Recurrent RARB Translocations in Acute Promyelocytic Leukemia Lacking RARA Translocation

Tomoo Osumi1,2, Shin-ichi Tsujimoto2,3, Moe Tamura4, Meri Uchiyama1, Kazuhiko Nakabayashi5, Kohji Okamura6, Masanori Yoshida1,3, Daisuke Tomizawa2, Akihiro Watanabe2, Hiroyuki Takahashi8, Tsukasa Hori9, Shohei Yamamoto10, Kazuko Hamamoto11, Masahiro Migita12, Hiroko Ogata-Kawata5, Toru Uchiyama13, Kazuko Hamamoto11, Masahiro Migita12, Hiroko Ogata-Kawata5, Toru Uchiyama13, Hiroe Kizawa14, Hitomi Ueno-Yokohata1, Ryota Shirai1,3, Masafumi Seki15, Kentaro Ohki1, Junko Takita15, Takeshi Inukai16, Seishi Ogawa17, Toshio Kitamura4, Kimikazu Matsumoto2, Kenichiro Hata5, Nobutaka Kiyokawa1, Susumu Goyama4, and Motohiro Kato1,2

Abstract

Translocations of retinoic acid receptor-α (RARA), typically PML–RARA, are a genetic hallmark of acute promyelocytic leukemia (APL). However, because a small fraction of APL lacks translocations of RARA, we focused here on APL cases without RARA translocation to elucidate the molecular etiology of RARA-negative APL. We performed whole-genome sequencing, PCR, and FISH for five APL cases without RARA translocations. Four of five RARA-negative APL cases had translocations involving retinoic acid receptor-β (RARB) translocations, and TBL1XR1–RARB was identified as an in-frame fusion in three cases; one case had an RARB rearrangement detected by FISH, although the partner gene could not be identified. When transduced in cell lines, TBL1XR1–RARB homodimerized and diminished transcriptional activity for the retinoic acid receptor pathway in a dominant-negative manner. TBL1XR1–RARB enhanced the replating capacity of mouse bone marrow cells and inhibited myeloid maturation of human cord blood cells as PML–RARA did. However, the response of APL with RARB translocation to retinoids was attenuated compared with that of PML–RARA, an observation in line with the clinical resistance of RARB-positive APL to ATRA. Our results demonstrate that the majority of RARA-negative APL have RARB translocations, thereby forming a novel, distinct subgroup of APL. TBL1XR1–RARB as an oncogenic protein exerts effects similar to those of PML–RARA, underpinning the importance of retinoic acid pathway alterations in the pathogenesis of APL.

Significance: These findings report a novel and distinct genetic subtype of acute promyelocytic leukemia (APL) by illustrating that the majority of APL without RARA translocations harbor RARB translocations. Cancer Res; 78(16):4452–8. ©2018 AACR.

Introduction

Acute promyelocytic leukemia (APL) accounts for 5% to 10% of acute myeloid leukemia (AML; ref. 1), which is characterized by unique morphological features typically including abnormal promyelocytes containing azurophilic granules. APL was once considered as the most intractable form of AML because of life-threatening coagulopathy and high relapse rate. The genomic basis of APL is characterized by the t(15;17)(q22;q21) translocation, which induces a fusion of promyelocytic leukemia (PML) and retinoic acid receptor-α (RARA; ref. 2). PML–RARA is a potent oncoprotein that deregulates transcription and transforms cells ex vivo and in vivo (3). APL cells with PML–RARA respond well to all-trans retinoic acid (ATRA; ref. 4), providing one of the most successful examples of targeted therapy for cancer. Furthermore, arsenic trioxide (ATO) also exerts extraordinarily potent anti-APL effects (4), and treatment for cancer. Furthermore, arsenic trioxide (ATO) also exerts extraordinarily potent anti-APL effects (4), and...
RARβ Translocations in APL

www.aacrjournals.org Cancer Res; 78(16) August 15, 2018 4453

Patients and Methods

Patients

A 2-year-old boy presented with leukocytosis and coagulopathy (unique patient identifier, UPN1). Bone marrow aspiration revealed the presence of blasts with azurophilic granules, and APL was morphologically diagnosed (Fig. 1A), but treatment with ATRA was ineffective. PCR for PML–RARα was negative, and break-apart FISH analysis for RARA did not show a split signal (Fig. 1B).

Furthermore, we collected four additional cases morphologically diagnosed as APL lacking a RARA translocation. As shown in Supplementary Fig. S1, all cases had proliferation of promyelocytes with azurophilic granules, which could not be distinguished from APL with PML–RARα, although few faggot cells were observed in all RARA-negative APL cases. Most of the cases had a similar pattern of surface markers with typical APL, such as positivity for CD13 and CD33, and negativity for CD34 and HLA-DR. As in the first case, all the additional cases were refractory to ATRA; hence AML-based multiagent chemotherapy was given. However, in total, four of five cases suffered a relapse or failed to achieve remission (Supplementary Fig. S2). The details of the characteristics and clinical course of the cases are shown in Supplementary Table S1. The clinical course of one case (UPN5) has previously been reported elsewhere (22).

Genomic analysis

For whole genome sequencing, DNA sample from leukemic cells was sonicated to produce random short fragments. Library reportedly cures up to 70% of patients when used alone (5). Recent clinical trials demonstrated that a combination of ATRA and ATO achieved excellent outcomes, with more than 90% event-free survival for typical APL with PML–RARα, even without conventional cytotoxic chemotherapy (4).

Indeed, translocated RARA genes are a hallmark of APL. Although a minority of APL cases (<5%) lacks canonical PML–RARα (6), most had a translocation between RARA and other partners including ZBTB16 (PLZF; ref. 6), NPM1 (7), NUMA1 (8), STAT5B (9), ZBTB16 (PLZF; ref. 6), NPM1 (7), NUMA1 (8), STAT5B (9), FNDC3B (10), PRAR1A (11), BCR (12), FIP1L1 (13), NABP1 (14), GTF2I (15), IRF2BP2 (16), and TBL1XR1 (17). Almost all known, APL-associated translocations involve RARA, which accounts for 99% of APL (6). A study showed whole genome sequencing could detect a cryptic PML–RARα, which were missed by conventional cytogenetics or FISH (18); however, genomic analyses focusing on RARA translocation are incapable of detecting RARA rearrangement in the remainder of APL cases (6). A recent study with whole exome sequencing revealed detailed genomic landscape of APL with PML–RARα (19), but few studies have been performed to identify novel fusions in RARA-negative APL cases. We herein focused on APL cases without RARA translocation, performed a high-throughput sequencing analysis, identified a novel recurrent fusion of retinoic acid receptor-β (RARβ) in the majority of patients with RARA-negative APL, and elucidated the features of this novel fusion in the pathogenesis of APL.

Figure 1.

Morphologic and cytogenetic features of the APL case with TBL1XR1–RARβ translocation (UPN1) and the model of the fusion gene. A, Morphologic features of RARβ-positive APL blasts in the bone marrow (May-Giemsa staining). Hypergranular promyelocytic blast cells with irregular nuclear membranes and prominent, heavily stained granules lacking Auer rods. B, Break-apart FISH analysis for RARB. Arrows, split signal for one allele; arrowhead, fusion signal of the wild-type allele. C and D, Reverse transcription PCR (C) and Sanger sequencing (D) confirming the in-frame TBL1XR1–RARβ fusion.
construction was performed with NEBNext Ultra II DNA Library Prep Kit according to the manufacturer's protocol. All the libraries were sequenced using the Illumina HiSeq 2500. In the use of Integrative Genomics Viewer 2.3.66, coloring by chromosome on which their mates were mapped can visualize the candidate mutations. Custom Perl and C programs, which were used in this analysis, are available upon request.

For whole exome sequencing, library construction was performed with SureSelect Human All Exon Kit V5 or V6, Agilent Technology, according to the manufacturer's protocol. Enriched fragment libraries were then sequenced on an Illumina HiSeq 2500 in 101-bp paired-end mode. Image analyses and base calling were performed using CASAVA 1.8.4 with default parameters. To validate the mutations detected by whole-exome sequencing, Sanger sequencing of each genes was performed.

Luciferase assay
Transcriptional activity of TBL1XR1–RARB was analyzed by luciferase assay. Retinoic acid-responsive element (RARE) signal reporter (Qiagen) including transcription factor responsive construct and constitutively expressing Renilla luciferase construct, and respective EX-B0004-M07 expression vectors (Mock, RARB, RARA, TBL1XR1–RARB, PML–RARA) were transiently cotransfected into HEK293 cells. Different concentration of ATRA were added after 24 hours and incubated 24 hours.

Coimmunoprecipitation and immunoblotting analysis
For coimmunoprecipitation, expression vectors with MYC- or HA-tag proteins were transiently transfected into HEK293 cells. After 24 hours, ATRA, tamibarotene, and ATO were added and incubated. For immunoblotting analysis, extracted proteins from the cell lines were separated in polyacrylamide gel electrophoresis and transferred to nitrocellulose membrane. The proteins were analyzed by immunoblotting using each antibody.

Flow cytometry and morphologic analysis
U937, which is a cell line widely used for the biological studies including the preceding study for TBL1XR1–RARA, were infected with prepared retrovirus (Mock, RARB, TBL1XR1–RARB, PML–RARA). ATRA-treated or nontreated U937 cell lines were incubated with FITC-anti-human CD11b antibody (BioLegend) and examined by flow cytometer. For morphologic analysis, cytopsin slides of 0 and 5 days after ATRA-treated U937 cells were stained with Wright-Giemsa staining solution.

Human cord blood cell culture
Human umbilical cord blood (CB) cells were obtained from Riken BRC or the Japanese Red Cross Kanto-Koshinetsu Cord Blood Bank (Tokyo, Japan). CD34+ cells were separated and those transduced with vector, TBL1XR1–RARB, PML–RARA, or RUNX1–RUNX1T1 were cultured.

Colony replating assay
Mouse bone marrow progenitors (c-Kit+ cells) were separated. The c-Kit+ cells transduced with vector, TBL1XR1–RARB, PML–RARA, or RUNX1–RUNX1T1 were plated in M3234 methylcellulose. Colony counting and replating were performed every 5 days.

Institutional Review Board approval
This study was approved by the institutional ethics board of the National Center for Child Health and Development (#1035), and required written informed consent was obtained from the patients or guardians. This study was conducted in accordance with the Declaration of Helsinki.

Results
Genomic analysis of RARA-negative APL
We first performed whole-genome sequencing to identify the potential genomic alterations in the initial case (UPN1). Intriguingly, we successfully identified an in-frame fusion between TBL1XR1 and RARB, which was confirmed by FISH and PCR (Fig. 1C and D; Supplementary Tables S2 and S3). RARB encodes RARB, a paralog of RARA, both of which are members of the nuclear receptor superfamily. We searched further for RARB alterations in the four additional cases of RARA-negative APL, using whole-genome sequencing, RT-PCR and FISH according to sample availability (Supplementary Table S1). Strikingly, two additional cases had the TBL1XR1–RARB fusion with a break point identical to that of UPN1, whereas one case had a RARB rearrangement detected by FISH although the partner gene could not be identified. No structural variant in RARA, RARB, and RARG was observed in UPN5 by whole-genome sequencing. In total, four of five (80%) RARA-negative APL cases had RARB translocations.

Previous reports showed that PML–RARA alone was insufficient to develop leukemia, requiring additional molecular events such as internal tandem duplication (ITD) of FLT3 (19). We thus performed whole-exome sequencing for UPN1 and UPN2, for which diagnostic and remission samples were available, to investigate additional somatic mutations other than the RARB translocation. Although we failed to find recurrent mutations in our cohort (Supplementary Table S4), it should be noted that one case had a somatic mutation of TBL1XR1 in a remaining allele that had not fused to RARB (Supplementary Fig. S3).

Effects of TBL1XR1–RARB on retinoic acid pathway and cell differentiation in vitro
The recurrent TBL1XR1–RARB fusion had a construct similar to that of TBL1XR1–RARA (17) and PML–RARA (Supplementary Fig. S5). To investigate the functional effect of this novel fusion on the etiology of APL, we transduced the HEK293 cell lines with TBL1XR1–RARB, PML–RARA, RARG, and RARA. The luciferase assay demonstrated that the transcriptional activity of TBL1XR1–RARB for a RARE was reduced. The fusion also suppressed transactivation of the reporter induce by RARA or RARB in response to ATRA (Fig. 2A and B), suggesting a dominant negative effect against both RARA and RARB.

All reported partners of RARA fusion in APL shared the ability to form homodimers, and the lissencephaly type-1-like homology motif (Lis1) domain of TBL1XR1 was capable of homodimerization. We therefore assessed the dimerization ability of the TBL1XR1–RARB fusion protein. As with PML–RARA and TBL1XR1–RARA (17), MYC-tagged TBL1XR1–RARB was able to coimmunoprecipitate with the HA-tagged fusion protein whereas wild-type RARB was not (Fig. 2C). The homodimerization of TBL1XR1–RARB was weakened by the therapeutic dose of ATRA but the effect remained partial. The impact of tamibarotene, a synthetic retinoic acid with enhanced biological functions, was also insufficient, even though both retinoids had a potent effect on PML–RARA. ATO had also no effect on TBL1XR1–RARB transduced cell lines.
TBL1XR1–RARB also failed to differentiate the U937 cell line whereas wild-type RARB did with increased expression of CD11b (Fig. 2D). ATRA was able to differentiate TBL1XR1–RARB-transduced U937 although the effect was much smaller than the PML–RARA-transduced effect. The blockage of differentiation by TBL1XR1–RARB and the partial neutrophil differentiation in response to ATRA was confirmed by morphologic changes (Supplementary Fig. S5).

Effects of TBL1XR1–RARB on cell differentiation and proliferation ex vivo

To assess the leukemogenic activity of TBL1XR1–RARB ex vivo, we first performed a colony replating assay using mouse bone marrow (BM) cells and the results were compared with those obtained for PML–RARA and RUNX1–RUNX1T1 (ETO). a(8;21)-derived fusion protein shown to promote self-renewal of hematopoietic progenitor cells (Fig. 3A and B; Supplementary Fig. S6A). Vector-transduced cells did not produce colonies beyond the third passage, whereas TBL1XR1–RARB-, PML–RARA-, and RUNX1–RUNX1T1-transduced cells acquired extensive serial replating capacity. TBL1XR1–RARB-expressing colonies contained many Gr-1<sup>+</sup>/c-Kit<sup>low</sup>/CD<sub>0</sub> myeloblasts and resembled PML–RARA-expressing colonies but were immunophenotypically distinct from RUNX1–RUNX1T1-expressing colonies (Fig. 3C; Supplementary Fig. S6B).

We next transduced these fusions into human CB CD34<sup>+</sup> cells and cultured them with cytokines designed to induce myeloid differentiation (Supplementary Fig. S7A). TBL1XR1–RARB and PML–RARA both showed increased frequency of myeloblasts and inhibited myeloid differentiation towards CD66<sup>+</sup> granulocytes and CD14<sup>+</sup> monocytes in the culture (Fig. 4A and B). Unlike RUNX1–RUNX1T1, TBL1XR1–RARB did not increase the frequency of primitive CD34<sup>+</sup> cells under these culture conditions (Supplementary Fig. S7B). Thus, TBL1XR1–RARB enhances the replating capacity of mouse BM cells and inhibits myeloid maturation of human CB cells as PML–RARA does.
When PML–RARA- and TBL1XR1–RARB-transduced CB cells were cultured in the presence of ATRA, ATRA inhibited the growth of TBL1XR1–RARB- and PML–RARA-expressing CB cells as evidenced by the decrease of GFP+ cells in the culture (Fig. 4C). A therapeutic dose of ATRA induced myeloid differentiation of PML–RARA-expressing CB cells to the same extent as they did in vector-transduced CB cells. In contrast, ATRA did not fully induce differentiation of TBL1XR1–RARB-expressing CB cells, indicating their attenuated response to ATRA (Fig. 4D; Supplementary Fig. S7B). Surprisingly, tamibarotene showed only modest effects on inhibiting the growth and promoting the differentiation of TBL1XR1–RARB cells while showing potent effects on PML–RARA cells (Fig. 4D; Supplementary Fig. S7C).

The attenuated response of TBL1XR1–RARB cells to tamibarotene was also confirmed in the murine colony-forming cell assay (Supplementary Fig. S8).

Discussion

We found that the majority of RARA-negative APL had a RARB translocation, and that TBL1XR1–RARB was the first recurrent fusion observed in APL other than the RARA fusions. RARB is located at 3p24 and TBL1XR1 at 3q26, thus fusion would involve a t(3;3) or an inv(3) and might therefore be very hard to recognize by standard karyotype analysis.

RARB has a function similar to that of RARA as a member of retinoic acid receptors (RAR) family of proteins. RARB forms heterodimers with retinoid X receptors (RXR), binds to RAREs, and recruits corepressors and histone deacetylases to target genes in resting cells. The RARS/RXR heterodimers are activated by physiologic concentration of retinoic acid, which mediates cellular signaling in embryonic morphogenesis, cell growth, and differentiation. Our study demonstrated that TBL1XR1–RARB was an oncogenic protein having effects similar to those of PML–RARA; it homodimerized, diminished transcriptional activity for the retinoic acid pathway with a dominant negative effect on both RARA and RARB, blocked cell differentiation, and induced proliferation. Our findings underscored the fact that alterations in the RAR pathway play a central role in APL pathogenesis, as repeatedly observed for PML–RARA-positive APL.

The effect of therapeutic retinoids on TBL1XR1–RARB was significantly smaller than that observed in PML–RARA, which agreed with the finding of clinical resistance to ATRA in RARB-positive cases. Recent studies of the combination of ATRA and ATO reported excellent outcomes for PML–RARA positive APL, but the RARB-positive cases in our cohort showed resistance not only to ATRA but also to multiagent chemotherapy. There is no case treated with ATO, but ATO had no effect on TBL1XR1–RARB transduced cell lines in vitro because the ATO targets the PML moiety of PML–RARA by preventing multimerization and inducing degradation.

Of note, two of the five RARA-negative APL cases had an extramedullary relapse, an extremely rare event in classical APL (1). Furthermore, our results suggested that tamibarotene, which is effective against relapsed APL (23), was shown to be less effective to RARB-transduce cells in vitro and ex vivo. Considering the dramatic success of precision medicine for APL with PML–RARA, a novel, small molecules/compounds capable of differentiating APL with the RARB translocation is required to improve outcomes in this resistant subgroup.

We demonstrated that TBL1XR1 is the recurrent partner gene of RARB. TBL1XR1 is reportedly a partner of RARA translocation in APL (17). The other known fusion partners of RARA, such as PML, NPM1, and NUMA1, share the characteristic of having a dimerization/oligomerization domain. The dimerization activity of the Lsh domain in TBL1XR1 contributes to homodimerization and the dominant negative effect of RARs. Interestingly, the deletion and mutations in TBL1XR1 have been reported in various malignancies (24), and a previous study showed that TBL1XR1 was a component of the protein complex regulating the retinoic acid pathway.

Figure 3.

Effects of TBL1XR1–RARB on differentiation and proliferation in mouse bone marrow. A, Colony numbers of vector, PML–RARA, TBL1XR1–RARB, or RUNX1–RUNX1T1-transduced mouse bone marrow c-Kit+ cells. Shown are weekly colony counts per 5 × 10⁶ replated cells. B and C, Wright-Giemsa staining (B) and Gr-1 and c-Kit expression (C) in cells at the third round. All vector-transduced cells were c-Kit+ mastocytes, whereas oncogene-transduced colonies contained myeloblasts. The percentages of Gr-1- and c-Kit- cells are indicated.
pathway (25). Considering an interesting result that one case had a mutation of TBL1XR1, alteration of TBL1XR1 may simultaneously contribute to the pathogenesis of APL.

Our study included only five pediatric cases of RARA-negative APL, and notably, most of our cases were young child with median of 2.6 years of age. In terms of additional mutations, no cases in our cohort had FLT3-ITD, which is detected commonly in adult APL. APL in young child might have distinct genetic landscape, and RARB-translocation is a candidate for genetic hallmark of APL in younger age. Further investigation in larger cohorts including older age and adult cases is required to assess frequency, clinical characteristics, and outcomes of RARB-translocation in APL. Further molecular investigation, such as transcriptome comparison between RARA-positive and RARB-positive APLs, would be informative to elucidate underlying mechanism of APLs.

In conclusion, we found that the majority of RARA-negative APL had RARB translocation, and thereby formed a novel, distinct subgroup of APL. Unlike wild-type RARB, both the TBL1XR1–RARB and PML–RARA proteins homodimerize, suppress the retinoic acid pathway, and have oncogenic potency. Our study confirmed that the dysregulation of the retinoic acid pathway was a hallmark of APL; however, the response of APL with RARB translocation to retinoids was partial and was in line with the clinical finding of the resistance of RARA-negative APL to ATRA. Thus, in the context of precision medicine, RARB alteration should be screened for in RARA-negative APL cases, and further investigation is required to improve outcomes for RARB-positive APL.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: T. Osumi, H. Takahashi, T. Uchiyama, M. Kato
Development of methodology: T. Osumi, M. Uchiyama, K. Hata, M. Kato

Figure 4.
Effects of TBL1XR1–RARB on differentiation and proliferation in human cord blood. A and B, Human CB CD34+ cells were transduced with vector, PML–RARA, TBL1XR1–RARB, or RUNX1–RUNX1T1 and were cultured with cytokines (SCF, TPO, FLT3L, IL3, IL6, each 10 ng/mL) to induce myeloid differentiation. The frequency of myeloblasts and mature myeloid cells (A) and the frequency of CD66+ and CD14+ cells (B) in human CB cells transduced with vector, PML–RARA or TBL1XR1–RARB at 10 days (A) and 24 days (B) of culture, respectively. TBL1XR1–RARB and PML–RARA increased the frequency of myeloblasts and decreased CD66/CD14 expression compared with the vector control, indicating their effects on inhibiting myeloid maturation. C, Human CB cells transduced with vector, PML–RARA, or TBL1XR1–RARB were cultured in cytokine-containing media. The mixed transduction culture containing both transduced GFP(+) and untransduced GFP(−) cells were passaged to score the frequency of GFP(+) cells by flow cytometric analysis as a measure of the impact of the transduced gene on the cellular proliferation rate. The initial frequency of GFP(+) cells immediately after transduction was set at 1. Both ATRA and tamibarotene showed a substantial growth-inhibitory effect on PML–RARA cells. TBL1XR1–RARB cells were relatively resistant to the retinoid treatments, especially to tamibarotene. D, CD66 and CD14 expression in cultures described in 10 days after addition of ATRA or tamibarotene. The retinoids induced myeloid differentiation of PML–RARA cells efficiently but showed only limited effects on TBL1XR1–RARB cells.

www.aacrjournals.org Cancer Res; 78(16) August 15, 2018 4457
Published OnlineFirst June 19, 2018; DOI: 10.1158/0008-5472.CAN-18-0840

Downloaded from cancerres.aacrjournals.org on March 9, 2021. © 2018 American Association for Cancer Research.

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H. Takahashi, T. Uchiyama, K. Ohki, N. Kiyokawa, M. Kato

Study supervision: H. Takahashi, K. Matsumoto

Acknowledgments

The authors would like to thank Ms. Natsuko Arizumi, Ms. Shinobu Kobayashi, and Ms. Yoshie Fukumasa for their technical assistance. The authors thank Mr. James R. Valera of the Department of Education for Clinical Research of the National Center for Child Health and Development for his assistance in proofreading and editing this manuscript. The authors are responsible for all final modifications to the manuscript prior to submission.

This study was supported in part by the Japan Society for the Promotion of Science (JSPS) through a Grant-in-Aid for Scientific Research (grant number 17H04234 to M. Kato), by a grant from the National Center for Child Health and Development (grant numbers 28-5 to M. Kato and 27-4 to D. Tomizawa), a grant from the Japan Foundation for Pediatric Research (to M. Kato), a grant from the Kawano Memorial Foundation for the Promotion of Pediatrics of Japan (to S. GoyaM), and a grant from The Cell Science Research Foundation (to S. GoyaM).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received March 26, 2018; revised May 17, 2018; accepted June 14, 2018; published first June 19, 2018.

References


Osumi et al.
## Recurrent RARB Translocations in Acute Promyelocytic Leukemia Lacking RARA Translocation

Tomoo Osumi, Shin-ichi Tsujimoto, Moe Tamura, et al.


<table>
<thead>
<tr>
<th>Updated version</th>
<th>Access the most recent version of this article at: doi:10.1158/0008-5472.CAN-18-0840</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplementary Material</td>
<td>Access the most recent supplemental material at: <a href="http://cancerres.aacrjournals.org/content/suppl/2018/06/19/0008-5472.CAN-18-0840.DC1">http://cancerres.aacrjournals.org/content/suppl/2018/06/19/0008-5472.CAN-18-0840.DC1</a></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cited articles</th>
<th>This article cites 25 articles, 12 of which you can access for free at: <a href="http://cancerres.aacrjournals.org/content/78/16/4452.full#ref-list-1">http://cancerres.aacrjournals.org/content/78/16/4452.full#ref-list-1</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>Citing articles</td>
<td>This article has been cited by 2 HighWire-hosted articles. Access the articles at: <a href="http://cancerres.aacrjournals.org/content/78/16/4452.full#related-urls">http://cancerres.aacrjournals.org/content/78/16/4452.full#related-urls</a></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>E-mail alerts</th>
<th>Sign up to receive free email-alerts related to this article or journal.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reprints and Subscriptions</td>
<td>To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at <a href="mailto:pubs@aacr.org">pubs@aacr.org</a>.</td>
</tr>
<tr>
<td>Permissions</td>
<td>To request permission to re-use all or part of this article, use this link <a href="http://cancerres.aacrjournals.org/content/78/16/4452">http://cancerres.aacrjournals.org/content/78/16/4452</a>. Click on &quot;Request Permissions&quot; which will take you to the Copyright Clearance Center's (CCC) Rightslink site.</td>
</tr>
</tbody>
</table>