A Pharmacologic Inhibitor of the Protease Taspase1 Effectively Inhibits Breast and Brain Tumor Growth

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Abstract

The threonine endopeptidase Taspase1 has a critical role in cancer cell proliferation and apoptosis. In this study, we developed and evaluated small molecule inhibitors of Taspase1 as a new candidate class of therapeutic modalities. Genetic deletion of Taspase1 in the mouse produced no overt deficiencies, suggesting the possibility of a wide therapeutic index for use of Taspase1 inhibitors in cancers. We defined the peptidyl motifs recognized by Taspase1 and conducted a cell-based dual-fluorescent proteolytic screen of the National Cancer Institute diversity library to identify Taspase1 inhibitors (TASPIN). On the basis of secondary and tertiary screens the 4-[[4-arsnonophenyl]methyl][phenyl] arsionic acid NSC48300 was determined to be the most specific active compound. Structure–activity relationship studies indicated a crucial role for the arsenic acid moiety in mediating Taspase1 inhibition. Additional fluorescence resonance energy transfer–based kinetic analysis characterized NSC48300 as a reversible, noncompetitive inhibitor of Taspase1 (Ki = 4.22 μmol/L). In the MMTV-neu mouse model of breast cancer and the U251 xenograft model of brain cancer, NSC48300 produced effective tumor growth inhibition. Our results offer an initial preclinical proof-of-concept to develop TASPINs for cancer therapy. Cancer Res; 72(3); 736–46. ©2011 AACR.

Introduction

Site-specific proteolysis offers spatiotemporal controls over fundamental aspects of organismal and cellular physiology (1–9). Accordingly, the identification and characterization of regulatory proteases in the context of human diseases has fueled the discovery of therapeutic interventions targeted at respective proteases (10). The best examples are the use of angiotensin-converting enzyme inhibitors, HIV protease inhibitors, and 26S proteasome inhibitors to treat hypertension, AIDS, and multiple myeloma, respectively (2, 11, 12).

Taspase1 (threonine aspartase) encodes a highly conserved 50 kDa α-β proenzyme that undergoes autoproteolysis, generating a mature αβ22 heterodimeric protease that displays an overall α/β/β/α structure (13, 14). Taspase1 was initially purified as the protease that cleaves MLL to regulate the expression of HOX genes (13, 15). Subsequent studies identified additional Taspase1 substrates, including MLL2 (also known as MLL4 in the GenBank database; ref. 8), TFIIBα-β, and ALF (TFIIA-like factor; ref. 16). The cloning of Taspase1 founded a novel class of endopeptidases that employs conserved amino terminal threonine of the mature β subunit to cleave peptide bonds after P1 aspartate (13).

Taspase1 is the only protease within the family of enzymes that possesses an asparaginase2 (PF01112) homology domain, whereas other members, including α-asparaginase and glycosylasparaginase, participate in the metabolism of asparagines and the ordered breakdown of N-linked glycoproteins, respectively (13, 17). Taspase1-mediated cleavage follows a distinct aspartate residue of a conserved QXD/GXDD motif (15), suggesting that Taspase1 evolved from hydrolyzing asparagines and glycosylasparaginases to cleaving polypeptides after aspartates (13). In addition to MLL, MLL2, TFIIA, and ALF, Taspase1 also proteolyzes Drosophila HCF (dHCF), whereas mammalian HCF is cleaved by O-GlcNac transferase due to the loss of GXD/GXDD motif during the evolution (18, 19).

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Initial characterization of Taspase1−/− mice discovered a critical role of Taspase1 in cell-cycle control (8). In the absence of Taspase1, cell cycle is disrupted with decreased expression of Cyclins and increased expression of CDK inhibitors (CDK; ref. 8). Consequently, Taspase1−/− mouse embryonic fibroblasts (MEF) are resistant to oncogenic transformation (8). Furthermore, Taspase1 is overexpressed in primary human cancers and required for tumor maintenance in many cancer cell lines (20). Knockdown of Taspase1 disrupts proliferation in the majority of cancer cells within which a subset of cell lines also displays enhanced apoptosis (20). Of note, Taspase1 is expressed at high levels in many cancer cells (8, 21, 22) and in general increased expression positively correlates with the cellular dependence on Taspase1 (8, 20). These data suggest that Taspase1 is co-opted to promote and sustain tumorigenesis. Therefore, inhibition of Taspase1 may offer a new anticancer strategy. Here, we present our endeavors in (i) establishing the safety of Taspase1 inactivation in adult mammals using a genetically well-defined mouse model, (ii) characterizing the consensus cleavage motif of Taspase1, (iii) developing an in vivo, dual fluorescent, Taspase1 proteolytic screen, (iv) screening, confirming, and characterizing a small molecule TASPIN NSC48300, [4-[(4-arsonophenyl) methyl]phenyl] arsonic acid, and (v) examining the efficacy of NSC48300 in treating cancers using 2 different preclinical mouse tumor models.

Materials and Methods

Animal studies

Animal studies were approved by the Animal Studies Committee at Washington University School of Medicine. Mice carrying straight and conditional knockout alleles of Taspase1 (8), MMTV-neu (23), and MMTV-wnt (24) transgenes have been described. Tumor mass followed by bioluminescence imaging using an IVIS 100 system has been previously described (25).

Constructs, recombinant proteases, cell lines, cell culture, knockdown, and Western blot analyses

The dual fluorescent Taspase1 proteolytic reporter (DFPR) was constructed by sequentially inserting cDNA encoding enhanced green fluorescent protein (eGFP), 2XNES of MAPKK, aa 2,400 to 2,900 of hMLL, 3XNL5 of SV40 large T antigen, and dsRED2 into the pMSCVpuro (Clontech) vector. Amphotropic retrovirus was produced as described (26) and utilized to infect 293T cells. The generation of recombinant Taspase1 and caspase8 has been described (13, 27). NCI60 cell lines were obtained from National Cancer Institute (NCI) Developmental Therapeutic Program (DTP). BT-474 was obtained from American Type Culture Collection. Taspase1−/− MEFs have been described (20) and were authenticated by both PCR genotyping and Western blot analysis. All of the cell lines were expanded, frozen, and used for no more than 2 months after the resurrection of frozen aliquots. Cell culture, Taspase1 knockdown, and Western blot analyses were carried out as previously described (28).

Results

Acute deletion of Taspase1 in adult mice does not incur overt toxic phenotypes

Our in vitro studies using Taspase1 knockdown and knockdown cells showed an important role of Taspase1 in tumor initiation and maintenance, suggesting that Taspase1 inhibitors (TASPINs) may be developed and utilized in cancer therapy (20). Because Taspase1 plays critical roles in mouse embryonic development, the application of TASPINs in children and pregnant women would be inadvisable (8). On the other hand, the few Taspase1−/− mice alive at weaning age went on to live a normal lifespan (Westergard and colleagues, unpublished data), implicating the safe use of TASPINs in adults. To evaluate whether Taspase1 can be safely inactivated in developed mammals, we induced a global deletion of the Taspase1 gene in 7-week-old Mx-Cre;Taspase1F−/− mice by administering polyinosine-polycytidine (pIpC; ref. 29). No discernible toxicities were observed in these Taspase1−/− mice (Fig. 1A–D). The efficiency of Taspase1 deletion in bone marrow, spleen, and thymus was determined (Fig. 1E). These results indicated that inactivation of Taspase1 through pharmacologic means is likely to be tolerated by adult mammals.

Critical amino acid residues within the Taspase1 cleavage consensus motif

To provide mechanistic insights about how Taspase1 recognizes its substrates, we characterized the Taspase1 cleavage motif. MLL contains 2 Taspase1 cleavage sites (CS1 and CS2) that are positioned 53 amino acids (aa) apart, among which CS2 is more conserved and more efficiently processed (Fig. 2A; refs. 13, 30). In vitro transcribed/translated (IVTT), 35S-methionine labeled, human MLL aa 2,500 to 2,800 fragment containing a mutated CS1 site was employed as a CS2-specific cleavage reporter (p45MLLCS2). Alanine scanning mutagenesis across the CS2 site (PKISQLD/GVDDG) of p45MLLCS2 was done and the cleavage of individual reporters by recombinant Taspase1 (rTaspase1) was examined. P1 aspartate and P1' glycine are essential, P2 leucine, P3 glutamine, and P5 isoleucine are important, and P3' and P4' aspartates are dispensable (Fig. 2B). These data are consistent with the fact that P1 aspartate and P1' glycine are absolutely conserved, P2, P3, and P5 hydrophobic residues are highly conserved, and P4 is variable, among known Taspase1 cleavage motifs (Fig. 2A). Surprisingly, highly conserved P3' and P4' aspartate residues are dispensable, indicating that these residues may play an indirect role in presenting substrates for Taspase1 recognition. In summary, the IXQL(V)D/G sequence represents the best Taspase1 cleavage recognition motif.
general classes of proteases (31), ISQLD-aldehyde and ISQLD-cmk were minimally active in inhibiting Taspase1 (IC50 > 100 μmol/L; Supplementary Fig. S1A). Of all proteases in mammals, the β subunit of the 20S proteasome (32) is the only known N-terminal threonine endopeptidase other than Taspase1 (13). Bortezomib—a boronic acid containing chemotherapeutic drug that targets the active site threonine of the 26S proteasome (11)—has no activity against Taspase1 (Supplementary Fig. S1B), which is consistent with the lack of activity of ISQLD-borate in inhibiting Taspase1 (33). Altogether, these data highlighted the unique mechanistic processes about Taspase1-mediated proteolysis.

A cell-based, dual-fluorescent, proteolytic screen identifies small molecule TASPINs in the NCI diversity set library

To identify bioactive, small molecule inhibitors of Taspase1, we developed an in vivo screen in which 293T HEK (human embryonic kidney) cells were engineered to stably express a DFPR (Fig. 3). The DFPR (eGFP-2XNES-p75MLL-3XNLS-dsRED2) consists of the CS1 and CS2 spanning human MLL polypeptide (aa 2,400–2,900, p75MLL) that is flanked by amino-terminal eGFP-NES (nuclear export signal) and carboxy terminal NLS (nuclear localization signal)-dsRED2 fusions. Taspase1-mediated cleavage of DFPR results in the

Figure 1. Induced genetic deletion of Taspase1 is well tolerated in adult mice. A, mice bearing an IFN-inducible cre recombinase (Mx-Cre) transgene and the indicated alleles of Taspase1 (+, wild type; -, knockout; F, conditional allele) at 7 weeks of age were subjected to 5 doses of plpC injection. Baseline measurements were obtained at 6 weeks of age. Mice were sacrificed 16 weeks after the last dose of plpC to obtain posttreatment measurements and harvest bone marrow (BM), spleen, and thymus for genotypic analysis. B–D, genetic deletion of Taspase1 does not affect the parameters of white blood cell (B), platelet (C), and red blood cell counts (D). E, genotyping of the indicated tissues shows a complete deletion of Taspase1 after the plpC treatment.
reporters were generated by single alanine substitution of individual amino acid across P7 to P5 (recombinant Taspase1) for 30 minutes at 30°C. NSC48300 inhibits Taspase1 were further investigated. We extended periods of time did not enhance inhibition, favoring an IC50 around 7.5 mol/L (Fig. 4A). The mechanisms by which NSC48300 inhibits Taspase1 were investigated. We employed HTI-9 (ISQLAGVDD), a weak, CS2-based, competitive peptide inhibitor of Taspase1 (Supplementary Fig. S3A) to further characterize these small molecule TASPINs, a tertiary specificity screen using an established Taspase1-based in vitro cleavage assay was carried out (13). Among candidate inhibitors, 5 showed appreciable inhibition of Taspase1-mediated cleavage of p75MLL in vitro (Fig. 3; Supplementary Table S1). To further characterize these small molecule TASPINs, a tertiary specificity screen was employed, in which caspase8-mediated cleavage of p22Bid was utilized to identify dual Taspase1–caspase8 inhibitors (27). Caspase8 was chosen because caspases, like Taspase1, proteolyze their substrates after P1 aspartate. The IVTT-based, caspase8–p22Bid in vitro cleavage assay was optimized (Supplementary Fig. S2). Interestingly, 4 of the 5 TASPINs were dual Taspase1–caspase8 inhibitors and compound #4 (NSC48300) specifically targeted Taspase1 (Fig. 3). Taken together, our cell-based screen, followed by in vitro confirmation and specificity assays, identified NSC48300 as a specific TASPIN.

**Characteristics and structure–activity relationships of TASPIN NSC48300**

The activity of TASPIN NSC48300 was evaluated using the IVTT, 35S methionine-labeled p75MLL reporter, which showed an IC50 around 7.5 μmol/L (Fig. 4A). The mechanisms by which NSC48300 inhibits Taspase1 were further investigated. We first determined whether it acts as a reversible or irreversible inhibitor. Preincubation of Taspase1 with NSC48300 for extended periods of time did not enhance inhibition, favoring a reversible mechanism (Fig. 4B). As NSC48300 is an arsonic acid, we assessed whether free arsenic acid can inactivate Taspase1. Up to 1 mmol/L of arsenic acid was utilized and no detectable inhibition of Taspase1 was observed (Fig. 4A). Nevertheless, the arsenic acid moiety of NSC48300 seems to be essential in that its analogues NSC74084, NSC47905, NSC23953, and NSC352678, have no demonstrable activity (Fig. 4C). In addition, modifications of the benzene ring, as illustrated in arylarsonic acid NSC49855, resulted in a 10-fold decrease of the inhibitory activity (Fig. 4C). In summary, NSC48300 functions as a reversible TASPIN and arsenic acid may serve as an active functional group against Taspase1 when correctly conjugated onto appropriate chemical backbones.

**Kinetic analysis of NSC48300 using a FRET-based cleavage reporter**

To enable detailed kinetic analysis of TASPINs, we modified an in vitro FRET (fluorescence resonance energy transfer)-based Taspase1 cleavage assay (14). The FRET-based Taspase1 proteolytic reporter (FRPR, MCA-KISQLGDVDD-DNP) consists of the 10 aa CS2 consensus sequence conjugated with a fluorogenic coumarin (MCA) group and a quenching 2,4-dinitrophenyl (DNP) group at the amino and carboxy terminus, respectively (Fig. 5A). Upon Taspase1-mediated cleavage, MCA is no longer quenched by DNP, resulting in a linear increase of fluorescence emission that is excited at 328 nm and detected at 393 nm (Fig. 5B). The apparent KM of FRPR is 9.06 ± 2.80 μmol/L (Fig. 5C). By incubating varying concentrations of FRPR and NSC48300, we showed NSC48300 as a noncompetitive TASPIN (KM = 4.22 ± 0.46 μmol/L; Fig. 5C). To confirm the noncompetitive nature of NSC48300 in inhibiting Taspase1, we employed HTI-9 (ISQLAGVDD), a weak, CS2-based, competitive peptide inhibitor of Taspase1 (Supplementary Fig. S3A).
and S3B). The fact that NSC48300 and HTI-9 cooperated to inhibit Taspase1 indicates that these 2 inhibitors function at distinct sites. It confirms NSC48300 as a nonsubstrate competitive inhibitor and reveals the presence of a yet to be characterized allosteric site on Taspase1 (Fig. 5D).

NSC48300 does not inhibit the intramolecular autoproteolysis of Taspase1

Our data thus far support a working model in which NSC48300 targets an allosteric site and thereby noncompetitively inhibits Taspase1. However, it remains plausible that NSC48300 can further function by interfering the maturation step of Taspase1, that is, autoproteolysis. Taspase1 is translated as a nonprocessed αβ precursor enzyme which undergoes autoproteolysis to generate mature αβ protease (13). Taspase1 precursors can be activated by intramolecular autoproteolysis. However, whether these precursors can also be activated by mature Taspase1 through intermolecular autoproteolysis remains undetermined. To probe into the mechanism by which Taspase1 matures, radiolabeled Taspase1 precursor (p50T1α-β) was incubated in cleavage buffer alone. A slow rate of autoproteolysis with approximately 50% maturation after 6 hours of incubation was observed (Fig. 5E). If p50T1α-β can be activated by intermolecular autoproteolysis, the addition of purified rTaspase1 would greatly expedite the maturation of labeled p50T1α-β. No enhanced cleavage of p50T1α-β was observed upon the addition of up to 100 ng of rTaspase1, indicating the lack of intermolecular autoproteolysis (Supplementary Fig. S4).

Altogether, Taspase1 precursors undergo intramolecular, but not intermolecular, autoproteolysis to generate mature Taspase1 enzyme. With this information in hand, increasing amounts of NSC48300 were added to the autoproteolysis reaction, and no impact on the Taspase1 autoproteolysis was observed (Fig. 5F), excluding the possibility that NSC48300 inhibits Taspase1 through disrupting autoproteolysis.

The expression level of Taspase1 in breast and brain cancer cell lines correlates with sensitivity to NSC48300

With a specific TASPIN in hand, we explored its potential application in treating cancer cells. Knockdown studies using cancer cell lines showed that cells with higher expression of Taspase1 are more dependent on Taspase1 (20). Hence, we...
envisioned that certain cancer types might exhibit a strong correlation between their Taspase1 expression and sensitivity to NSC48300. To examine this hypothesis, we first employed SV40-transformed Taspase1−/− MEFs that are stably reconstituted with Taspase1 (Taspase1+/−:Taspase1). When compared with the congenic parental Taspase1−/− cell line, Taspase1−/−:Taspase1 cells displayed increased sensitivity to NSC48300, indicating that the presence of Taspase1 renders treatment sensitivity (Fig. 6A). We next integrated the Taspase1 protein expression profile and the NSC48300 growth inhibition database of the NCI60 cancer cell lines (8, 34). According to the NCI DTP database (35), NSC48300 produces a distinct pattern of growth inhibition in the NCI60 in vitro anticancer drug screen (Supplementary Table S2). The sensitivity to NSC48300-mediated growth inhibition is in general agreement with the protein level of Taspase1 in many human cancer cell lines (Supplementary Table S2), among which an especially tight correlation is detected in breast and brain cancer cells (Fig. 6B and C, left panels). The differential sensitivity to NSC48300 within these 2 cancer types was confirmed (Fig. 6B and C, right panels), supporting Taspase1 as a target of NSC48300 in treating cancer cells. However, when high concentrations of NSC48300 were utilized, nonspecific toxicity became evident, which could relate to its intrinsic arsenic acid moiety or effects on other cellular enzymes. NSC48300 was recently shown to inhibit autotaxin, an extracellular enzyme that converts lysophosphatidylcholine to lipophosphatic acid (LPA; ref. 36). The lysophospholipase D activity of autotaxin can be inhibited by NSC48300, resulting in the disruption of LPA production and a subsequent decrease of the in vitro invasiveness of cancer cells, which can be reversed by adding extracellular LPA (36). To examine whether
our observed cancer cell sensitivity to NSC48300 can be partially attributed to the inhibition of autotaxin, we treated cancer cells with NSC48300 in the presence or absence of LPA. Because the relative cell number curves basically overlaid irrespective of LPA in both NSC48300 sensitive and insensitive lines, we excluded autotaxin as a target for NSC48300-mediated growth inhibition, which is consistent with the known extracellular expression and action of autotaxin (Fig. 6B and C, right panels).

**NSC48300 inhibits the growth of MMTV-neu mouse breast cancers and U251 brain tumor xenografts**

Following up on the cell line data, we wished to evaluate the in vivo efficacy of NSC48300 in treating cancers. Because NSC48300 is an arsenic acid and no data with regard to its in vivo safety have been reported, we determined its toxicity profile. Short-term, instead of long-term, tail vein injections were given due to observed fibrotic damages of regional vessels at injection sites upon repetitive treatment of NSC48300. Histologic examination of major organs did not reveal any obvious abnormalities, whereas blood chemistry revealed a significant decrease in the LDH level and certain alterations in electrolytes and kidney functions (Supplementary Fig. S5 and Table S3). Complete blood counts were also done, which showed a decrease in white blood cell counts and hemoglobin levels (Supplementary Fig. S6A–C).

To enable a scientific selection of the most relevant breast cancer model for in vivo experiments, we expanded our breast cancer cell line repertoire to incorporate an estrogen receptor (ER) negative, Her2/neu overexpressing cancer cell line, BT474 (37), and conducted genetic knockdown experiments (Supplementary Fig. S7). Among these 3 breast cancer cell lines, MDA-MB-231 cells, an ER negative, Her2/neu negative cell line (38) that expresses a very low level of Taspase1, were least affected by the deficiency of Taspase1 (Fig. 7A and Supplementary Fig. S7; ref. 20). By contrast, cells with higher levels of Taspase1, including MCF7, an ER positive, Her2/neu negative cell line (39), and BT-474 cells were more dependent on Taspase1 (Fig. 7A and Supplementary Fig. S7). On the basis of these in vitro data, Her2/neu-driven tumors may be more dependent on Taspase1 and would be more sensitive to NSC48300 treatment, whereas ER negative, Her2/neu negative breast cancers would be resistant. To examine this hypothesis in vivo, we treated MMTV-neu (23) or MMTV-wnt (ER-, Her2-; ref. 24) breast cancer–bearing mice with NSC48300. Indeed, NSC48300 consistently disrupted the growth of MMTV-neu breast cancers, whereas it exhibited no effects on the growth of MMTV-wnt breast cancers (Fig. 7B).
To test the efficacy of NSC48300 in treating brain tumors, we employed a brain cancer xenograft model using U251 cells based on 2 reasons. First, knockdown of Taspase1 in U251 cells impaired cell proliferation (20). Second, among the NCI60 brain tumor cell lines, U251 cells express high levels of Taspase1 and are most sensitive to NSC48300 treatment in vitro.

To monitor treatment response in live animals, U251 cells were first engineered to stably express firefly luciferase (fLuc) before subcutaneous implantation into mice. Bioluminescent imaging showed a stasis of tumor growth in NSC48300-treated compared with vehicle-treated tumors (Fig. 7C). In summary, our in vivo preclinical efficacy trials showed an antitumor effect of NSC48300 in both MMTV-neu breast cancer and U251 GBM xenograft models.

Discussion

In the evolution of cancer, individual cells must overcome a multitude of challenges and eventually exhibit hallmarks of cancer, a process demanding multiple oncogenes and nononcogenes that function cooperatively to achieve and maintain the aberrant, oncogenic state. The evolved reliance of oncogenes on particular sets of subordinate nononcogenes during tumorigenesis, offering a novel anticancer treatment strategy aiming at tumor dependent, nononcogenes (40). Thus far, well-characterized tumor dependent, nononcogenic actors are scarce, including the 26S proteasome, HSF1 (41), and IRF4 (42), which handles the rapid protein turnover, mediates the stress response, and maintains expression of the MYC oncogene, respectively. The fact that the proteasome inhibitor bortezomib is effective against multiple myeloma in human patients substantiates this new anticancer therapeutic concept (11). Successful application of this strategy in cancer therapy relies on the identification and characterization of tumor dependent, nononcogenic factors that cancer but not normal cells heavily rely on.

Taspase1 by itself or in conjunction with MYC, RAS, or E1A fails to transform NIH/3T3 cells or primary MEFs, respectively.
yet is required for efficient cancer initiation and maintenance (20). Hence, Taspase1 functions as a tumor dependent, non-oncogenic protease, whose inhibition may offer an anticancer strategy. However, Taspase1 is highly conserved and known to regulate embryonic development (8). The severe perinatal lethality resulting from the embryonic loss of Taspase1 suggests that inactivation of Taspase1 by genetic or pharmacologic means is inadvisable in pregnant females and children to avoid potential developmental sequelae. In contrast, inactivation of Taspase1 in fully developed adult mammals seems to be well tolerated. Cancer commonly hijacks key developmental pathways during tumorigenesis and thus frequently exhibits unique properties, which may underlie the preferential therapeutic benefit conferred by targeting Taspase1 to treat cancers.

Through proteolytic processing of nuclear transcription regulators, Taspase1 controls cellular proliferation by suppressing and activating the expression of CDKIs and cyclins, respectively (8, 20). Although knockdown of Taspase1 affects proliferation in many cancer cell lines, candidate cancer types that may respond to Taspase1 inhibition in vivo remain unclear. Unlike activating mutations of epidermal growth factor receptor and BCR-ABL that can serve as powerful biomarkers for selecting responsive cancers (43), Taspase1 does not function like a classical oncogene and is not mutated in cancers (COSMIC, Sanger Institute). Data thus far indicate a positive correlation between Taspase1 protein levels and the underlying dependence, which provides a useful primary tool in identifying potentially susceptible cancers. Our prior knockdown experiments showed an in vitro and in vivo reliance of U251 brain cancer cells on Taspase1 for a full cancer phenotype (20). Here, we show treatment response of U251 cells to TASPIN NSC48300 in both cell culture and xenograft models. Hence, genetic knockdown experiments in cancer cells could reflect the cellular dependence on Taspase1 and thus provide assessment of in vivo responsiveness of individual cancers to TASPINs.
In addition to brain tumors, we expanded our in vivo studies to incorporate breast cancer models. Deficiency in Taspase1 by genetic knockdown disrupts the proliferation of Her2/neu+ human BT-474 breast cancer cells, suggesting that Her2-neu+ breast cancer may be sensitive to the inactivation of Taspase1. Indeed, de novo breast cancers developed in MMTV-neu mice responded to the treatment with NSC48300, whereas MMTV-wnt breast cancers were insensitive. Consistent with the treatment effects, mammary tissue–specific knockout of Taspase1 disrupts MMTV-neu–driven, but not MMTV-wnt–driven, breast carcinogenesis (Van Tine and colleagues, unpublished data). Taspase1 seems to play an important role in the Her2/neu growth factor signaling pathway, whereas WNT signals apparently bypass Taspase1-mediated oncogenic events. These data highlight the heterogeneity underlying individual tumorigenesis and the importance of selecting the responsive cancers that may benefit from treatment with TASPINs. Further studies with regard to the involvement of Taspase1 in various oncogenic pathways and the pathogenesis of subtypes of cancers would offer a better target cancer selection for the potential use of TASPINs in the future.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interests were disclosed.

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