

Shared pathogenesis of human and canine tumors - an inextricable link between cancer and evolution

Review Article

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Summary

The etiological significance of pathognomonic molecular cancer signatures cannot be understated. Since the discovery that the Philadelphia Chromosome was present recurrently in karyotypes from human chronic myelogenous leukemia patients, the identification of other structural and/or numerical chromosomal abnormalities that are associated recurrently with specific tumors have implicated a long list of genes in the pathogenesis of hematological and solid tumors. Still, a gap remains in our understanding of the mechanisms driving these changes. We recently showed that morphologically equivalent cancers of humans and dogs share homologous molecular signatures. This not only confirms the causal relationship between defined genetic abnormalities and specific tumor types, but it also provides novel mechanistic insights for these events. Here, we review these findings and propose that at least some of these abnormalities occur due to promiscuous reassembly between fragile sites connecting chromosomal segments with preserved gene order. We also advance the notion that cancer susceptibility and tumor progression are the result of comparable gene-environment interactions in humans and dogs, thus allowing us to leverage the unique genetic structure of purebred dog populations to identify heritable factors that contribute to the pathogenesis of cancer in both species.

I. Introduction

Spontaneous tumors that are morphologically and clinically equivalent occur in humans and dogs. This allows for comparative studies in both species as a means to understand the pathogenesis of these conditions. Among the advantages that dogs provide for this approach are the greater prevalence of some types of cancer in dogs than in people, thus providing ready access to case materials, and the fact that cancer progression follows a compressed time course in dogs, allowing for the timely assessment of new interventions (MacEwen, 1990; Hansen and Khanna, 2004; Khanna et al, 2006; Paoloni and Khanna, 2008). In addition, there is a distinct, significant and reproducible

breed predisposition for certain canine cancers, including lymphoma and soft tissue sarcomas (Dorn et al, 1968; Priester and McKay, 1980; Onions, 1984). In the case of lymphoma, familial clustering has been reported in dogs, suggesting that genetic risk (or protective) factors for the disease have segregated with breed-specific traits. Finally, the status of both humans and dogs as “post-genomic” species (Lander et al, 2001; Kirkness et al, 2003; Lindblad-Toh et al, 2005) also opens a new window of opportunity for comparative cancer genomics.

Despite the strong similarities in histological appearance, biological behavior and response to conventional therapies, a major obstacle that remained to fully validate the relevance of canine cancers to the human

condition was the uncertainty as to whether naturally occurring canine tumors would recapitulate the molecular pathogenesis of their human counterparts. Hence, we decided to test the hypothesis that homologous cancers of humans and dogs would harbor evolutionarily conserved and pathognomonic genetic abnormalities. For this purpose, we used chronic myelogenous leukemia (CML), Burkitt lymphoma, and chronic lymphocytic leukemia (CLL), all of which occur spontaneously in humans and dogs, and each of which have well defined chromosomal aberrations in people; that is, the t(9;22) Philadelphia (Ph') chromosome in CML, t(8;14) translocations superimposing c-Myc onto the immunoglobulin heavy chain enhancer in Burkitt lymphoma, and del13q14 deletion in CLL (Breen and Modiano, 2008).

II. The “Raleigh Chromosome” in canine chronic myelogenous leukemia

Various lines of evidence support the causal association between t(9;22) translocations that generate the Ph' chromosome and the consequent BCR-ABL fusion proteins with CML. First, this group of aberrations is seen in as many as 95% of adult patients with CML (Kurczok et al, 2003). Second, chimeric BCR-ABL proteins have constitutively elevated tyrosine kinase activity that is crucial to their oncogenic potential, as demonstrated by the ability to induce and maintain remission of CML patients with imatinib mesylate (Gleevec®), an antagonist of BCR-ABL kinase activity (Kurczok et al, 2003). Third, adoptive transfer of hematopoietic stem cells carrying the BCR-ABL fusion product into lethally irradiated mice recapitulates the disease (Wertheim et al, 2002).

Given the similarities between human and canine CML, we surmised that an homologous translocation might be detectable in dogs with naturally occurring CML. We established that the genomic locations for the canine ABL and BCR loci are at CFA 9q25dist-q26 and CFA 26q24-q25, respectively, and used canine BAC clones representing these loci to examine if a translocation involving these genes was detectable in canine CML. Indeed, we identified the canine equivalent of the Ph chromosome, which we refer to as the “Raleigh chromosome” recurrently associated with canine CML cells (Breen and Modiano, 2008). Not only was the translocation evident by fluorescence *in situ* hybridization (FISH) analysis of metaphase and interphase nuclei, but also, immunoprecipitation of Abl proteins followed by reciprocal immunoblotting with anti-BCR antibodies verified the presence of a BCR-Abl fusion protein that was comparable to the common ~190 kDa form seen in human CML. The Ph' chromosome is also seen in a limited number of human acute lymphoblastic leukemias (ALL). Intriguingly, we also identified the Raleigh chromosome in a case of canine ALL, although leukemic cells from this dog did not harbor a BCR-Abl fusion protein, suggesting a distinct variant form of the translocation event in canine ALL (Breen and Modiano, 2008).

III. Overexpression of c-Myc in sporadic Canine Burkitt Lymphoma

Compelling evidence supports an essential role for deregulated c-Myc expression in BL (Hecht and Aster, 2000). A translocation of C-MYC from HSA 8q24 to HSA 14q32 places c-Myc under the control of the immunoglobulin heavy chain enhancer in virtually every case of BL or L3 subtype acute B-cell ALL, and in a small number of T-cell lymphomas and leukemias (Croce et al, 1984; McKeithan et al, 1986; Shima et al, 1986; MacEwen 1990; Navid et al, 1999).

BL in humans occurs most frequently as the endemic form associated with Epstein Barr virus infection, although the molecular pathogenesis of the endemic and the sporadic forms are indistinguishable. In dogs, BL only occurs sporadically, thus this condition is somewhat less common than in humans (Fosmire et al, 2007; Modiano et al, 2007). As we did for BCR and ABL in canine CML, we established the locations for the MYC and IGH loci at CFA 13q12 and CFA 8q33, respectively, to examine if a translocation involving these genes was detectable in canine BL. As we predicted, using BAC clones for these loci to probe cells from canine BL patients, we demonstrated that they harbored a translocation that juxtaposed MYC and the IGH enhancer, leading to constitutive expression of c-Myc in canine BL (Breen and Modiano, 2008).

IV. Loss of CFA 22 as a Marker of Canine CLL

The most common chromosome abnormality in adult CLL is hemizygous deletion of the q14 region of HSA 13 at the RB-1 locus (Dohner et al, 2000). A causal relationship was recently identified between CLL and the 13q14 deletion in humans, which was due to loss of two microRNAs (mir-15 and mir-16) with strong tumor suppressor activity (Cimmino et al, 2005; Calin and Croce, 2006). Both mir-15 and mir-16 are Bcl-2 antagonists, and reduced levels of mir-16 in NZB mice is responsible for the characteristic lymphoproliferative disease in these mice that recapitulates human CLL (Raveche et al, 2007). Two observations suggested this abnormality might be evolutionarily conserved in canine CLL. First, deletion of 13q14 is prognostically significant, defining the group with most favorable outcomes among human CLL patients. In dogs, CLL is an indolent disease that also shows favorable response to therapy with extended remissions. Second, the structure of the chromosomal region that includes *RB-1* locus and miR15/16 at HSA13q14 is evolutionarily conserved back to zebrafish. In dogs, a deletion homologous to HSA 13q14 would manifest as a deletion within CFA 22q11.2. As we did for CML and BL, we examined if deletion of CFA 22q11.2 (based on use of a BAC clone containing RB-1) was a common occurrence in canine CLL. Perhaps not surprisingly, eight of nine cases of CLL we tested had hemizygous or homozygous deletions of CFA 22 that always included at least one copy of the RB-1 locus with reduced or absent expression of Rb, indicating that loss of

this region of CFA 22 was a functionally significant event (Breen and Modiano, 2008).

One intriguing difference between CLL in humans and dogs is worth noting: in people, CLL is predominantly a disease of B-cells and T-CLL is a rare entity (Schlegelberger et al, 1994); whereas in dogs, CLL presents frequently as a disease of T-cells (Burnett et al, 2003). The observation that loss of syntenic DNA is a shared cytogenetic feature between the canine and humans diseases further supports the notion that this event facilitates proliferation of both B- and T-cells without overt malignant transformation, resulting in similar disease phenotypes and underscoring the essential role of mir-15 and mir-16 in lymphocyte cell cycle control.

V. Mechanistic insights for the pathogenesis of chromosomal abnormalities in naturally occurring cancers of humans and dogs

Our results show that comparable, recurrent chromosomal aberrations were identifiable in these naturally occurring tumors, supporting a strong causal association between each abnormality and the respective tumor phenotype. In addition, the data suggest that each of these pathognomonic lesions leads to similar consequences in both species; *i.e.*, the translocations or the deletion seemed to generate a fusion protein, promote overexpression of a homologous protein, or silence a tumor suppressive activity. Curiously, the aberrations in CML and CLL involved chromosomal regions located near areas of genome reorganization (Breen et al, 1999; O'Brien et al, 1999; Yang et al, 1999), suggesting the existence of evolutionarily conserved, intrachromosomal fragile sites that are used for reassembly in different species, and which may be particularly susceptible to pathologic rearrangements.

Our understanding of genome reorganization during speciation remains rudimentary. A comparative genomic map of 19 mammals representing various placental orders illustrates strong associations between large segments of chromosomal DNA, such as regions that comprise portions of chromosomes 12 and 22 in humans, but which are joined in a single chromosome in lower primates (lemur and tree shrew), carnivores, ungulates (including cetaceans), bats, and rodents (O'Brien et al, 1999). In contrast, there does not appear to have been strong selection to retain the full structural association of the chromosomes involved in the CML (HSA 9 and HSA 22) translocation and the CLL deletion (HSA 13). Instead, these regions are near putative sites of genomic reorganization and may be susceptible not only to translocation or deletion, but also to other abnormalities that are presumably acquired during DNA replication and mitosis (such as a peculiar inversion at CFA 22q11). Similar mechanisms appear to be operative for rearrangements involving *AML1* and *ETO* (on chromosomes 8 and 21 in humans and chromosomes 13 and 31 in dogs) in acute myelogenous leukemia and for deletion of *PTEN* (on HSA 10 and CFA 26) in various types of tumors. Together, this supports a notion that such

sites of genomic reorganization are inherently fragile and may serve as evolutionary retained 'hot spots' for chromosomal reassembly associated with tumorigenesis (Pfeiffer et al, 1995; Murphy et al, 2005). There are recurrent cancer-associated translocations that do not involve such sites, such as the t(8;14) translocation observed in human (and canine) Burkitt lymphoma. Thus, other underlying mechanisms probably contribute to the pathogenesis of this disease in both species (Finger et al, 1986; Reiter et al, 2003).

VI. Canine cancers and heritable traits that control risk and progression

Familial cancer syndromes associated with single gene defects have been characterized in humans, rats, and dogs (Fearon 1997; Hino, 2003; Lingaas et al, 2003), and myriad cancers have been modeled in laboratory mice using genetic engineering. It is curious that cancer syndromes with spontaneous mutation of the *BHD* gene have been identified in each of these species (Comstock et al, 2004; Okimoto et al, 2004). Nevertheless, most cancers occur sporadically as complex diseases where multiple genes with variable penetrance interact with environmental factors to progressively endow a single "tumor initiating cell" with malignant properties.

Lymphoid cancers are among the most common cancers of dogs (Modiano et al, 2005a). Lymphoma and leukemia account for 20-25% of all canine tumors with an average lifetime risk of ~1 in 15 (Modiano et al, 2005a). Generally, lymphomas are "treatable," but not "curable." The median survival for dogs with lymphoma treated with standard-of-care is 10-14 months, and most will die from their disease. However, while lymphoma and leukemia can affect dogs of all breeds and all ages, there are significant breed-associated differences in the age of onset for these diseases (Figure 1) and in their incidence, and disease progression and response to therapy are different among distinct histologic and phenotypic subtypes of lymphoma and leukemia (Modiano et al, 2007).

The breed predisposition for lymphoid tumors in dogs suggests that heritable risk factors for the disease have segregated with breed-specific traits. A number of dog breeds, including Boxers, Golden Retrievers, Labrador Retrievers, Cocker Spaniels, Bassett Hounds, and others are at increased risk for lymphoid cancer, while other breeds, such as Chihuahuas, Dachshunds and Pomeranians show a lower incidence of the disease (Priester and McKay 1980). To put this in proper context, the estimated lifetime risk for any dog to develop lymphoma or leukemia is approximately 1 in 15. The lifetime risk for Boxers is more than four times higher (Priester and McKay, 1980), while that for Golden Retrievers is approximately 1 in 8 (Glickman et al, 2000). The contribution of heritable factors to the risk for lymphoma and leukemia is further supported by the familial clustering observed in certain Rottweiler and Scottish Terrier lines (Teske et al, 1994), and by the fact that breed type can influence response to therapy (Garrett et al, 2002).

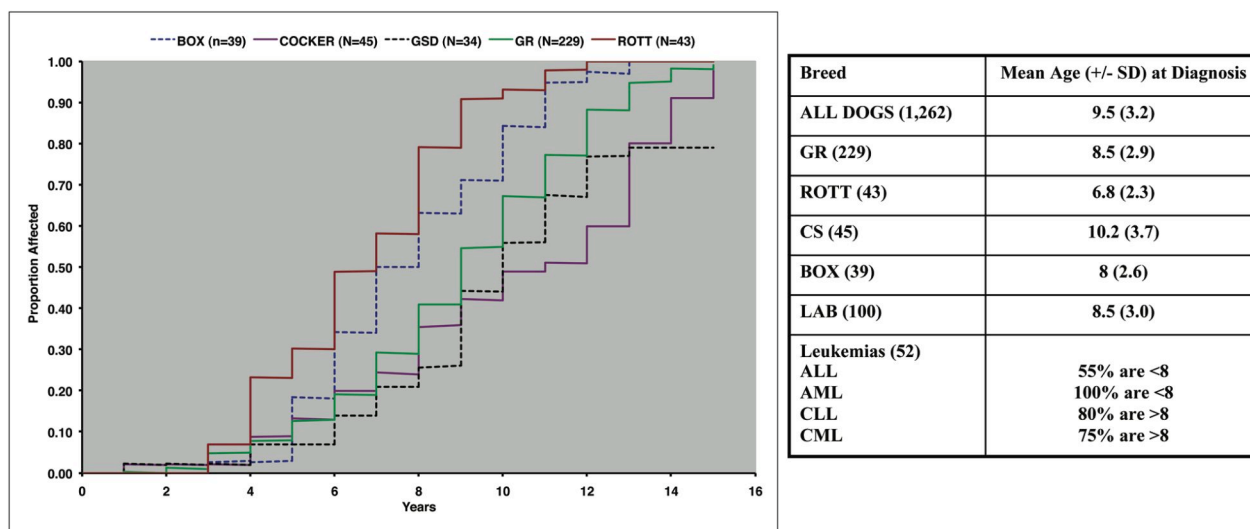


Figure 1. Age at onset for canine lymphoma differs according to breed. The proportion of affected dogs for five dog breeds from a large collaborative study (Modiano et al, 2005b) is shown as a function of the age at which dogs were diagnosed (in years). There are significant differences among breeds, with three groups becoming readily apparent: dogs that develop lymphoma at a significantly younger age than the mean (and median) for all dogs (exemplified by Rottweilers and Boxers), dogs that are diagnosed when they are younger than the mean (and median) for all dogs, but older than the first group (exemplified here by Golden Retrievers, but also including Labrador Retrievers), and dogs that are representative for the mean (and median) of all breeds (exemplified here by German Shepherd Dogs). When all purebred dogs were considered together, this group was not significantly different from mixed breed dogs. This suggests that mix breed dog populations indeed approximate the mean of all the heritable contributions of purebred dogs, and that these contributions may have variably penetrance with few, if any, dominant alleles. In this sample set, Cocker Spaniels seem to be diagnosed even later than the mean (and median) for all dogs, but the difference between these groups was not statistically significant.

Recently, we showed that this breed predisposition could be extended to specific phenotypes (“B-cell” and “T-cell”) of lymphoid tumors (Modiano et al, 2005b). We showed that Spitz breeds and Asian “lap dogs” belonging to the oldest domestic dog groups (Parker et al, 2004) almost exclusively develop T-cell tumors, and some European breeds like Cocker Spaniels and Bassett Hounds almost exclusively develop B-cell tumors. Boxers and Golden Retrievers also show more T-cell disease than what is seen as an average for the population, and as shown in **Figure 1**, lymphoproliferative diseases occur earlier in life (Modiano et al, 2007), but in these breeds, the frequency of B-cell and T-cell phenotypes is more balanced (Modiano et al, 2005b). The prevalence of B-cell and T-cell lymphoma and leukemia in most other breeds is not significantly different from the average of all dogs or from that seen in dogs of mixed breeding (*i.e.*, ~2:1 B-cell to T-cell).

Environmental factors cannot account for the observed breed predilections for lymphoid tumors. There is, therefore, a basis for the hypothesis that heritable risk factors predispose dogs to develop B-cell or T-cell malignancies. Such risk factors are presumably mutations or epigenetic changes in genes that regulate lymphocyte development, although the involvement of genes that regulate the fidelity of the genome cannot be ruled out, as familial non-Hodgkin lymphomas in people are largely associated with conditions such as the p53 mutation in Li-Fraumeni syndrome (Segel and Lichtman, 2004; Siddiqui et al, 2004). Whether these factors arose ancestrally, or

developed more recently during the process of breed derivation, they are now firmly embedded in the genome, and their identification will provide information that will be valuable for prevention and treatment. While this task may seem difficult, we already have documented the occurrence of breed-specific cytogenetic changes in lymphoid tumors from Golden Retrievers (Modiano et al, 2005b), and analyses of pedigrees from Golden Retriever families suggest there is shared susceptibility to specific forms of lymphoma or leukemia among family members. The use of genomic and expression arrays provide contemporary tools that will allow us to refine familial data by tumor type and clinical response, thus providing greater insight and statistical power to find specific genes that are important in disease susceptibility and progression. For example, preliminary analyses of gene expression signatures of lymphoma samples from Golden Retrievers and non-Golden Retrievers shows a ~1.2 to 1.4-fold decrease in expression of genes that localize to CFA 14, and a corresponding increase in expression of genes that localize to CFA 15 in the Golden Retrievers samples, with *p* values <0.05 (**Table 1**). The data suggest there is a significant difference in expression levels, but the extent of the reduction is small. Several non-mutually exclusive possibilities can account for the small magnitude of these changes, including low levels of expression for genes along these chromosomes, loss of a single copy (or portion thereof) of CFA 14 in tumors from Golden Retrievers, leading to haploinsufficiency rather than absolute deletion of genes encoded therein, or epigenetic modification.

Table 1. Gene Expression Profiles from Golden Retriever Lymphomas Reflect Genomic Aberrations^a

CFA	GENBANK	PATHWAY	P-VALUE	FOLD-CHANGE
14	CO600038	RNA processing	0.007	-1.2
14	PMRNA4890	Cell division	0.013	-1.2
14	PMRNA4163	IDDM	0.016	-1.2
14	PMRNA5677	DNA binding and repair	0.019	-1.1
14	CX008060	Fatty acid metabolism and apoptosis	0.041	-1.2
14	PMRNA3988	gamma-HCCH/Bisp A degradation	0.044	-1.2
15	CF406744	Transcriptional control	0.005	1.3
15	CO706972	Pantothenate and CoA biosynthesis	0.014	1.1
15	DN362831	Ischemia/ reperfusion	0.018	1.1
15	PMRNA4530	Glucose transport	0.020	1.1
15	PMRNA7126	MAPK/Insulin signaling	0.037	1.1
15	PMRNA9940	?Chromosome segregation	0.040	1.1

^aSignificantly different genes show reduced expression along CFA 14 and increased expression along CFA 15 in tumors from Golden Retrievers as compared to tumors from non-Golden Retrievers. The Genbank identifier and the biochemical pathways in which the genes are known to participate are shown, as is the p value from Student's T-test after normalization and the fold-change. The small magnitude of change reflects consistent values with little variance, and may be due to relatively low levels of expression or epigenetic regulation.

Intriguingly, complementary experiments suggest that loss of CFA 14 may be a more widespread occurrence in multiple types of tumors from Golden Retrievers. This is the subject of additional investigation by our groups.

New molecular and phenotypic classification of lymphoma and leukemia also may assist in predictions regarding the course of disease and outcomes in response to treatment. A series of genomic microarrays have been developed to analyze DNA copy number aberrations associated with canine cancers (Thomas et al, 2003a,b, 2005, 2007), and are being used now to investigate the cytogenetics of numerous canine cancers, including lymphoma and leukemia, brain tumors, osteosarcoma, and soft tissue sarcomas. The genome assembly-integrated nature of the most recent iterations of these genomic arrays facilitates rapid transition from the identification of a region within a chromosome associated with cancer presentation to the precise location with the canine genome assembly, and thus to the identification of specific genes within those regions (Thomas et al, 2007). Simultaneously, studies are underway to assess the predictive ability of gene expression profiles for lymphoma and leukemia. We are using chromosome-specific FISH to directly define and catalogue altered genome organization within individual cells comprising the malignant mass (Thomas et al, 2001, 2003c; Milne et al, 2004; Breen and Modiano 2008). We also are using canine comparative genomic hybridization (CGH) analysis to determine DNA copy number status across the entire genome (Dunn et al, 2000; Thomas et al, 2001, 2003c, 2005, 2007). FISH analysis also has enabled the precise definition of structural chromosome aberrations that show a convincing evolutionary history between homologous cancers of dogs and people (Breen and Modiano, 2008).

These analyses can be used to predict both tumor origin and response to therapy. For instance, gain of dog chromosome 13 (CFA 13), particularly in a region syntenic to human chromosome 4q (HSA 4q), occurs in ~70% of canine diffuse B-cell lymphoma (Thomas et al, 2003c). This suggests that this region of the genome contains heretofore-unidentified genes that are etiologically and prognostically significant for this disease. There are also indications that gain of CFA 13 also is predictive for chemotherapy response (Hahn et al, 1994; Thomas et al, 2003c), perhaps because amplification of the *c-myc* and *c-kit* oncogenes, both of which are encoded in this region of CFA 13, increases the proliferative rate of the malignant cells and consequently their susceptibility to anti-mitotic compounds. These approaches also can be used to define specific chromosomal regions that are associated with heritable risk by identifying unique tumor genomes that segregate with selected breeds or groups, and that can pinpoint new regions for gene discovery (Modiano et al, 2005b).

Although they are at earlier stages of development, tools such as arrays that can be used to analyze global gene expression are making rapid progress toward clinical application. The ability to analyze thousands of genes at once maximizes the efficiency with which we can identify genetic alterations associated with tumor pathogenesis, as well as prognostic "gene signatures" (Rosenwald et al, 2002). Importantly, even simple tests that can be used as surrogates for cytogenetic changes such as the deletion of CFA11 in high-grade T-cell lymphomas have predictive value for this disease (Modiano et al, 2007).

A contemporary area of emphasis is to define whether these properties are inherent to cancer cells or whether they arise by natural selection and clonal

evolution. We can use molecular tools to predict risk, prognosis, and response to therapy in some cancers of companion animals, and we believe the availability and usefulness of such tools in clinical practice will expand rapidly. Therefore, as we improve our understanding of basic mechanisms that account for malignant transformation and tumor progression, we will be able to design even better strategies for cancer prevention and therapy.

VII. Conclusions

In summary, we describe the identification of evolutionarily conserved chromosomal abnormalities associated with specific leukemia and lymphoma phenotypes in humans and dogs. We propose that a previously unidentified mechanism, which involves inappropriate reassembly of chromosomal regions that are involved in genome reorganization across species, may account for some of these abnormalities, offering a plausible explanation for the remarkable consistency and specificity of these cytogenetic signatures in human and canine tumors.

Moreover, the predisposition of dog breeds to develop select types of cancer (Modiano et al, 2005b) suggests that, through the process of breed development, dogs have acquired genomes that render specific cells distinctly susceptible to malignant transformation. The presence of evolutionarily conserved chromosome aberrations in naturally occurring cancers will thus allow us to determine if the apparent structural 'instability' in these regions is indeed a conserved feature of the ancestral genome. Furthermore, a thorough investigation of recurrent breakpoint regions in canine tumor genomes, especially those that have high breed specificity, offers the potential to define regions that contain genes that have so far not been associated with tumorigenesis in humans or other species (Modiano et al, 2005b). Evaluation of these genes may hence shed light on the observed disparity in response to treatment among different dog breeds (Garrett et al, 2002) and among people diagnosed with leukemia and lymphoma.

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