

subgroups will specify the interactions of the JAK2 mutation in AML and will reveal insights into its prognostic impact and into its relationship with previous chemo- or radiotherapy.

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References

- 1 Lee JW, Kim YG, Soung YH, Han KJ, Kim SY, Rhim HS *et al.* The JAK2 V617F mutation in *de novo* acute myelogenous leukemias. *Oncogene* 2006; **25**: 1434–1436.
- 2 Steensma DP, McClure RF, Karp JE, Tefferi A, Lasho TL, Powell HL *et al.* JAK2 V617F is a rare finding in *de novo* acute myeloid

leukemia, but STAT3 activation is common and remains unexplained. *Leukemia* 2006; **20**: 971–978.

- 3 Schnittger S, Bacher U, Kern W, Haferlach T, Schoch C. The role of the JAK2 mutations: a study in 1103 patients with CMPD and in 196 patients with AML. *Blood* 2006 (Suppl): (in press).
- 4 Gilliland DG. Hematologic malignancies. *Curr Opin Hematol* 2001; **8**: 189–191.
- 5 Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR *et al.* A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med* 2005; **352**: 1779–1790.
- 6 Kuchenbauer FS, Susanne L, Thomas G, Gary T, David H, Torsten H *et al.* Identification of additional cytogenetic and molecular genetic abnormalities in acute myeloid leukemia with t(8;21)/AML1-ETO. *Br J Haematol* 2006; **134**: 610–619.
- 7 Schessl C, Rawat VP, Cusan M, Deshpande A, Kohl TM, Rosten PM *et al.* The AML1-ETO fusion gene and the FLT3 length mutation collaborate in inducing acute leukemia in mice. *J Clin Invest* 2005; **115**: 2159–2168.
- 8 Desta F, Christiansen DH, Andersen MK, Pedersen-Bjergaard J. Activating mutations of JAK2V617F are uncommon in t-MDS and t-AML and are only observed in atypic cases. *Leukemia* 2006; **20**: 547–548.

Predictive value of p16 or Rb inactivation in a model of naturally occurring canine non-Hodgkin's lymphoma

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We report here the use of naturally occurring non-Hodgkin's lymphoma (NHL) as a model to investigate heritable risk and predictive factors for response in this disease. Dogs are at risk to develop virtually all subtypes of sporadic NHL seen in humans (no transforming lymphotropic viruses such as Epstein–Barr virus or human T-cell leukemia virus type 1 have been conclusively identified in dogs); these tumors not only have morphological similarities between both species, but they also share a similar natural history and clinical behavior^{1–3} (Table 1). NHL is the most common life-threatening cancer of dogs, accounting for up to 24% of all malignancies and over 80% of all hematopoietic cancers.¹ Untreated cases, especially those of high-grade type, rarely survive beyond 3 months after diagnosis, but the disease is generally responsive to standard of care using CHOP-based chemotherapy protocols, increasing both the length and quality of affected dogs' lives.¹ With standard of care, remission can be achieved in >85% of canine NHL cases, and median survival ranges between 9 and 12 months.^{4,5} This represents approximately 10% of an average canine lifetime, thus providing a fair target to compare the outcome between dogs and people. Predictive factors for survival in dogs include immunophenotype, clinical substage and histologic subtype. However, as is true for NHL in humans, additional criteria are required to generate more refined schemes that can accurately predict outcomes.

Beyond these similarities, peculiar properties of canine NHL increase its value as a model for the human disease. Specifically, the age-adjusted incidence of NHL (excluding other lymphoid neoplasms) is estimated at 107 per 100 000 dog-years,⁶ a rate approximately five times higher than that reported for humans by the SEER program (~22 per 100 000).⁷ In addition, the relative risk for this disease in some breeds is up to four times higher than the average for all dogs,^{6,8,9} suggesting that heritable risk factors for the disease have been firmly established in the derivation of specific breeds.

To further investigate heritable risks, we examined how breed could influence the age of onset for this disease. We previously

reported a unimodal association between age and lymphoproliferative disease in dogs, with the mean age among all dogs equal to 9.1 years (s.d. 3.2 years).⁸ Here, we compared the age of onset between the single largest breed group in that study (Golden Retriever, *N*=229) and all other dogs ('non-Golden Retriever', *N*=1033). The results show a 1-year difference, which is highly statistically significant (*P*<0.001) between the two groups (Figure 1). In addition, the standard deviation of the mean age was smaller for Golden Retrievers than for all other dogs, underscoring that age of onset is more similar within this single high-risk breed than when all dogs are considered together. Other evidence to support the existence of strongly embedded (albeit complex) heritable risk factors for this disease in dogs can be inferred by familial clustering observed in Bull mastiff, Rottweiler and Scottish Terrier lines,^{10,11} and by the fact that breed also can influence response to therapy.⁵

The breed predisposition for NHL in dogs extends to specific phenotypes ('B cell' and 'T cell').⁸ The average frequency of B- and T-cell neoplasms for the population as a whole is respectively ~60 and 40%. We showed that Spitz breeds and Asian 'lap dogs' belonging to the oldest domestic dog groups developed almost exclusively T-cell tumors, and some European breeds like Cocker Spaniels and Bassett Hounds developed almost exclusively B-cell tumors. Boxers and Golden Retrievers also show more T-cell disease than average, but in these breeds the frequency is more balanced (~50% of each).⁸ B- and T-cell neoplasms from Golden Retrievers also have peculiar cytogenetic changes that are seen significantly less frequently or not at all in tumors from other dogs.^{8,12}

In contrast to this, more than 85% of NHL diagnosed in humans consist of mature B-cell tumors and fewer than 10% represent T-cell tumors;¹³ the incidence rates for NHL are lower in people of Asian descent and there appears to be predominance for T-cell neoplasms in non-white individuals. Even though improved understanding of NHL biology has allowed therapy for this disease to evolve in humans, the genetic structure of humans and the prevalence of NHL subtypes make it difficult, respectively, to define heritable risk and to accrue enough cases to define prognostic and predictive factors for

Table 1 Comparison of common NHL subtypes in humans and dogs according to the WHO classification^a

Tumor classification	Comments	
	Human	Dog ^b
B-cell NHL		
Follicular lymphoma	More than 85–90% of cases	Approximately 60% of cases
Diffuse large B-cell lymphoma	Accounts for 20–25% of cases Most common subtype; accounts for up to 40% of cases	Fewer than 5% of cases Most common subtype; accounts for up to 40% of cases
Mantle cell lymphoma	Accounts for approximately 6% of cases	Rare (probably less than 1% of cases)
Marginal zone lymphoma	Most commonly diagnosed as a splenic tumor, about 2% occur in lymph nodes	Most common indolent B-cell NHL subtype in dogs; seen frequently as a nodal tumor ³
Burkitt's lymphoma	Most common form is endemic (EBV-associated), sporadic form not associated with EBV	Only occurs as sporadic form, a gamma-herpesvirus of dogs has not been identified
Small lymphocytic lymphoma	Solid counterpart to CLL	Solid counterpart to CLL, more often has T-cell phenotype
T-cell NHL		
Precursor T-lymphoblastic lymphoma	Fewer than 10% of cases Associated with poor prognosis	Approximately 40% of cases Associated with poor prognosis, may be more common in dogs than people
Peripheral T-cell lymphoma – not otherwise specified	Associated with poor prognosis	Associated with poor prognosis, may be more common in dogs than people
Angioimmunoblastic lymphoma	Mature T-cell NHL subtype with the worst prognosis	Appears to be rare in dogs
Anaplastic large cell lymphoma	Mature T-cell NHL subtype with best prognosis, forms include CD30 ⁺ and CD30 ⁻ , associated with inappropriate expression of Alk	Can have a B-cell phenotype, expression of CD30 and Alk as yet undefined
T-zone lymphoma	Among few types of indolent T-cell lymphoma	Most common type of indolent T-cell lymphoma ³
Small lymphocytic lymphoma	T-cell phenotype is rare	Most common solid counterpart to canine CLL, indolent progression and good prognosis

Abbreviations: ALK, activated lymphocyte kinase; CLL, chronic lymphocytic leukemia; EBV, Epstein-Barr virus; NHL, non-Hodgkin's lymphoma. ^aAn abbreviated list of NHL subtypes is presented, excluding leukemias, plasma cell tumors and NHL types that occur predominantly outside the lymph nodes (cutaneous lymphoma, enteric lymphoma, etc.).

^bA multi-institution, systematic assessment of the prevalence of different canine NHL subtypes classified according to WHO is currently under way, which, once completed, will allow more precise estimates of frequency for each subtype.

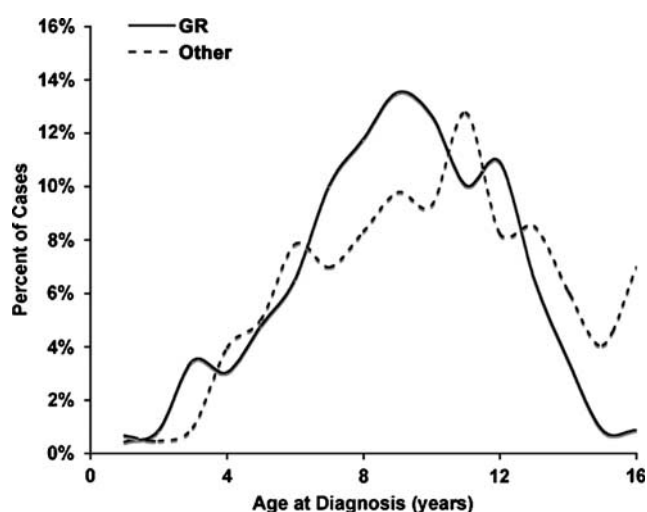


Figure 1 Influence of breed on age of diagnosis for canine NHL. We used a cohort of 1262 dogs with a diagnosis of lymphoproliferative disease⁸ to analyze the influence of breed on age at diagnosis. The single most common pure breed group in this cohort was Golden Retrievers ('GR', $N=229$). This group was separated from all other dogs ('Other'), and percent of total cases was plotted as a function of age in yearly intervals. The means \pm s.d. for each group were 8.5 ± 2.9 for Golden Retrievers and 9.5 ± 3.5 for all other dogs. The ages of onset for disease are statistically significantly different ($P < 0.001$) between these two populations.

infrequently encountered tumors.¹⁴ This situation offers unique opportunities to use canine NHL as a robust model to investigate these aspects of the disease.

The value of the spontaneous canine NHL to investigate heritable risk is illustrated above; to show an example of how canine NHL can be used to investigate prognosis and prediction for conditions that occur uncommonly in humans, we examined the predictive value of p16 and retinoblastoma protein (Rb) inactivation in nodal canine NHL using a prospective cohort designed to focus on a high-risk breed that would include approximately equal numbers of B- and T-cell NHL. It is known that these proteins contribute to the regulation of normal lymphocyte proliferation, possibly providing mechanisms that enforce quiescence,¹⁵ but their precise role in the pathogenesis of NHL remains incompletely understood. In human NHL, alterations leading to p16 inactivation (deletion or promoter methylation) are commonly seen in blastoid mantle cell lymphoma; they occur less frequently in association with progression from mucosal associated lymphoid tissue lymphoma or follicular lymphoma to diffuse large B-cell lymphoma (DLBCL), and only sporadically in high-grade tumors. The inappropriate activation of cyclin D1 in mantle cell lymphoma also results in Rb inactivation. The prognosis for human mantle cell lymphoma is generally poor, but the prognostic significance of p16 deletions and Rb inactivation for other NHL subtypes remains uncertain.¹⁶

We designed our sample collection strategy around Golden Retrievers with nodal (stage II–IV) NHL, allowing us to evaluate tumors within a consistent background of heritable risk, and we included samples from other dog breeds to distinguish abnormalities that might be breed-specific from those associated with disease pathogenesis (not breed-specific). We analyzed the frequency of p16 inactivation (deletion or methylation) and its functional consequences, including Rb phosphorylation, in 48

cases representing four common types each of nodal T- and B-cell canine NHL. These included high-grade tumors (lymphoblastic T-cell lymphoma, peripheral T-cell lymphoma – not otherwise classified, DLBCL, sporadic Burkitt's lymphoma and anaplastic large B-cell lymphoma) and indolent tumors (T-zone lymphoma, small T-cell lymphoma and marginal zone B-cell lymphoma). Our results showed that inactivation of p16/Rb occurred predominantly in high-grade tumors and was less prevalent in low-grade tumors (Fosmire *et al.*, manuscript submitted). Rb inactivation, evidenced by phosphorylation at sites homologous to Ser249/Thr252 and/or Thr826, was seen in tumors with deletions or methylation of p16, consistent with constitutive activation of CDK4 and/or CDK6. However, this pattern of Rb inactivation was also seen in tumors that retained p16, suggesting that other mechanisms might lead to Rb phosphorylation (for example, overexpression of c-Myc secondary to gain of chromosome 13¹²). This suggests that Rb phosphorylation may represent a rate-limiting event in malignant transformation or for tumor progression of peripheral lymphocytes in high-grade NHL.

Outcome data were available for 40 dogs that were treated with standard of care. In this group of dogs, overall median survival was 9 months. As shown in Figure 2, p16 status and Rb phosphorylation were the only variables that showed statistically significant differences in outcome among all tumors; histologic grading was predictive only for T-cell tumors. The median survival for dogs with detectable phosphorylated Rb was 4 months, whereas it was >24 months for dogs with unphosphorylated (or hypophosphorylated) Rb, a timeframe roughly equivalent to >7–10 years for humans with the same disease.

In summary, we show here the potential of naturally occurring canine NHL to explore heritable factors that contribute to NHL and to identify prognostic factors for this disease, especially in subtypes that occur infrequently in people and thus cannot be easily studied in a systematic fashion. Our results showing that Rb phosphorylation at canonical sites for CDK4 can be readily detected using routine immunohistochemical approaches and that it has high predictive value in canine NHL make it reasonable to consider its

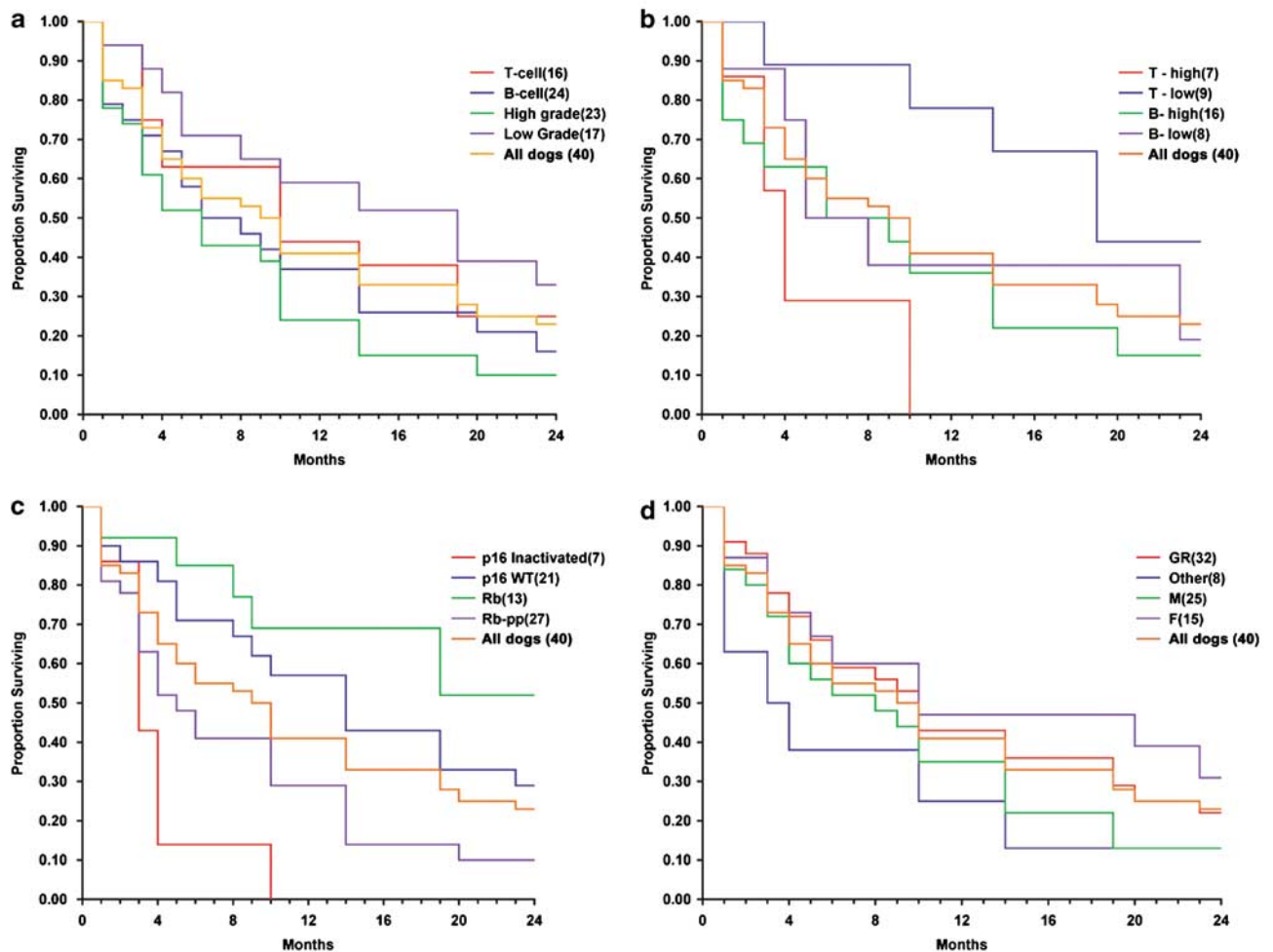


Figure 2 Predictive value of variables associated with canine NHL. Kaplan–Meier survival curves for 40 cases of dogs with NHL, treated with standard of care, classified according to the WHO based on (a) immunophenotype or histologic grade, (b) immunophenotype and histologic grade, (c) p16 status ('inactivated' or 'wild type') or Rb phosphorylation ('Rb' or 'Rb-pp') and (d) breed (Golden Retriever or 'GR' vs 'other') or gender ('male' vs 'female'). The numbers in parentheses to the right of each legend represent sample size for the groups. Median survival for all dogs was 9 months. None of the groups had a statistically different probability (based on two-tailed Fisher's exact test) to survive significantly longer than the median for all dogs. However, dogs with inactivation of p16 (red line, panel c) had significantly shorter ($P=0.02$) survival than dogs with wild-type p16 (blue line, panel c), and dogs with phosphorylated Rb (Rb-pp, purple line, panel c) had significantly shorter ($P=0.04$) survival than dogs with unphosphorylated Rb (green line, panel c). Among the dogs with T-cell tumors, dogs with high-grade tumors (red line, panel b) had significantly shorter survival ($P=0.03$) than dogs with low-grade tumors (blue line, panel b).

utility as an indicator of prognosis or as a predictor of outcome in human NHL patients.

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References

- Hansen K, Khanna C. Spontaneous and genetically engineered animal models; use in preclinical cancer drug development. *Eur J Cancer* 2004; **40**: 858–880.
- Valli VE, Jacobs RM, Parodi AL, Vernau W, Moore PF. *Classification of Hematopoietic Tumors of Domestic Animals*, 2nd Series edn. AFIP – American Registry of Pathology: Washington, DC, 2002.
- Valli VE, Vernau W, de Lorimier LP, Graham PS, Moore PF. Canine indolent nodular lymphoma. *Vet Pathol* 2006; **43**: 241–256.
- MacDonald VS, Thamm DH, Kurzman ID, Turek MM, Vail DM. Does L-asparaginase influence efficacy or toxicity when added to a standard CHOP protocol for dogs with lymphoma? *J Vet Intern Med* 2005; **19**: 732–736.
- Garrett LD, Thamm DH, Chun R, Dudley R, Vail DM. Evaluation of a 6-month chemotherapy protocol with no maintenance therapy for dogs with lymphoma. *J Vet Intern Med* 2002; **16**: 704–709.
- Edwards DS, Henley WE, Harding EF, Dobson JM, Wood JLN. Breed incidence of lymphoma in a UK population of insured dogs. *Vet Comp Oncol* 2003; **1**: 200–206.
- Morton LM, Wang SS, Devesa SS, Hartge P, Weisenburger DD, Linet MS. Lymphoma incidence patterns by WHO subtype in the United States, 1992–2001. *Blood* 2006; **107**: 265–276.
- Modiano JF, Breen M, Burnett RC, Parker HG, Inusah S, Thomas R *et al.* Distinct B-cell and T-cell lymphoproliferative disease prevalence among dog breeds indicates heritable risk. *Cancer Res* 2005; **65**: 5654–5661.
- Priester WA, McKay FW. The occurrence of tumors in domestic animals. *Natl Cancer Inst Monogr* 1980; **54**: 1–210.
- Teske E, de Vos JP, Egberink HF, Vos JH. Clustering in canine malignant lymphoma. *Vet Q* 1994; **16**: 134–136.
- Onions DE. A prospective survey of familial canine lymphosarcoma. *J Natl Cancer Inst* 1984; **72**: 909–912.
- Thomas R, Smith KC, Ostrander EA, Galibert F, Breen M. Chromosome aberrations in canine multicentric lymphomas detected with comparative genomic hybridisation and a panel of single locus probes. *Br J Cancer* 2003; **89**: 1530–1537.
- Müller AM, Ihorst G, Mertelsmann R, Engelhardt M. Epidemiology of non-Hodgkin's lymphoma (NHL): trends, geographic distribution, and etiology. *Ann Hematol* 2005; **84**: 1–12.
- Escalon MP, Liu NS, Yang Y, Hess M, Walker PL, Smith TL *et al.* Prognostic factors and treatment of patients with T-cell non-Hodgkin lymphoma: the M.D. Anderson Cancer Center experience. *Cancer* 2005; **103**: 2091–2098.
- Modiano JF, Mayor J, Ball C, Fuentes MK, Linthicum DS. Cdk4 expression and activity are required for cytokine responsiveness in T cells. *J Immunol* 2000; **165**: 6693–6702.
- Gronbaek K, de Nully Brown P, Moller MB, Nedergaard T, Ralfkiaer E, Moller P *et al.* Concurrent disruption of p16INK4a and the ARF-p53 pathway predicts poor prognosis in aggressive non-Hodgkin's lymphoma. *Leukemia* 2000; **14**: 1727–1735.

PPAR β -mediated growth suppression of baicalein and dexamethasone in human myeloma cells

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Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that are originally reported to regulate the expression of genes involved in fatty acid uptake and oxidation, lipid metabolism and inflammation.¹ The PPARs consist of three subtypes, PPAR α , PPAR β/δ (PPAR β) and PPAR γ ; PPAR α is expressed primarily in the liver, brown adipose tissue, kidney, heart and skeletal muscle, PPAR γ is expressed mainly in white and brown adipose tissue, and PPAR β is ubiquitously expressed; PPAR(s) have recently been reported to be involved in tumorigenicity.² As for the inflammation, PPARs are considered to be anti-inflammatory, and PPAR agonists induce the downregulation of nuclear factor-kappa B (NF- κ B) activity. As the constitutive higher activation of NF- κ B is considered to be important for the survival and proliferation in B-cell malignancies, including multiple myeloma (MM), it has been extensively explored as to which compounds can inhibit NF- κ B activity in the MM.³

In this paper, we first examined which subtype of PPAR was expressed in myeloma cells and whether PPAR activation could inhibit the proliferation of myeloma cells *in vitro*. Primary myeloma cells from newly diagnosed MM patients as well as

myeloma cell lines (U266, ILKM2, ILKM3, ILKM8, AMO1, NOP2 and KMS5) predominantly expressed the PPAR β gene and also showed the weak expression of PPAR γ 1 (Figure 1a). We also found the suppressive effect of carbacyclin (PPAR β agonist) or troglitazone (PPAR γ agonist), but not Wy14643 (PPAR α agonist) on primary myeloma cells as well as myeloma cell lines, as shown in Figure 1b and c.

Although myeloma cells predominantly expressed PPAR β , we evaluated which compounds showed PPAR β -stimulating activity in the PPRE β -luciferase assay.⁴ We screened the PPAR β -stimulating activity of prostaglandins, hydroxy-eicosatetraenoic acids (HETEs), the hormones of the adrenal cortex, derivatives of cholesterol and flavonoids. Among the compounds we examined, interestingly, the hormones of the adrenal cortex, dehydroepiandrosterone sulfate DHEA-S and hydrocortisone (dexamethasone (Dex)), showed the strong PPAR β -stimulating activity (Figure 2a), and one of the flavonoids from *Scutellaria radix*, baicalein,⁵ also showed strong PPAR β -stimulating activity (Figure 2b). Furthermore, we found the cooperative effect of Dex and baicalein on the PPAR β -stimulating activity in the PPRE β -luciferase assay (Figure 2c). We examined whether Dex and baicalein showed the cooperative effect on the expression of PPAR response element (PPRE)-mediated target genes, *ILK* and *PPAR γ 1* in myeloma cell lines (U266 and ILKM2) by reverse