Review Article

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Fetal Microchimerism in Cancer Protection and Promotion: Current Understanding in Dogs and the Implications for Human Health

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Abstract. Fetal microchimerism is the co-existence of small numbers of cells from genetically distinct individuals living within a mother's body following pregnancy. During pregnancy, bi-directional exchange of cells occurs resulting in maternal microchimerism and even sibling microchimerism in offspring. The presence of fetal microchimerism has been identified with lower frequency in patients with cancers such as breast and lymphoma and with higher frequency in patients with colon cancer and autoimmune diseases. Microchimeric cells have been identified in healing and healed tissues as well as normal and tumor tissues. This has led to the hypothesis that fetal microchimerism may play a protective role in some cancers and may provoke other cancers or autoimmune disease. The long periods of risk for these diseases make it a challenge to prospectively study this phenomenon in human populations. Dogs get similar cancers as humans, share our homes and environmental exposures, and live compressed life-spans, allowing easier prospective study of disease development. This review describes the current state of understanding of fetal microchimerism in humans and dogs and highlights the similarities of the common cancers mammary carcinoma, lymphoma, and colon cancer between the two species. Study of fetal microchimerism in dogs might hold the key to characterization of the type and function of microchimeric cells and their role in health and disease. Such an understanding could then be applied to preventing and treating disease in humans.

KEYWORDS: cancer; comparative; dog; microchimerism; pregnancy.

INTRODUCTION

Microchimerism is the co-existence of a small population of cells from genetically distinct individuals living within the body of another individual [1, 2]. In humans, during pregnancy, cells traffic bi-directionally across the placental interface between fetus and mother and establish permanent residence, resulting in fetal or maternal microchimerism. Trafficked cells may even include those from a twin during pregnancy or older sibling from prior pregnancy. A persistent microchimeric state can also result from medical intervention, such as organ transplantation or blood transfusion [3]. Ultimately, the result is the persistence of minor cell populations that may play roles in the development of autoimmune disease and some cancers, reduction of risk of other cancers, and enhancement of engraftment rates following hematopoietic stem cell or organ transplantation with a reduction in graft vs host disease (GVHD) [1, 4, 5]. While

significant epidemiological data supports differential risk for certain diseases between women with demonstrated fetal microchimerism and those without, the diseases are multifactorial in cause and often late to develop in life [4, 6]. These conditions create significant challenges in designing prospective studies to identify clear differences in risk and elucidate specific mechanisms by which microchimerism might alter that risk. The ideal model to bridge these challenges would study naturally occurring cancers in a species that shares environmental exposures with humans, has similar outbred genetics, and lives a compressed life-span to speed data accrual. Companion dogs appear well suited to fill the role as such a model species [7, 8]. Dogs experience cancers that are both biologically and molecularly similar to humans [8–10]. Companion dogs share our home environment with the attendant chemical, food, and environmental factors experienced by humans [8]. The compressed life-span of dogs and relatively high-quality medical care they receive allow for rapid accumulation of detailed health and disease observations. Recently, male fetal microchimerism was identified in parous companion golden retrievers [11]. Subsequent work has also identified the phenomenon in Dachshunds, with evidence for the existence of sibling microchimerism from prior litters [12]. The suitability of these companion dogs as models is enhanced by the ease of serial sampling through local veterinary practices and breed specialty events. These

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ABBREVIATIONS: GVHD, Graft vs host disease; nHL, Non-Hodgkin Lymphoma.

dogs that share our lives could help us define the mechanisms of development and activity of fetal microchimerism. However, little is yet known of the role of microchimerism in canine disease risk.

Microchimerism in human health and disease was recently reviewed [13]. Microchimeric cells have been reported to be identified more frequently by various means in women with systemic sclerosis, rheumatoid arthritis, juvenile myositis, type I diabetes mellitus, systemic lupus erythematosus, Sjögren's syndrome, and thyroid autoimmune disease. In humans, microchimeric cells have been identified in tissues of the brain, lymph node, thyroid, blood, heart, skin, spleen, kidney, pancreas, liver, gallbladder, intestine, bone marrow, and cervix [14, 15]. Microchimeric cells in these tissues have been demonstrated to express lineage-specific molecules, including lymphocyte differentiation molecules and cytokeratins, appropriate to the tissue [13]. Combining this evidence with the epidemiologic evidence of potential cancer protection or risk, it is not unreasonable to suspect that these cells play an active role in the carcinogenesis and pathogenesis of these diseases. As early as 1960, maternal lymphocytes were postulated to participate in Hodgkin lymphoma of offspring [16]. Women who are microchimeric in the peripheral blood appear to be at lower risk for the development of breast cancer [17–19]. Male microchimeric cells have been demonstrated in higher frequency in normal and benign breast tissues than in malignant breast lesions [19]. Similarly, lower frequency of chimerism detected in peripheral blood was identified in women with papillary thyroid cancer and hematological malignancies, including non-Hodgkin lymphoma (NHL) [6, 20–23]. Evidence of lower frequency or degree of chimerism has been interpreted as a protective effect of the chimeric cells against these cancers in women. Conversely, chimerism has been identified more frequently in women with colon cancer [24]. The mechanism of this association is unclear. In the case of melanoma, microchimeric cells have been suggested to contribute directly to lymphangiogenesis and tumor growth [25]. Because of the multiple factors known to contribute to these cancers over decades of life, the interacting mechanisms involving microchimerism are likely to be elusive.

CANCER MODELS AND HUMAN MICROCHIMERISM

This manuscript will review the comparative aspects of mammary cancer, lymphoma, and colon cancer between dogs and humans. A summary of the known associations between the detection of fetal microchimerism and the development of cancer in humans is presented in Table I. The link between fetal microchimerism and cancer in dogs has not yet been explored.

Mammary Cancer

Mammary cancer in dogs is thought to have similar molecular origins and hormonal influences as its human corollary. Frequency of mammary carcinoma is reported to be as high as 205/100,000 dogs/year in the UK with a lower incidence in the USA of 199/100,000 dogs/year [26]. The discrepancy is almost certainly due to differences in neutering practices, with the majority of US dogs undergoing ovariectomy at relatively early ages. Rather than menstrual cycles of women, dogs undergo estrus cycles twice

yearly. Dogs which remain intact throughout life carry a fourfold risk of mammary cancer compared to those ovariectomized before 2 years of age. Relative risk of mammary cancer is 26% in dogs after 2 estrus cycles, whereas it is 0.5 and 8% in dogs before 1 and 2 cycles, respectively [27]. As in women, adiposity of dogs and their diet has been associated with mammary cancer risk [28]. Dogs which were thin at a young age had a 96% reduction in mammary cancer risk if ovariectomized [29]. The majority of canine mammary tumors are epithelial in origin. A much smaller proportion are sarcomas, including fibrosarcoma and osteosarcoma, or carcino-sarcomas [30]. Much work has described the histology of canine mammary carcinomas, and grading schemes similar to those in women have been defined. Histological grade has shown to correlate with prognosis [31].

The role of hormones in canine mammary carcinoma is well established by the epidemiological association of the disease with estrus cycles. Dogs undergoing ovariectomy at the time of mammary tumor resection have been shown to have better outcome than those developing tumors more than 2 years after ovariectomy or remaining intact after surgery [32]. Estrogen receptor (ER) expression in canine mammary carcinoma has been documented, with higher ER expression observed in lower grade carcinomas. Expression of ER α is infrequent in mammary carcinomas and more common in benign mammary lesions [30, 33]. ER β is expressed in the majority of canine mammary carcinomas [33]. Similarly, benign tumors are more likely to express progesterone receptors (PR) in tandem with ER α . Malignant tumors appear more likely to be ER α negative and PR negative. Dogs with PR negative tumors have been reported to have a worse survival outcome [30]. Expression characteristics have been used to classify canine mammary carcinomas as luminal A, luminal B, basal, and HER-2 overexpressing tumors using a modified human classification scheme [34]. Selective ER modulators (SERMs) have been administered to dogs with mixed results. Tamoxifen has been administered to dogs following mastectomy for epithelial neoplasia [35]. Of 18 dogs on therapy, 7 were discontinued because of estrogenic effects, including vulvar swelling and discharge [35]. In normal laboratory dogs, similar effects were observed with the addition of pyometra in sexually intact dogs [36].

Environment and diet appear to play a role in mammary carcinogenesis of dogs. Obesity at 1 year of age has been associated with increased risk of mammary cancer [28]. Homemade diets were also associated with higher mammary cancer risk than commercial diets [28]. This finding was attributed to higher fat content in the diet and possibly red meat, potentially paralleling human dietary effects on breast cancer risk [28]. Like humans, serum retinol was higher in controls than in cases with mammary carcinoma [28].

Like women, a small percentage of dogs develop inflammatory mammary carcinoma. In one hospital population in Spain, inflammatory mammary carcinoma cases represented 7.6% of all mammary masses in dogs [37]. In the USA, this is likely an overestimation. Dogs present with erythema, edema, and pain in association with mammary masses, often mistaken initially for mastitis. A retrospective study has found a survival advantage to medical therapy, but both treated and untreated groups of dogs had median

Table I. Summary of the Results of Studies Examining Rates of Microchimerism in Women With and Without Selected Cancers as well as Parous Dogs and Newborn Female Puppies

Population	Species	Frequency of microchimerism (%)	Hypothesized disease influence	Reference
Cancer-free women	Human	69.90	See below	[24]
Women with breast cancer	Human	40.40	Reduced risk of breast cancer	[24]
Women with colon cancer	Human	89.60	Increased risk of colon cancer	[24]
Women without breast cancer	Human	56	Protective against breast cancer	[18]
Women with breast cancer	Human	26	Lack of protection against breast cancer	[18]
Normal breast tissue	Human	63	Protective against breast cancer	[40]
Breast cancer tissue	Human	26	Lack of protection against breast cancer	[40]
Parous women without cancer	Human	57	Microchimerism confers immunological tolerance of donor	[6]
Non-parous women without cancer	Human	7.40	Microchimerism confers immunological tolerance of donor	[6]
Parous women with solid tumors	Human	28	Microchimerism confers immunological tolerance of donor	[6]
Parous women with hematological malignancies	Human	47	Microchimerism confers immunological tolerance of donor	[6]
Parous dogs	Dog	36	Fetal microchimerism exists in dogs	[11]
Female puppies	Dog	100	Sibling microchimerism occurs from male siblings in prior pregnancy	[12]

For most solid tumors and hematological malignancies, the frequency of microchimerism is lower than in women without cancer. Colon cancer appears to be a significant exception to this rule, with a much higher frequency of microchimerism identified in women with colon cancer

survivals less than 50 days [38]. These striking similarities in mammary carcinoma between humans and dogs support the potential utility of studying the effects of fetal microchimerism on this group of diseases.

It has long been accepted that nulliparous women are at greater risk for breast cancer than parous women. The examination of peripheral blood fetal microchimerism in matched women with and without breast cancer identified a higher rate (56%) of male microchimerism in unaffected women than in affected women (26%) for invasive breast cancer [18] and 85% for women without carcinoma *in situ* and 64% in women with *in situ* breast cancer [39]. The median number of fetal genomes per million maternal genomes was also higher in women without breast cancer compared to those with the disease [18]. Further, the microchimeric cells have been identified more frequently in healthy breast tissue than in tumor tissue [40]. It is worth noting that only male microchimerism has been evaluated using a quantitative PCR assay of Y chromosomal DNA or fluorescent *in situ* hybridization. Comparison of parous women with and without solid tumors found a similar association of lower frequency of fetal microchimerism in women with breast cancer [6]. Other solid tumors studied included lung, colon, uterine, ovarian, and cervical cancers, which all had similarly low rates of chimerism identified [6]. A group of 272 cancer-free Danish women was found to have a 70% rate of microchimerism [24]. Of these women, 89 went on to develop breast cancer, only 40% of whom had detectable microchimerism [24]. In a follow-on study, these same women with detectable microchimerism had a reduced hazard ratio of all-cause mortality of 0.42 [41]. Overall, fetal microchimerism has been associated with a protective role in breast cancer, although a mechanism has not been identified.

Lymphoma

Non-Hodgkin lymphoma (nHL) is the third most common cancer that occurs in companion dogs [42]. Neoplastic transformation of lymphocytes may occur in the bone marrow prior to differentiation, in the germinal center of lymph nodes, or in the post-germinal phase of B cell biology [43]. To facilitate comparative examination of the diseases that arise from these various stages of development, the human Kiel, Working Formulation, and World Health Organization classification systems have been applied to canine nHL [44, 45]. The Kiel classification is based on cytological criteria defined by cell size, nuclear morphology, cytoplasmic characteristics, and degree of cellular variability [45]. Immunophenotype further subdivides nHLs in this system, but is difficult to identify based on cytological criteria alone [45]. The Working Formulation is a histologic system classifying nHLs using their location of origin within the node, cellular morphology, degree of differentiation, and mitotic rate [45, 46]. See Table II for classification categories. This system has been found to be adequate to grade canine nHLs with few modifications [45, 46]. In a study of 134 canine nHLs, Fournel-Fleury and others found that approximately 26% met the criteria for low-grade malignancy and 74% intermediate to high-grade malignancy in the Kiel system [45]. Using the Working Formulation in the same cases, 20% of the cases were low grade and 80% intermediate to high grade [45]. Carter and others found only 5% of 285 cases of canine nHL to be low grade, with 28% intermediate and 67% high grade using the Working Formulation [46]. With the advent of immunohistochemistry, veterinary pathologists could identify T cell nHL using anti-CD3, anti-CD4, anti-CD5, and anti-CD8 polyclonal antibodies and mAbs [45]. However, anti-CD19 and anti-CD20 pan-B antibodies that are in wide use in humans do not react with the canine antigens [45]. As such,

Table II. Comparison of Three Human Lymphoma Classification Schemes Applied to Canine Lymphoma

Grade	Working formulation	Kiel formulation	World health organization (abbreviated)
Low	Diffuse, small lymphocytic Follicular, predominately small cleaved cells Follicular, mixed	Lymphocytic Lymphoplasmacytic Lymphoplasmacytoid Centrocytic, follicular	Indolent B-cell Marginal zone Mantle cell Follicular Low-grade B cell Diffuse large B cell T cell-rich large B cell B cell small lymphocytic B cell chronic lymphocytic leuk. Diffuse intermediate B cell Indolent T cell T zone Low-grade T cell T cell anaplastic large cell Enteric T cell Cutaneous T cell
Intermediate	Follicular, predominately large Diffuse, small cleaved Diffuse, mixed Diffuse, large cleaved Diffuse, large non-cleaved	Centroblastic/centrocytic, follicular, small cells Centrocytic, diffuse Centroblastic/centrocytic, diffuse, small cells Centrocytic, diffuse, large cells Centrocytic monomorphous Centroblastic polymorphous	Diffuse large B-cell mid-centroblastic Diffuse large B-cell mid-immunoblastic Plasmacytoma Lymphoplasmacytoid lymphoma
High	Immunoblastic Lymphoblastic Small non-cleaved	Immunoblastic Lymphoblastic	Diffuse large B cell high grade Burkitt like B-anaplastic large cell B cell lymphoblastic B cell lymphoblastic cleaved Plasmablastic lymphoma Peripheral T cell T cell lymphoblastic T cell lymphoblastic cleaved

Histology and immunohistochemistry protocols exist that allow classification of canine lymphomas for modeling of human disease. While low, intermediate, and high-grade lymphomas are present in dogs as in humans, the distribution observed is somewhat different *leuk.* leukemia

the development of anti-B cell antibodies anti-CD21, anti-immunoglobulin, anti-CD79a, and, most recently, anti-canine CD20 antibody have allowed the reliable identification of B cells [45, 47]. In the Fournel-Fleury study, a subset of 92 cases was evaluated histologically and immunohistochemically. Of these cases, 24 (26%) were found to be of T cell origin, of which 15 were low-grade nHLs [45]. The frequency of T cell disease in dogs prompted the investigators to identify the centrocytic designation as problematic in a comparative study, as human centrocytic disease is of B cell origin [45]. Carter also noted hypercalcemia and a mediastinal location associated with lymphoblastic nHL, evidence that they were of T cell origin [46]. Valli and others, examining exclusively low grade canine nHL, found only 10 of 66 cases to be of T cell origin, however [48]. Additionally, those tumors identified as immunoblastic demonstrated nuclear size smaller than that typical of human immunoblastic forms [46]. The category of small non-cleaved lymphomas is also problematic, in that the Burkitt's form, common in this class of human nHL, is extremely rare in dogs, without a causal virus like Epstein-Barr [46–49]. This led to a modified subtype of plasmacytoid or clear cell that is not commonly employed in the human classification of this form of nHL [46]. Intermediate to high-

grade lymphomas occur most frequently in mature dogs, with diffuse large cell being the most common, followed by immunoblastic nHL [45].

Most recently, the World Health Organization criteria were applied to canine lymphomas. Application of this classification system proved reliable [50]. Immunophenotyping was judged to be necessary for accurate classification of canine nHL [50]. Compared to human case distribution, follicular nHL was rare in the 300 cases examined [50]. Subsequently, the clinical significance of this grading scheme for dogs with nHL has been confirmed, with high-grade lymphomas leading to more rapid mortality [51]. Application of the current human grading scheme underscores the utility of naturally occurring nHL in dogs as models for the human disease correlates in the evaluation of the impact of fetal microchimerism on lymphomagenesis.

Similar to breast cancer, the frequency of microchimerism in women has been identified to be lower in patients with nHL [6]. To date, microchimerism in women with lymphomas has been less studied than breast cancer. The therapeutic use of donor lymphocyte infusions in the treatment of humans with nHL makes microchimerism interesting therapeutically for this group of diseases [52, 53]. In the

limited study performed, therapy did not reduce the frequency of observed microchimerism [6]. The presence of fetal microchimerism has not been examined as a prognostic variable in patients with nHL.

Colon Cancer

Colon cancer is relatively rare in dogs compared to humans. Adenocarcinomas are the most common tumor of the colon in both species. As in humans, there is evidence that polyps can progress to malignant neoplasms over time in dogs. Most recently, dachshunds in Japan have been shown to develop multiple polyps with progression to adenocarcinoma [10]. The tumor suppressor p53 is overexpressed in benign and malignant colonic lesions of dogs. Dysregulation of β -catenin has been demonstrated in sporadic canine colorectal tumors, similar to that seen in human tumors [54]. The clonality of canine colorectal lesions has not been explored, but more polyclonal lesions in humans carry a higher risk of progressing to malignancy [55]. Further investigation of this group of cancers in dogs is warranted to assess the role of fetal microchimerism in this naturally occurring disease as a model for the human condition.

The reported association of microchimerism with the development of colon cancer in humans is conflicting. An early report combined colon cancers with other solid tumors and found a significantly lower rate of microchimerism in women with solid tumors [6]. Of the previously described 272 Danish women, 67 developed colon cancer during the course of the study. Nearly 90% were detectably microchimeric [24]. This high frequency was associated with an odds ratio of 3.93 for the development of colon cancer [24]. This is an interestingly strong association and does not appear to be confounded by diet or other factors [24]. Recent work suggests that human intestinal tumors can be polyclonal in origin [56]. Perhaps microchimeric cells can participate in or enhance the process of polyclonal recruitment. Further work is necessary to clarify the relationship between detectable microchimerism and colon cancer.

Microchimerism in Dogs

Recently, our laboratory undertook an examination of peripheral blood DNA samples from 90 parous golden retrievers with a history of male puppies in at least one litter [11]. A nested PCR assay for Y chromosomal DNA was developed with a sensitivity of male cell identification between 1 in 60,000 and 90,000 female nucleated cells. Of the 90 dogs, 38 had evidence of Y chromosomal DNA in peripheral blood DNA samples. Following further owner query, 9 of the 38 positive samples had been collected prior to delivering any litter of puppies. Each of these nine dogs was reported to have had a male littermate, similar to what has been reported in nulliparous women with male siblings [6]. This group of dogs, tested with this particular nested PCR assay, revealed a microchimerism rate of 36%. The suggestion of sibling microchimerism appeared in these dogs as well. Our group also evaluated a litter comprised entirely of female puppies for evidence of chimerism [12]. The dam had given birth to prior litters containing male puppies, but the evaluated litter contained only females. Peripheral blood

DNA from the dam was positive for a Y chromosomal band by nested PCR assay. Peripheral blood DNA from two female puppies was also positive. Interestingly, the amplified band from one was stronger than the dam and the other was less strong. The differential degree of chimerism raises questions about the mechanisms of establishment and maintenance of the chimeric state.

CLINICAL MICROCHIMERISM QUESTIONS

Factors that contribute to the development of fetal and maternal chimerism and the resulting interplay of the chimeric state with health and disease are currently unclear. In the case of the female puppies, it is unknown if each was exposed to a different dose of male cells, whether the placental interface may be more or less permissive to cell transfer, or if fetal immune reactions to haplotype differences greatly impact the quantity of identifiable microchimeric cells [12]. A large exchange of maternal and fetal cells occurs in the creation of the uterine blood vessel network that sustains the pregnancy with 12% of uterine vessel endothelium deriving from the fetus [57]. This exchange of cells occurs against the backdrop of pregnancy-associated immune tolerance. The result is the development of fetal regulatory T cells that tolerize the offspring to maternal antigens [58]. This tolerance can be very persistent. As an example, an Rh-negative woman gestating an Rh-positive child may be less likely to generate anti-Rh antibodies if she was born from an Rh-positive mother [59]. In the transplantation setting, children receiving maternal hematopoietic stem cell (HSC) grafts for acute leukemia have improved survival compared to those receiving paternal grafts, likely due to pre-existing tolerance [52]. The presence of microchimerism in the recipient lowers the rate of rejection in humans receiving HLA-haploidentical renal transplants [60]. Human patients who are microchimeric and receive mismatched HSC transplant are more likely to engraft and have less graft vs host disease (GVHD) than patients who are not microchimeric [53–61].

Evidence exists that chimerism is more likely with maternal haploidentical cells than with mismatched haplotype cells [62]. However, tolerization of mismatched cells is described [63]. It is unclear how chimerism changes over a lifetime. In multiparous individuals, do initial colonizing cells persist as the dominant population over a life-time, or do cells of subsequent offspring compete and displace initial chimeric cells? Is there a process of immune tolerization and optimization that results in certain lines being selected? Does this process play a role in health and disease directly? These questions could be investigated in dogs with naturally occurring cancers and immune-mediated diseases.

DISCUSSION

Differential rates of chimerism are associated with cancers and immune-mediated diseases of humans as described above. In women, fetal cells have been identified in many maternal tissues and associated with cancer tissue. What these associations do not demonstrate is causative or preventive mechanism in the disease process. It is unknown whether detectable levels of chimeric cells modify disease or are

modified by disease in women. At least one prospective study evaluating microchimerism in Danish women a decade prior to the advent of cancer has supported the opposite associations of the microchimeric state with breast and colon cancer [24]. Further prospective studies are needed to characterize and follow chimeric cells in circulation over the time course of disease risk to determine the mechanism of protection from or promotion of disease. Such an undertaking is a huge challenge in human populations, particularly given the very long time-span of risk for many of the diseases of interest. With their compressed life-span and period of risk for the same diseases, dogs offer a tremendous opportunity for study in a naturally occurring model. With projects like the Golden Retriever Lifetime Study (<http://caninelifetimehealth.org/>), prospective collection of samples has already begun with careful collection of disease and exposure histories. Dogs offer the opportunity to quantify and characterize microchimeric cells before, during, and after disease manifestation. Mechanistic understanding in dogs would streamline the hypothesis generation and testing in humans.

Microchimerism appears to play a role in the pathophysiology of important diseases to human health. Understanding how to manipulate chimerism may open new pathways to disease prevention and therapy. Identifying chimerism using advanced methods like genotyping non-shared polymorphisms between mother and children could allow for more optimal selection of donor for immune reconstitution in cancer therapy. In an interesting single case report, a microchimeric mother was treated with an infusion of her daughter's lymphocytes for thymic carcinoma, resulting in a remission of her cancer [60]. Infusion of immune cells from microchimera donors could improve outcome for other cancers. Reduction of microchimeric cells circulating in individuals with auto-immune diseases might improve outcome for those patients. Until the mechanistic role of these microchimeric cells is better understood, therapeutic exploitation of fetal microchimerism likely will remain an untapped potential. Prospective modeling in naturally occurring diseases could speed understanding of this interesting and complex process. A One Health approach evaluating companion dogs offers a potential solution to the currently daunting challenge of elucidating the role of shared maternal-fetal cells in health and disease.

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