Augmentation of Therapeutic Responses in Melanoma by Inhibition of IRAK-1,-4

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Abstract

Toll-like receptors (TLR) are expressed by a variety of cancers, including melanoma, but their functional contributions in cancer cells are uncertain. To approach this question, we evaluated the effects of stimulating or inhibiting the TLR/IL-1 receptor-associated kinases IRAK-1 and IRAK-4 in melanoma cells where their functions are largely unexplored. TLRs and TLR-related proteins were variably expressed in melanoma cell lines, with 42% expressing activated phospho-IRAK-1 constitutively and 85% expressing high levels of phospho-IRAK-4 in the absence of TLR stimulation. Immunohistochemical evaluation of melanoma tumor biopsies (n = 242) revealed two distinct patient populations, one that expressed p-IRAK-4 levels similar to normal skin (55%) and one with significantly higher levels than normal skin (45%). Levels of p-IRAK-4 levels did not correlate with clinical stage, gender, or age, but attenuated IRAK-1,-4 signaling with pharmacologic inhibitors or siRNA-enhanced cell death in vitro in combination with vinblastine. Moreover, in a xenograft mouse model of melanoma, the combined pharmacologic treatment delayed tumor growth and prolonged survival compared with subjects receiving single agent therapy. We propose p-IRAK-4 as a novel inflammation and prosurvival marker in melanoma with the potential to serve as a therapeutic target to enhance chemotherapeutic responses. Cancer Res; 72(23); 6209–16. ©2012 AACR.

Introduction

The incidence of melanoma has been on the rise in the United States and worldwide over the last 30 years and has the fastest rising cancer incidence in the United States (1–3). Melanoma is the 5th/6th most common cancer in men and women, respectively (1–3). The median survival of patients with advanced disease is approximately 6 months and the survival rate at 5 years is 6% and 45% for Stage III patients (1,2). Treatment failure is largely attributed to melanoma’s resistance to all existing forms of cancer therapies.

Recent reports indicate that Toll-like receptors (TLR) signaling within nonimmune cells, including several types of human cancers, can contribute to cancer progression (4–7). TLRs recognize infectious microorganisms as well as endogenous signals released by dying or stressed cells. The engagement of all known TLRs, except TLR3, initiates interleukin (IL)-1 receptor-associated kinase (IRAK) signaling (8–10). IL-1, IL-18, and IL-33 can also activate IRAK signaling. IRAK-4 kinase activity is regulated by autophosphorylation (Ser346, Thr342, and Thr34; refs. 8–11), which in turn can activate IRAK-1. IRAK-4,-1 activation results in the downstream activation of various kinases and transcription factors including JNK, AP-1, NF-κB, and p38 mitogen-activated protein kinase (MAPK), leading to the production of a mixture of chemokines and proinflammatory cytokines including TNF-α, IL-1, IL-6, and IL-8 (12). IRAK signaling can also induce the expression of several proteins involved in cell survival and division (13).

In the current study, we examined the TLR and TLR signal-related protein expression profile on various melanoma cell lines as well as the expression of the activated (phosphorylated) form of IRAK-4 on patient biopsies. Cytokine production and cell survival in response to stimulating or inhibiting IRAK-1,-4 in melanomas was examined in vitro. In vivo, the therapeutic efficacy of combinatorial treatment with IRAK-1,-4 inhibitor plus chemotherapy was investigated in mice with an established human melanoma tumor.

Materials and Methods

Melanoma cell lines

Human melanoma cell lines were obtained from American Type Culture Collection (ATCC) within 2 years of manuscript submission. Melanoma cell lines were initially expanded and cryopreserved within 1 month of receipt. Cells were typically used for 6 months, at which time a fresh vial of cryopreserved cells was used. Malme-3M cells were maintained in Iscove’s...
Modified Dulbecco’s Medium, SK-MEL-2, WM115, C32, and RPMI-751 in Eagle’s Minimum Essential Medium, A375 in Dulbecco’s Modified Eagle’s Medium, and G361 in McCoy’s 5a Medium Modified. All media were purchased from Invitrogen Life Technologies and supplemented with FBS, penicillin, and streptomycin according to culture media recommended by ATCC.

Western blotting and immunohistochemical staining

Total cell extracts were prepared from melanoma cells. Proteins (20–30 μg/lane from cell lines) were resolved in Tris-glycine SDS gels and transferred to polyvinylidene difluoride membranes. The membrane was blocked for 4 hours with 5% milk in PBS and 0.05% Tween-20, followed by incubation with antibodies against TLR1 (N-23), phosphorylated (p)-IRAK-1 (Ser 376), total IRAK-1 (H-273; Santa Cruz Biotechnology); TLR2, TLR4, TLR7, TLR9, TRIF, IRAK-M, IRAK-4, Tollip, glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 14C10), and β-actin (Cell Signaling); TLR3/CD283, TLR5 (IMAGEX); TLR6 (6D10H11), TLR8 (4IC143), TLR10, MyD88 (Abcam), PARP, caspase-3 (Cell Signaling), overnight at 4°C and, subsequently, incubated with horseradish peroxidase-conjugated secondary antibody, and detected using enhanced chemiluminescence (ECL Plus; Amersham Pharmacia Biotech). Melanoma tissue arrays were purchased from US Biomax, Inc. For immunohistochemical staining, samples were stained with mouse monoclonal anti-human phosphor-IRAK-4 antibody (Cell Signaling; dilution, 3.5 μg/mL) or normal mouse serum. Tissue histology sections (5-μm-thick) were deparaffinized, hydrated, heated in boiling 10 mmol/L sodium citrate (pH 6.0), for antigen retrieval, for 30 minutes, and washed in Tris buffer. Peroxide blocking was done with 3% H2O2 in methanol at room temperature for 15 minutes, followed by 10% FBS in TBST for 30 minutes at room temperature. Primary antibody incubation with antibodies against TLR1-TLR2 agonist (Pam3CysK4) or TLR5 (Flagellin) was performed overnight at 4°C. Secondary antibody incubation with horse anti-mouse (1:200; Vector Laboratories) was done for 1 hour, followed by application of diaminobenzidine chromogen for 5 minutes. The slides were then counterstained with haematoxylin and topped with a coverslip. Immunohistochemistry (IHC) specimens were analyzed using "quick score" system in which the intensity of the immunohistochemical reaction was scored by multiplying the intensity 1 (weak), 2 (moderate), and 3 (strong) and the proportion of cells staining positively 0 (<10%), 1 (10%–25%), 2 (26%–50%), 3 (51%–75%), or 4 (>75%). In some experiments, A375 cells were transfected via electroporation (Amaxa, Program X-001, Lonza buffer L) with siRNA-hIRAK-1 and siRNA-hIRAK-4 (5 μg/mL; Invivogen).

A375 melanoma xenograft model

NOD.Cg-Prkdcead.IItg2rgm1Hsd/SzJ IL2RG (NSG) mice (Jackson Laboratories) were subcutaneously injected with 2.5 × 106 A375 melanoma cells delivered in PBS. The Institutional Animal Care and Use Committee approved use of mice. When tumors reached a size of approximately 50 mm2 mice were injected intratumorally with 35 mg/kg of IRAK-1, -4 inhibitor or an equal volume of vehicle (dimethyl sulfoxide; DMSO). Mice were injected via intraperitoneal injection with vinblastine (0.25 mg/kg) every 2 days for 5 days starting on the same day that mice received with IRAK-1, -4 inhibitor. Tumor sizes were analyzed using a mixed model approach for repeated measurements and mouse survival data were analyzed with the exact log-rank test.

TLR agonists, IRAK inhibitor, chemotherapies, and flow cytometry

The TLR1-TLR2 ligand tripalmitoyl-S-(bis[palmitoyloxy])propyl-Cys-Ser-Lys-Lys-Lys (Pam3CysK4) was purchased from Invivogen. In some experiments we used the IRAK-1/-4 inhibitor (EMD Millipore), which is a cell-permeable benzimidazole compound that selectively inhibits IRAK-1 and -4 and exhibits little activity against a panel of 27 other kinases. In some experiments cells were treated with the indicated concentrations of IRAK-1, -4 inhibitor and vinblastine, cisplatin, and 5-fluorouracil (Sigma-Aldrich), and apoptosis was quantitated by flow cytometry after staining cells with fluorescein isothiocyanate-labeled Annexin-V (BD Pharmingen) and propidium iodide (PI; Sigma-Aldrich).

Results and Discussion

TLR expression profiles on melanoma cell lines

Previous reports have shown the presence of TLR mRNA transcripts and a limited number of TLRs by flow cytometry on human melanomas (4, 6). We examined in greater detail the expression profile of TLR1 through TLR10 and several TLR-related signaling proteins on various melanoma cell lines by Western blotting. With the exception of C32 and Malme-3M, all melanoma cells expressed relatively high but variable levels of TLR1 (Fig. 1A). Appreciable levels of TLR2 and TLR3 were detected in SK-Mel-2 and A375. TLR3 was also moderately expressed on RPMI-7951 and G361 cells. Most cell lines expressed variable levels of TLR4 and TLR7. TLR5 was strongly expressed on SK-Mel, WM115, and A375, moderately expressed on G361 and Malm-3M, but weakly detected on C32 and RPMI-7951. TLR8 levels were low on SK-Mel-2 and A375. TLR9 was strongly expressed on SK-Mel-2 and WM115 and moderately expressed on Malm-3M and G361. TLR10 expression was weak and variable on the different cell lines. All cell lines expressed the TLR adapter molecules MyD88 and TRIF.

IRAK-1 and IRAK-4 play a central role in TLR-mediated signaling. All of the melanoma lines expressed high levels of IRAK-4 and variable levels of IRAK-1 (Fig. 1A). Interestingly, total IRAK-1 as well as the activated form of IRAK-1 (phosphorylated at serine 376; p-IRAK-1) was strongly expressed in Malme-3M, SK-MEL-2, and A375, specifically in the absence of exogenous TLR agonists. Similarly, variable levels of p-IRAK-4 (at threonine 345) were detected in melanoma cells (Fig. 1A). We also examined whether TLR stimulation could augment p-IRAK levels in cells that expressed IRAK-1 and IRAK-4. However, p-IRAK levels in A375 remained unchanged following TLR1-TLR2 stimulation suggesting that phosphorylated levels may already be at or near the maximum. Supplementary Fig. S5B. In G361 cells, which express low levels of p-IRAK-1 (and which express TLR5), neither TLR1-TLR2 agonist (Pam3CysK4) nor TLR5 (Flagellin)
stimulation increased or induced p-IRAK-4 or p-IRAK-4 expression levels. Inexplicably, however, the TLR5 agonist flagellin reduced total and p-IRAK-4 levels in both cell lines. This is the first report showing the expression of constitutively phosphorylated IRAK-1 and IRAK-4 on human cutaneous melanoma cells. These data also represent a comprehensive protein expression profile of TLRs and TLR-signaling proteins on melanoma cells and highlight the differences in the expression of these proteins in different melanoma lines. It is worth noting however, that Western blotting was used to detect total TLR proteins levels versus flow cytometry, which detects surface TLRs.

Cytokine/chemokine production by melanoma cells following activation or inhibition of IRAK-1,-4

The stimulation of TLR-MyD88 or IL-1/18/33–MyD88 activates IRAK-1,-4 resulting in the expression of various chemokines and cytokines involved in cell survival and division as well as factors capable of promoting tumor growth such as angiogenic and inflammatory cytokines. We compared the cytokine/chemokine profile between A375 cells stimulated with the TLR1-TLR2 agonist Pam3CysK4 and untreated cells. TLR stimulation significantly augmented the production levels of various factors including those associated with angiogenesis such as VEGF, the melanoma growth factor chemokine ligand-1 (CXCL1), and IL-8, which promote cell survival and proliferation (Fig. 1B; P < 0.05; ANOVA; refs. 14–16). The levels of granulocyte macrophage colony-stimulating factor (GM-CSF) and IP-10 were also increased following addition of the TLR1-TLR2 agonist (Fig. 1B; P < 0.001; ANOVA). TLR1-TLR2 also increased MCP-1 and IL-6 levels but appeared to reduce fractalkine concentrations. To further confirm that the TLR-IRAK signaling pathway was intact in melanoma cells and that changes in cytokines/chemokines are a result of activating this pathway, we transiently overexpressed IRAK-1 in G361 melanoma cells and compared changes in cytokine/chemokine...
levels with control G361 cells. Overexpressing IRAK-1 increased the levels of various cytokines/chemokines including VEGF, CXCL1, G-CSF, and IL-12p40. IRAK-1 expression also induced the expression of IP-10, G-CSF, and PDGF-AA but had no effect on EGF production, as shown in Supplementary Fig. S1. Collectively, these data indicate that melanoma cells express a functional TLR-IRAK signaling pathway and that the activation of this pathway might play a role in promoting cell survival or proliferation in part through the production and chemokines/cytokines.

On the basis that melanoma cells exhibited increased levels of phosphorylated IRAK-1 and IRAK-4 and IRAK signaling results in the activation of various transcription factors, we examined the outcome of inhibiting IRAK signaling in melanoma cells. Melanoma cells cultured in the presence of an IRAK-1,-4 inhibitor showed marked reduction of phosphorylated NF-κB (p-NF-κB) in all 4 melanoma cell lines tested as compared with cells treated with vehicle alone (DMSO), Fig. 1C. Furthermore, IRAK-1,-4 inhibition reduced the production of VEGF more than 90% and diminished CXCL1, monocyte chemotactic protein-1 (MCP1), platelet-derived growth factor alpha (PDGF-A), and fibroblast growth factor (FGF-2) levels in A375 cells (Fig 1D, P < 0.05). The fact that that IRAK-1,-4 inhibition reduced the levels of these molecules in the absence of exogenous TLR agonists, suggests that IRAK-1,-4 contributes greatly to cell function through the production of various factors. It is worth highlighting that the addition of IRAK-1,-4 inhibitors also decreased the effects of TLR1-TLR2 agonist (Supplementary Fig. S2), further confirming that changes in chemokines/cytokines occurred in a TLR-MyD88-IRAK fashion.

Collectively, these findings support the contention that the activation of IRAK-1,-4 signaling on melanoma cells in vitro might contribute to cancer progression by inducing the expression of various chemokines and cytokines beneficial to cancer cell survival, division, and/or angiogenesis. Furthermore, the inhibition of IRAK-1,-4 drastically reduced the production of several cytokines/chemokines, highlighting the possibility that IRAK-1,-4 signaling plays a central role in cytokine/chemokine production even in absence of exogenous TLR agonists. However, these data do not exclude the possibility that TLRs recognize endogenous TLR agonists or that other cytokines produced in response to IRAK signaling might further potentiate this signaling pathway.

Combining IRAK inhibition with certain chemotherapies augments melanoma cell apoptosis in vitro and in vivo

Melanoma cells become resistant to a variety of chemotherapies by altering their survival signaling pathways during cancer progression (17). Various studies have documented the prosurvival effects that TLR signaling have on different cell types (13). Considering the impact that inhibiting IRAK-1,-4 had on NF-κB activation and chemokine/chemokine production, we examined whether IRAK-1,-4 inhibitor might be used as a means to augment the toxic effects of certain chemotherapies, which alone are not very effective. The melanoma cell lines A375 and Malme-3M were cultured in the presence of IRAK-1,-4 inhibitor and various concentrations of vinblastine, 5-fluorouracil, and cisplatin. The data in Fig. 2A show increased sensitivity of both cell lines to vinblastine (left panels). The addition of 5'-fluorouracil and IRAK-1,-4 inhibitor also appeared to enhance the apoptosis of Malme3M cells but had no effect on A375 cells (Fig. 2A, middle panels). IRAK-1,-4 inhibition did not increase cisplatin’s cytotoxicity of either cell line (Fig. 2A, right panels). We examined in greater detail the role that IRAK had in mediating the chemoprotective effects. A375 cells engineered to knockdown IRAK-1 expression were treated with vinblastine, and apoptosis was measured 48 hours later. psi-RNA reduced IRAK protein levels more than 95%; a representative image is shown in Fig. 2B. Reducing IRAK-1 or IRAK-4 expression levels augmented the toxic effects of vinblastine, as compared with cells transfected with the control plasmid, Fig. 2B (right panel). Cells coexpressing psi-RNA-hIRAK-1 and -4 exhibited a higher percentage of death than cells expressing either of these vectors alone, Fig. 2B (right panel). It is worth noting that IRAK inhibitor alone or cells expressing psiRNA-IRAK moderately increased melanoma apoptosis (in the absence of vinblastine), suggesting that IRAK signaling plays an important role in cell survival.

IRAK-1,-4 inhibition resulted in reduced levels of activated NF-κB (Fig. 1C). Therefore, we examined whether IRAK–NF-κB signaling is linked to chemoresistance by treating A375 melanoma cells with and without NF-κB inhibitor and in the absence and presence of vinblastine. Interestingly, NF-κB inhibition did not alter A375’s sensitivity to vinblastine (Supplementary Fig. S3A; P = 0.14). Furthermore, because the toxic effects of doxorubicin have been shown to involve NF-κB signaling, we also examined the effects of inhibiting NF-κB in A375 melanoma cells (18). In sharp contrast to the combinatorial effects of NF-κB inhibition plus vinblastine, NF-κB inhibition increased the toxic effects of doxorubicin on A375, Supplementary Fig. S3A. These data suggest that although TLR-IRAK signaling can activate NF-κB and inhibiting IRAK signaling can reduce p-NF-κB levels, the chemoprotective effects observed appear to be mediated via a mechanism independent of NF-κB. Because IRAK signaling results in the activation of various transcription factors including c-jun/Fos, Elk-1, and CAM-responsive element binding protein (CREB), we postulate that perhaps IRAK signaling is linked to chemoresistance via the activation of one of these factors. The possibility that these transcription factors could impart melanoma cells a prosurvival signal is substantiated by several studies highlighting that these transcription factors play a critical role in melanoma progression (19–22).

Alternatively, cytokines/chemokines produced in response to TLR-IRAK signaling might also contribute to the chemoprotective effects observed. Therefore, supernatant from TLR1-TLR2-stimulated or unstimulated A375 melanoma cells was added to the C32 melanoma cells in the presence or absence of vinblastine. Another group of cells were treated with TLR1-TLR2 ligand and apoptosis was examined by flow cytometry. The Supplementary Fig. S3B show that supernatant from TLR-stimulated A375 cells moderately reduced vinblastine-induced apoptosis as compared with untreated cells (P <
0.05; ANOVA), whereas TLR1-TLR2 ligand had little effect. Furthermore, neither IL-1 or CXCL1 appeared to be the cytokines/chemokines responsible for the observed chemoprotective effects, Supplementary Fig. S3C. These data suggest that cytokines/chemokines produced in response to TLR-IRAK stimulation can contribute to cell survival; however, other IRAK-mediated factors appear to play a more important role. It is also worth noting that different melanoma cell lines might be more or less sensitive to these molecules.

As an independent biochemical assay to confirm that A375 melanoma cells triggered apoptosis, we also conducted Western blot analysis using antibodies specific for caspase-3 and PARP. Active caspase-3, which is cleaved to yield catalytically active subunits, was detected following the addition of 10 μmol/L IRAK-1,-4 inhibitor (Fig. 2C). Enhanced accumulation of cleaved PARP, which is targeted for caspase-dependent proteolysis, was also observed in IRAK-1,-4 inhibitor-treated cells. Increased levels of cleaved PARP and caspase-3 occurred in a concentration-dependent manner (Fig. 2C). As shown in Fig. 2D, the combination of vinblastine and IRAK-1,-4 inhibitor also increased the levels of cleaved PARP and caspase-3. In contrast, treatment with vinblastine or IRAK-1,-4 inhibitor alone had little effect on the levels of these molecules.

We also sought to determine whether inhibitor-treated cells exhibited changes in the levels of apoptosis-related molecules. We compared the expression of various apoptosis-related genes in A375 melanoma cell line treated with DMSO vehicle (control), IRAK inhibitor, and vinblastine and observed a gene profile that favored apoptosis in the presence of inhibitor alone or vinblastine (Supplementary Fig. S4). Taken together with data presented in Fig. 2, these gene array data confirm that inhibiting the IRAK pathway in melanoma cells is important for their survival and indicate that interfering with this pathway can sensitize melanoma cells or at least function in concert with certain chemotherapies to enhance their toxic effects. These data also show that some chemotherapeutic drugs, such as vinblastine, that have been deemed ineffective might be rendered therapeutically useful when combined with a TLR/IRAK inhibitor.

The antitumor effects of combinatorial therapy using vinblastine and IRAK-1,-4 inhibitor were tested in vivo. A375 cells were subcutaneously injected into NSG mice and grown to 30 to 50 mm². Mice were injected intraperitoneally with...
vinblastine or intratumorally with IRAK-1,-4 inhibitor (35 mg/kg) or with vinblastine or IRAK-1,-4 inhibitor alone. Mice receiving IRAK-1,-4 inhibitors plus vinblastine showed a marked reduction in tumor growth and improved mouse survival (median survival: 38 days) as compared with mice receiving vehicle (DMSO) plus vinblastine (median survival: 22 days) or mice receiving IRAK-1,-4 inhibitor (median survival: 19 days), Fig. 3A and B. Mice receiving single therapy for IRAK-1,-4 inhibitor or vinblastine showed modest tumor growth delay but similar survival as compared with control mice (median survival: 17 days). Despite a tumor growth delay, mice receiving IRAK-1,-4 inhibitors plus vinblastine succumbed to tumor challenge 40 days after initiation of treatment. Nonetheless, these results emphasize the antitumor effects of combinational therapy and highlight the need for further optimization. Identifying novel signaling pathways that can improve the efficacy of chemotherapeutic drugs to treat melanoma patients is critical, considering that advanced melanoma is highly resistant to all known chemotherapies (17). The synergistic effects of IRAK-1,-4 inhibitor and vinblastine may reside in the mechanism of action by vinblastine. Whereas, vinblastine kills cells by suppressing microtubule dynamics, cisplatin and 5fluorouracil damage DNA by inducing DNA (and protein) cross-linking, and by irreversibly inhibiting thymidylate synthase, respectively. Ongoing studies by our group are focused on identifying additional chemotherapeutic drugs that are enhanced when used in combination with IRAK-1,-4 inhibitor.

Altogether, these findings corroborate a link between IRAK-1,-4 signaling in melanoma cells and survival and suggest a phenotype supportive of melanoma progression in cancer patients. The importance of IRAK-1/IRAK-4 signaling and cell survival is further highlighted in immune cells in which TLR engagement enhances the expression of various antiapoptotic genes (13).

**Phosphorylated IRAK-4 in melanoma biopsies**

Phosphorylated IRAK-4 expression was analyzed by IHC in normal skin and in melanoma tissue derived from patients at various clinical stages. Although little staining was observed in normal skin, p-IRAK-4 was highly expressed in melanoma samples (representative staining are shown in Fig. 4A). We sought to determine the association between p-IRAK-4 expression and the clinical stage. As shown in Fig. 4B, p-IRAK-4 levels were not linked to melanoma stage nor was there a correlation between p-IRAK-4 levels and metastasis. Unexpectedly, however, quantification of the staining revealed 2 distinct groups in melanoma samples in all clinical stages; one group of melanoma samples expressed p-IRAK-4 levels similar to those on normal skin, and another group expressed significantly higher p-IRAK-4 levels (Fig. 4B; \( P < 0.005; \text{ANOVA} \)). Samples from stage I through IV melanoma also showed a distinct division between high and low/no expression of phosphorylated IRAK-4 (Fig. 4B; \( P < 0.05; \text{ANOVA} \)). Of the 242 melanoma samples analyzed, nearly half expressed elevated levels of p-IRAK-4. It is worth noting that the elevated levels of p-IRAK-4 in melanoma patients did not correlate with patient age or gender (Fig. 4C).

Currently, there is no effective cure for patients with advanced melanoma. This is due in large part to their high resistance to chemotherapeutic drugs. Ongoing studies by our group are focused on identifying the association between total and phosphorylated IRAK-1 and IRAK-4 expression levels with clinical outcome including response to treatment and survival. The immunohistochemical data presented here are in line with the Western blotting data showing that a subset (42%; 3 of 7) of melanoma cell lines express p-IRAK. Ongoing studies are focused on determining the use of total and/or p-IRAK-1,-4 as biomarkers for prediction of therapy resistance and patient survival. It is important to note that recent studies support our contention that IRAK signaling contributes to cancer progression. Boukerche and colleagues found that IRAK-1 gene transcript levels in melanoma patients were associated with highly metastatic melanoma individuals, whereas Li and colleagues observed that reexpression of miR-146a in pancreatic cancer lines downregulated IRAK-1 expression as well as NF-kB activity that blocked pancreatic cancer cell invasiveness and metastasis (23, 24).
The studies presented here highlight the possibility of identifying new chemotherapeutic agents that might be currently in use to treat other cancers or other disorders but might be repurposed against melanoma when joined with IRAK inhibitors. Although new drugs such as vemurafenib (BRAF enzyme inhibitor), ipilimumab (anti-CTLA-4), BMS-936558 (anti-PD1), and BMS-663513 (anti-CD137; ref. 25) show antitumor activity, the effects are quite moderate, prolonging survival 2 to 6 months. In addition, patients eventually develop a resistance to vemurafenib. For this reason, Plexxikon and Roche are currently testing vemurafenib in combination with other signaling pathway inhibitors such as mitogen-activated protein inhibitors in an attempt to prevent the development of tumor chemoresistance. Unfortunately, no other therapies to date have proven effective at improving overall survival of these patients (3, 26, 27), and the development of new therapeutic agents has not kept up with the increased melanoma incidence (28). We propose that understanding how IRAK inhibition sensitizes melanoma to chemotherapies (or augments their toxic effect) might provide novel insights and allow the discovery of more effective therapies to treat melanoma patients.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Conception and design: Z. Li, E. Davila
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