques whereby extracted RNA was converted to a fluorescently labelled cDNA and hybridised to DNA coding sequences attached to a solid surface platform (Nambiar et al., 2005); either cDNA fragments (Buishand et al., 2013) or short synthesised oligonucleotide segments (25-60bp) representing the whole canine genome (Klopfleisch et al., 2010b; Scott et al., 2011). The

location and intensity of the cDNA identified which genes were expressed and their level of expression (Nambiar et al., 2005).

Since microarray techniques rely on hybridisation between nucleic acids, cross-hybridisation makes analysis of highly related sequences difficult, prior knowledge of gene sequences being investigated is necessary to attach these to the array, and detection of low abundance genes is difficult (Shendure, 2008). More recently, massively parallel sequencing of nucleic acids (next generation sequencing, NGS) has proved more popular because it does not rely on hybridisation and provides extra information on splice variants, polymorphisms and possible mutations. Interpreting the significance of mutations can be challenging, however, since many are bystander mutations which do not lead to cell transformation and in addition, redundancy in cancer pathways means that the biological impact of gene modifications is not always immediately apparent as an oncogenic effect. For transcriptomic NGS (RNA-Seq), RNA is converted to cDNA fragments, adaptors are attached to one (single-end sequencing) or both ends (pair-end sequencing) and fragments are sequenced to give reads of 30-400base pairs depending on the technology used. Resulting reads are either compared to a reference genome or assembled without the genomic sequence to give detail of gene expression and transcript structure. With decreasing costs, NGS is now preferred over microarray methods (Wang et al., 2009), although both techniques require enormous bioinformatic input for manipulation and interpretation of the data.

Genomic profiling - common veterinary tumour types

GEP has been conducted in a variety of canine tumour types (Table 1), although the data obtained are not yet in diagnostic use in most cases.

Lymphoma

Conventional diagnosis of canine lymphoma is based on either cytological or histological interpretation of cell morphology and architecture, often using human classification systems (Teske et al., 1994; Fournel-Fleury et al., 1997; Vezzali et al., 2010; Valli et al., 2011). Application of the revised WHO classification scheme has identified the most common canine subtypes as being diffuse large B cell (DLBCL; 48%), peripheral T cell lymphoma not otherwise specified (PTCL-NOS; 14%), T-zone lymphoma (13%; TZL), T-cell lymphoblastic lymphoma (T-LBL; 4%) and marginal zone lymphoma (4%; MZL) (Valli et al., 2011) although additional criteria such as phenotype, cytogenetic and molecular changes are rarely available for dogs as part of their routine diagnostic investigations.

Molecular profiling of canine lymphoma is still in its infancy, however, a microarray analysis of 35 lymphomas of the six most common subtypes (Frantz et al., 2013) was able to split the tumours into B (DLBCL, MZL, Burkitt and Burkitt-like lymphoma [BL]) and T cell phenotypes (T -LBL, TZL, PTCL-NOS) according to gene expression. Furthermore a clear difference in gene signature split the T cells into low and high grade tumours but the B cell tumours were less easily separated. Mindful of the need for their data to be diagnostically relevant, the researchers developed a benchtop diagnostic test based on qRT-PCR of four genes that could reliably classify an independent cohort of 17 canine lymphomas into the three main subgroups. To distinguish B or T phenotype, the ratio of *CD28* to *ABCA5* expression was calculated with a value of >1 indicating T cell, and <1 indicating B cell. To separate each T cell tumour according to grade, the expression ratio for *CCDC3* to *SMOC2* was calculated with a value of >1 indicating low grade T cell tumour and <1 indicating high grade. Although technically possible to carry out qRT-PCR within the scope of a diagnostic laboratory, it remains to be seen whether this gene expression test is adopted commercially.

Other research groups have focussed on molecular subtyping of DLBCL, the most common subtype in both dogs and man. Human DLBCL is further divided into activated B cell (ABC) or germinal centre B cell (GCB) on the basis of gene expression, an important prognostic classification with only 16% of ABC patients alive at 5 years compared to 76% of GCB patients (Richards et al., 2013; Richards and Suter, 2015). The ABC subtype is characterised by B cell receptor pathway signalling and constitutive canonical NF-kB activity and a similar subtype with NF-kB activity has been identified in dogs (Gaurnier-Hausser et al., 2011; Mudaliar et al., 2013; Richards et al., 2013). Although the gene signatures that typically separate human ABC and GCB DLBCL do not separate dog DLBCL as reliably, the pathways and biologic processes that distinguish GC and post GC groups are shared between species (Richards et al., 2013). A canine specific set of differentially expressed genes separates two distinct groups with significantly different survival as do IgH somatic hypermutations (ongoing or static). Immunohistochemical algorithms based on antigens expressed by GC or post GC cells (CD10, BCL6 and MUM1) are used to identify the ABC/GCB subtypes in man as a diagnostically more useful surrogate for GEP, however, in dogs IHC seems less useful since only CD10 stained a moderate number of DLBCL samples, with BCL6 and MUM1/IRF4 rarely expressed (Richards et al., 2013). Unfortunately this means diagnostic application of DLBCL subtyping by IHC will be more complicated in the dog until new markers can be identified and tested.

The molecular classification of canine lymphoma holds promise for future therapeutic developments. Intra-nodal injection of an NF-º B essential modulator-binding domain peptide in four dogs with relapsed DLBCL expressing NF-º B, produced a marked reduction in tumour mass in three cases by inhibiting NF-º B expression (Gaurnier-Hausser et

al., 2011), and oral administration of the bruton tyrosine kinase inhibitor PCI-32765 (ibrutinib) to block B cell receptor signalling in eight treatment naïve or relapsed lymphoma dogs produced a partial response (3/8) or stable disease (3/8) (Honigberg et al., 2010). Thus with more accurate classification of canine tumours based on gene expression, more tailored and targeted drug therapy should be possible for individual patients.

Mammary tumours

GEP of canine mammary tumours is still in its infancy but has much potential when compared to molecular classification of human breast cancer. Although the IHC detection of oestrogen receptor (ER), progesterone receptor (PR), and Human epidermal growth factor receptor 2 (HER2) in human breast cancer has been routine diagnostic practice for decades to assist with prognosis and therapeutic intervention, genomic profiling has greatly refined their usage. GEP of human breast tumours using microarray analysis first identified four major groups with different gene signatures: luminal, HER2 enriched, basal-like and normal (Perou et al., 1999; Perou et al., 2000). Subsequent studies split the luminal group into luminal A (high ER expression) and luminal B (low to moderate ER expression), and showed gene expression patterns could be linked to overall survival, disease relapse, metastasis and chemotherapy response (Toss and Cristofanilli, 2015). Since then, these molecular subtypes have been adopted in routine clinical practice, but for ease of clinical diagnostics, they are identified using the three basic IHC markers, with treatment tailored accordingly e.g. endocrine therapy for luminal A and B, and HER2 therapy for HER2-enriched. The basal-like subtype, recognised as carrying a poor prognosis was originally defined as being triple (ER, PR, HER2)-negative (TN), but more recently it has been emphasised that the two terms are not synonymous, with basal-like tumours forming only a proportion of triple negative tumours (Foulkes et al., 2010). Molecular classification of TN tumours has identified six gene

expression subtypes: two basal-like, an immunomodulatory, a mesenchymal, a mesenchymal stem-like and a luminal androgen receptor (AR) subtype (Lehmann et al., 2011; Turner and Reis-Filho, 2013). Since the AR luminal subtype has a better overall survival, it is important to distinguish these from basal-like tumours especially since recent clinical trials with androgen receptor antagonists have shown therapeutic benefit.

Since malignant canine mammary tumours share many clinical properties with their human counterparts, several IHC studies have examined whether the same molecular subtypes can be identified in dogs. One study (Gama et al., 2008) used an IHC panel based on five markers (ER, HER2, cytokeratin 5, p63 and P-cadherin) to evaluate 96 malignant carcinomas (simple or complex) and carcinosarcomas and identified the four main human subtypes: luminal A (44.8%), luminal B (13.5%), HER2 (8.3%) and basal (29.2%). In contrast, a smaller IHC study on 44 mammary carcinomas (simple or complex) and one squamous cell carcinoma identified luminal A and B and basal subtypes, but no HER2 enriched tumours (Sassi et al., 2010). The basal subtype was associated with a poor prognosis (shorter overall and disease-free survival) in the first, but not the second study. A more recent study focussed on canine mammary tumours which were phenotypically TN in an attempt to distinguish these from the basal-like breast cancer molecular subtype (Kim et al., 2013). Of the 45 TN tumours (33 simple or mixed carcinomas and 12 special types) examined, 43 expressed at least one basal marker (CK14, 5/6, p63 or EGFR), and 34 expressed more than two markers and were therefore considered to have a basal-like phenotype with a worse prognosis (Kim et al 2013). Although these three studies show that there may be some overlap of the molecular subtypes between dog and man, it has yet to be shown that the full molecular signatures are similar between the two species. More studies, as well as standardisation of methodologies in terms of antibody clone and dilution, and antigen

retrieval treatment are needed before diagnostic laboratories are likely to adopt IHC with these markers as routine procedure. While subtyping of human breast tumours determines treatment selection, the benefit of anti-estrogens and antiHER2 therapy has not been established in dogs, meaning that subtyping may have less therapeutic value in this species.

GEP studies on small numbers of canine mammary tumours have been conducted; however, data are limited and have not yet translated to routine diagnosis. Using a dog specific cDNA microarray, one study examined gene expression in 21 mammary tumours compared to normal tissue and progesterone-induced mammary hyperplasia (Rao et al., 2009). The mammary tumours differentially expressed genes involved in cell motility, cytoskeleton organisation and extracellular matrix production, although the expression signatures of benign (n = 4) and malignant (n = 17) tumours, which might be of more use diagnostically, were not compared (Rao et al., 2009). A microarray study on 18 mammary carcinomas compared gene expression to histological grade (Pawlowski et al., 2013b). Five key genes were identified as being either upregulated (*sehrl, mipep, relaxin*) or downregulated (*magi3, zfp37*) in high grade tumours, and protein expression was verified by IHC to demonstrate that this gene set has potential to distinguish high grade malignancy (Pawlowski et al., 2013c). Using different microarrays, the same researchers also compared six low and six high grade tumours and found that poorly differentiated tumours often contain upregulated chemokine and cytokine mediated signalling pathways (Pawlowski et al., 2013a), consistent with conventional histological grading of tumours which often takes into account presence of inflammatory response as one criterion of malignancy (Ehrhart et al., 2013). Although canine metastatic carcinomas can be differentiated from both normal mammary gland and non-metastatic carcinomas by global gene expression (Klopfleisch et al., 2010b, 2011), a small number of key genes that could be detected by IHC in a diagnostic setting to

predict metastatic potential, has not been finalised as yet. Further microarray studies on isolated populations of cancer cells separated from background stromaare needed to clarify the genetic contribution of each cell type to breast cancer progression.

Canine mammary tumours may be complex (epithelial and myoepithelial components) or mixed (osseocartilaginous) as well as forming simple carcinomas as in human breast cancer and the underlying molecular mechanisms for each still need to be established.. Using paired-end whole genome sequencing, whole-exome sequencing and RNA-seq, Liu et al (2014) showed that while mammary carcinomas have extensive genomic aberrations, complex tumours contain mostly epigenomic aberrations (Liu et al., 2014). A further study using microarray analysis separated gene expression of canine mammary carcinomas from sarcomas and identified homeobox genes as overexpressed in the latter (Wensman et al., 2009). Such studies may have diagnostic potential in particularly undifferentiated tumours and further work may relate these gene signatures to prognosis or to key pathways for targeted chemotherapy.

In human medicine, five commercial multi-gene profiling tests have been developed to analyse individual patient breast tumour samples and provide prognostic information based on more reliable, reproducible and less subjective techniques than IHC (Toss and Cristofanilli, 2015). Some just predict low or high risk e.g. MammaPrint (70 genes), but the Prediction Analysis of Microarray (PAM50–50 genes) and BluePrint (80 genes) determine the four major intrinsic subtypes (Luminal A/B, HER2-enriched or basal-like) which is helpful for treatment decisions and prognosis. The comparative predictive value of these tests is still being evaluated in independent clinical trials. Ultimately, similar tests could be developed for dogs, if GEP of mammary tumours reveals defined subtypes.

Soft tissue sarcomas

Peripheral nerve sheath tumours (PNST) and fibrosarcomas (FSA) can be difficult to distinguish by morphological characteristics alone or using IHC markers. Klopfleisch et al (2013) therefore looked at gene expression in five PNST and five FSA using microarrays and identified 45 gene products that were differentially expressed (Klopfleisch et al., 2013). Seven genes known to be specifically expressed in neuroectodermal tissues were upregulated in PNSTs compared to eight genes associated with carcinogenesis which were more highly expressed in FSA. Interestingly, when the same group tried to validate RT-PCR assays for these genes to differentiate PNST from FSA which had been classified using conventional IHC markers (S100, laminin and PGP9.5), the potential PNST markers *GLI1* and *CLEC3B* were able to differentiate the two tumour types with a sensitivity of 89% and specificity of 87%, while the potential FSA markers *FHL2-Ex4* and *FHL2-Ex9* were unable to separate the tumour types reliably (sensitivity 50%, specificity 88%) (Meyer and Klopfleisch, 2014).

As well as distinguishing between different types of soft tissue sarcoma, genomic profiling can assist subclassification within sarcoma types. Microarray analysis of 15 Histiocytic sarcomas in the Flatcoated retriever identified nine genes that separated tumour from normal spleen (five downregulated; four upregulated) (Boerkamp et al., 2013) but also identified further genes that were differentially expressed between the two clinical presentations of the disease: the soft tissue form affecting limb muscles and joints and the visceral form affecting internal organs with widespread dissemination (Boerkamp et al., 2014). qRT-PCR confirmed the differential expression of three genes: *C6* upregulated and *VLEC12A* and *CCL5* downregulated in the visceral compared to the soft tissue form. Whilst this could be adopted as a useful assay in a diagnostic laboratory, it is arguable that the two

forms of the disease are generally distinguished quite easily by clinicians, without the need for such a test.

GEP of canine haemangiosarcomas has also helped identify tumour subtypes. After candidate gene investigations had failed to show abnormalities in the PTEN/Akt pathway, *VHL* or *Ras* genes, microarray analysis was used to examine global gene expression of ten haemangiosarcoma samples cultured to enrich for endothelial cells prior to analysis and compared to four splenic haematomas (normal endothelium) (Tamburini et al., 2010). Gene expression data indicated that the two main processes central to the pathogenesis of canine haemangiosarcoma were inflammation and angiogenesis and it was hypothesised that, rather than the inflammatory component being recruited to the tumour microenvironment, it may be derived from a shared haematopoietic/endothelial progenitor i.e. a single lineage gives rise to both endothelial and haematopoietic progenitors. Subsequent genome-wide expression profiling of 24 canine haemangiosarcoma tissue samples using NGS supported the idea of a multipotent progenitor differentiating into three distinct subtypes: angiogenesis, inflammation and adipogenesis (Gorden et al., 2014). The three gene signatures were then validated in 47 other tumour samples using RNA-seq analysis. It remains to be seen whether these subtypes have different clinical outcomes and whether diagnostic assays to differentiate them would be of value.

Osteosarcoma

Gene expression studies of canine osteosarcoma (OSA) have focussed on the comparative nature of the disease with its human counterpart. Microarray analysis of 15 dog and 15 paediatric osteosarcomas was not able to distinguish the human and canine diseases by their gene expression signatures and two genes consistently expressed in dog tissues (*IL-8*

and *SLC1A3*) were associated with a poor outcome in an independent group of children with OSA (Paoloni et al., 2009). The heterogeneous nature of the tumours has hampered genomic analysis, so another study of 26 OSA used low-passage cells lines to filter out stromal contributions (Scott et al., 2011). This identified two groups of tumours with robustly different gene signatures and which had significantly different survival times. The gene signatures were used to segregate reliably, further data sets from dogs and humans, suggesting there is potential for this to be applied in a diagnostic setting, if the key genes in the signature could be narrowed down. A previous microarray study on 32 OSA frozen tissue samples was also able to distinguish two groups based on long and short survival times with upregulated genes in the short survivors involved in proliferation, drug resistance or metastasis (Selvarajah et al., 2009).

Mast cell tumour

Differentiation of low grade from high grade mast cell tumours (MCT) is difficult based on histological appearance alone despite the used of different grading systems (Kiupel et al., 2011; Patnaik et al., 1984). A recent microarray study of 51 canine MCT was unable to separate the samples clearly into well-defined groups on the basis of unsupervised gene expression clustering because of too much sample variability, however comparison of differentiated and undifferentiated tumours based on histological criteria, identified clear differences in gene expression relating to cell cycle, DNA replication, p53 signalling, nucleotide excision repair and pyrimidine metabolism (Giantin et al., 2014). The two groups had significantly different survival times, and four genes in particular, *FOXM1*, *GSN*, *FEN1* and *KPNA2* were linked to MCT-related mortality. For practical diagnostic purposes, qRT-PCR assays were developed for 13 transcripts which most reliably separated differentiated

and undifferentiated tumours, and the authors suggest these could be developed as a useful and cheap benchtop diagnostic test to predict MCT outcome regardless of histological grade.

Proteomic profiling - techniques

Protein expression profiling in clinical practice has lagged behind genomic techniques in both human and veterinary fields (Kycko and Reichert, 2014; Maes et al., 2015) but has great potential, particularly in identifying cancer biomarkers to assist with diagnosis, treatment selection and monitoring of patients.

To detect proteins of both high and low abundance, protein extracts must be fractionated using techniques such as liquid chromatography (LC) or gel electrophoresis (GE) (Maes et al., 2015; Matharoo-Ball et al., 2008). Two dimensional polyacrylamide gel electrophoresis (2D-PAGE) which separates according to charge (isoelectric focusing) before molecular weight achieves better resolution than 1D-PAGE. To identify the separated proteins, bands or spots visualised by staining are excised, digested with proteases to small fragments which are then ionized and analysed by mass spectrometry (MS). Electrospray ionization (ESI) is usually combined with LC in tandem (LC-MS/MS) whereas matrix-assisted laser desorption/ionization (MALDI) or surface enhanced laser desorption/ionization (SELDI) are often combined with time-of flight (TOF) MS. The peptide sequences obtained by MS are matched to known protein databases to identify the relevant proteins in a 'bottom-up' approach. The alternative 'top-down' approach in which intact proteins are identified without prior enzymatic digestion (Gregorich and Ge, 2014) results in reduced sample complexity but suffers from molecular weight limitations in protein solubility, separation and MS analysis (Matharoo-Ball et al., 2008; Gregorich and Ge, 2014).

353 To overcome the low throughput limitations of 2D-PAGE (one sample on one gel),
354 two dimensional differential gel electrophoresis (2D- DIGE) has been developed, in which
355 two samples and an internal control are labelled with different fluorescent cyanine dyes, then
356 run together on a single gel to allow the ratio of the sample proteins to be determined at each
357 separated spot on the gel (Matharoo-Ball et al., 2008; Maes et al., 2015).
358
359 **Proteomic Profiling – common veterinary tumour types**
360 A limited number of veterinary studies have used proteomics (Table 2), most
361 comparing gene expression in tumour to normal tissue. Formalin fixed paraffin embedded
362 sections are less reliable than fresh-frozen tissue, particularly for the high molecular weight
363 subproteome (Tanca et al., 2012; Tanca et al., 2013). Blood and other body fluids including
364 dog tears have also been examined in the search for biomarkers to diagnose and monitor
365 cancer patients (de Freitas Campos et al., 2008).
366
367 *Lymphoma*
368 *Tissue*
369 Only one study has examined canine lymphoid tissue using proteomics, comparing
370 11 lymph nodes from dogs with B cell lymphoma to 13 from normal dogs using 2D-PAGE.
371 Ninety-three differentially expressed spots analysed by MALDI-TOF MS revealed three
372 down regulated (prolidase, triosephosphate isomerase and glutathione S transferase) and one
373 upregulated protein (macrophage capping protein) in lymphoma (McCaw et al., 2007).
374 Further studies are needed to confirm whether these proteins could represent potentially
375 useful biomarkers for diagnosis or classification.
376
377 *Blood*

In contrast, several proteomic studies have examined serum from dogs with lymphoma. One, comparing serum samples from 29 dogs with B cell lymphoma and 87 control dogs (healthy, with non-cancer diseases, or non-lymphoma cancers) using ion exchange chromatography and SELDI-TOF MS identified three protein peaks with biomarker potential (Gaines et al., 2007). These separated the lymphoma cases from the controls in a classification tree with positive and negative predictive values of 78% and 99% (97% sensitivity, 91% specificity, 92% accuracy), however the identity of the proteins was not given.

A second larger study using the same proteomic technique compared serum samples from 87 lymphoma patients and 92 non-lymphoma samples (diseased or healthy). Although 19 serum peaks were differentially expressed, two biomarkers in particular were able to differentiate control and lymphoma patients and when tested on a separate cohort of 96 patients gave positive and negative predictive values of 80% and 88% (sensitivity 75%, specificity 91%) (Ratcliffe et al., 2009). These were later revealed as the acute phase proteins, haptoglobin and C-reactive protein (CRP) which have been combined with a unique algorithm to produce the canine lymphoma blood test (cLBT) (Alexandrakis et al., 2014). Although these proteins are not specific to lymphoma, the cLBT score appears useful in predicting prognosis and monitoring remission status whilst on treatment and is marketed as a commercial diagnostic test (Avacta) for lymphoma.

Using a different 2D gel-based approach combining agarose and PAGE techniques with MALDI-TOF MS, another study (Atherton et al., 2013) examined the sera of three lymphoma cases compared to two normal healthy dogs. Ten proteins were identified in at least one lymphoma case but not in control dogs, with haptoglobin detected in all three

lymphoma cases. Other acute phase proteins, ±2 macroblobulin, inter-±-trypsin inhibitor and ±-chymotrypsin were identified in lymphoma patients, consistent with an inflammatory component being present. Kininogen was present in control sera but absent from all three lymphoma cases. Although interesting, the data need to be verified in a larger cohort of dogs to see whether these proteins have diagnostic potential in the clinical setting.

Another small study on three dogs with lymphoma, two with transitional cell carcinomas of the bladder and seven control dogs, looked specifically at serum glycoproteins using lectin affinity capture prior to global internal standard technology (GIST) stable isotope labelling, LC and MALDI-TOF MS (Wilson et al., 2008). Eleven fucosylated proteins were common to all three dogs with lymphoma and upregulated over 50% compared to controls. In one dog, the fucosylated protein levels fluctuated in response to chemotherapy treatment, suggesting the potential for disease monitoring. Further studies are needed, however, before these proteins can be used diagnostically.

Mammary tumours

Tissue

To search for protein expression differences between long and short survivors with mammary tumours, Klopfleisch et al (2010) compared the proteome of six mammary carcinomas with lymph node metastasis at presentation (survival time < six months) to six mammary carcinomas without metastasis at presentation (metastasis-free survival time > two years). Using 2D-DIGE and MALDI-TOF MS, they identified 11 upregulated and ten downregulated proteins in metastatic tumours, 19 of which were reported previously in metastatic human tumours including breast (Klopfleisch et al., 2010a). Most proteins were involved in cellular functions related to metastatic spread such as proliferation, cell adhesion,

extracellular matrix remodelling, and hypoxia resistance. In a similar study comparing six normal, six benign, six non-metastatic and six metastatic carcinomas, step wise changes in protein expression were identified, with the biggest change in protein expression detected by the switch to metastasis (Klose et al., 2011). Eleven of 48 proteins increased in metastatic tumours were involved in proliferation (PCNA, RAN binding protein 1, phosphoglycerate mutase 1, siderophilin and Rho-GTPase activating protein) and cell motility (tropomyosin 1 and 3, myosin light chain 2, gelsolin and calumenin) and have been identified in the previous proteomic or genomic studies (Klopfleisch et al., 2010a; Rao et al., 2009), suggesting they are promising candidates for prognostic markers or therapeutic targets.

Blood

In a slightly different approach, serum from 15 dogs with mammary tumours and 15 healthy controls (Zamani-Ahmadmahmudi et al., 2014) was hybridised by western blot to a membrane transferred from a 2D-PAGE gel on which proteins extracted from a mammary tumour cell line had been separated. Four immunoreactive autoantigens (manganese-superoxide dismutase, triose phosphate isomerase, alpha-enolase, phosphoglycerate mutase1) were identified by cutting out spots from the original gel and subsequent MALDI-TOF MS, and all four showed higher expression in tumour tissue compared to control samples using IHC and western blotting and stimulated autoantibody responses in human breast cancer. Further work is needed to determine whether these proteins are useful in diagnostic classification or as prognostic biomarkers in dogs.

Prostatic tumours

Tissue

A 2D-DIGE and MALDI-TOF MS study of three canine prostatic tumours compared them to normal prostate or bladder and identified nine proteins differentially upregulated over 2.5 fold; three being significantly overexpressed: keratin 7, glucose-regulated protein GRP78 and endoplasmin (GRP74) (LeRoy et al., 2007). Keratin 7 assists classification of anaplastic human prostatic neoplasms that are of transitional origin, so has diagnostic potential for the dog. A previous IHC study in dogs, however, did not find it helpful to discriminate prostatic neoplasms from transitional cell carcinoma (LeRoy et al., 2004) although others found it correlated with castration status in younger dogs (Sorenmo et al., 2003).

Mast cell tumours

Tissue

In an attempt to find better diagnostic methods to subclassify canine MCT, a proteomic study using 2D-DIGE and MALDI-TOF MS identified 13 proteins which were differentially expressed, with four stress response proteins upregulated in high grade tumours, and proteins associated with cell motility and metastasis either increased or decreased (Schlieben et al., 2012). Only five low and five high grade tumours were examined but their grade was confirmed using both Kiupel and Patnaik grading systems and reduced expression of tryptase on proteomic analysis of the high grade tumours was consistent with their dedifferentiation. This proteomic study was performed prior to the genomic study discussed above (Giantin et al., 2014), however, there was no overlap in the genes identified by the two different methodologies. Further studies are needed to find proteins that reliably differentiate the biological grade of mast cell tumours and can be used in clinical diagnosis.

Conclusion

Although case numbers are small, the existing genomic and proteomic canine studies have already identified key genes and gene signatures which have potential for diagnostic use. Further validation of these marker genes on more clinical samples is now needed before applying them to diagnostic use in clinical pathology laboratories. Although progress is slow, the possibility of individualised patient care based on molecular or proteomic profiling of clinical samples may become reality in the not-too-distant future.

Conflict of interest statement

The author has no financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the review.

References

Alexandrakis, I., Tuli, R., Ractliffe, S.C., Tappin, S.W., Foale, R.D., Roos, A., Slater, K.J., 2014. Utility of a multiple serum biomarker test to monitor remission status and relapse in dogs with lymphoma undergoing treatment with chemotherapy. Veterinary Comparative Oncology doi: 10.1111/vco.12123.

Atherton, M.J., Braceland, M., Fontaine, S., Waterston, M.M., Burchmore, R.J., Eadie, S., Eckersall, P.D., Morris, J.S., 2013. Changes in the serum proteome of canine lymphoma identified by electrophoresis and mass spectrometry. The Veterinary Journal 196, 320-324.

Bidard, F.C., Weigelt, B., Reis-Filho, J.S., 2013. Going with the flow: From circulating tumor cells to DNA. Science Translational Medicine 5, 207ps214.

Boerkamp, K.M., van der Kooij, M., van Steenbeek, F.G., van Wolferen, M.E., Groot Koerkamp, M.J., van Leenen, D., Grinwis, G.C., Penning, L.C., Wiemer, E.A., Rutteman, G.R., 2013. Gene expression profiling of histiocytic sarcomas in a canine model: the predisposed flatcoated retriever dog. Public Library of Science One 8, e71094.

Boerkamp, K.M., van Steenbeek, F.G., Penning, L.C., Groot Koerkamp, M.J., van Leenen, D., Vos-Loohuis, M., Grinwis, G.C., Rutteman, G.R., 2014. The two main forms of histiocytic sarcoma in the predisposed Flatcoated retriever dog display variation in gene expression. Public Library of Science One 9, e98258.

Buishand, F.O., Kirpensteijn, J., Jaarsma, A.A., Speel, E.J., Kik, M., Mol, J.A., 2013. Gene expression profiling of primary canine insulinomas and their metastases. The Veterinary Journal 197, 192-197.

Chantrill, L.A., Nagrial, A.M., Watson, C., Johns, A.L., Martyn-Smith, M., Simpson, S., Mead, S., Jones, M.D., Samra, J.S., Gill, et al. Australian Pancreatic Cancer Genome, I., Individualized Molecular Pancreatic Cancer Therapy Trial Management Committee of the Australasian Gastrointestinal Trials, G., 2015. Precision Medicine for Advanced Pancreas Cancer: The Individualized Molecular Pancreatic Cancer Therapy (IMPaCT) Trial. Clinical Cancer Research 21, 2029-2037.

de Freitas Campos, C., Cole, N., Van Dyk, D., Walsh, B.J., Diakos, P., Almeida, D., Torrecilhas, A., Laus, J.L., Willcox, M.D., 2008. Proteomic analysis of dog tears for potential cancer markers. Research in Veterinary Science 85, 349-352.

Ehrhart, E.J., Kamstock, D.A., Powers, B.E., 2013. The Pathology of Neoplasia. In Withrow and MacEwen's Small Animal Clinical Oncology Eds: Withrow, S.J., Vail, D.M., Page, R.L. 5th Edition, Elsevier, Missouri, p55.

Forshew, T., Murtaza, M., Parkinson, C., Gale, D., Tsui, D.W., Kaper, F., Dawson, S.J., Piskorz, A.M., Jimenez-Linan, M., Bentley, D., Hadfield, J., May, A.P., Caldas, C., Brenton, J.D., Rosenfeld, N., 2012. Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. Science Translational Medicine 4, 136ra168.

Foulkes, W.D., Smith, I.E., Reis-Filho, J.S., 2010. Triple-negative breast cancer. New England Journal of Medicine 363, 1938-1948.

Fournel-Fleury, C., Magnol, J.P., Bricaire, P., Marchal, T., Chabanne, L., Delverdier, A., Bryon, P.A., Felman, P., 1997. Cytohistological and immunological classification of canine malignant lymphomas: Comparison with human non-Hodgkin's lymphomas. Journal of Comparative Pathology 117, 35-59.

Frantz, A.M., Sarver, A.L., Ito, D., Phang, T.L., Karimpour-Fard, A., Scott, M.C., Valli, V.E., Lindblad-Toh, K., Burgess, K.E., Husbands, B.D., et al. 2013. Molecular Profiling Reveals Prognostically Significant Subtypes of Canine Lymphoma. Veterinary Pathology 50, 693-703.

Gaines, P.J., Powell, T.D., Walmsley, S.J., Estredge, K.L., Wisnewski, N., Stinchcomb, D.T., Withrow, S.J., Lana, S.E., 2007. Identification of serum biomarkers for canine B-cell lymphoma by use of surface-enhanced laser desorption-ionization time-of-flight mass spectrometry. American Journal of Veterinary Research 68, 405-410.

Gama, A., Alves, A., Schmitt, F., 2008. Identification of molecular phenotypes in canine mammary carcinomas with clinical implications: application of the human classification. Virchows Archives 453, 123-132.

Gaurnier-Hausser, A., Patel, R., Baldwin, A.S., May, M.J., Mason, N.J., 2011. NEMO-binding domain peptide inhibits constitutive NF-kappaB activity and reduces tumor burden in a canine model of relapsed, refractory diffuse large B-cell lymphoma. Clinical Cancer Research 17, 4661-4671.

Giantin, M., Granato, A., Baratto, C., Marconato, L., Vascellari, M., Morello, E.M., Vercelli, A., Mutinelli, F., Dacasto, M., 2014. Global gene expression analysis of canine

cutaneous mast cell tumor: could molecular profiling be useful for subtype classification and prognostication? Public Library of Science One 9, e95481.

Gorden, B.H., Kim, J.H., Sarver, A.L., Frantz, A.M., Breen, M., Lindblad-Toh, K., O'Brien, T.D., Sharkey, L.C., Modiano, J.F., Dickerson, E.B., 2014. Identification of three molecular and functional subtypes in canine hemangiosarcoma through gene expression profiling and progenitor cell characterization. American Journal of Pathology 184, 985-995.

Gregorich, Z.R., Ge, Y., 2014. Top-down proteomics in health and disease: Challenges and opportunities. Proteomics 14, 1195-1210.

Hoeppner, M.P., Lundquist, A., Pirun, M., Meadows, J.R., Zamani, N., Johnson, J., Sundstrom, G., Cook, A., FitzGerald, M.G., Swofford, R., et al. 2014. An improved canine genome and a comprehensive catalogue of coding genes and non-coding transcripts. Public Library of Science One 9, e91172.

Honigberg, L.A., Smith, A.M., Sirisawad, M., Verner, E., Loury, D., Chang, B., Li, S., Pan, Z., Thamm, D.H., Miller, et al. 2010. The Bruton tyrosine kinase inhibitor PCI-32765 blocks B-cell activation and is efficacious in models of autoimmune disease and B-cell malignancy. Proceedings of the National Academy of Science USA 107, 13075-13080.

Kim, N.H., Lim, H.Y., Im, K.S., Kim, J.H., Sur, J.H., 2013. Identification of triple-negative and basal-like canine mammary carcinomas using four basal markers. Journal of Comparative Pathology 148, 298-306.

Kiupel, M., Webster, J.D., Bailey, K.L., Best, S., DeLay, J., Detrisac, C.J., Fitzgerald, S.D., Gamble, D., Ginn, P.E., Goldschmidt, M.H., et al. 2011. Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behavior. Veterinary Pathology 48, 147-155.

Klopfleisch, R., Klose, P., Weise, C., Bondzio, A., Multhaup, G., Einspanier, R., Gruber, A.D., 2010a. Proteome of metastatic canine mammary carcinomas: Similarities to and differences from human breast cancer. Journal of Proteome Research 9, 6380-6391.

Klopfleisch, R., Lenze, D., Hummel, M., Gruber, A.D., 2010b. Metastatic canine mammary carcinomas can be identified by a gene expression profile that partly overlaps with human breast cancer profiles. BioMedCentral Cancer 10, 618.

Klopfleisch, R., Lenze, D., Hummel, M., Gruber, A.D., 2011. The metastatic cascade is reflected in the transcriptome of metastatic canine mammary carcinomas. Veterinary Journal 190, 236-243.

Klopfleisch, R., Meyer, A., Lenze, D., Hummel, M., Gruber, A.D., 2013. Canine cutaneous peripheral nerve sheath tumours versus fibrosarcomas can be differentiated by neuroectodermal marker genes in their transcriptome. Journal of Comparative Pathology 148, 197-205.

Klose, P., Weise, C., Bondzio, A., Multhaup, G., Einspanier, R., Gruber, A.D., Klopfleisch, R., 2011. Is there a malignant progression associated with a linear change in protein expression levels from normal canine mammary gland to metastatic mammary tumors? Journal of Proteome Research 10, 4405-4415.

592 Kycko, A., Reichert, M., 2014. Proteomics in the search for biomarkers of animal cancer.
593 Current Protein and Peptide Science 15, 36-44.

594 Lehmann, B.D., Bauer, J.A., Chen, X., Sanders, M.E., Chakravarthy, A.B., Shyr, Y.,
595 Pietenpol, J.A., 2011. Identification of human triple-negative breast cancer subtypes
596 and preclinical models for selection of targeted therapies. Journal of Clinical
597 Investigation 121, 2750-2767.

598 LeRoy, B., Painter, A., Sheppard, H., Popiolek, L., Samuel-Foo, M., Andacht, T.M., 2007.
599 Protein expression profiling of normal and neoplastic canine prostate and bladder
600 tissue. Veterinary Comparative Oncology 5, 119-130.

601 LeRoy, B.E., Nadella, M.V., Toribio, R.E., Leav, I., Rosol, T.J., 2004. Canine prostate
602 carcinomas express markers of urothelial and prostatic differentiation. Veterinary
603 Pathology 41, 131-140.

604 Lindblad-Toh, K., Wade, C.M., Mikkelsen, T.S., Karlsson, E.K., Jaffe, D.B., Kamal, M.,
605 Clamp, M., Chang, J.L., Kulbokas, E.J., 3rd, Zody, M.C., et al. 2005. Genome
606 sequence, comparative analysis and haplotype structure of the domestic dog. Nature
607 438, 803-819.

608 Liu, D., Xiong, H., Ellis, A.E., Northrup, N.C., Rodriguez, C.O., Jr., O'Regan, R.M., Dalton,
609 S., Zhao, S., 2014. Molecular homology and difference between spontaneous canine
610 mammary cancer and human breast cancer. Cancer Research 74, 5045-5056.

611 Maes, E., Mertens, I., Valkenborg, D., Pauwels, P., Rolfo, C., Baggerman, G., 2015.
612 Proteomics in cancer research: Are we ready for clinical practice? Critical Reviews in
613 Oncology/ Hematology.

614 Matharoo-Ball, B., Miles, A.K., Creaser, C.S., Ball, G., Rees, R., 2008. Serum biomarker
615 profiling in cancer studies: A question of standardisation? Veterinary Comparative
616 Oncology 6, 224-247.

617 McCaw, D.L., Chan, A.S., Stegner, A.L., Mooney, B., Bryan, J.N., Turnquist, S.E., Henry,
618 C.J., Alexander, H., Alexander, S., 2007. Proteomics of canine lymphoma identifies
619 potential cancer-specific protein markers. Clinical Cancer Research 13, 2496-2503.

620 Meyer, A., Klopfleisch, R., 2014. Multiple polymerase chain reaction markers for the
621 differentiation of canine cutaneous peripheral nerve sheath tumours versus canine
622 fibrosarcomas. Journal of Comparative Pathology 150, 198-203.

623 Monks, N.R., Cherba, D.M., Kamerling, S.G., Simpson, H., Rusk, A.W., Carter, D., Eugster,
624 E., Mooney, M., Sigler, R., Steensma, M., et al. 2013. A multi-site feasibility study for
625 personalized medicine in canines with osteosarcoma. Journal of Translational Medicine
626 11, 158.

627 Mudaliar, M.A., Haggart, R.D., Miele, G., Sellar, G., Tan, K.A., Goodlad, J.R., Milne, E.,
628 Vail, D.M., Kurzman, I., Crowther, D., et al. 2013. Comparative gene expression
629 profiling identifies common molecular signatures of NF-kappaB activation in canine
630 and human diffuse large B cell lymphoma (DLBCL). Public Library of Science One 8,
631 e72591.

Nambiar, P.R., Boutin, S.R., Raja, R., Rosenberg, D.W., 2005. Global gene expression profiling: a complement to conventional histopathologic analysis of neoplasia. Veterinary Pathology 42, 735-752.

Paoloni, M., Davis, S., Lana, S., Withrow, S., Sangiorgi, L., Picci, P., Hewitt, S., Triche, T., Meltzer, P., Khanna, C., 2009. Canine tumor cross-species genomics uncovers targets linked to osteosarcoma progression. BioMedCentral Genomics 10, 625.

Paoloni, M., Webb, C., Mazcko, C., Cherba, D., Hendricks, W., Lana, S., Ehrhart, E.J., Charles, B., Fehling, H., Kumar, L., et al. 2014. Prospective molecular profiling of canine cancers provides a clinically relevant comparative model for evaluating personalized medicine (PMed) trials. Public Library of Science One 9, e90028.

Patnaik, A.K., Ehler, W.J., MacEwen, E.G., 1984. Canine cutaneous mast cell tumor: morphologic grading and survival time in 83 dogs. Veterinary Pathology 21, 469-474.

Pawlowski, K.M., Homa, A., Bulkowska, M., Majchrzak, K., Motyl, T., Krol, M., 2013a. Expression of inflammation-mediated cluster of genes as a new marker of canine mammary malignancy. Veterinary Research Communications 37, 123-131.

Pawlowski, K.M., Maciejewski, H., Dolka, I., Mol, J.A., Motyl, T., Krol, M., 2013b. Gene expression profiles in canine mammary carcinomas of various grades of malignancy. BioMedCentral Veterinary Research 9, 78.

Pawlowski, K.M., Maciejewski, H., Majchrzak, K., Dolka, I., Mol, J.A., Motyl, T., Krol, M., 2013c. Five markers useful for the distinction of canine mammary malignancy. BioMedCentral Veterinary Research 9, 138.

Perou, C.M., Jeffrey, S.S., van de Rijn, M., Rees, C.A., Eisen, M.B., Ross, D.T., Pergamenschikov, A., Williams, C.F., Zhu, S.X., Lee, J.C., et al. 1999. Distinctive gene expression patterns in human mammary epithelial cells and breast cancers. Proceedings of the National Academy of Science USA 96, 9212-9217.

Perou, C.M., Sorlie, T., Eisen, M.B., van de Rijn, M., Jeffrey, S.S., Rees, C.A., Pollack, J.R., Ross, D.T., Johnsen, H., Akslen, L.A., et al. 2000. Molecular portraits of human breast tumours. Nature 406, 747-752.

Rao, N.A., van Wolferen, M.E., Gracanin, A., Bhatti, S.F., Krol, M., Holstege, F.C., Mol, J.A., 2009. Gene expression profiles of progestin-induced canine mammary hyperplasia and spontaneous mammary tumors. Journal of Physiology and Pharmacology 60 Suppl 1, 73-84.

Ratcliffe, L., Mian, S., Slater, K., King, H., Napolitano, M., Aucoin, D., Mobasheri, A., 2009. Proteomic identification and profiling of canine lymphoma patients. Veterinary Comparative Oncology 7, 92-105.

Richards, K.L., Motsinger-Reif, A.A., Chen, H.W., Fedoriw, Y., Fan, C., Nielsen, D.M., Small, G.W., Thomas, R., Smith, C., Dave, S.S., et al. 2013. Gene profiling of canine B-cell lymphoma reveals germinal center and postgerminal center subtypes with different survival times, modeling human DLBCL. Cancer Research 73, 5029-5039.

671 Richards, K.L., Suter, S.E., 2015. Man's best friend: what can pet dogs teach us about non-
672 Hodgkin's lymphoma? Immunological Reviews 263, 173-191.

673 Sassi, F., Benazzi, C., Castellani, G., Sarli, G., 2010. Molecular-based tumour subtypes of
674 canine mammary carcinomas assessed by immunohistochemistry. BioMedCentral
675 Veterinary Research 6, 5.

676 Schaefer, D.M., Forman, M.A., Kisseberth, W.C., Lehman, A.M., Kelbick, N.T., Harper, P.,
677 Rush, L.J., 2007. Quantification of plasma DNA as a prognostic indicator in canine
678 lymphoid neoplasia. Veterinary Comparative Oncology 5, 145-155.

679 Schlieben, P., Meyer, A., Weise, C., Bondzio, A., Einspanier, R., Gruber, A.D., Klopfleisch,
680 R., 2012. Differences in the proteome of high-grade versus low-grade canine cutaneous
681 mast cell tumours. Veterinary Journal 194, 210-214.

682 Scott, M.C., Sarver, A.L., Gavin, K.J., Thayanithy, V., Getzy, D.M., Newman, R.A., Cutter,
683 G.R., Lindblad-Toh, K., Kisseberth, W.C., Hunter, L.E., et al. 2011. Molecular
684 subtypes of osteosarcoma identified by reducing tumor heterogeneity through an
685 interspecies comparative approach. Bone 49, 356-367.

686 Selvarajah, G.T., Kirpensteijn, J., van Wolferen, M.E., Rao, N.A., Fieten, H., Mol, J.A.,
687 2009. Gene expression profiling of canine osteosarcoma reveals genes associated with
688 short and long survival times. Molecular Cancer 8, 72.

689 Shendure, J., 2008. The beginning of the end for microarrays? Nature Methods 5, 585-587.

690 Sorenmo, K.U., Goldschmidt, M., Shofer, F., Goldkamp, C., Ferracone, J., 2003.
691 Immunohistochemical characterization of canine prostatic carcinoma and correlation
692 with castration status and castration time. Veterinary Comparative Oncology 1, 48-56.

693 Tamburini, B.A., Phang, T.L., Fosmire, S.P., Scott, M.C., Trapp, S.C., Duckett, M.M.,
694 Robinson, S.R., Slansky, J.E., Sharkey, L.C., Cutter, G.R., et al. 2010. Gene expression
695 profiling identifies inflammation and angiogenesis as distinguishing features of canine
696 hemangiosarcoma. BioMedCentral Cancer 10, 619.

697 Tanca, A., Pagnozzi, D., Burrai, G.P., Polinas, M., Uzzau, S., Antuofermo, E., Addis, M.F.,
698 2012. Comparability of differential proteomics data generated from paired archival
699 fresh-frozen and formalin-fixed samples by GeLC-MS/MS and spectral counting.
700 Journal of Proteomics 77, 561-576.

701 Tanca, A., Pisanu, S., Biosa, G., Pagnozzi, D., Antuofermo, E., Burrai, G.P., Canzonieri, V.,
702 Cossu-Rocca, P., De Re, V., Eccher, A., et al. 2013. Application of 2D-DIGE to
703 formalin-fixed diseased tissue samples from hospital repositories: results from four case
704 studies. Proteomics Clinical Applications 7, 252-263.

705 Teske, E., Wisman, P., Moore, P.F., van Heerde, P., 1994. Histologic classification and
706 immunophenotyping of canine non-Hodgkin's lymphomas: unexpected high frequency
707 of T cell lymphomas with B cell morphology. Experimental Hematology 22, 1179-
708 1187.

709 Toss, A., Cristofanilli, M., 2015. Molecular characterization and targeted therapeutic
710 approaches in breast cancer. Breast Cancer Research 17, 60.

711 Turner, N.C., Reis-Filho, J.S., 2013. Tackling the diversity of triple-negative breast cancer.
712 Clinical Cancer Research 19, 6380-6388.

713 Valli, V.E., San Myint, M., Barthel, A., Bienzle, D., Caswell, J., Colbatzky, F., Durham, A.,
714 Ehrhart, E.J., Johnson, Y., Jones, C., et al. 2011. Classification of canine malignant
715 lymphomas according to the World Health Organization criteria. Veterinary Pathology
716 48, 198-211.

717 Vezzali, E., Parodi, A.L., Marcato, P.S., Bettini, G., 2010. Histopathologic classification of
718 171 cases of canine and feline non-Hodgkin lymphoma according to the WHO.
719 Veterinary Comparative Oncology 8, 38-49.

720 Wang, Z., Gerstein, M., Snyder, M., 2009. RNA-Seq: A revolutionary tool for
721 transcriptomics. Nature Reviews Genetics 10, 57-63.

722 Wensman, H., Goransson, H., Leuchowius, K.J., Stromberg, S., Ponten, F., Isaksson, A.,
723 Rutteman, G.R., Heldin, N.E., Pejler, G., Hellmen, E., 2009. Extensive expression of
724 craniofacial related homeobox genes in canine mammary sarcomas. Breast Cancer
725 Research and Treatment 118, 333-343.

726 Wilson, C.R., Regnier, F.E., Knapp, D.W., Raskin, R.E., Andrews, D.A., Hooser, S.B., 2008.
727 Glycoproteomic profiling of serum peptides in canine lymphoma and transitional cell
728 carcinoma. Veterinary Comparative Oncology 6, 171-181.

729 Zamani-Ahmadmahmudi, M., Nassiri, S.M., Rahbarghazi, R., 2014. Serological proteome
730 analysis of dogs with breast cancer unveils common serum biomarkers with human
731 counterparts. Electrophoresis 35, 901-910.

732

733 **Table 1**
734 Veterinary studies using genomic profiling.
735

Tumour type	Cases	Technique	Findings	Reference
LSA	17 T cell 17 B cell 1 nonT-nonB cell	Microarray Canine genome 2.0 GeneChip [a]	GEP separates B and T cell tumours and low and high grade T cell tumours. Ratio *CD28:ABCA5* >1 predicts T cell Ratio *CCD3:SMOC2* <1 predicts High grade T	Frantz et al. (2013)
LSA	58 B cell	Microarray Canine genome 2.0 GeneChip [a]	Two separate groups of DLBCL with different progression free and overall survival times, similar to ABC and GCB subtypes in human DLBCL	Richards et al. (2013)
LSA	23 DLBCL 10 normal LNs	Microarray Canine genome 2.0 GeneChip [a]	NF-kBp65 canonical pathway activated as in human DLBCL	Mudaliar et al. (2013
MT	21 tumours (4 benign, 17 malignant) 8 normal mammary tissue 8 progesterone-induced hyperplasia	Microarray Dog specific cDNA	Tumours had ‘ expression of ECM, cell motility and invasion genes (*FN1, SPARC, CTHRC1, FHL2*) and “ expression of cytoskeletal organisation genes (*DES, ACTN1, NEB, DAG1*)	Rao et al. (2009)
MT	18 CA (6 well, 6 moderately, 6 poorly differentiated)	Microarray Dog specific cDNA (as in Rao et al, 2009)	High grade CA have ‘ expression of *Sehrl, mipep, relaxin* and “ expression of *Magi3, zfp37*	Pawlowski et al. (2013b, 2013c)
MT	12 CA (6 well, 6 poorly differentiated)	Microarray Oligo-microarray[b]	High grade CA express myeloid specific antigens and have ‘ expression of chemokine and cytokine mediated pathways (e.g. *S100P*), and ECM/inflammatory response genes (*MMP1 and 3*)	Pawlowski et al. (2013a)
MT	13 LN +ve simple CA 14 LN –ve simple CA	Microarray Canine genome 2.0 GeneChip [a]	Metastatic CA have ‘ expression of cell cycle, matrix modulation, protein folding and proteasomal degradation genes and “ expression of cell differentiation, growth factor pathway and actin organization genes	Klopfleisch et al. (2010)
MT	13 LN +ve simple CA 12 corresponding normal glands	Microarray Canine genome 2.0 GeneChip [a]	Metastatic CA have ‘ cell division and invasion genes (*MMP, SERPINE1, TIMP3*) and“ differentiation (*EGF, EGFR, MAP2K6, STAT 5*), cell adhesion (*CLDN5, CTNNAL1, MUC1, PECAM1*), angiogenesis (*ANGPT 2, ANGPTL1-4, FIGF, TIE1*) and membrane receptor (*EGFR, FGFR1, GHR, PDGFR, TGFBR, TIE1*) genes	Klopfleisch et al. (2011)
MT	7 simple CA 4 complex CA	NGS [C]	Extensive genomic aberrations in simple CA. Epigenomic changes in complex CA.	Liu et al. (2014)
MT	7 simple CA 6 FSA 5 OSA 4 normal tissues	Microarray Canine genome 2.0 GeneChip [a]	CA expressed epithelial markers and cell adhesion genes. SA expressed mesenchymal differentiation genes and homeobox genes previously linked to craniofacial bone formation	Wensman et al. (2009)
STS	5 FSA 5 PNST	Microarray Canine genome 2.0 GeneChip [a]	Neuroectodermal genes (*FMN2, KIF1B, GLI1, ROBO1, NMUR2, DOK4* and *HMG20B*) ‘ in PNSTs. Carcinogenesis genes (*FHL2, PLAGL1, FNBP1L, BAG2, HK1, CSK* and *Cox5A*) ‘ in FSAs	Klopfleisch et al. (2013)
HisSA (FCR)	7 visceral tumours 6 soft tissue (limb)	Microarray Canine Gene	*PPBP, SpiC, VCAM1, ENPEP, ITGAD* “ and *GTSF1, Col3a1, CD90* and *LUM* ‘ in both	Boerkamp et al. (2013)

	tumours 6 normal spleen	Expression V1 [b]	forms of HisSA compared to normal tissue	
HisSA (FCR)	8 visceral tumours 7 soft tissue (limb) 8 pooled normal organs	Microarray Canine Gene Expression V1 [b]	*C6* was ‘ and *CLEC12A* and *CCL5* were “ in the visceral compared to the soft tissue form	Boerkamp et al. (2013)
HSA	10 tumour samples 4 non tumour (low passage cell culture)	Microarray Canine genome 2.0 GeneChip [a]	‘ of genes involved in inflammation, angiogenesis, adhesion, invasion, metabolism, cell cycle, signaling, and patterning.	Tamburini et al. (2010)
HSA	12 tissue samples & 18 cell lines (microarray) 35 tissue samples (NGS), 12 samples (microarray and NGS)	Microarray Canine Gene Expression[b] and NGS[C]	Three distinct tumor subtypes associated with angiogenesis (group 1), inflammation (group 2), and adipogenesis (group 3). Possibly a common progenitor.	Gorden et al. (2014)
OSA	15 dog tumours 15 human tumours	Microarray Canine genome v1.0 Human Genome U133A[a]	Expression signatures could not distinguish the canine and human diseases	Paoloni et al. (2009)
OSA	32 tumours	Microarray Dog specific cDNA	Gene expression identifies two prognostic groups based on survival time < or >6 months. ‘ of proliferation, drug resistance or metastasis genes in short survivors	Selvarajah et al. (2009)
OSA	26 low passage cell lines 1 osteomyelitis 6 tumours	Microarray Canine genome 2.0 GeneChip [a]	Differential gene expression segregates dog samples into two groups with differential survival probabilities and applies to human datasets	Scott et al. (2011)
MCT	51 tumours (5 undifferentiated, 13 differentiated reference samples)	Microarray Canine Gene Expression V2[b]	13 genes involved in cell cycle, DNA replication, p53 signaling pathway, nucleotide excision repair and pyrimidine metabolism can separate undifferentiated and differentiated MCT. *FOXM1, GSN, FEN1* and *KPNA2* expression related to increased mortality	Giantin et al. (2014)

ABC – activated B cell, CA – carcinomas, DLBCL -Diffuse large B cell, ECM –extracellular matrix, FCR - flatcoated retriever, FSA-fibrosarcoma, GCB –germinal centre B cell, GEP- Gene expression profiling, LSA – lymphoma, MT – mammary tumour, HSA – haemangiosarcoma, HisSA – histiocytic sarcoma, MCT –mast cell tumour, SA – sarcomas, STS – soft tissue sarcoma, NGS-next generation sequencing, OSA- osteosarcoma, PNST –peripheral nerve sheath tumour, +ve -positive, -ve – negative, *ABCA5- ATP-Binding Cassette, Sub-Family A (ABC1), Member 5; CCD3-cytoplasmic cluster of differentiation 3, SMO2-small organ 2, NF-kB – Nuclear factor kappa B; FN1-fibronectin1; SPARC –secreted protein acidic cysteine rich, CTHRC1- Collagen Triple Helix Repeat Containing 1, FHL2 - Four And A Half LIM Domains 2, DES -desmin, ACTN1-actinin alpha 1, NEB -nebulin, DAG1-dystroglycan 1, MMP –matrix metalloproteinase, SERPINE1- Serpin Peptidase Inhibitor Clade E, TIMP –tissue inhibitor of metalloproteinase, EGF –epidermal growth factor, EGFR – epidermal growth factor receptor, MAP2K6- Mitogen-Activated Protein Kinase Kinase 6, STAT- signal transducer and activator of transcription, CLDN5-claudin 5, CTNNAL1- Catenin Alpha-Like 1, MUC1-mucin 1, PECAM1- Platelet/Endothelial Cell Adhesion Molecule 1, ANGPT 2- Angiopoietin 2, ANGPTL1-4- Angiopoietin-like 1-4, FIGF- C-Fos Induced Growth Factor, TIE1- Tyrosine Kinase With Immunoglobulin-Like And EGF-Like Domains 1, FGFR1 –fibroblast growth factor receptor 1, GHR, PDGFR – platelet derived growth factor receptor, TGFBR –transforming growth factor beta receptor, FMN2-formin 2, KIF1B- Kinesin Family Member 1B, GLI1- GLI Family Zinc Finger 1, ROBO1- Roundabout 1, NMUR2- Neuromedin U*

754 *Receptor **2**, DOK4- Docking Protein 4, HMG20B- High Mobility Group 20B, PLAGL1- Pleiomorphic*
755 *Adenoma Gene-Like 1, FNBP1L- Fructose-1,6-Bisphosphatase 1-like, BAG2- BCL2-Associated Athanogene 2,*
756 *HK1- Hexokinase 1, CSK- c-src tyrosine kinase, Cox5A- Cytochrome C Oxidase Subunit Va, PPBP- Pro-*
757 *Platelet Basic Protein, SpiC- Spi-C Transcription Factor, VCAM1- Vascular Cell Adhesion Molecule 1,*
758 *ENPEP- Glutamyl Aminopeptidase, ITGAD- Integrin, Alpha D, GTSF1- Gametocyte Specific Factor 1, Col3a1-*
759 *Collagen, Type III, Alpha 1, CD90-cluster of differentiation 90, LUM-lumican, CLEC12A- C-type lectin-like*
760 *domain family 12, member A ,CCL5- Chemokine (C-C Motif) Ligand 5, FOXM1- Forkhead Box M1, GSN-*
761 *gelsolin, FEN1- Flap Structure-Specific Endonuclease 1, KPNA2- Karyopherin Alpha 2.*
762 [a] Affymetrix.
763 [b] Agilent Technologies.
764 [c] Illumina.
765

766 **Table 2**
767 Veterinary studies using proteomic profiling.
768

Tumour type	Cases	Technique	Findings	Reference
LSA	Lymph nodes from 11 B cell 13 normal	2D-PAGE and MALDI-TOF MS	Prolidase (proline dipeptidase), triosephosphate isomerase, and glutathione S-transferase down-regulated and macrophage capping protein up-regulated in lymphoma samples	McCaw et al. (2007)
LSA	Serum from 29 dogs –B cell 87 dogs – healthy or nonLSA cancer	IEC and SELDI-TOF MS	Three biomarker protein peaks identified which could separate control from B cell lymphoma dogs	Gaines et al. (2007)
LSA	Serum from 87 dogs –LSA 92 dogs – healthy or diseased	IEC and SELDI-TOF MS	Two biomarker protein peaks identified which could separate lymphoma and non-lymphoma patients.	Ratcliffe et al. (2009)
LSA	Serum from 3 dogs – LSA 2 dogs –healthy	Agarose and PAGE electrophoresis and MALDI-TOF MS	Haptoglobin identified in all three lymphoma dogs Kininogen absent in all three lymphoma dogs but present in the sera of healthy dogs	Atherton et al. (2013)
LSA	Serum from 3 dogs – LSA 2 dogs – TCC bladder 7 dogs - control	LA capture GIST stable isotope labelling, LC and MALDI-TOF MS	11 fucosylated peptides ‘ by >50% in all 3 lymphoma cases compared to normal. In one lymphoma case, 46 upregulated fucosylated peptides “ post-chemotherapy, and then subsequently ‘ upon recurrence and 9 ‘ post-chemotherapy and “ upon recurrence	Wilson et al. (2008)
MT	6 LN +ve carcinomas (<6 months survival) 6 LN –ve carcinomas (>2 years survival)	2D-DIGE and MALDI-TOF MS	Proteins ‘ in LN +ve carcinomas : proliferating cell nuclear antigen, ferritin light chain, bomapin, tropomyosin 3, thioredoxin-containing domain C5, adenosin, ornithine aminotransferase, coronin 1A, RAN-binding protein 1,3-phosphoglycerate dehydrogenase, & eukaryotic translation elongation factor 1. Proteins “ in LN +ve carcinomas : calretinin, myosin, light chain 2, peroxiredoxin 6, maspin, ibrinogen beta chain, vinculin, isocitrate dehydrogenase 1, tropomyosin 1, annexin A5, and Rho GTPase activating protein 1	Klopfleisch et al. (2010)
MT	6 normal tissues 6 adenomas 6 LN –ve carcinomas 6 LN +ve carcinomas	2D-DIGE and MALDI-TOF MS	Differences in malignancy are associated with a stepwise but not linear change in protein expression levels Acquisition of metastatic potential is associated with the strongest changes in protein expression levels	Klose et al. (2011)
MT	Serum from 15 dogs with tumours 15 healthy controls	Hybridisation to cell line sep by 2D-PAGE, and MALDI-TOF MS	Four autoantigens: manganese-superoxide dismutase, triose phosphate isomerase, alpha-enolase, and phosphoglycerate mutase 1 with ‘ immunoreactivity in tumour samples identified as biomarker candidates	Zamani-Ahmadmahmudi et al. (2014)
PT	3 prostate carcinomas 6 normal prostate 6 normal bladder	2D-DIGE and MALDI-TOF MS	Three proteins (keratin 7, GRP78, and endoplasmin) were ‘ in the carcinomas compared with normal prostate or bladder	LeRoy et al. (2007)
MCT	5 Low grade 5 High grade	2D-DIGE and MALDI-TOF MS	Four stress response proteins (HSPA9, PDIA3, TCP1A, TCP1E) were ‘ in high-grade tumours. Proteins associated with cell motility and metastasis were either ‘ (WDR1, ACTR3, ANXA6) or “ (ANXA2, ACTB)	Schlieben et al. (2012)

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+ve -positive, -ve – negative, 2D-DIGE – 2 dimensional differential gel electrophoresis, 2D – PAGE - 2dimensional polyacrylamide gel electrophoresis, GIST – global internal standard technology, IEC-Ion exchange chromatography, LA – lectin affinity, LC – liquid chromatography, LN –lymph node, LSA-lymphoma, MCT –mast cell tumour, MT-mammary tumour, PT-prostate tumour, MALDI-TOF MS– matrix-assisted laser desorption/ionisation with time of flight mass spectrometry, SELDI-TOF MS – surface-enhanced laser desorption/ionisation with time of flight mass spectrometry, sep-separated , TCC – transitional cell carcinoma, GRP78-glucose regulated protein 78, HSPA9- Heat Shock 70kDa Protein 9 (Mortalin), PDIA3-Protein Disulfide Isomerase Family A, Member 3, TCP1A- T-complex polypeptide 1 alpha, TCP1E- T-complex polypeptide 1 epsilon, WDR1- WD Repeat Domain 1, ACTR3- Actin-Related Protein 3 Homolog, ANXA6-annexin A6, ANXA2-annexin A6, ACTB-actin beta, RAN - RAs-related Nuclear protein.