Brain-derived Neurotrophic Factor Activation of TrkB Protects Neuroblastoma Cells from Chemotherapy-induced Apoptosis via Phosphatidylinositol 3’-Kinase Pathway

Jerry Jaboin,1 Chong Jai Kim, David R. Kaplan, and Carol J. Thiele2

Pediatric Oncology Branch, National Cancer Institute, Bethesda, Maryland 20892 [J. J., C. J. T.]; Department of Pathology, Seoul National University College of Medicine, Seoul, Korea 100-799 [C. J. K.]; and Brain Tumor Research Centre, Montreal Neurological Institute, Montreal, Quebec, Canada, H3A 2B4 [D. R. K.]

ABSTRACT

Neuroblastoma (NB) tumors expressing high levels of brain-derived neurotrophic factor (BDNF) and TrkB are associated with poor 5-year survival outcomes. Our previous studies indicated that BDNF blocked the cytotoxic effects of vinblastine on NB cells. Here we evaluated the ability of BDNF to decrease the chemosensitivity of NB cells to a number of common chemotherapeutic agents. Two SH-SY5Y NB cell lines (TB3 and TB8) expressing TrkB under the control of a tetracycline (Tet)-repressible promoter element were generated, and used to assess apoptosis resulting from treatment with cisplatin, doxorubicin, etoposide, and vinblastine. BDNF treatment of high TrkB-expressing TB8 and TB3 cells blocked drug-induced cell death in a dose-dependent manner. Only high-dose BDNF (100 ng/ml) could block the effects of chemotherapy in low TrkB-expressing cells. The ability of BDNF to rescue the cells from chemotherapeutic agent-induced cell death was inhibited by treatment with the Trk tyrosine kinase inhibitor K252a or the phosphatidylinositol 3’-kinase (PI3K) inhibitor LY294002, but not by the mitogen-activated protein kinase kinase inhibitor PD98059 or the peritoneal lymphocyte γ inhibitor U73122, indicating that both TrkB and PI3K activities are required for the survival-promoting effects of BDNF. BDNF also protected TrkB-expressing NGP and KCNR NB cells from chemotherapeutic agent-induced cell death, and LY294002 inhibited this protection. These results suggest that TrkB and BDNF can contribute to the chemoresistance of poor prognosis tumors, and that suppression of PI3K activity might improve the ability of these agents to induce the death of NB tumors.

INTRODUCTION

NB1 is a pediatric solid tumor derived from neural crest precursor cells (1). It can be classified into good and poor prognostic categories based on age, DNA ploidy, N-myc amplification, tumor location, and TrkA expression (1). Favorable-prognosis NB tumors express relatively high levels of TrkA, whereas cell lines and tumors from patients with poor prognosis are associated with expression of BDNF and its receptor, TrkB (1-3). Several studies indicate that BDNF increases NB cell survival (2, 4, 5), neurite extension (2, 6), and cell invasion (4, and protects cells from chemotherapy (7, 8). This indicates that NB cell survival (2, 4, 5), neurite extension (2, 6), and cell invasion with poor prognosis are associated with expression of BDNF and its receptor, TrkB, whereas cell lines and tumors from patients with poor prognosis are associated with expression of BDNF and its receptor, TrkB. These results were extended to include other chemotherapeutic agents used in the treatment of NB (8). However, it was not clear using these model systems whether the effects of BDNF were solely dependent on TrkB or whether BDNF could block the effects of chemotherapy in low TrkB-expressing cells. Our previous studies indicated that BDNF blocked the cytotoxic effects of vinblastine on NB cells. Here we evaluated the ability of BDNF to decrease the chemosensitivity of NB cells to a number of common chemotherapeutic agents.

MATERIALS AND METHODS

Construction of Vectors and Establishment of Stable Transfectants. A 3.1-kb fragment of rat TrkB spanning a full coding region was subcloned into the pBSTRI1 vector (23) and transfected into the human NB cell line SH-SY5Y using LipofectAMINE (Life Technologies, Inc.). Stable transfectants (TB3, TB8, and AS-TB8) were obtained after selection with puromycin (0.5 μg/ml) and characterized in detail by Kim et al. (23).

Cell Culture. Cells were cultured in RPMI 1640 containing 10% fetal bovine serum, 2 mM glutamine, and antibiotics as described previously (23). To maintain selection pressure the TB3 and TB8 cells were cultured in puromycin (0.5 μg/ml), and in the presence of Tet (1 μg/ml) for repression of the transfected gene.

Reagents. Recombinant human BDNF (100 ng/μl; PeproTech, Inc.) and 2.5S mouse NGF (100 ng/μl; Upstate Biotechnology) stocks were prepared as directed and stored at −20°C. The chemotherapeutic agents, Cis, Doxo, Etop,
Forty MAPK path. Total Akt by Western blot analysis of protein lysate was analyzed for P-Ser-Akt and membrane. Phoresed in a 1.2% formaldehyde gel and subsequently transferred to a nitrocellulose 1m M phenylmethylsulfonyl fluoride, 10 M, and K252a (1 M) were obtained from Sigma, and reconstituted according to manufacturer’s specification.

Northern Blot Analysis. RNA isolation and hybridization were performed as described previously (24). Thirty μg total RNA for TrkB was electrophoresed in 1% agarose-6% formaldehyde gels. Gels were stained with 2 mg/ml ethidium bromide. Hybridization was performed with 32P-labeled insert DNA isolated from a plasmid containing TrkB (23).

Immunoblotting/Immunoprecipitation. For protein analysis, cells were washed in PBS, centrifuged, and cell pellets kept in −70°C until analysis, and prepared as described previously (25). The cells were then lysed in NP40 lysis buffer containing 20 mM Tris (pH 7.4), 150 mM NaCl, 1% NP40, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, and 500 μM sodium orthovanadate in ice-cold PBS. Cells were lysed on ice for 30 min, and protein concentrations were determined using the Bradford assay kit (Bio-Rad). For immunoprecipitation of phosphorylated TrkA and TrkB receptors, 500 μg of protein from each lysate was immunoprecipitated with polyclonal rabbit anti-pan-TrkB antibody (C-14; Santa Cruz) and protein A agarose (Life Technologies, Inc.). Immunoprecipitates were electrophoresed in 8% Tris-glycine bis-acrylamide gels, and probed with mouse monoclonal antiphosphotyrosine antibody, and subsequently reprobed with anti-Cas antibody to determine total Cas protein levels. Akt and ERK phosphorylation changes were analyzed by Western blotting analysis of 40 μg of protein loaded onto 12% Tris-glycine bis-acrylamide gels, and probed with rabbit monoclonal antibodies to phospho-Akt (Cell Signaling) and phospho-p44/p42 (Cell Signaling). Total Akt and ERK protein levels were determined by Western blotting analysis of 40 μg of protein with rabbit monoclonal antibodies to Akt and p44/p42. Signals were detected using enhanced chemiluminescence reagents (Pierce).

Apoptosis Assay. To assess a parameter of apoptosis, TB3 or TB8 cells were cultured as indicated in “Results,” washed, fixed in 4% paraformaldehyde, and stored in ethanol. Cells were end-labeled with TdT-FITC using the ApoDirect kit (PharMingen) according to the manufacturer’s recommendations and analyzed using a FACScan Flow Cytometer (Becton Dickinson). Experiments were performed in triplicate, plates were combined, and each set of 10,000 cells were collected for each condition. The data were acquired and analyzed by the CELLQuest software (Becton Dickinson).

Cell Survival Analysis. Ten-thousand cells per well were plated into 96-well plates. After 24 h, the cells were treated with control medium or BDNF (100 ng/ml). Twenty-four h later, indicated concentrations of chemotherapeutic drugs were added for 2 h. After 2 h the culture supernatant was removed and fresh medium added for the remainder of the culture period. Similar results were obtained when the drug was cultured for the duration of 48 h without a medium change. Cell number was assessed 48 h later using the MTT assay. Each value represents six replicates, and each experiment was repeated two to three times (26).

RESULTS

Tet Suppresses BDNF-mediated Signal Transduction in SY5Y NB Cells Expressing TrkB Under the Regulation of a Tet-responsive Promoter Element. The previous studies evaluating the effects of TrkB on chemosensitivity were limited by cell model systems that used stable cell lines that constitutively overexpressed TrkB (7) or used retinoids to induce endogenous TrkB expression (7, 8). To assess TrkB function in the absence of effects of receptor overexpression or of retinoids, we generated SY5Y NB cell lines in which the levels of TrkB function in the absence of effects of receptor overexpression or of retinoids, we generated SY5Y NB cell lines in which the levels of TrkB are controlled by a Tet-regulatable promotor element (23). In two cell lines, TrKB-SY5Y (TB3) and TrKB-SY5Y (TB8), the levels of TrkB mRNA were markedly repressed when the cells were cultured in Tet (1 μg/ml; Fig. 1). TrkB mRNA expression was completely suppressed by Tet (1 μg/ml), and TrkB levels increased 4.6-fold in the absence of Tet (Fig. 1). The time course of BDNF-induced TrkB autophosphorylation, and

![Graph](image-url)
the phosphorylation of the signaling proteins AKT, ERK1/2, and Cas were investigated in TB8 cells in the presence or absence of Tet. TB8 cells expressed 2-fold higher TrkB levels in the absence of Tet as compared with the presence of Tet (Fig. 2A). Three major signaling pathways are activated after BDNF activation of the TrkB tyrosine kinase: the PI3k pathway, the Ras/MAPK pathway, and the PLC-γ pathway (27). We used Akt, ERK1/2, and Cas phosphorylation as indicators of the activation of the PI3k, Ras/MAPK, or PLC-γ pathway, respectively. In the presence of Tet (Tet+), Trk, Akt, and ERK1/2 phosphorylation were maximal at 5 min of BDNF treatment of cells, and declined to basal levels by 30 min for Trk and Akt, and 60 min for ERK1/2 (Fig. 2, A–C). In the absence of Tet (Tet−), Akt and ERK1/2 phosphorylation were also maximal at 5 min but persisted for at least 180 min (Fig. 2, A–C). Cas phosphorylation was sustained in both the presence and absence of Tet addition, although phosphorylation was quantitatively more in the absence of Tet (Fig. 2D).

BDNF Rescues TrkB-expressing Cells from Chemotherapy-induced Apoptosis. Scala et al. (7) showed previously that BDNF addition to NB cells induced to express TrkB by RA treatment could rescue the cells from Vbl-induced cytotoxicity. We assessed apoptosis in the Tet-responsive cells lines treated either with either Vbl or with three other chemotherapeutic agents commonly used to treat patients with NB, Cis, Doxo, or Etop. All four of the agents appeared to induce the death of TB3 cells in the absence of BDNF treatment (Fig. 3). Both Cis and Etop treatment resulted in detachment and loss of cell adherence (Fig. 3, top panel). Vbl treatment caused a detachment and loss of adhesion of some cells, whereas others seemed to have a more differentiated morphology (Fig. 3, top panel). The IC50 of these agents that was required to induce the apoptosis of TB8 cells was 3 μg/ml for Cis, 3 μg/ml for Etop, 0.05 μg/ml for Doxo, and 3 ng/ml for Vbl (data not shown). The IC50 for the drugs were similar in TB3 cells, and there was no statistical difference if cells were cultured in the absence or presence of Tet (data not shown).

To determine whether BDNF would rescue the TrkB-expressing
cells (Tet−) from chemotherapy-induced cell death, TB3 cells were cultured with BDNF (100 ng/ml) for 24 h, and Cis (3 μg/ml), Etop (3 μg/ml), Doxo (30 ng/ml), or Vbl (6 ng/ml) were added for 48 h. The agents were removed, and apoptosis was assessed after an additional 48 h. BDNF did not affect the cell growth or morphology of the TB3 cells as compared with control medium (Fig. 3, bottom left panel). However, pretreatment with BDNF prevented the chemotherapy-induced reduction in cell number. These data indicate that BDNF can protect NB cells from cell death caused by a variety of chemotherapeutic agents.

To confirm that the reduction in cell number after treatment with the chemotherapeutic agents was because of an increase in apoptosis and not a cell cycle arrest, we performed TdT labeling to assess apoptosis and flow cytometry to examine cell growth. Treatment of TB3 cells (Tet−) with Cis, Etop, and Vbl at the IC50 dose for 2 h followed by a 48-h incubation in control medium resulted in 58, 59, and 61% increases in TdT labeling as compared with 10% TdT labeling in cells not treated with the agents (Fig. 4A). TdT labeling was markedly decreased (16, 19, and 23%) if TB3 cells were pretreated for 24 h with BDNF (100 ng/ml). Similarly, in TB8 cells, Cis, Etop, and Vbl induced 56, 55, and 35% apoptosis, and BDNF pretreatment dramatically reduced apoptosis (Fig. 4B). In the presence of the chemotherapeutic agents, TdT labeling was detected in cells throughout the cell cycle, indicating that the agents induced apoptosis regardless of the phase of the cell cycle (data not shown). In addition, under these conditions the agents did not induce a cell cycle arrest (data not shown). These data indicate that the BDNF can protect TrkB-expressing NB cells from chemotherapy-induced apoptosis.

**Dose Response of BDNF Required for Protection from Chemotherapy-induced Cell Death.** We first determined whether the BDNF-induced protection from cell death was dependent on the level of expression of the TrkB receptor. TB8 cells were cultured in the presence (Tet+) or absence (Tet−) of Tet for 24 h, treated with various concentrations of BDNF (10, 33, and 100 ng/ml) for 24 h,
followed by a 2-h treatment with the chemotherapeutic agents. Cell number was assessed after an additional 48 h in control medium. BDNF at 10 or 33 ng/ml could not rescue the Tet+ cells from cell death resulting from treatment with the agents (Fig. 5, A1–D1). However, there was a complete and statistically significant rescue from cell death when low TrkB-expressing cells were pretreated with BDNF at 100 ng/ml (Fig. 5, A1–D1). In each case the values for the BDNF rescue of cells were significantly different from the values in the drug-treated cells (Fig. 5, *), and for Cis, Doxo, and Vbl, the values were not statistically different from the untreated or control cells (Fig. 5, A–D). Thus, in TB8 cells expressing low levels of TrkB, only BDNF (100 ng/ml) could protect from chemotherapeutic-induced cell death. NGF (100 ng/ml), which binds both the p75NTR and low levels of TrKA expressed in SY5Y cells, could not protect the cells from chemotherapeutic-induced cell death.

In TB8 (Tet−) cells, which express higher levels of TrkB as compared with TB8 (Tet+) cells, a lower dose of BDNF (33 ng/ml) protected the cells from chemotherapeutic agent-induced cell death (Fig. 5, A2–D2). In the high TrkB (Tet−) expressing TB8 cells there was a dose-dependent increase in the ability of BDNF to protect cells from Cis-induced cell death. For Cis and Vbl, as little as 10 ng/ml BDNF could significantly protect the cells from chemotherapeutic agent-induced cell death (Fig. 5, A2 and D2). Again, NGF could not protect the cells from death induced by the agents. These data indicate that the protection from cell death depends on both the levels of TrkB and the concentration of BDNF.

**BDNF Protects TrkB-expressing NB Cells from Chemotherapeutic Agent-induced Cell Death via the PI3K Pathway.** To confirm that TrkB and not the p75NTR mediate the BDNF-induced protection from cell death, and to identify the signal transduction pathway(s) required for protection, we assessed the ability of BDNF to protect TB8 cells from cell death in the presence of pharmacological inhibitors of Trk, PI3k, ERK1/2, and PLC-γ. First we determined the concentrations of inhibitors required to suppress their respective pathways. K252a, a relatively selective Trk tyrosine kinase inhibitor, prevented BDNF activation of TrkB autophosphorylation in TB8 (Tet−) cells at 500 nm (Fig. 6A). LY294002, a selective inhibitor of PI3k, blocked the phosphorylation of the Akt at 10 μM (Fig. 6B). PD98059, an inhibitor of mitogen-activated protein kinase kinase, inhibited the phosphorylation of ERK1/2 at 50 μM (Fig. 6C). U73122, a selective inhibitor of PLC-γ, completely blocked the phosphorylation of Cas activation at 10–20 μM (Fig. 6D). Each of the inhibitors was added to assess the contribution of the above proteins in the BDNF rescue from chemotherapeutic agent-induced cell death. The inhibitors were added to the TB8 (Tet−) cells 30 min before the addition of BDNF. After 24 h the cells were treated with Etop (3 μg/ml) for 2 h, the Etop was removed, and cell number was assessed after 48 h. Treatment of BDNF-stimulated cells with the inhibitors did not significantly affect cell survival under these conditions (Fig. 7). The Trk tyrosine kinase inhibitor K252a was able to completely block the ability of BDNF to rescue cells from Etop-induced cell death (Fig. 7A). Similarly, the PI3k inhibitor LY294002 completely abrogated the ability of BDNF to protect cells from Etop-induced cell death (Fig. 7B). In contrast, neither the mitogen-activated protein kinase kinase inhibitor PD98059 nor the PLC-γ inhibitor U73122 altered the ability of BDNF to protect cells from Etop-induced cell death (Fig. 7, C and D). These data indicate that the activities of TrkB and of PI3k are required for BDNF to protect the NB cells from Etop-induced cell death. LY294002 also blocked the ability of BDNF to rescue cells from Doxo-, Vbl-, and Cis-induced cell death (data not shown). Similar results were found in the AS-TB8 cell lines, another TrkB-transfected NB cell line derived from SK-N-AS (Fig. 7E, inset). Increasing concentrations of BDNF protect AS-TB8 cells from Etop-induced cell death (Fig. 7E), and the protection can be blocked by using LY294002 (Fig. 7F).

To determine whether inhibition of PI3k activity would sensitize other NB cell lines to chemotherapeutic agent-induced cell death, we treated KCNR and NGP NB cell lines, which constitutively express BDNF (25), with physiological concentrations of all-trans-RA (10 nm) to induce TrkB expression. This concentration of RA does not significantly alter cell growth, although cells do extend neurites (4). LY294002 (10 μM) was able to cause a 29% and 36% increase in cell death in RA and Etop-treated cells (KCNR IC_{50} = 4.1 ng/ml; NGP IC_{50} = 2.3 ng/ml) when compared with cells not treated with LY294002 (KCNR IC_{50} = 5.8 ng/ml, Fig. 8A; NGP IC_{50} = 3.6 ng/ml, Fig. 8, A and B). LY294002 also shifted the dose response of Etop-induced cell death of BDNF and RA-treated NB cells. The IC_{50} was increased nearly 2-fold in KCNR (IC_{50} = 8.14 ng/ml; Fig. 8C) and 2.5-fold in NGP (IC_{50} = 8.5 ng/ml; Fig. 8D). Yet in each case, treatment with LY294002 blocked the effects of BDNF (KCNR IC_{50} = 3.9 ng/ml, Fig. 8C; NGP IC_{50} = 3.1 ng/ml, Fig. 8D).

**DISCUSSION**

Because NTs protect normal neurons from a variety of chemical and physical insults (28), we hypothesized that the expression of NTs...
by tumors of neuroectodermal origin may antagonize the effects of chemotherapy and contribute to chemoresistance (7). A number of studies have shown that NTs such as BDNF promote the survival (2, 4, 5) and chemoprotection of NB cells (7, 8), although the mechanisms remain unclear. In this report, we demonstrate that BDNF via activation of PI3K can block the apoptosis of NB cells treated with Etop, Doxo, and Cis, chemotherapeutic agents commonly used in the treatment of NB.

Our finding that the mechanism by which BDNF blocks chemotherapy-induced apoptosis is TrkB-dependent is based on three findings: (a) the inability of NGF to protect cells (Fig. 5); (b) the findings that K252a could block the ability of BDNF to protect cells from chemotherapy-induced cell death (Fig. 7); and (c) that greater protection was afforded at high TrkB levels with low concentrations of BDNF (Fig. 5). In our model, NGF, which binds TrkA and p75 but not TrkB, is unable to protect cells from chemotherapy-induced apoptosis. This is in contrast to the finding that the ability of NGF to protect SY5Y cells from neocarzinostatin-induced apoptosis is mediated by a
Furthermore, both the MAPK and PI3K pathways have been implicated for mediating BDNF rescue from hypoxic-ischemic injury (34). Rescued neurons from death in culture with low KCl (33) through the signaling intermediaries of the Trk tyrosine kinase have been shown to inhibit the PI3K path is able to prevent BDNF rescue. Depending on the model system and insult used, different downstream signaling mechanisms of action include changes in microtubule polymerization, inhibition of topoisomerase activity, and intercalation or alkylation of DNA. In our NB model system, in which Tet represses the levels of TrkB, we showed that the ability of low doses of BDNF (10–33 ng/ml) to protect NB cells from apoptosis was dependent on the level of TrkB expression (Fig. 5). Moreover, at a constant TrkB level, BDNF protection was dose-dependent (Fig. 5). This is of interest because we have shown that in drug-resistant NB cells there is a direct correlation between increasing drug resistance and BDNF mRNA, whereas TrkB levels remained constant (7). This indicates that TrkB-expressing NB cells that constitutively express high levels of BDNF or metastasize to sites rich in BDNF or other NTs that activate TrkB may be more resistant to chemotherapy. In rodents, BDNF is expressed at high levels in adult brain, lung, and muscle. NB patients with metastases to these sites have a shortened event-free survival (30). Furthermore, BDNF is highly expressed in active osteoblasts in bone, and in the proliferating and mature zones of the epiphyseal growth plate (31) raising the possibility that BDNF may contribute to the chemoresistance of bony lesions in NB (32).

Our finding that K252a, a relatively selective Trk tyrosine kinase inhibitor, is able to block the ability of BDNF to rescue cells from chemotherapy-induced cell death indicates that activation of the Trk tyrosine kinase is necessary to mediate the protective effects of BDNF. Using a pharmacologic approach we have also shown that inhibition of the PI3K path is able to prevent BDNF rescue. Depending on the model system and insult used, different downstream signaling intermediaries of the Trk tyrosine kinase have been shown to mediate the protective effects of BDNF. For example, BDNF has rescued neurons from death in culture with low KCl (33) through the PI3K pathway. In neonatal brains, the MAPK pathway was responsible for mediating BDNF rescue from hypoxic-ischemic injury (34). Furthermore, both the MAPK and PI3K pathways have been implicated in the ability of BDNF to rescue cerebellar granule neurons from oxidative stress (35) and retinal ganglion cells from axotomy-induced cell death (36). In fact, the survival of NB cells in serum-free medium requires activation of the PI3K path (37). Despite the differing mechanisms of action of our chemotherapeutic agents, we have shown that BDNF is able to mediate rescue. This suggests that the ability of BDNF activation of TrkB to rescue NB cells from chemotherapy may be the result of modulation of a key survival signaling molecule(s), which is able to block the apoptosis-inducing signals of each of these drugs.

Abrogation of Trk tyrosine kinase activity may be an important tool in improving chemotherapeutic efficacy, as seen by the effect of K252a on BDNF activation of TrkB. Although K252a has not been used clinically, CEP-751, a K252a derivative with selectivity for Trk tyrosine kinase, has been shown to inhibit growth in TrkB-expressing NB xenografts in nude mice (38). Inhibition of Trk tyrosine kinase would abrogate all of the downstream signaling paths (MAPK, PLC-γ, and PI3K). We find that inhibition of the PI3K path is the only one required to block the ability of BDNF/TrkB to make cells resistant to chemotherapy. Utilization of an inhibitor with more restricted specificity to a downstream signaling path might be expected to have less or different toxicities. In the future we will analyze whether differential TrkB expression affects NB tumor growth in an in vivo NB model (39) and assess the effects of the PI3K inhibitor LY294002. LY294002 has been used in an in vivo ovarian cancer model to demonstrate growth inhibition with minimal toxicities (40). Our future studies will be aimed at determining the downstream targets of PI3K that are necessary to mediate BDNF/TrkB chemoprotection because a more precise delineation of the specific signaling path will lead to more targeted therapy for the treatment of NB.

REFERENCES


Brain-derived Neurotrophic Factor Activation of TrkB Protects Neuroblastoma Cells from Chemotherapy-induced Apoptosis via Phosphatidylinositol 3′-Kinase Pathway

Jerry Jaboin, Chong Jai Kim, David R. Kaplan, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/62/22/6756

Cited articles
This article cites 39 articles, 11 of which you can access for free at:
http://cancerres.aacrjournals.org/content/62/22/6756.full#ref-list-1

Citing articles
This article has been cited by 22 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/62/22/6756.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.