Priority Report

Tumor Suppressor RARRES1 Interacts with Cytoplasmic Carboxypeptidase AGBL2 to Regulate the α-Tubulin Tyrosination Cycle

Ziad J. Sahab¹, Michael D. Hall¹, You Me Sung¹, Sivanesan Dakshanamurthy¹, Yun Ji¹, Deepak Kumar², and Stephen W. Byers¹,²

Abstract

Even though it is among the most commonly methylated loci in multiple cancers, the retinoic acid–induced tumor suppressor retinoic acid receptor responder 1 (RARRES1) has no known function. We now show that RARRES1 is lost in many cancer cells, particularly those with a mesenchymal phenotype, and is a transmembrane carboxypeptidase inhibitor that interacts with ATP/GTP binding protein-like 2 (AGBL2), a cytoplasmic carboxypeptidase. Knockdown of AGBL2 results in a failure of the cell to detyrosinate the C-terminal EEY region of α-tubulin and indicates that it is a candidate for the long sought-after tubulin tyrosine carboxypeptidase important in the regulation of microtubule dynamics. In contrast, knockdown of RARRES1 increases the level of detyrosinated α-tubulin consistent with a role as the cognate inhibitor of AGBL2. We conclude that RARRES1, its interacting partners AGBL2, Eg5/KIF11, another EEY-bearing protein (EB1), and the microtubule tyrosination cycle are important in tumorigenesis and identify a novel area for therapeutic intervention.

Cancer Res; 71(4); 1219–28. ©2011 AACR.

Introduction

Retinoic acid receptor responder 1 (RARRES1), also known as tazarotene-induced gene 1, was first identified as a novel retinoid-responsive gene in skin. It is induced in a retinoic acid receptor–specific manner in a variety of human skin-related systems (¹). The RARRES1 promoter region is methylated in primary prostate cancers compared with normal tissues or benign hyperplasias, and its decreased expression is associated with an increase in the malignant potential of prostate carcinoma cells (²). RARRES1 is among the most commonly methylated loci in multiple cancers and is often described as a putative tumor suppressor gene (³). In addition, RARRES1 also plays a role in the proliferative/differentiative switch in adult adipose-derived mesenchymal stem cells. Notably, a RARRES1 family member, latexin (LXN), has been implicated in controlling aspects of stem cell biology, in which an inverse and direct relationship between LXN expression and the size of the hematopoietic stem cell population in mice has been reported (⁴). LXN was initially described as the only known mammalian carboxypeptidase inhibitor and is involved in the regional specification of neurons (⁵–⁸). Despite extensive evidence for a tumor suppressor role of RARRES1, no mechanism for its biological function has been determined.

Here, we show that RARRES1 is a type III membrane protein found in a complex with several proteins involved in the regulation of microtubule function and reveal that ATP/GTP binding protein-like 2 (AGBL2; a carboxypeptidase) and RARRES1 (an inhibitor) regulate the tubulin tyrosination cycle. Our findings point to a role for RARRES1, AGBL2, and the tubulin tyrosination cycle in cancer and identify a novel avenue for potential therapeutic intervention.

Materials and Methods

Cells

Immortalized human prostate epithelial PWR-1E cells (a gift from Dr. S.C. Chauhan, University of South Dakota), PC-3 human prostate cancer cells, SKBR-3 human breast cancer cells, and HEK 293 human embryonic kidney cells were maintained according to the recommendation of American Type Culture Collection.

Antibodies and reagents

Primary antibodies targeting the following antigens were used: goat anti-human RARRES1 (catalogue no. AF4255, R&D Systems), antityrosinated tubulin, and anti-Eg5 (Abcam),
anti-E-cadherin (BD-Transduction Laboratories), anti-cyclin D1 (EMD-Calbiochem), anti-HA (Millipore), anti-histone-H4 (Cell Signaling), anti-detyrosinated tubulin (AbD Serotec), and anti-AGBL2, and anti-pan-cadherin (Sigma-Aldrich). The following inhibitory RNAs (RNAi; Dharmacon) were used: for RARRES1 knockdown, GUACACGGCUCAUCGAGAA and UGGACAGAGUAAGUUC. For AGBL2 knockdown: GCA-CACUCUCAACCCAUAA and UGGAAGGAUGUAAGUUC. For pEYFP-N1 vector (Clontech). N-RARRES1 (Hs99999905_m1,AAGL1/Nna1 – master mix and the following inventoried primer/probe sets: HT system (Applied Biosystems), using TaqMan universal PCR kit (Qiagen). Single-stranded cDNA was prepared from 400 ng of RNA by TaqMan reverse transcription reagents (Applied Biosystems). Total RNA was extracted from indicated cell lines with Trizol reagent (Invitrogen) and isolated with an RNeasy purification kit (Qiagen). Cell fractionation and tandem affinity purification

A stable clone expressing a level of exogenous pGlue RARRES1 close to the endogenous level of this protein was grown in 5 dishes (150 mm each). At 90% confluency, medium was discarded and each dish was lysed in 0.5 mL of a lysis buffer, composed of 10% glycerol, 50 mmol/L HEPES-NaOH, pH 8.0, 100 mmol/L NaCl, 2 mmol/L EDTA, 0.1% NP-40, 2 mmol/L DTT, 10 mmol/L NaF, 5 mmol/L calyculin A, 50 mmol/L β-glycerophosphate, and 1× Complete Mini protease inhibitor (Roche). Lysates were harvested by scraping and 2 freeze-thaw cycles were done to improve protein recovery. Lysates were then centrifuged at 15,000 × g for 15 minutes, and supernatant were recovered and incubated for 4 hours with 100 μL of streptavidin beads (Streptavidin Sepharose High Performance, GE Healthcare) prewashed 3 times with the lysis buffer. The slurry was then centrifuged at 1,500 × g for 2 minutes, and the precipitate containing the streptavidin beads was recovered and washed 3 times with lysis buffer and 2 times with the TEV buffer, supplied with the ActEvr Protease kit (catalogue no. 12575-015; Invitrogen). Streptavidin beads were then incubated with 200 units of TEV protease in 150 μL of TEV buffer overnight at 4°C. The slurry was then centrifuged at 1,500 × g for 2 minutes, and the supernatant was recovered. The precipitated beads were washed twice with 20 μL of TEV buffer, centrifuged, and supernatants were pooled. The final pool volume was diluted 1:1 (v/v) with calmodulin-binding buffer composed of 10 mmol/L β-mercaptoethanol, 10 mmol/L HEPES-NaOH, pH 8.0, 150 mmol/L NaCl, 1 mmol/L MgOAc, 1 mmol/L imidazole, 0.1% NP-40, and 2 mmol/L CaCl2. The mixture was then incubated for 90 minutes at 4°C with 100 μL of calmodulin beads (Calmodulin Sepharose 4B; GE Healthcare) prewashed 3 times with calmodulin-binding buffer. The slurry was then centrifuged at 1,500 × g for 2 minutes, the supernatant was discarded, and the precipitated beads were washed twice with a calmodulin-rinsing buffer composed of 50 mmol/L ammonium bicarbonate, pH 8.0, 75 mmol/L NaCl, 1 mmol/L MgOAc, 1 mmol/L imidazole, and 2 mmol/L CaCl2. Each wash was followed by centrifugation and supernatant shedding. A total of 150 μL of a calmodulin- elution buffer composed of 50 mmol/L ammonium bicarbonate, pH 8.0, and 25 mmol/L EGTA was then added to the calmodulin resin. The slurry was then vortex mixed, centrifuged at 1,500 × g, and the supernatant was collected. This elution step was repeated twice and supernatants were pooled together (Supplementary Fig. S1).

Trypsin digestion and liquid chromatography/tandem mass spectrometric analysis

The pull-down sample was vacuum-dried and reconstituted with 20 μL of a buffer composed of 500 mmol/L triethylammonium bicarbonate, pH 8.5. The protein sample was then denatured by adding 1 μL of a 2% SDS solution followed by the addition of 2 μL of a reducing reagent composed of 50 mmol/L...
Protein identification

Protein identification was done using ProteinPilot software with the following settings (11): sample type: identification; cysteine alkylation: MMTS; digestion: trypsin; instrument: QSTAR ESI; species: Homo sapiens; MS/MS fragment tolerance of 0.3 Da; cursor tolerance 75 ppm; maximum missed cleavage: 1; and MS/MS fragment tolerance of 0.3 Da.

Western blotting

Western blotting was done as described previously (12, 13). Cells were lysed with RIPA buffer and loaded onto 4% to 12% gradient polyacrylamide gel. Amounts of proteins loaded were as described previously (10). Nano liquid chromatography/tandem mass spectrometry (LC/MS-MS) was done using a Q-Star Elite (Applied Biosystems), equipped with a nanoAcquity UPLC system (Waters). Sample separations were done with a 1.7-μm nanoAcquity BEH130 C18 column (100 μm × 100 mm) at a flow rate of 400 nL/min. Tryptic digests were eluted by the following gradient: 100% of solvent A [97.9% water, 2% acetonitrile, 0.1% formic acid (v/v/v)] for 1 hour; then from 100% solvent A to 100% solvent B [2% water, 97.8% acetonitrile, 0.1% formic acid (v/v/v)] in 2 hours; finally, A 100% solvent B flow was maintained for 1 hour, followed by a return to 100% of solvent A flow in 15 minutes. Mass spectrometric settings were as follows: ion spray voltage, 2,300 V; interface heater temperature, 220°C; cone voltage, 20 V; and collision energy, 8 V.

Homology modeling

AGBL2 structure was predicted with human carboxypeptidase A1 (PDB code: 1V77) as a template. The sequence identity of AGBL2 and CPA1 is 27%. The missing loops were built using the "loop model" building option in the Modeler9v7. The model was refined further by molecular dynamics (MD) simulations, followed by energy minimization using SANDER module of AMBER 10.0. The quality of the refined model was checked with PROCHECK. Docking of the 'EY' peptide motif was carried out with the SurFlexDock. MD simulations and energy minimization were done using the AMBER10.0 package.

Immunofluorescence

To assess the levels of detyrosinated tubulin in control versus AGBL2, RARRES1 knockeddown, and/or paclitaxel-treated HEK 293 cells, 50,000 cells were plated on cover slides (Fisher brand microscope cover glass #12-545-100 18CIR-1). After 24 hours, cells were treated with paclitaxel or dimethyl sulfoxide at a final concentration of 5 μmol/L for 2 hours. HEK 293 cells were then fixed in 3.7% paraformaldehyde/PBS for 10 minutes at room temperature, followed by washing the cells 3 times with PBS. Postpermeabilization was done by adding PBST (PBS/Tween 20) and incubating the cells for 5 minutes at room temperature. Cells were then washed 3 times with PBS, followed by the addition of the primary antibody that consisted of a rabbit polyclonal anti–detyrosinated tubulin (ABD Sero tec) with a final dilution of 1:250. Cells were then washed 3 times with PBS before adding the secondary antibody that consisted of an Alexa 488–conjugated anti-rabbit IgG diluted 1:300 (Invitrogen). 4.6-Diamidino-2-phenylindole (DAPI) was also added at a 1:50 dilution for nuclei detection. Cells were washed 3 times with PBS, and images were obtained using a 60× oil lens on the Olympus FV 300 confocal microscope. Consistent laser intensity or camera exposure levels for each fluorescent marker in each experiment were used. For image analysis and quantification, measurements were made using Metamorph Image analysis software ver. 7.0. Average intensity was calculated from integrated intensity and area for each selected area. Quantitation of fluorescence signals from 5 random fields for each treatment was done. An example of an original image used for quantitation is included in the supplementary materials (Supplementary Fig. S2).

Results

RARRES1 organization

RARRES1 is related to the putative carboxypeptidase inhibitor LXX, and both genes are adjacent to one another on
chromosome 3, suggesting that they arose as a result of gene duplication (ref. 14; Fig. 1A). Similar molecules exist in all vertebrates examined; in zebrafish and several other more ancient vertebrates, only one orthologue exists, and this is more closely related to RARRES1 than to LXN (Fig. 1B). These data indicate that RARRES1 is the ancestral gene and in zebrafish, may, at least, fulfill the function of both LXN and RARRES1. We found that RARRES1 exists as 2 mRNA splice-variants: a low abundance variant and an abundant form which encodes for an additional 66 amino acids at the C-terminus (Fig. 1C–E).

RARRES1 mRNA is decreased in prostate cancer and further in metastatic prostate cancer compared with normal prostate tissue and is induced after neoadjuvant therapy (2). RT-PCR analysis shows that RARRES1 is expressed in normal prostate and breast cell lines and more differentiated breast cancer cell lines and is very low in aggressive prostate and breast cancer cells with a mesenchymal phenotype (Fig. 2A; Supplementary Table S1). HEK 293 cells express low but still detectable levels of RARRES1. In PWRE-1E cells, RARRES1 is further induced both by retinoic acid (RA) and vitamin D.

Identification of the RARRES1 interactome

We utilized a tandem affinity purification (TAP) LC/MS-MS approach to characterize the RARRES1 interactome in HEK 293 cells. Nano-LC-Q-TOF/TOF mass spectrometry and a ProteinPilot software search revealed 9 proteins (including RARRES1) identified with a CI of more than 95% (Table 1). Proteins that were also present in a complex isolated from cells stably expressing the empty vector control were removed from the analyses. Several proteins in the RARRES1 complex regulate microtubule function. These are as follows: the mitotic spindle-associated kinesin eg5/KIF11, an emerging target for cancer therapy; EB1, a microtubule plus end-binding protein; and the Rhesus Monkey X001101220 and Human TIG1 Long Isoform proteins.
protein that is regulated by RARRES1 (15); a novel human cytosolic carboxypeptidase member of the ABGL/CCP family (AGBL2), and α-tubulin. The presence of AGBL2, Eg5, and EB1 in the exogenously and endogenously expressed RARRES1 complex was confirmed by Western blot after TAP, RARRES1 immunoprecipitation, and reverse AGBL2 immunoprecipitation (Fig. 2C). Two other interactors, ANKRD26-like family member 1A and Crk-like protein kinase, are likely involved in

<table>
<thead>
<tr>
<th>Table 1. RARRES1 interactome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Confidence</strong></td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>98</td>
</tr>
<tr>
<td>97</td>
</tr>
</tbody>
</table>

NOTE: List of proteins identified at more than 95% confidence as being present in the RARRES1-TAP produced complex. Proteins that were also present in a complex isolated from cells stably expressing the empty vector control were removed from this list.
RARRES1 is a type III membrane protein

An affinity purified polyclonal antibody raised to residues 43 to 294 of human RARRES1 protein recognizes an approximately 38-kDa protein from PWR-1E whole cell lysate which is present at much reduced levels in PC-3 cells, consistent with mRNA data. The antibody recognizes TAP-tagged RARRES1, DN-RARRES1, and RARRES1-EYFP at their respective predicted molecular masses but does not detect the variant isoform of RARRES1-EYFP. Cell fractionation analysis reveals that approximately 38-kDa RARRES1 is membrane bound; a second, higher molecular weight pool (~50 kDa) is present in the nuclear/cell debris pool, indicating a form which is highly modified and/or insoluble in nonionic detergents (Fig. 2B). Although RARRES1 has a transmembrane domain, its directionality once inserted into the membrane is unclear (www.expasy.ch). We addressed RARRES1 orientation by examining its N-glycosylation status via incubation with PNGase F glycosidase. Unlike E-cadherin, RARRES1 does not undergo a shift in molecular weight when exposed to PNGase F (Fig. 2B), suggesting that, even though it possesses several putative sites for glycosylation, it is not N-glycosylated and that the C-terminal likely faces the cytoplasm and not lumen of the membrane compartment (a type III transmembrane protein). This is consistent with its association with several cytoplasmic proteins (Table 1).

Regulation of α-tubulin tyrosination

Other than its presence in GenBank, nothing is known of human AGBL2, though a likely family member, mouse Nna-1, is important in cerebellar degenerative disorders (17). Nna-1 knockout mice have high levels of tyrosinated tubulin in the
degenerative cerebellum, and it was suggested that Nna-1 might have the characteristics of a tubulin carboxypeptidase (18). However, it is now thought that Nna-1 is involved in the degradation of proteosomally generated peptides (19, 20).

Although significant levels of AGBL2 and other family members exist in HEK 293, PC-3, and PWR-1E cells, Nna-1 (AGTPBP) was not detectable by quantitative PCR (Fig. 2D) and neither itself nor the other AGBL family members were found in the RARRES1 complex (Table 1).

To gain some insight into its potential carboxypeptidase activity, we predicted AGBL2 structure using human CPA1 (PDB code: 1V77) as template. Although the overall sequence identity is only 27% (active site 21%), the predicted tertiary structure of AGBL2 is remarkably similar to CPA1 (Fig. 3A). Importantly, the residues predicted to interact with the zinc coordination atom and the folding in this catalytic site is almost identical. Although the structure of the catalytic site is very similar, the substrate binding cavity of AGBL2 is lengthy and narrow whereas it is bulky and wide for CPA1 (Fig. 3B and C). These structural predictions strongly indicate that AGBL2 is likely to have carboxypeptidase activity. Importantly, extensive molecular simulations indicate that the putative C-terminal α-tubulin peptide EYE substrate can be docked precisely into the predicted AGBL2 binding pocket (Fig. 3D).

To further test whether AGBL2 has the characteristics of a tubulin carboxypeptidase, we carried out Western blots using antibodies specific for the tyrosinated and detyrosinated forms of α-tubulin on proteins extracted from HEK 293 control cells and cells in which AGBL2 was knocked down. Several predicted AGBL2 variants and/or possibly degradation fragments are detected in Figure 4A. AGBL2 siRNA resulted in marked knockdown of all AGBL2 variants and fragments. B, RARRES1 immunoblot depicting exogenous pGlue-RARRES1 (45 kDa) and endogenous RARRES1 (35 kDa), using a mouse monoclonal antibody. C and D, immunoblot for detyrosinated α-tubulin in HEK 293 cells in which RARRES1 or AGBL2 was knocked down or exogenously expressed. Control, control pGlue, Myc-AGBL2, and RARRES1-pGlue transfections were done in duplicate. Loading control was done using a monoclonal anti-α-tubulin antibody.

Figure 4. RARRES1 and AGBL2 regulate α-tubulin tyrosination in HEK 293 cells. A, AGBL2 immunoblot depicting the full-length protein (104 kDa) and other predicted variants (or degradation fragments) at 72, 64, 38, 33, and 31 kDa. Myc-AGBL2 transfection results in a low amount of full-length exogenous protein expression. AGBL2 knockdown results in the disappearance of all AGBL2 variants and fragments. B, RARRES1 immunoblot depicting exogenous pGlue-RARRES1 (45 kDa) and endogenous RARRES1 (35 kDa), using a mouse monoclonal antibody. C and D, immunoblot for detyrosinated α-tubulin in HEK 293 cells in which RARRES1 or AGBL2 was knocked down or exogenously expressed. Control Myc, control pGlue, Myc-AGBL2, and RARRES1-pGlue transfections were done in duplicate. Loading control was done using a monoclonal anti-α-tubulin antibody.

RARRES1 (Fig. 4B) were detected using a mouse monoclonal antibody (Ab92884; Abcam). Consistent with their role in tubulin detyrosination, AGBL2 knockdown and RARRES1 overexpression reduced the level of detyrosinated tubulin. In contrast, knockdown of RARRES1 and exogenous expression of AGBL2 increased the level of detyrosinated tubulin, consistent with RARRES1 inhibition of endogenous AGBL2 activity (Fig. 4C and D). Similar results were found following the analysis of detyrosinated and tyrosinated tubulins by mass spectrometry (not shown). These findings were further validated using immunofluorescent staining of detyrosinated tubulin (Fig. 5A, Supplementary Fig. S3). The relative abundance of detyrosinated tubulin was significantly upregulated after RARRES1 knockdown and expression of exogenous AGBL2, and significantly downregulated after exogenous expression of RARRES1 or AGBL2 knockdown, when compared with the appropriate control (Fig. 5B and C). Although knockdown of AGBL2 did not completely abolish detyrosinated tubulin, as measured by immunocytochemical quantitation of whole cells, examination of individual micrographs reveals a more significant loss of microtubule-associated detyrosinated tubulin. Diffuse cytoplasmic staining observed under all conditions makes the threshold of this particular assay rather high in HEK 293 cells. The association between detyrosinated tubulin and microtubules is shown in Supplementary Figure S3. Interestingly, treatment of HEK 293 cells with paclitaxol does not result in a major increase in the levels of detyrosinated tubulin, as observed previously in HeLa cells (21). This may be a result of the known drug-resistant phenotype of HEK 293 cells, an embryonically derived cell line. Although immunoblots show an increase in detyrosinated tubulin on transfection of AGBL2, immunofluorescence staining reveals a more significant increase at the single cell level. Taken together, these data provide strong evidence that...
Figure 5. RARRES1 and AGBL2 regulate the detyrosination cycle of α-tubulin in HEK 293 cells. A, immunofluorescence signal of Alexa 488 probing the detyrosinated α-tubulin (green) and DAPI probing the nucleus (blue) in HEK 293 controls, RARRES1 knockdown, AGBL2 knockdown, RARRES1 overexpression, and AGBL2 overexpression with and without taxol treatment. The color balance and the contrast of all the images combined were optimized using Photoshop CS3 to allow for visualization of both DAPI and detyrosinated tubulin staining on an 8-bit resolution monitor. An example of the original image used for quantitation is included in the supplementary materials (Supplementary Fig. S1); B, average signal intensities of detyrosinated α-tubulin in HEK 293 cells following knockdown or exogenous expression of RARRES1 with and without taxol treatment (*, P < 0.05 and #, P < 0.001); C, average signal intensities of detyrosinated α-tubulin in HEK 293 cells, following knockdown or exogenous expression of AGBL2 with and without taxol treatment (*, P < 0.05; **, P < 0.01; #, P < 0.001; ##, P < 0.0001).
AGBL2 and RARRES1 are α-tubulin tyrosine carboxypeptidase and carboxypeptidase inhibitor, respectively.

Discussion

Previous studies indicated a role for RARRES1 in some aspect of cellular proliferation and tumorigenicity (3). However, these groups utilized the variant version of RARRES1 in their studies (the only form identified at the time), and we have shown that the variant mRNA species is present in very small quantities and cannot verify that this transcript is translated into a functional protein. Consequently, it is likely that the major function of RARRES1 is carried out by the full-length isoform.

The RARRES1 paralogue LXN, was initially described as a carboxypeptidase inhibitor in the rodent brain and regulates hematopoietic stem cell numbers and lifespan in mice (4, 22). RARRES1 also regulates proliferation and differentiation in adipose-derived mesenchymal stem cells and proliferation and motility in HK1 cells, suggesting a role for this family of genes in the differentiation of stem cells from several different tissues (23). Recombinant LXN can inhibit the activity of the lysosomal carboxypeptidase A (CPA) family members in vitro and can be cocrySTALLized with them; however, structural analyses and localization patterns of LXN clearly suggest that it is a cytoplasmic protein and thus it is not likely to interact with members of the lysosomal CPA family (24). Because RARRES1 is a type III transmembrane protein, the cytoplasmic localization of its putative carboxypeptidase inhibitor domain would preclude it too from interacting with the lysosomal CPAs. This suggests that its cognate carboxypeptidase(s) is likely active within the cytoplasm. Although little is known about cytoplasmic carboxypeptidases or their substrates, removal of the C-terminal tyrosine of α-tubulin by an unknown carboxypeptidase (the tyrosination cycle) is important in several aspects of microtubule function including kinesin interactions, spindle dynamics, mitosis, and neuronal specification (25). Microtubules containing large amounts of detyrosinated α-tubulin are more stable and resistant to depolymerization by nocodazole and other destabilizing agents (26). Detyrosinated α-tubulin is elevated in aggressive breast and prostate cancers which are often resistant to microtubule-targeted chemotherapeutics (27). Elevated levels of detyrosinated α-tubulin and Δ2-tubulin, a highly stable version generated from the precursor detyrosinated form, predominate in normal brain tissue but are also associated with tumors in other tissues, further suggesting a role for the tubulin tyrosination cycle in tumorigenesis (28). Recently, a role for detyrosinated tubulin in epithelial to mesenchymal transitions, important in development, stem cell differentiation, and tumor invasion has been proposed (29). Remarkably, although the tubulin tyrosination cycle has been known to occur for decades, the identity and regulation of tubulin carboxypeptidase have remained a mystery (30, 31).

Our demonstration that AGBL2 is a RARRES1-interacting protein that regulates the tubulin tyrosination cycle implicates both molecules in the regulation of this evolutionarily ancient process and identifies it as a novel target for intervention (Fig. 6).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors thank the Lombardi Cancer Center for the following core facilities (NIH P30 CA51008): microscopy, tissue culture, proteomics, genomics, and epigenomics.

Grant Support

This study was funded by NIH R01CA129813, NIH 1 P01 CA130821, R01 DK58196 (S.W. Byers), and NIH U56 LCCC/UDC Partnership (D. Kumar and S.W. Byers). Y. Ji was partly supported by an NIGMS T32 grant.

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 June 28, 2010; revised December 13, 2010; accepted December 15, 2010; published OnlineFirst February 8, 2011.

References


Tumor Suppressor RARRES1 Interacts with Cytoplasmic Carboxypeptidase AGBL2 to Regulate the \( \alpha \)-Tubulin Tyrosination Cycle

Ziad J. Sahab, Michael D. Hall, You Me Sung, et al.

Cancer Res 2011;71:1219-1228. Published OnlineFirst February 8, 2011.

Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-10-2294

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2011/02/07/0008-5472.CAN-10-2294.DC1

Cited articles
This article cites 31 articles, 12 of which you can access for free at:
http://cancerres.aacrjournals.org/content/71/4/1219.full.html#ref-list-1

Citing articles
This article has been cited by 7 HighWire-hosted articles. Access the articles at:
/content/71/4/1219.full.html#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.