ATAD2 Is a Novel Cofactor for MYC, Overexpressed and Amplified in Aggressive Tumors

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Abstract

The E2F and MYC transcription factors are critical regulators of cell proliferation and contribute to the development of human cancers. Here, we report on the identification of a novel E2F target gene, ATAD2, the predicted protein product of which contains both a bromodomain and an ATPase domain. The pRB-E2F pathway regulates ATAD2 expression, which is limiting for the entry into the S phase of the cell cycle. We show that ATAD2 binds the MYC oncogene and stimulates its transcriptional activity. ATAD2 maps to chromosome 8q24, 4.3 Mb distal to MYC, in a region that is frequently found amplified in cancer. Consistent with this, we show that ATAD2 expression is high in several human tumors and that the expression levels correlate with clinical outcome of breast cancer patients. We suggest that ATAD2 links the E2F and MYC pathways and contributes to the development of aggressive cancer through the enhancement of MYC-dependent transcription.

Introduction

The retinoblastoma protein (pRB) pathway has a central role in the control of cell proliferation (1). In fact, most human cancers harbor mutations in core members of the pRB pathway, and as a consequence, E2F-regulated genes are aberrantly expressed. Interestingly, E2F target genes such as MYC, CCNE1 (cyclin E1), B-MYB, c-MYB, and EZH2 are also bona fide oncogenes, frequently amplified and overexpressed in primary human tumors (2–5).

MYC is one of the most studied oncogenes, which contributes to the malignancy of many different aggressive and undifferentiated human cancers (4). The pathologic effect of MYC has been ascribed to its ability to control many cellular processes such as cell growth, differentiation, apoptosis, and, more recently, DNA damage response, genomic instability, angiogenesis, and tumor invasiveness (6). MYC acts as a potent, sequence-specific transcription factor (6) interacting both with the SWI/SNF chromatin remodeling complexes (7) and with the histone acetyltransferases CBP/p300 (8), GCN5 (9, 10), and Tip60 (11).

Here we report on a hitherto uncharacterized E2F target gene, ATAD2. ATAD2 is highly expressed and genetically amplified in several types of human cancer, and we show that high levels of ATAD2 correlate with unfavorable prognosis in breast cancer patients. Furthermore, we show that ATAD2 works as an important cofactor for MYC-dependent transcription, suggesting that ATAD2 contributes to tumor development, in part, through enhancing the transcriptional activity of MYC.

Materials and Methods

Tissue culture. Cell lines were grown in DMEM supplemented with 10% fetal bovine serum, 2 mmol/L glutamine, 100 units/mL penicillin, and 100 units/mL streptomycin. Transfections were done with standard CaPO4 method. Crystal violet staining and quantification were done as described (12). HEK293 cells were synchronized by double-thymidine block (13) and released from the block in the presence of 100 ng/mL nocodazole.

RNA interference. Small interfering RNA (siRNA) and short hairpin RNA (shRNA) against ATAD2 were generated targeting two different sequences: GGATCTCTCTTCATTAAT and GTGCGTGGAAATGTAGGA, respectively. siRNA duplexes from annealed pairs of 21 ribonucleotides (Dharmacon Research) were transfected into TIG3 fibroblasts using Oligofectamine (Invitrogen). shRNAs were expressed from lentiviruses generated using pLL3.7 vector (14). As a control, we used siRNA or shRNA duplex targeting firefly luciferase.

Antibodies. We raised and affinity purified a polyclonal antibody against ATAD2 by immunizing rabbits with a MBP NH2-terminal portion of the protein (amino acids 1–447). In addition, the following antibodies were used: anti-vinculin (h-VIN1, Sigma), anti-HA (HA-11, BABCO), anti-FLAG (M2, Sigma), anti-CCNA2 (C-19, Santa Cruz Biotechnology), anti-MYC (N-262, C-33 Santa Cruz Biotechnology), mouse anti-bromodeoxyuridine (BrdUrd; Becton Dickinson), anti-E2F1 (KH20; ref. 15), anti-H3 (ab1791, Abcam), anti-acetylated H3 (Upstate), anti-α-tubulin (DM-1A, Sigma), anti–lamin A/C (sc-7292, Santa Cruz Biotechnology), anti-cyclin E1 (M20, Santa Cruz Biotechnology), anti-TIP60 (11), and cyclin B1 (H-20, Santa Cruz Biotechnology). Antibodies used in chromatin immunoprecipitation assays are described below.

Chromatin immunoprecipitation. Chromatin immunoprecipitations were done and analyzed essentially as described (16). The antibodies used were immunopurified polyclonal anti-ATAD2, anti-MYC (N-262, Santa Cruz Biotechnology), anti-E2F1, anti-E2F2, anti-E2F3 (sc-193, sc-633, sc-878, Santa Cruz Biotechnology), and anti-HA (HA-11, BABCO) as a control. Primer sequences for amplification of ATAD2 promoter are available on request. Primer sequences for amplification of MYC target promoters and acetyl-choline receptor promoter were obtained from Fernandez and colleagues. (17).

Sequential nuclear extraction. Sequential nuclear extraction was done as described in ref. 18. In the first fractionation step, soluble proteins were removed by extraction with Triton X-100. Chromatin proteins were then released by DNase I digestion followed by 0.25 mol/L ammonium sulfate treatment, which resulted in the release of most of the histones. After a 2 mol/L NaCl wash, the last fraction was enriched in nuclear matrix–associated proteins.

Recombinant protein production. Recombinant glutathione S-transferase (GST)-ATAD2 was obtained from sf9 cells as previously described (19).
Recombinant core and tailless H3 and H4 histones were kindly donated by Elisabetta Citterio (FIRC Institute of Molecular Oncology, Milan, Italy). Recombinant core and tailless H3 and H4 histones were kindly donated by Cancer Research and by the use of shRNA or siRNA against ATAD2.

**Table 1. ATAD2 is highly expressed and amplified in human cancer**

<table>
<thead>
<tr>
<th>Tumor</th>
<th>No. of samples</th>
<th>Highly expressed, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast carcinoma</td>
<td>25</td>
<td>9 (36)</td>
</tr>
<tr>
<td>Colon carcinoma</td>
<td>27</td>
<td>10 (37)</td>
</tr>
<tr>
<td>Lung carcinoma</td>
<td>22</td>
<td>5 (23)</td>
</tr>
<tr>
<td>Prostate carcinoma</td>
<td>24</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Stomach carcinoma</td>
<td>21</td>
<td>9 (43)</td>
</tr>
<tr>
<td>Uterus carcinoma</td>
<td>20</td>
<td>4 (20)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>12</td>
<td>2 (17)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>11</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>10</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Total</td>
<td>172</td>
<td>40 (23)</td>
</tr>
</tbody>
</table>

**ATAD2 copy number in breast carcinomas**

<table>
<thead>
<tr>
<th>Lesion</th>
<th>No. of samples</th>
<th>≥5 copies, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroadenoma</td>
<td>8</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td>70</td>
<td>12 (17)</td>
</tr>
</tbody>
</table>

**ATAD2 protein expression in breast carcinomas**

<table>
<thead>
<tr>
<th>Tumor</th>
<th>No. of samples</th>
<th>Highly expressed, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast carcinoma</td>
<td>66</td>
<td>26 (39)</td>
</tr>
</tbody>
</table>

**Results**

**Identification of ATAD2.** By combining gene expression profiles done in our lab and publicly available databases (Supplementary Fig. S2), we identified a new E2F target gene, ATAD2, which is predicted to be overexpressed and amplified in human cancer. The ATAD2 gene is mapped to chromosome 8q24 and codes for a predicted protein of 1,391 amino acids that contains a double AAA ATPase domain (21) and a bromodomain (22). ATAD2 is highly conserved between human, mouse, and rat, and it also has a human homologue, called ATAD2B. Recently, Zou and colleagues (23, 24) described ATAD2 as being a cofactor for the estrogen and androgen receptors with a potential role in the survival and proliferation of tumor cells.

**Table 2. Correlation between ATAD2 tissue expression and clinicopathologic characteristics of primary breast cancer**

<table>
<thead>
<tr>
<th>No. of women</th>
<th>ATAD2 protein expression</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>All</td>
<td>349</td>
<td>217</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;45</td>
<td>92</td>
<td>53</td>
</tr>
<tr>
<td>&gt;45</td>
<td>255</td>
<td>162</td>
</tr>
<tr>
<td>Pathologic stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT1</td>
<td>213</td>
<td>127</td>
</tr>
<tr>
<td>pT2-pT4</td>
<td>134</td>
<td>88</td>
</tr>
<tr>
<td>Tumor grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>72</td>
<td>54</td>
</tr>
<tr>
<td>G2</td>
<td>145</td>
<td>86</td>
</tr>
<tr>
<td>G3</td>
<td>101</td>
<td>55</td>
</tr>
<tr>
<td>Nodal status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>199</td>
<td>123</td>
</tr>
<tr>
<td>Positive</td>
<td>146</td>
<td>91</td>
</tr>
<tr>
<td>Hormone receptor status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>98</td>
<td>61</td>
</tr>
<tr>
<td>Positive</td>
<td>244</td>
<td>150</td>
</tr>
<tr>
<td>K&lt;sup&gt;67&lt;/sup&gt; expression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20%</td>
<td>203</td>
<td>139</td>
</tr>
<tr>
<td>≥20%</td>
<td>127</td>
<td>67</td>
</tr>
<tr>
<td>HER2/Neu status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>297</td>
<td>187</td>
</tr>
<tr>
<td>Positive</td>
<td>49</td>
<td>28</td>
</tr>
</tbody>
</table>

**Association between ATAD2 expression and risk of distant recurrence in breast cancer patients**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients</th>
<th>ATAD2 protein expression</th>
<th>OR1 (95% CI)</th>
<th>OR2 (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low</td>
<td>High</td>
<td>(%)</td>
</tr>
<tr>
<td>No event (control)</td>
<td>216</td>
<td>144</td>
<td>72</td>
<td>1</td>
</tr>
<tr>
<td>Distant metastasis* (case)</td>
<td>69</td>
<td>35</td>
<td>34</td>
<td>1.94</td>
</tr>
</tbody>
</table>

**NOTE:** Odds ratios (OR) and 95% confidence intervals (95% CI) were obtained from logistic regression models. Data were adjusted for age, pathologic stage, tumor grade, hormone receptor status, nodal status, K<sup>67</sup>, and HER2 status.

*Eleven patients had simultaneous loco-regional relapse or contralateral cancer.
**ATAD2 in Aggressive Tumors**

ATAD2 is highly expressed and amplified in human cancer. By searching published gene expression studies of primary human tumors, we found that ATAD2 is highly expressed in several types of tumors (25–29). Moreover, we found that ATAD2 is located in an area of the genome that is frequently amplified in human cancer (30). Based on this, we determined a broad range of primary human tumors by mRNA expression levels in a significant number of breast cancers. ATAD2 expression is located in an aggressive tumor and is associated with increased risk of distant recurrence in breast cancer.

When the same TMA was analyzed for ATAD2 expression by immunohistochemistry, we found that ATAD2 is highly expressed in 39% of 66 breast carcinomas analyzed (Table 1; Supplementary Fig. S3). Again, and consistent with the analysis of mRNA expression and amplification data, we found a significant correlation also between ATAD2 protein levels and DNA copy number (Supplementary Fig. S4; $P = 0.01$). Taken together, these results show that ATAD2 is amplified in a significant number of breast cancers and that there is high correlation between the copy number and the levels of expression.

ATAD2 levels are associated with increased risk of distant recurrence in breast cancer. To determine if ATAD2 expression levels are associated with patient outcome, we analyzed a collection of 349 breast carcinomas. ATAD2 was generally higher expressed in grade 3 and Ki67-positive tumors (Table 2). In univariate analysis, high ATAD2 levels (immunohistochemistry score ≥20) were found to correlate with a higher risk of distant recurrence (odds ratio, 1.94; 95% confidence interval, 1.12–3.37). After adjustment for other prognostic factors, ATAD2 expression levels maintained a significant independent predictive power (Table 2). Taken together, these results indicate that ATAD2 levels are associated with increased risk of distant recurrence in breast cancer patients.

ATAD2 is a direct target of the pRB/E2F pathway. To show that ATAD2 is a direct transcriptional target of E2F, total RNA was extracted from serum-starved human diploid fibroblasts (WI38) expressing the ER-E2F1 fusion protein (31). Activation of E2F1

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**Figure 1.** ATAD2 is an E2F target gene. A, quantitative PCR analysis of ATAD2 transcript levels. Left, WI38 fibroblasts expressing inducible ER-E2F1 were serum starved, and total RNA was extracted 4 h after the addition of 4-hydroxytamoxifen (OHT) and/or cycloheximide (CHX), as indicated. Right, total RNA was extracted from U2OS osteosarcoma cells expressing inducible ER-E2F1, ER-E2F2, or ER-E2F3 grown in the presence or absence of tamoxifen for 4 h. B, ATAD2 promoter. Arrow, putative transcriptional start site deduced from the most 5′ nucleotide of the longest expressed sequence tag identified in the National Center for Biotechnology Information database; black rectangle, ATG; circles, E2F binding site; filled gray circles, E2F binding sites conserved between human, mouse, and rat; white rectangles, position of PCR amplicons in chromatin immunoprecipitation experiments relative to ATG (A1, 1600; A2, 700; A3, 300; A4, +300; A5, +900). Bottom, different promoter constructs used in C, C, E2F1 activates the ATAD2 promoter. Luciferase reporter assay in U2OS ER-E2F1 cells transfected with different promoter constructs and grown for 0, 4, and 8 h in the presence or absence of tamoxifen. *, $P < 0.05$, ns, not significant (see Supplementary data for details). D, the E2Fs associate with the ATAD2 promoter. Chromatin immunoprecipitation analysis of HeLa S3 cells done with antibodies against E2F1, E2F2, and E2F3 or FLAG peptide as a control. The resulting DNA was analyzed by quantitative PCR with primers corresponding to the amplicons shown in B. Primers amplifying the acetylcholine receptor promoter were used as a negative control (ctr).
led to a 5-fold increase in ATAD2 mRNA levels independently of de novo protein synthesis (Fig. 1A, left), suggesting that ATAD2 is a direct target of the E2Fs. Similar results were obtained in human osteosarcoma cells U2OS (Fig. 1A, right). In addition, ATAD2 mRNA levels were induced by deletion of Rb-1 and by the expression of the E1A oncoprotein (Supplementary Fig. S5). These data strongly suggest that ATAD2 is a physiologic direct target of the pRB/E2F pathway.

We identified the putative ATAD2 promoter based on the 5’ end of the longest cDNA present in the National Center for Biotechnology Information database and the presence of a CpG island. The
ATAD2 mRNA and protein levels are significantly reduced on mi- 
croblotting (Supplementary Fig. S7) showed that endogenous E2fs are specifi- 
cally bound to the

Moreover, the promoter-containing construct, as endogenous

results show that the pRB-E2F pathway controls the expression of

ATAD2 binds histones through its bromodomain. A, ATAD2 is highly expressed in S phase. Immunofluorescence of SKBR3 cells labeled with 

Figure 3. ATAD2 associates with histones through its bromodomain. A. ATAD2 is highly expressed in S phase. Immunofluorescence of SKBR3 cells labeled with 15-min pulse of BrdUrd and probed with anti-ATAD2 and anti-BrdUrd antibodies. DAPI, 4',6-diamidino-2-phenylindole. B, SKBR3 cells were subjected to

ATAD2 binds directly to histone H3. Left, HeLa S3 cell lysates were

binding is increased on TSA treatment, and in agreement with this,

which is highly expressed in S-phase cells and present in the his-

ATAD2 binds to the histones, we ectopically expressed ATAD2 and

In the

ATAD2 promoter contains seven potential E2F DNA binding sites, spanning a region of 1.8 kb (Fig. 1B). The reporter construct contain- 
ing the −1497/−20 fragment of the ATAD2 promoter is activated by E2F1, and the two E2F sites in the −487/−20 region are required for E2F-dependent regulation of the ATAD2 promoter (Fig. 1C, Δ−472−380). In the minimal, the additional −487/−20 region encompassing these sites maintains the ability to respond to E2F (Fig. 1C).

Moreover, the promoter-containing construct, as endogenous ATAD2, is activated by serum addition to quiescent cells (Supplement- 
yary Fig. S6). Finally, chromatin immunoprecipitation experi- ments in HeLa S3 cells (Fig. 1D) and TIG3 human fibroblasts (Supplementary Fig. S7) showed that endogenous E2Fs are specifi- 
cally bound to the ATAD2 promoter at the E2F consensus sites

300 bp upstream of the putative start site. Taken together, these results show that the pRB-E2F pathway controls the expression of

Consistent with being downstream of the pRB/E2F pathway, 

proliferation. Whereas the inhibition of ATAD2 expression in expen- 
sionally growing cells did not lead to a detectable difference in proliferation rate or cell cycle profile of TIG3-T cells and U2OS cells (data not shown), it significantly reduced the ability of TIG3-T and U2OS cells to form colonies when plated at low density (Fig. 2C). In this setting, lack of cell-cell adhesion and integrin signaling leads to downregulation of G1 cyclins and delayed S-phase entry (32), which is functionally similar to the physiologic condition of quiescent 
cells. In agreement with this finding, inhibition of ATAD2 expres- 
sion significantly reduced the number of quiescent TIG3 cells reen- 
tering the S phase after serum stimulation (Fig. 2D). Taken together, these results show that ATAD2 is required for quiescent cells to enter the cell cycle and for the proliferation of cells grown at low density.

ATAD2 binds histones through its bromodomain. In the breast carcinoma cell line SKBR3, where ATAD2 is predicted to be overexpressed and amplified (21), ATAD2 is a nuclear protein, which is highly expressed in S-phase cells and present in the histone and nuclear matrix fractions (Fig. 3A and B). To investigate if ATAD2 binds to the histones, we ectopically expressed ATAD2 and 

Inhibition of ATAD2 expression affects the growth of normal and cancer cells. Because ATAD2 is highly expressed in primary tumors and several E2F target genes are required for normal proliferation, we tested if downregulation of ATAD2 affects

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Figure 4. ATAD2 binds MYC and MYC-associated proteins. A, ATAD2 interacts \textit{in vivo} with MYC. Left, Phoenix cells transfected with expression plasmids for FLAG-ATAD2, HA-MYC, or HA-TIP60 were subjected to immunoprecipitation with FLAG antibody followed by Western blotting with anti-FLAG or anti-HA antibodies (IN, input). Middle, endogenous ATAD2 binds MYC. HeLa S3 cell lysates were immunoprecipitated with antibody to ATAD2 and to HA peptide as a control. Immunoprecipitated material was analyzed by Western blotting with the indicated antibodies (*, immunoglobulin chains). Right, the NH$_2$ terminus of ATAD2 is required for binding to MYC. Lysates from Phoenix cells transfected with expression plasmid for HA-MYC in the presence or absence of either full-length or Δ$^{337}$ FLAG-ATAD2 deletion mutant.

B, ATAD2 binds to MYC target genes. Chromatin immunoprecipitation of P493-6 cells using antibodies against ATAD2 and MYC. HA was used as a control. The enrichment of the specific sequences was analyzed by quantitative PCR with primers surrounding the E-BOX region of the indicated MYC target gene promoters (NUC, nucleolin; GPAT, glycerol-3-phosphate acetyltransferase; CCND2, cyclin D2). Primers amplifying acetylcholine receptor promoter (ctr) were used as negative control.

C, left, ATAD2 is limiting for MYC-dependent transcription. U2OS MYC-ER cells were infected with a lentiviral vector encoding shRNA to ATAD2 (shATAD2) or to luciferase as a control (ctr). RNA was extracted from cells grown in the presence (+) or absence (−) of tamoxifen for 8 h, and the levels of the indicated target genes were assessed by quantitative PCR (UBC, ubiquitin). Right, ATAD2 stimulates MYC-dependent transcription. WI38 MYC-ER fibroblasts were transfected with FLAG-ATAD2. After puromycin selection, cells were grown in the presence (+) or absence (−) of tamoxifen for 16 h. RNA was extracted and the levels of the indicated target genes were assessed by quantitative PCR.

D, a model for the role of ATAD2 in cancer. See Discussion for details.
I1074A). As expected, the ability of the Y1063A/I1074A mutant to bind to histone H3 is impaired both in TSA-treated and in untreated samples (Fig. 3C, left, lanes 9 and 12). The residual binding may be caused either by regions of ATAD2 outside the bromodomain or by the ATAD2 mutant forming a dimer with endogenous wild-type ATAD2 (Supplementary Fig. S8).

Immunoprecipitation analysis using an antibody specific for ATAD2 on HeLa S3 cells lysates showed that endogenous ATAD2 coprecipitates with histone H3, which is also acetylated (Fig. 3D, left). In addition, a Far Western blot using purified GST-ATAD2 showed that ATAD2 binds specifically to histone H3 (Fig. 3D, middle). Significantly, when bacterial purified tailless histone H3 was used, ATAD2 binding was completely abrogated (Fig. 3D, right). Taken together, these data show that ATAD2 preferentially binds to the tail of histone H3, and suggest that the in vivo interaction depends on an intact bromodomain.

ATAD2 binds to MYC and the TRRAP complex in vivo. Because ATAD2 does not contain a predicted DNA binding domain, we speculated that it would be recruited to chromatin by sequence-specific transcription factors. Interestingly, the MYC oncogene is mapped on chromosome 8q24, 4.3 Mb telomeric to ATAD2, and they are often found co-amplified in human cancers (27, 34, 35). Thus, we tested if ATAD2 and MYC can form a complex, and as shown in Fig. 4A and Supplementary Fig. S8, both ectopically expressed ATAD2 and endogenous ATAD2 interact with MYC in vivo dependent on the NH2 terminus of ATAD2.

MYC is present in distinct macromolecular complexes interacting via the core subunit TRRAP and the acetyltransferases GCN5 or TIP60 (11). As shown in Fig. 4A and Supplementary Fig. S8, ATAD2 can associate with TIP60, TRRAP, GCN5, and the MYC partner MAX. These results suggest that ATAD2 can be part of a transcriptionally active MYC complex.

ATAD2 binds to MYC target genes and cooperates with MYC in transcriptional activation. Next, we performed experiments to test the importance of the interaction between ATAD2 and MYC. First, we showed that ATAD2 is bound to the E-box region of several different MYC target genes (Fig. 4B). Second, we showed that ATAD2 is required for efficient activation of a number of MYC target genes (Fig. 4C; Supplementary Fig. S9). Third, we showed that the expression of ATAD2 can cooperate with MYC in inducing transcription of several MYC target genes (Fig. 4C; Supplementary Fig. S9). Interestingly, transcription can be also induced by ATAD2 expression alone, further suggesting that endogenous ATAD2 levels are limiting for MYC-dependent transcription. In summary, these data show that ATAD2 is required for the efficient transcriptional activation of a subset of MYC target genes, and importantly, that ATAD2 can cooperate with MYC in activating transcription.

Discussion

Deregulation of the pRB-E2F pathway is one of the hallmarks of human tumors and an obligatory event for the development of human cancer (1). Significantly, in addition to being essential for normal proliferation, a small subset of E2F-regulated genes also contribute to the development of human tumors and have been classified as bona fide oncogenes.

We have shown that ATAD2 is expressed at high levels in a significant proportion of human tumors, but not in benign lesions or normal tissues. Furthermore, gene expression arrays have shown that ATAD2 is highly expressed in several different types of human tumors (25–29, 36). Strikingly, we have shown that high levels of ATAD2 correlate significantly with higher risk of distant metastasis and grade 3 tumor in breast cancer patients.

The high levels of ATAD2 observed in many tumor types are likely acquired through deregulation of the pRB pathway and/or through specific amplification of the ATAD2 locus. Importantly, the copy number of ATAD2 and the levels of expression correlate, strongly suggesting that genomic amplification results in high levels of ATAD2. Recent reports have also shown that the genomic region containing ATAD2 is amplified in hepatocellular carcinoma (34) and breast carcinoma (30, 35, 37). Cytogenetic amplifications occurring at high frequency suggest that genes mapped to these regions are positively involved in cancer progression and are selectively driving the amplification. Interestingly, ATAD2 is mapped to chromosome 8q24.13, a genomic region frequently amplified in multiple cancer types, only 4.3 Mb centromeric to the MYC oncogene that contributes to the genesis of different human tumors and is highly expressed in aggressive and poorly differentiated human cancers (4). Even if the 8q amplification is therefore normally ascribed to MYC, recent results suggest that this is not always the case. For instance, although MYC is often amplified in oral cancers (38), there are several examples of oral cancers carrying amplification of 8q21–24 that does not lead to amplification of MYC (39). Moreover, the genomic region containing MYC is frequently amplified in hepatocellular carcinomas with MYC expression remaining unchanged, whereas ATAD2 gene is highly expressed and correlates with amplification (34). This suggests that ATAD2 could be the driving oncogene. In addition, two reports indicate that ATAD2 and MYC are both selectively overexpressed and amplified during the transition from a preinvasive to an invasive and malignant stage of breast carcinomas (27, 35).

ATAD2 function: the MYC connection. ATAD2 contains two conserved domains: a double AAA+ class ATPase domain and a bromodomain. AAA ATPase domains are found in proteins involved in a variety of cellular activities based on the formation and activation of protein-DNA complex, such as DNA replication (CDC6, MCM, and RFC), chromatin remodeling and transcription (TIP48 and TIP49), and DNA damage (VCP; ref. 21). The bromodomain contributes to the high-affinity recognition of acetylated lysines (22), which are often present in histone tails, and is frequently found in chromatin associated proteins with a role in ATP-dependent chromatin remodeling and histone acetylation (40). We have shown that ATAD2 is a nuclear protein associated with both chromatin and the nuclear matrix. In addition, we have shown that ATAD2 is found in a complex with histone H3 and that it associates preferentially with the histone H3 tail, and that the integrity of the bromodomain affects its ability to interact with acetylated histones in vitro. However, thus far, we have been unable to conclusively show that ATAD2 has chromatin remodeling activity in vitro, and the exact mechanism by which ATAD2 contributes to transcriptional activation therefore still remains to be determined.

The finding that ATAD2 is expressed at high levels in aggressive tumors, localizes on the same chromosome arm as MYC, and is co-amplified with MYC in several tumors led us to test whether ATAD2 binds to MYC. Intriguingly, we found that ATAD2 is in a complex with MYC, and consistent with a role in regulating MYC-dependent transcription, ATAD2 binds to MYC target genes. Furthermore, we have shown that ATAD2 cooperates with MYC in activating transcription and that it is also limiting for the ability of MYC to transactivate genes.
Based on these results, we make a proposal for the role of ATAD2 in normal proliferation and tumorigenesis (Fig. 4D). In normal proliferating cells, ATAD2 levels are tightly controlled by mitotic factors through the pRB pathway. ATAD2 is a cofactor for MYC, and it contributes significantly to the transcription of these genes, possibly by changing the chromatin accessibility at MYC target loci. Consistent with this, ATAD2 is required for the entry into the S phase of the cell cycle. During tumorigenesis, however, ATAD2 levels are increased as a result of specific alterations in core members of the pRB pathway or amplification of the ATAD2 locus, thereby contributing to MYC-dependent transcriptional activity and tumor development. Importantly, the association of ATAD2 with other oncogenic transcription factors, such the androgen and estrogen receptors (23, 24), may also contribute to the oncogenic activity of ATAD2.

In this work, we have also shown a significant correlation between ATAD2 expression and poor clinical outcome. Future work will be aimed at establishing the prognostic value of ATAD2 in breast cancer and validate it as a novel therapeutic target for anticancer therapy.

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

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