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Dogs: Active Role Model for Cancer Studies—A Review

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ABSTRACT

Many studies have been done and many results have been established for studying cancers in human and the ways of treating it. However, one thing that remains relevant is the study model that is used to diagnose, cure or conclude treatment methods for human cancers. The scientists have tried some ways to link the data and tried to analyze the malicious disease in various animal models in order to solve the problem for humans. Out of all the models, scientists have preferred dogs as the most suitable model and conducted studies on them. Our article will review the reason for preferences given to dog as a study model and what the previous studies have tried to conclude by considering the dreaded disease in dogs. Our article has focused on most of the recent observations and tried to elucidate the reasons/preferences for studying cancer disease in dogs (scientific name; Canis Lupus familiaris). We will also talk about the idea of comparative oncology programs that many centers adapt in order to study the disease called cancer.

1. Introduction

The most common type of tumor in unspayed female dogs is the mammary tumor especially between the ages of five to 10 years. Some male dogs do develop this type of breast cancer, but due to the hostility of this type of breast cancer, the prognosis is not good. Mammary tumors in dogs range in size and often grow quickly with an irregular shape. These malignant tumors can also root bleeding and ulceration [1]. There were an estimated 12.7 million cancer cases around the world reported in 2008, out of which 6.6 million cases were in men and 6.0 million in women. This number is expected to increase to 21 million by 2030 [2]. According to the statistics in 2011, 230,480 new cases of invasive breast cancer are estimated to be diagnosed in women in the US, along with 57,650 new cases of non-invasive (*in situ*) breast cancer [3].

Breast cancer being the most common cancer in women, is responsible for almost 20% of all cancer deaths in women. Increase in awareness and routine mammograms has helped women to get diagnosed in earlier stages of breast cancer thereby increasing the chances of curing it. The statistics state that for every 100 women, one man is also diagnosed with this disease. The disease is more common in women above 40. It is also more frequent in women of a higher social-economic class [4].

2. The Reason behind Dog

The inspiration behind comparative oncology is simple. If a model is very similar to human beings then why can't we investigate it to introduce quality improvement for human healthcare? Dogs get many cancers that strike humans, including lymphoma, breast cancer and bone cancer. Contrasting to genetically altered laboratory animals that we used to test potential new cancer treatments, dogs develop this disease naturally. We can measure the same things in dog that we do in humans, like heart changes, organ function and blood pressure. Hence, these data can be applied directly to human diseases. With dogs, we can ask many questions that one cannot ask in mouse preclinical models of cancer and cannot answer in

human clinical trials.

Treating naturally occurring diseases of dogs does not create an ethical issue as seen with experimentally induced disease in models and hence it provides a more robust model. The physiology of dog allows responding to drug metabolism in way comparable to humans, therefore the dog is used routinely for pharmaceutical toxicological studies [5]. Cancer is the most recurrent canine disease. Dogs develop spontaneous tumors that display behavior and histo-pathological characteristics to human tumors [6-8]. For example, in dogs, studying about the role of inhibition of telomere size shortening plays in cell immortalization and proliferation is relevant, because dog chromosomal telomeres closely resemble human telomeres than murine telomeres [9]. Among the practical benefits of clinical investigation of cancer in dogs is the fact that due to their shorter life span, clinical intervention is easily studied over a condensed period of time. The dog cancer cases have the survival rate over one year, rather than five, as in human oncology [8], thus the possibility to attain comparatively rapid results when performing clinical trials and monitoring disease progression in dogs.

2.1. Why Not Mouse as Models?

Murine models have been used in many studies, but *in vivo* murine model such as xenografts and transgenics fail to reiterating essential features and characteristics (such as heterogeneity, tumor microenvironment and dependence on steroid hormones) of human breast cancer [10,11]. In addition to the inherent evolutionary remoteness between mice and humans, further differences can originate due to induced genetic modifications (transgenic mice) or from altered presence of adjacent normal tissue, stromal cells, vasculature and immune system components (xenografts) [12-16]. Jointly these factors explain the limited values of murine model for studying cancer pathogenesis, progression and therapy and embody a major obstacle in identifying of reliable predictive molecular biomarkers and effective therapeutic agents development [10,17].

2.2. Studying Cancer with Dog Models

Of all the disorders for which dogs are likely to enlighten human health, canine cancer is likely to have the greatest effect [18]. Cancer is the most frequent cause of disease-associated death in canines, and naturally occurring cancers have been well described in several breeds [19-21]. Although, substantial effort has been spent in studying common cancers, the dog has always serves as a model for rare tumors studies, including aggressive histiocytic sarcoma and lethal dendritic-cell neoplasms [22].

A mammary tumor is the common kind of tumor in unspayed female dogs. Similarly, breast cancer is the most widespread cancer in women, striking 1/8th women during their lifetime. Not all dogs or even women succumb to the disease. Many individuals have benign slow growing tumors, which are curable. In other cancerous cells grow profusely and metastasize. But like in studies done in rats or mice, caner researchers don't induce caner in dogs. Instead, the cancer is so commonly found in these models that the researchers compassionately treat them naturally. Another reason why canines render help in studying mammary tumor is the fact that each female has eight to ten mammary glands thereby making it probable to study several tumors—each cropping up separately from the other and therefore genetically unique-in one individual. However, studying separate breast tumors in humans is usually not possible because it is rare for a woman to develop more than one spontaneous tumor in the breast [23].

2.3. Need for More Predictive Preclinical Models

Due to cancer complexity and limited knowledge available in this arena, many animal models fall short of predictive. The information we try to decipher from these models comes to a limited scope every now and then. Also, it can be said that even humans are not prognostic models when it come to cancer studies. When considering the evaluation of novel therapy in a species distinct from humans, it should be essential to ensure that questions asked in preclinical models can be answered and certify the interpretation of answers within the totality of information available [24]. Murine cancer models have proved to be useful for analyzing the complicated pathways involved in cancer initiation, promotion and progression. However, they have proved incapable in defining features occurring in humans like long latency periods, genetic instability, and heterogeneity of tumor cells. Most important of all; the complex biology of cancer, recurrence and metastasis, which is an outcome in human patients, fails to be explained in the conventional mouse models and in cancer drug development. Thus, the needs for additional models that represent the human disease in an enhanced way are needed [24].

According to leading cancer researchers, dogs have proved to be good research subjects as they develop the disease spontaneously, and many of the modern breeds have developed over the past few hundred years using restricted gene pools. Due to this selective breeding, the breed genetics have been preserved and has made some breeds more susceptible to certain cancers. These aspects along with high grade of similarity between dog and human genomes have helped the researchers to compare their genomes and study the evolutionary genetic changes associated with cancer. These factors, coupled with high degree of similarity between the genomes of

dogs and humans, which provide the researchers with an opportunity to compare the genomes and study the evolutionary genetic changes associated with cancer [25].

Another basis for using them as study models deals with how human drug trials are conducted. The benefit would be to study and test the treatment strategies in canines with aggressive tumors and monitor how they respond to the treatment. In this way, the comparative oncologists can test new treatment ideas against early-stage of cancers and delivering the drugs just as they would ultimately be in cancer patients [25]. This means more effective treatments can be developed more quickly, with less adverse health risks, for human trials and ultimately viable human treatments. Dogs also have shorter life spans than humans (most unfortunate) meaning scientists can more quickly determine whether a prevention strategy or therapy has a good chance of improving human survival rates [18]. Now moving on from the physiological similarities to genetic level, the chance of studying health and disease in the dog (Canis lupus familiaris) was significantly expanded with the release of the first public draft of the canine genome [26-28]. This prospect was advanced further with the development of high throughput technologies, such as expression and SNP microarrays that are commercially available for the dog nowadays [26,29,30].

Last many years showed the sequencing of entire dog genome (99% complete, ~2.5 billion base pairs) and the confirmation of its close similarity with the human genome [31]. For many gene families and those genes linked to cancer, the relationship between dog and human gene sequences is found to be much closer than any other counterparts [32]. When molecular cytogenetic analysis of canine tumor cells was carried out from hematological malignancies, it was revealed that the ancestral chromosomal aberrations were preserved in comparable cancers of human and dog [33,34]. Altered expression of ERBB2 and TP53 genes in mammary carcinomas is similar to the two species hence, proving alike roles in carcinogenesis and prospective use as prognostic indicators [35-37]. Similar mutations in oncogenes resulted in different cancer in humans and dogs, a study suggested [38]. For example, a similar mutation in KIT, a tyrosine kinase growth factor receptor, is recognized in both human gastrointestinal stromal tumors (GIST) and dog mast-cell cancers [38]. Likewise, the intratumoral (cell-to-cell) heterogeneity in human breast tumors also takes place in cognate dog tumors [39]. Thus the natural consequences of this heterogeneity that causes the deadly features of human cancers like acquired resistance to therapy, recurrence and metastasis.

For nearly two decades now, we have seen a rise in canine genome utilization to understand the genetic foundation of disorders that are difficult to unravel in humans [40-42]. Their large size makes the canine families amenable to conventional linkage mapping. This has been well exemplified in the canine gene search for hereditary multifocal renal cystadenocarcinoma and nodular dermatofibrosis (RCND) in German shepherds [43]. Also the canine genome provided an accessible set of validated expression profiles for human gene expression on both oligonucleotide and cDNA array platforms which, benefited researchers with several datasets that were publicly available through web-based interactive analytical tools [26,44-46]. Using similar approach, the availability of online database of canine normal tissue gene expression profiles will help in silica analysis of canine diseases. This will promote more rigorous comparative genomic analysis for humans, rat and dog tissues [47]. These comparative oncology studies enable identification of common gene regulatory regions as well as evolutionary conserved gene expression networks. Remarkable similarities have been found during the analysis of canine and human orthologous gene expression in their respective matched tissues [48]. This tissue expression data demonstration has supported in expanding and redefining canine gene ontologies consequently allowing further robust assessment of biological functions. Common inherited human diseases occur due to complex interactions between multiple genes and environmental factors. Dogs share a common environment with man, so the selection of an authentic model for a multifactorial human disease becomes an easy choice.

3. Genomic Detailing

The position of the dog within mammalian evolutionary tree also makes it an important channel for comparative analysis of the human genome [47]. High prevalence of specific diseases that affect certain breeds suggests that there are limited numbers of loci underlying for each disease. This makes the genetic analysis potentially more traceable in dogs than humans [49]. Within the exception of human, dog is the most intensely studied animal in medical practice, with detailed family history and pathology data [50]. Using genetic resources developed over the past 15years [51-56], researchers have already identified mutations in genes underlying ~25 Mendelian diseases [57,58]. Dog is equally important for the comparative analysis of mammalian genome biology and evolution [47]. The important findings, after studying the dog's genome, its gene evolution, haplotype structure and phylogenetic etc showed the average transposon insertion rate of dog genome lower then humans. Also further comparison showed that ~5.3% of the human genome contained functional elements that have been under purifying selection in both lineages. Similar patterns of evolution were observed in functionally related gene sets in human and dog lineages [47]. Unique unambiguous

aligned sequences created multi-species synteny maps showed regions of conserved synteny between dog and human genome. The total number of breakpoints in humans was found to be substantially lower than that in dogs while more intra-chromosomal breakpoints were found in human lineages than dogs [47]. Although, the level of genomic rearrangement has been much higher in rodent than in human, comparison with dog shows that there are regions where the opposite is true. Human chromosome 17 is rich in segmental duplications and gene families, which may contribute to its genomic fragility [48].

Gene Expression Profiles of Metastatic Canine Mammary Carcinomas Overlaps with Human Breast Cancer Profile

Molecular mechanisms behind lymph node and distant metastasis in Canines still remain incomplete. Several studies have been done trying to cover this issue but significant metastasis associated and predictable expression patterns of single genes have still not been identified in canine mammary tumor (CMT) [35,59-61]. Global gene expression profiles that compare metastasizing versus non-metastasizing CMT are unavailable, whereas several studies on human breast cancer found to be significant metastasis associated expression profiles. The latter studies have identified several non-overlapping expression signatures, which are related to the development of lymph node and distant metastases and worse prognosis [62-65]. Moreover, comparison of canine and human expression profiles disclosed an overlap of deregulated genes in human and canine mammary tumors [66].

The greatest challenge faced by clinical scientists is the incomplete understanding of genetic basis for complex human disease [62]. Regardless of numerous technological advances in genetics, the progress in this field has been slow due to the intricate gene-gene interactions and poorly understood environmental effects [67]. Also, the identification of these interactions and environmental influences is difficult to scrutinize in humans due to high level of genetic heterogeneity [68]. Many genome-wide association studies (GWAS) identified a small fraction of genetic basis of complex diseases [69]. And yet disease heritability is critical to understanding disease risk, the effects of environment and lifestyle on disease development, and response to treatment.

In one of the studies conducted by Klofleisch *et al.*, a gene expression profile in CMT associated with early metastatic spread to lymph node was identified that could discriminate carcinomas with similar histological features but different metastatic potential. This expression profile contained several enriched functional gene classes and had significant overlaps with expression profiles of metastatic human breast cancer [35]. Med gene literature

mining also confirmed the similarities found between canine and human mammary tumors in terms of increased proliferation, altered cell differentiation status and decreased cell adhesion [59].

Approximately 25% of the deregulated genes in metastatic canine carcinomas were cited in association with human breast cancer. In this subset of cited genes, a significant enrichment of genes associated with cell cycle regulation, protein kinases, DNA integrity checkpoint and protein metabolism was observed [61]. It was therefore likely that gene expression profiles may also predict metastasis in CMT. Klofleisch *et al.* concluded that metastatic spread of CMT to the lymph nodes was associated with a gene expression profile of increased cell cycle progression, altered cell differentiation and decreased growth factor signaling. Several key characteristic of metastasis associated gene expression are therefore similar between human and canine mammary tumors [35].

4. Conclusions

Comparative oncology has proved to be useful for clinico-pathological and therapeutic study. It has been proved beneficial to analyze the particular disease pattern between two or more than two species. The comparative oncology program has also focused on genetic history and the pattern of inheritance in different species and their correlation. This in turn has proved useful to the research workers to go into the depth of a particular study of interest.

The goal of scientific research has been to better understand cancer biology in order to understand cancer diagnosis, treatment and prevention in humans and animals. It is due to the exchange of ideas and observations between researchers studying human and companion animal cancer that these studies have become more common and successful.

Clinical and translational studies in dogs don't face the same constraints as human trials and hence a platform is prepared by previous researchers to further continue and enhance these studies for human betterment. We tried to sum up previous discoveries and conclusions that were done by renowned researchers. The focus is to analyze till where have we succeeded in enabling ourselves utilize this model for prognostic cancer strategies in humans. With comparative oncology studies researchers have become more confident in development and validation of new medical devices. Dog models showed that helical tomotherapy devices could successfully image, position, and treat spontaneously occurring tumors [70].

The evolutionary history of dogs, their position as a family member in many households, and the high level of health care they receive in our world offer tremendous opportunities. Alongside this combined with recently developed genetic resources, makes dogs outstanding

models for the studying known genetic pathways, discovery of genetic and environmental contributions to disease, and translational studies in cancer risk, prevention, and treatments [70]. Increased gratitude of the inimitable and comparative values of dog as a model for diverse human diseases should promote and accelerate more researches leading to new better treatments and improved health care for both mankind and his best friend-the dog. Thus in conclusion our summation is that Dogs are exceptionally suited for use as an animal model of complex human disease due to their phenotypic diversity and naturally occurring disease resemblance to human conditions.

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