

REVIEW

Proteomics and naturally occurring animal diseases: Opportunities for animal and human medicine

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The exquisite sensitivity and selectivity of contemporary protein analysis means that proteomics is increasingly at the forefront of biomedical investigation. The molecular basis of diseases states can now be revealed at the protein level, complementing transcript and genomics data. Moreover, protein biomarkers are increasingly accessible to the scrutiny of the protein chemist. The focus of these studies has been on human systems but diseases that occur naturally in nonhuman mammalian species may provide additional perspectives on the pathophysiology of human disorders. As a result, veterinary research aimed at promoting animal health may lead to concomitant improvements in the characterisation, clinical management and treatment of human diseases. This review will focus on the use of proteomic approaches to study naturally occurring disease states in animals and discuss ways by which these investigations may advance the health of both animals and humans.

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1 Introduction

Proteomics offers the potential of defining changes in protein expression [1] and addressing the technically and conceptually challenging problems of protein–protein interactions [2], post-translational modifications [3] and proteome dynamics [4]. Proteomic strategies employ a combination of efficient and stringent separation technologies, high-resolution mass spectrometry and powerful bioinformatic tools to characterise and quantify proteins from body fluids and tissues [5–9].

Disease processes may be caused by altered nutritional status, inherited defects and exposure to infectious agents, toxins, xenobiotics or environmental stressors. The resulting pathophysiology will be reflected in an altered expression of a broad range of proteins. As a consequence, proteomics is

increasingly being used to identify diagnostic biomarkers, monitor novel therapeutic strategies and explore the pathophysiology of prevalent and important diseases such as cardiovascular disease [10–12], neurodegenerative disorders [13–15] and cancer [16–18].

Human disease research can be constrained by the type and scale of experiments that are feasible for most researchers. The symptoms of many diseases in humans may take a considerable period of time to manifest, which can limit the availability of suitable patients covering the complete trajectory of the disease pathology. In addition, the complex genetic background of human populations means that larger and better-controlled subject groups are required. There may also be a requirement to prevent age and gender bias in clinical research studies. These factors have the potential to impact on the rigorous statistical interpretation required in biomedical research. Critically, many experimental manipulations are not allowed to be performed on human patients due to ethical, legal and practical considerations. As a result numerous nonhuman mammalian species have been employed as models of human disease using a wide range of biochemical and biophysical techniques. This review will

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focus on the use of proteomics to study naturally occurring diseases in animals and discuss ways by which these investigations may improve the health of both animals and humans.

2 The role of comparative medicine

Many of the advances in human medicine have been forged by taking advantage of the unique experimental opportunities that are offered by animal models of human disease. At present, rats and mice are the most widely used animal models in biomedical research and they can provide a considerable level of experimental control particularly with the advent of transgenic technologies [19]. The use of rodents as model species for research into human disease has led to important developments in identifying the cellular and molecular components of many human disease states and in extrapolating how many of these elements function in a coordinated manner in physiological systems.

However, rodent models do have intrinsic limitations. Anatomical or physiological differences between rodents and humans can diminish their value, particularly in diseases where the clinical manifestations and therapeutic responses in the rodent fail to accurately reflect the human form of the disease [20]. For example, cystic fibrosis in humans is characterised by lung and gut pathology. Death from cystic fibrosis is usually as a result of respiratory infection caused by obstruction of the airways. Cystic fibrosis transmembrane conductance regulator has been cited as of critical importance in the disease. However, when the gene encoding the chloride channel was knocked out in mice, the resulting pathology was characterised by serious gastrointestinal effects, but with little or no consequence to the lung [21].

An alternative to rodent models is the study of naturally occurring diseases in larger animals. For the purpose of this review we will define larger animals as companion animals, for example, cats and dogs, and farm animals, which include cattle, sheep, pig and the horse. Naturally occurring diseases in large animals are generally discovered due to their clinical presentation and often share a similar molecular pathology with their human analogues [22, 23]. Studies of such diseases can shed light on pathological processes that so far have proved difficult to study for practical, ethical and biological reasons in humans and rodents. A comparative approach to studying diseases common in both human and animals can have an extensive impact on clinical practice and the development of treatments in veterinary and human medicine [24, 25].

Comparative medicine has many advantages for the study of genetic diseases. Due to the prevalence of inbred populations within specific breeds there are many naturally occurring inherited animal diseases that are of interest to human medicine [26–28]. This has led to the establishment of databases that catalogue inherited disorders in animals,

such as the Online Mendelian Inheritance in Animals (<http://omia.angis.org.au>) [29]. As more genetic diseases have been recognised in dogs than any other species except humans, a specialist scientific database of spontaneous inherited canine diseases has also been developed, the Inherited Disease in Dogs Database (<http://www.vet.cam.ac.uk/idid>) [30]. This database details diseases in dogs which are likely to be transmitted wholly or partly through a genetic mechanism, according to the breeds in which they have been described. The site is complementary to the Canine Inherited Disorders Database (<http://www.upei.ca/cidd/intro.htm>), a database that reviews canine inherited diseases aimed at dog owners and practicing veterinarians [31].

It is however, important to realise there are also practical issues when studying naturally occurring animal diseases in large animals. These include the availability of animal numbers, sample type and the ability to obtain relevant control groups. The life span and size of large animals (particularly in the case of farm animals), must also be considered, together with husbandry and clinical expertise and the costs of specialist facilities required to house and maintain the animals.

3 Proteomics of naturally occurring animal diseases

The technologies of proteomics permit the discovery of proteins that are differentially expressed in normal and disease conditions, and the identification of such proteins. In many diseases the molecular basis of cellular dysfunction is not fully understood. Undoubtedly, the use of high-throughput proteomic techniques in the study of naturally occurring animal diseases will help to decipher downstream cellular pathways that are affected in these disorders and help us to elucidate their pathological mechanisms and identify biomarkers of disease states. The following section highlights the application and examples of where naturally occurring animal diseases have been investigated using proteomic approaches. This research, whilst aimed at promoting animal health, may ultimately lead to concomitant improvements in the characterisation, clinical management and treatment of human diseases.

3.1 Canine diseases

Dogs were domesticated approximately 10–15 000 years ago [32], although the majority of breeds have emerged only in the last 250 years [33]. There is remarkable phenotypic variation displayed between different breeds of dogs [34] as exemplified by differences in size, for example, the Great Dane and Chihuahua. Nonetheless, individual breeds were derived from a small number of founder animals selected for particular desirable traits, which restricted genetic diversity [35, 36]. This has led to an over-representation of inherited

diseases in pure-bred canine populations, which match, phenotypically or genotypically, diseases that are of relevance to human medicine [22, 28–31].

Canine diseases can be extrapolated to the human equivalent of the disease due to similarities in physiology and clinical responses to treatments. Furthermore, dogs and humans share a common environment [22], and therefore, the underlying cause of certain human and canine diseases related to lifestyle, such as obesity [37] and cancer [38] may be comparable. Dogs are of immense value in understanding human cancer because they develop spontaneous tumours that share many physiological and pathophysiological characteristics with human malignancies [39, 40]. Acknowledgment of the role that dogs can play in human cancer research has led to the establishment of a comparative oncology programme within the US National Cancer Institute's Center for Cancer Research (<http://ccr.ncifcrf.gov/resources/cop/>) [41]. This programme is focused on canine tumour biology and treatment and will involve the development of proteomic assays aimed at predicting drug toxicity, efficacy and mechanisms in dogs [42]. This use of proteomic technologies to study canine cancer may ultimately result in a greater understanding of the disease processes in humans.

Lymphoma is a type of cancer defined by a proliferation of malignant lymphocytes within organs such as the lymph nodes, bone marrow, liver and spleen and is the common form of haemopoietic tumour in dogs [43]. Canine lymphoma is similar to human non-Hodgkin's lymphoma [43]. In a recent pilot study, protein expression in the lymph nodes from normal dogs was compared to dogs with multicentric B-cell lymphoma [44]. Soluble protein extracts from surgically removed lymph nodes were separated by 2-DE and the gels analysed for changes in protein expression levels between cancerous and control lymph nodes. Twenty-one proteins were differentially expressed between the lymphoma and control groups of samples, a number of which were characterised by MALDI-TOF-MS. The expression of prolidase (proline dipeptidase), triosephosphate isomerase and glutathione S-transferase were found to be decreased in lymphoma samples, whereas macrophage capping protein was up-regulated. The authors discussed that those proteins which were down-regulated had previously been implicated in pancreatic and breast cancer. However, a small number of samples were analysed in this study and only four of the differentially expressed proteins were identified.

A second recent proteomics-based study has focussed on the identification of protein biomarkers of canine lymphoma [45]. Sera were analysed from cohorts of dogs with B-cell lymphoma and control cohorts of healthy dogs, dogs with malignant cancers other than B-cell lymphoma and dogs with disorders unrelated to cancer. The serum samples were initially fractionated using anion exchange chromatography prior to analysis by SELDI-TOF-MS. The data revealed three major proteins as potential biomarkers for lymphoma in dogs although the identities of the proteins were not confirmed. Further investigations will be needed to determine

whether these biomarkers will be useful in both the screening of B-cell lymphoma in dogs and the monitoring of therapies.

3.2 Feline diseases

A number of feline diseases are of relevance to human health. In cats many of the clinical, physiological and pathological features of type 2 diabetes mellitus are strikingly similar to the human form of the disease [46, 47], including the association with obesity [48]. Cats are also susceptible to genetic diseases, such as muscular dystrophies [49]. It is perhaps surprising that global proteomic approaches have not been used more extensively to study naturally occurring feline diseases.

However, one study has employed proteomic strategies to investigate familial hypertrophic cardiomyopathy in Maine Coon cats [50]. This disease is highly prevalent in domestic cats and is also believed to be one of the most common causes of sudden cardiac death in young human adults [51]. The presentation and progression of the disease in cats mimics that of the human form [51, 52], however, whilst numerous causative mutations have been identified, the pathogenetic process is still poorly understood. The analysis of left ventricle muscle obtained *postmortem* from the Maine Coon cats indicated that there is a decrease in the expression of myosin binding protein C and myomesin. Moreover, sequencing of the myosin binding protein C gene in the hypertrophic cardiomyopathy affected cats revealed an alanine to proline mutation. This mutation is in a region of the protein believed to be involved in binding to myosin and/or actin. The authors postulated that as myomesin has an important structural and stabilisation role in myofibrillar assembly, *via* interaction with both myosin and titin, an altered expression or conformation of this protein could lead to incomplete or faulty assembly of the sacromeric structure. Further work will investigate the relevance of this mutation to the mechanism of familial hypertrophic cardiomyopathy.

3.3 Ovine diseases

As with many farm animals, one of the major health risks to sheep is from infectious diseases. Infectious agents of sheep that have been implicated in human disease are increasingly being subjected to proteomic investigations in an attempt to elucidate the mechanism of infection and to identify potential vaccine targets. The parasitic liver fluke *Fasciola hepatica* is known to infect sheep resulting in reduced productivity and increased mortality [53]. The parasite also infects large sections of the human population [54]. Little is known about the protein response to infection *in vivo*.

Morphew *et al.* [55] analysed bile fluid from naturally infected sheep and compared these to bile fluid from uninfected sheep. Flukes from the same infected sheep were also cultured and proteins from the excretory-secretory matrix compared to *in vivo* profiles. Protein extracts from each

sample were subjected to 2-DE, protein spots of interest excised and proteins identified by MALDI-TOF/TOF-MS. Comparison of the bile proteomes indicated six possible protein biomarkers of infection. To identify possible antigenic proteins, Western blotting was used to compare the antigenic pattern of the *in vivo* and *in vitro* sample sets. The antigenic proteins were almost exclusively identified as belonging to the cathepsin L protease family.

Paratuberculosis, also known as Johne's disease, is a significant economic problem in the sheep industry [56] but more recently has also been implicated as a possible factor in the development of Crohn's disease in humans [57]. In the study by Hughes *et al.* [58] naturally infected sheep were identified in the clinic and samples of the mycobacterium obtained from the terminal ilea postslaughter. 2-DE was performed on the protein extract from the clinical samples and compared with those cultured *in vitro* from the same sheep. Following gel comparison and MALDI-TOF-MS, ten proteins were identified as being up-regulated in the naturally infected samples. The majority of the proteins were postulated to have a role in nutrient scavenging and adaptation of the bacterium to adverse conditions in the pathogenic lesion. Whilst the primary aim of these studies has been the development of vaccines for animal health, the studies have provided fundamental knowledge of the host-parasite relationship and thus can act as model systems for the parallel human disease.

3.4 Bovine diseases

Cattle may not appear practicable for studying human diseases. This is mainly due to the expense of their husbandry and size of a mature adult. Nonetheless, naturally occurring diseases in cattle may be of use in investigating human disease analogues. A key example is dilated cardiomyopathy. Bovine hereditary dilated cardiomyopathy occurs in the Simmentaler-Red Holstein crossbreed of cattle and progresses rapidly to global heart failure in affected animals [59]. The disease aetiology in humans is multifactorial with prior viral infections, cardiac specific autoantibodies, toxic agents, genetic factors and sustained alcohol abuse believed to be contributing factors [60]. A proteomic approach has been applied to investigate the molecular pathogenesis of the bovine form of the disease [61]. In this study, the protein expression between the ventricular tissue from Simmentaler-Red Holstein crossbred cattle with dilated cardiomyopathy and control heart tissue was compared. Heart muscle proteins were separated by 2-DE and identified by a combination of PMF and amino acid compositional analysis. Densitometric analysis of gel images revealed 35 differentially expressed proteins. Many of the proteins were mitochondrial metabolic enzymes, however, a key cytosolic protein (ubiquitin C-terminal hydrolase) was significantly up-regulated. This suggests that inappropriate post-translational modification of proteins, namely ubiquitination, may play a role in the molecular pathogenesis of dilated cardiomyopathy. Poly-ubiqui-

tination of proteins acts as a signal for proteolysis by the 26S proteasome and it was postulated that an increase in this type of proteolysis may play a role in heart disease.

3.5 Equine diseases

Several spontaneous diseases in horses may impact upon human health. Osteoarthritis occurs naturally in horses and can be compounded by physical exertion particularly in horseracing [62, 63]. Comparative studies of equine gastrointestinal diseases may also have the potential to advance our understanding of the pathophysiology of human gut diseases [64]. Another area that is of interest to equine health is recurrent uveitis, which is an autoimmune disease affecting the eye [65]. Due to its high prevalence and resulting blindness the disease is considered a major problem for horses. The pathophysiology of equine recurrent uveitis is unclear, but the disease undoubtedly has an immune-mediated basis. The disease closely resembles human autoimmune uveitis regarding clinical and immune pathological aspects [66]. Identifying biological markers or pathways associated with this disease, which lead to retinal degeneration and consequent blindness may allow the understanding of its pathogenesis at a molecular level.

In order to explore the pathogenic mechanisms involved in equine recurrent uveitis Deeg *et al.* [67] analysed the retinal proteome in horses under normal and uveitic conditions. Proteins from horse retinas were separated by 2-DE and identified using MALDI-TOF-MS and MALDI-TOF/TOF-MS. Comparison of the healthy retinal proteome with two different stages of uveitic disease revealed marked differences between the overall protein patterns. Seventeen differentially expressed proteins were characterised. Several were identified as protein markers of blood-retinal barrier breakdown such as alpha-1-antitrypsin, albumin, transferrin, immunoglobulins and haemoglobin, the expression levels of which were increased with disease progression. Uveitic changes in the retina were also accompanied by up-regulation of glial fibrillary acidic protein. This finding was further confirmed by 1-D and 2-D Western blots. In contrast the proteins, glutamine synthase and pigment epithelium-derived factor were found to be down-regulated. The authors commented that the changes in expression of these proteins is indicative of activated Muller glial cells and suggest that these particular cells may play a critical role in the pathogenesis of equine recurrent uveitis and its human analogue.

4 Concluding remarks

In this review we have discussed the application of proteomic approaches to the investigation of naturally occurring diseases of animals including dogs, cats, sheep, cattle and horses and how these investigations may shed light on the corresponding disorders in humans. Importantly, the study of naturally occurring diseases of animals does not present

the same ethical dilemmas seen with experimental animal models. However, despite the variety of opportunities which would flow from collaborative research in human and veterinary medicine many issues remain. There is at present a lack of funding for long-term studies of naturally occurring animal diseases and securing the necessary resources to advance proteomic investigations in this area will therefore be an important goal. The use of proteomics strategies in animals also brings significant analytical challenges, which have yet to be fully optimised, especially where proteins are uncharacterised or unknown. A potential difficulty with animal proteomics is the lack of complete and annotated genome sequences of the majority of species of interest.

The identification of proteins by mass spectrometry depends on database searches of peptide masses. Whilst it is possible to identify proteins with high sequence conservation by PMF and cross-species matching, a single amino acid change in a protein can result in dramatically different peptide mass fingerprint. Therefore, to accurately determine the identity of proteins from animals often requires *de novo* sequencing of peptides by tandem mass spectrometry. This technique however still relies on sufficient sequence information being available from a homologous protein in another sequenced species. This situation will only improve as genome sequences of animals are completed and fully annotated, leading to further improved tools which will further enhance the use of spontaneous animal models of human diseases in biomedical research.

Undoubtedly the advancement of the proteomic technologies has added new dimensions to the analyses of clinically relevant samples and promises to enhance the way diseases will be managed in the future. The ability to obtain a profile of the biochemical responses at the protein level will provide a more comprehensive view of cellular control mechanisms, which may have direct outcomes in improving our understanding of animal and human disease states.

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