Evidence of a Role for Antizyme and Antizyme Inhibitor as Regulators of Human Cancer

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Abstract

Antizyme and its endogenous antizyme inhibitor have recently emerged as prominent regulators of cell growth, transformation, centrosome duplication, and tumorigenesis. Antizyme was originally isolated as a negative modulator of the enzyme ornithine decarboxylase (ODC), an essential component of the polyamine biosynthetic pathway. Antizyme binds ODC and facilitates proteasomal ODC degradation. Antizyme also facilitates degradation of a set of cell cycle regulatory proteins, including cyclin D1, Smad1, and Aurora A kinase, as well as Mps1, a protein that regulates centrosome duplication. Antizyme has been reported to function as a tumor suppressor and to negatively regulate tumor cell proliferation and transformation. Antizyme inhibitor binds to antizyme and suppresses its known functions, leading to increased polyamine synthesis, increased cell proliferation, and increased transformation and tumorigenesis. Gene array studies show antizyme inhibitor to be amplified in cancers of the ovary, breast, and prostate. In this review, we summarize the current literature on the role of antizyme and antizyme inhibitor in cancer, discuss how the ratio of antizyme to antizyme inhibitor can influence tumor growth, and suggest strategies to target this axis for tumor prevention and treatment.

Introduction

Regulation of the polyamine pathway by antizyme inhibitor and antizyme

Antizyme inhibitor and antizyme are critically important for maintaining polyamine homeostasis within the cell. Polyamines are multivalent, organic cations, and adequate polyamine levels are necessary for optimal cell growth. Within the cell, polyamines function in diverse processes, including regulating chromatin condensation, stabilizing the double helical structure of DNA, regulating cell differentiation, and regulating translation through a unique posttranslational modification known as hypusination (1). Polyamines are produced from the amino acid ornithine in a rate-limiting reaction catalyzed by the enzyme ornithine decarboxylase (ODC), as shown in Fig. 1. ODC is only functional as a homodimeric complex, and individual ODC monomers have no enzymatic activity (2). ODC monomers bind to each other with relatively low affinity, and under physiologic conditions, ODC dimers are in rapid equilibrium with inactive ODC monomers.

ODC is negatively regulated by antizyme. High intracellular polyamine levels induce a +1-frameshift that bypasses a stop codon and results in production of full-length antizyme protein. Depending on the start codon used, full-length antizyme protein can be either 29.5 kDa or 24 kDa in size. Antizyme decreases polyamine levels through 3 mechanisms, as summarized in Fig. 1. First, antizyme disrupts active ODC dimers, resulting in decreased polyamine synthesis. Second, antizyme targets ODC for degradation by the 26S proteasome in a reaction that does not require ubiquitin. ODC is already one of the most rapidly degraded proteins in mammalian cells, with a half-life of only 1 to 2 hours in the absence of antizyme. When antizyme is present, however, this half-life decreases to only a few minutes (3). Third, antizyme inhibits polyamine uptake from the microenvironment through a mechanism that has yet to be completely characterized. Thus, an increase in antizyme protein is associated with lower levels of ODC, decreased polyamine synthesis, and reduced rates of cell growth.

Antizyme activity is further regulated by the protein antizyme inhibitor, which is highly homologous to ODC, retaining 47.4% identity and 63.5% similarity at the amino acid level between human antizyme inhibitor and human ODC. Although antizyme inhibitor was originally thought to be a derivative of ODC (4), subsequent cloning and sequencing showed that antizyme inhibitor is a distinct protein (5, 6). Antizyme inhibitor acts as a positive regulator of the polyamine pathway by binding to antizyme and preventing antizyme-mediated ODC degradation (Fig. 1).

High levels of antizyme inhibitor correlate with increased ODC protein, increased polyamine synthesis, and greater cell proliferation. Whether antizyme inhibitor functions as a monomer or as a dimer has been examined in some detail. Of the 8 residues involved in dimer formation in ODC, 7 (87.5%) are also conserved in the antizyme inhibitor sequence, including a critical glycine residue (G387) necessary for ODC dimerization (5, 7). A comparison of the...
structure and conserved amino acids in antizyme inhibitor and ODC is shown in Fig. 2. Although early studies reported that antizyme inhibitor could form homodimers that lack ODC enzymatic activity (4, 8), more recent evidence from size exclusion chromatography and crystallography indicates that antizyme inhibitor functions as a monomer (9).

Isoforms of antizyme and antizyme inhibitor

The most predominant antizyme protein is antizyme-1, although there are multiple antizyme genes and at least 4 members of the antizyme family. All antizyme proteins inhibit ODC activity (10). Antizyme-2, like antizyme-1, has a wide tissue distribution in vertebrates, though it is expressed at much lower levels than antizyme-1 in most tissues. Antizyme-2 is more conserved evolutionarily than antizyme-1, suggesting it may have an important physiologic role.

Although antizyme-2 is structurally similar to antizyme-1, antizyme-2 does not promote proteasomal degradation of ODC in vitro, even though it does inhibit both ODC activity and polyamine uptake. In vivo, however, antizyme-2 promotes ODC degradation and inhibits both ODC activity and polyamine uptake (11). The difference in antizyme activity in vitro was subsequently mapped to 2 Asp amino acids in antizyme-2, replacing Arg^{131} and Ala^{135} in antizyme-1 (12). The physiologic role of antizyme-2 in facilitating protein degradation in vivo is not yet well understood, although it can promote ODC degradation in human embryonic kidney cells (13).

Expression of antizyme-3 is testis specific and is restricted to a late stage in sperm production. This highly restricted expression suggests that antizyme-3 is necessary to abruptly alter polyamine levels during sperm morphogenesis (3, 14). This idea is supported by reports that animals overexpressing ODC in the testes have defects in spermatogenesis, perhaps because the high level of ODC overwhelms the levels of antizyme-3 (15). Antizyme-3, like antizyme-2, has the ability to inhibit both ODC activity and polyamine uptake, but it does not target ODC for degradation (13). By yeast two-hybrid screen, antizyme-3 was found to interact with gametogenetin protein-1 (GGN-1), a germ cell-specific protein. All antizyme proteins inhibit ODC activity (10). Antizyme-2, like antizyme-1, has a wide tissue distribution in vertebrates, though it is expressed at much lower levels than antizyme-1 in most tissues. Antizyme-2 is more conserved evolutionarily than antizyme-1, suggesting it may have an important physiologic role.

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protein, although the functional consequences of this interaction are not known (16).

A putative fourth member of the antizyme family (antizyme-4) was originally isolated from a human brain cDNA library, but it has not been well characterized. Yeast two-hybrid assays showed that antizyme-4 could also bind to ODC and inhibit ODC enzymatic activity (17). The ability of antizyme-4 to promote ODC degradation or inhibit polyamine uptake has not been examined yet.

There are not only multiple isoforms of antizyme that contribute to the complex regulation of the polyamine pathway but also multiple isoforms of antizyme inhibitor. The most predominant antizyme inhibitor is antizyme inhibitor-1 (AZIN-1), which is ubiquitously expressed at high levels and has been the most studied. Antizyme inhibitor-2 (AZIN-2) was first identified in 2001 as an ODC paralog and termed ODCp or ODC-like (18). Subsequent studies established that ODCp lacked enzymatic activity and seemed to function as a tissue-specific antizyme inhibitor in the brain and testes, where it is expressed at 6-fold or 23-fold greater levels than antizyme inhibitor-1, respectively (19, 20). Human antizyme inhibitor-2 retains 45% identity and 66% similarity to antizyme inhibitor-1 at the amino acid level (21) and has been shown to interact with all 3 characterized antizymes (19, 22, 23). Similar to antizyme inhibitor-1, overexpression of antizyme inhibitor-2 has been shown to increase growth of NIH3T3 cells (23). In the future, it will be interesting to determine whether this growth advantage is mediated primarily through the polyamine pathway and dependent on an intact antizyme-binding domain in antizyme inhibitor-2. To date, the majority of studies on the role of antizyme inhibitor in tumors have been conducted on antizyme-1 and antizyme inhibitor-1. For the remainder of this article, antizyme refers to antizyme-1 and antizyme inhibitor refers to antizyme inhibitor-1.

Polymamines and cancer

Because of its key role in promoting cell proliferation, ODC is considered a potential oncogene. ODC is downstream of Myc and is one of the most rapidly induced genes upon growth stimulation (24). Elevated levels of ODC and polymamines have been associated with numerous types of neoplastic transformation, and ODC overexpression alone can induce cell transformation and in vivo tumor growth in NIH3T3 cells following subcutaneous implantation in nude mice (25). ODC activity is induced by a wide range of chemical, environmental, and genetic cancer risk factors, including ultraviolet light, asbestos, and exposure to chemical agents that induce skin carcinogenesis (26). ODC levels are also upregulated in intraepithelial neoplasias, the non-invasive precursors of epithelial cancers. ODC expression is regulated by androgens in the prostate gland, and ODC levels are highly elevated in human prostate cancer (27) and numerous other cancer types (28–30). Furthermore, a meta-analysis of multiple microarray datasets established that the polyamine pathway is often highly altered in human prostate cancer (31).

Although high levels of ODC and polymamines are associated with numerous types of neoplastic transformation, the role of antizyme and antizyme inhibitor in this process is just emerging. It is clear from in vitro studies that increasing the ratio of antizyme inhibitor relative to antizyme within the cell favors growth activation, whereas a decrease in the antizyme inhibitor/antizyme ratio favors growth repression. The pathways directly upstream of antizyme inhibitor expression are largely unknown, and the antizyme inhibitor promoter has not yet been studied in detail. Treatment with mitogens or growth factors leads to a very rapid increase in antizyme inhibitor, prior even to the increase observed in ODC, an immediate early gene (6). The antizyme inhibitor gene has been localized to chromosome 8q22.3 in human cells, a region previously recognized as a frequently amplified hotspot associated with decreased overall survival and decreased time to distant metastases in primary breast tumors (32). Antizyme inhibitor has been shown to be highly expressed in gastric cancer (33), as well as in tumors of the lung, liver, and ovary (33–35). In NIH3T3 cells, overexpression of antizyme inhibitor alone was sufficient to transform cells and promote tumor growth in vivo (36). Using siRNA to suppress antizyme inhibitor levels in A549 cells led to decreased cell proliferation and decreased polyamine levels; however, the ability of these cells to form tumors in vivo was not determined (37). These results suggest that decreasing the ratio of antizyme inhibitor to antizyme within the cell could have a significant inhibitory effect on tumor growth.

Evidence from transgenic mice suggests that elevated polyamine levels are associated with tumor development

Numerous transgenic mouse models are available to study the polyamine pathway, and they were summarized in a recent review (38). Several different mouse models with elevated ODC expression have been described. Transgenic mice overexpressing a C-terminally truncated ODC from the bovine keratin 5 (K5) and keratin 6 (K6) promoters have elevated ODC activity, as well as elevated levels of the polymamines putrescine and spermidine (39). These mice developed hair loss, formed dermal follicular cysts, and older mice frequently developed spontaneous squamous neoplasms. These experiments were done, however, on founder and F1 mice with a mixed genetic background, and subsequent breeding to either a B6 or FVB background did not result in spontaneous tumor formation (40). K6/ODC mice do develop tumors following treatment with carcinogens, exposure to UV radiation, or by breeding to transgenic mice that carry another oncogene. K5/ODC and K6/ODC mice were much more susceptible to tumor formation following a single treatment with the carcinogen 7,12 dimethylbenz(a)anthracene (DMBA), and treatment with the tumor promoter phorbol 12-myristate 13-acetate (PMA) was not required (40). Subsequent treatment of existing tumors in the K6/ODC mice with the irreversible ODC inhibitor alpha-difluoromethylornithine (DFMO) led to rapid tumor regression.
Tumor initiation in K6/ODC mice was not limited to DMBA treatment, suggesting that these mice may be a useful model to identify potential human carcinogenic compounds (42). Tumor development in K6/ODC mice was not limited to treatment with chemical carcinogens, because crossing K6/ODC mice with mice overexpressing v-Ha-ras led to development of spontaneous squamous cell skin tumors. Consistent with results from previous models, these tumors also regressed upon ODC inhibition with DFMO (43, 44). These studies show that ODC overexpression is sufficient to increase tumor development in multiple models. Furthermore, the ability of DFMO to reverse tumor growth suggests that interfering with polyamine homeostasis may prove to be an effective therapeutic strategy for tumor treatment. This theory is supported to some extent by results from clinical trials in which DFMO has been used in cancer therapy (27, 45).

The ability of antizyme to influence tumor growth in vivo has also been examined in some detail. Antizyme has been shown to have tumor-suppressive activity in numerous mouse models. In one model, antizyme expression was targeted to the skin using either the bovine K5 or K6 promoter (46). Transgenic K6/antizyme and K5/antizyme mice developed normally but showed delayed tumor onset and decreased tumor numbers relative to wild-type controls after exposure to the chemical carcinogen DMBA and the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA; ref. 46). Expression of the K5 and K6 promoters can sometimes occur in other epithelial cells, and K6/antizyme and K5/antizyme mice were also shown to have a decreased incidence of forestomach tumors induced by N-nitrosomethylbenzylamine (NMBA) (47).

To date, only a few studies have addressed the role of antizyme inhibitor in vivo, and many of these reports have focused on expression of antizyme inhibitor during normal development (48). Attempts to make antizyme inhibitor knockout mice established that homozygous antizyme inhibitor deletion results in neonatal lethality (49), and antizyme inhibitor+/− embryos are currently characterized to better understand normal antizyme inhibitor function (50). Antizyme inhibitor+/− heterozygote mice are fertile and have a normal lifespan; they have no morphologic abnormalities, although they do display lower ODC protein levels in the liver (49). One critical test of whether antizyme inhibitor functions as an oncogene in vivo will be to determine whether transgenic mice with tissue-specific antizyme inhibitor overexpression have an increased rate of tumor incidence, with or without prior carcinogen treatment. For direct comparison to previous results from ODC transgenic animals, such mice could be generated using either the K5 or K6 promoters to explore the role of antizyme inhibitor in skin carcinogenesis. It is likely that, similar to K6/ODC mice, K6/antizyme inhibitor mice would display elevated levels of ODC and polyamines, and they may develop skin tumors following treatment with carcinogenic compounds. Furthermore, it will be beneficial to determine whether treatment with DFMO would reverse tumor growth in K6/antizyme inhibitor mice.

Figure 3. Comparison of antizyme inhibitor and antizyme copy number in normal versus cancer tissue. A, changes in antizyme inhibitor and, B, antizyme copy number were determined by meta-analysis of Oncomine data. For antizyme inhibitor, samples analyzed include the following: 1, normal; 2, acute myeloid leukemia; 3, breast adenocarcinoma; 4, breast carcinoma; 5, ductal breast carcinoma; and 6, prostate cancer. For antizyme, the following tissue types were analyzed: 1, normal; 2, breast adenocarcinoma; 3, breast carcinoma; 4, deranged liposarcoma; 5, ductal breast carcinoma; 6, non-small cell lung carcinoma; 7, ovarian carcinoma; and 8, prostate carcinoma.

Generation of conditional mouse models for genes in the polyamine pathway would contribute to our understanding of how the balance among antizyme inhibitor, antizyme, and ODC influences tumor growth in vivo.

Expression of antizyme inhibitor and antizyme in human cancer

Genomic studies have previously determined that antizyme inhibitor expression is upregulated in human gastric cancer, ovarian cancer, breast cancer, and prostate cancer (32–35). Current data from the Oncomine database also suggest that antizyme inhibitor copy number is elevated in cancers of the bone marrow, breast, and prostate (Fig. 3A; ref. 51). Furthermore, studies collected in the Oncomine database suggest that antizyme inhibitor expression is elevated in cancers of the breast (ductal invasive breast carcinoma), testis (seminoma), liver (hepatocellular carcinoma), lung (adenocarcinoma and squamous cell carcinoma), skin (melanoma), and prostate (carcinoma). Studies of antizyme inhibitor expression in cancers of the brain (oligodendroglioma) and bladder (superficial bladder cancer) were inconclusive, with some arrays showing moderately increased expression and others showing moderately decreased expression. Because antizyme inhibitor is known to promote cell proliferation, increased antizyme inhibitor levels may directly enhance tumorigenesis.

Antizyme has been shown to function as a tumor suppressor in vivo, and it is possible that decreased antizyme levels will also contribute to tumor development. A meta-analysis of current data in Oncomine suggests that antizyme copy number is slightly decreased in several types of breast tumors compared with normal tissue (Fig. 3B). Antizyme copy number may also be decreased in tumors of the lung, ovary,
Antizyme and Antizyme Inhibitor in Cancer

Table 1. SAGE analysis of antizyme inhibitor and antizyme expression in normal versus cancer tissue

<table>
<thead>
<tr>
<th>Gene</th>
<th>Tissue</th>
<th>Normal</th>
<th>Cancer</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antizyme inhibitor</td>
<td>Prostate</td>
<td>4.67 ± 2.89</td>
<td>16 ± 7.55</td>
<td>3.43</td>
</tr>
<tr>
<td></td>
<td>Brain (ependymoma)</td>
<td>4.33 ± 2.89</td>
<td>14.20 ± 4.80</td>
<td>3.28</td>
</tr>
<tr>
<td></td>
<td>Breast</td>
<td>2.82 ± 2.38</td>
<td>7.05 ± 4.83</td>
<td>2.50</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>3.00 ± ND</td>
<td>7.33 ± 4.51</td>
<td>2.44</td>
</tr>
<tr>
<td></td>
<td>Brain (meningioma)</td>
<td>4.33 ± 2.89</td>
<td>7.50 ± 4.95</td>
<td>1.73</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>4.00 ± ND</td>
<td>6.67 ± 3.79</td>
<td>1.67</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>6.00 ± ND</td>
<td>7.75 ± 3.10</td>
<td>1.29</td>
</tr>
<tr>
<td></td>
<td>Brain (glioblastoma)</td>
<td>4.33 ± 2.89</td>
<td>5.58 ± 3.63</td>
<td>1.29</td>
</tr>
<tr>
<td>Antizyme</td>
<td>Bone Marrow</td>
<td>263.67 ± 32.59</td>
<td>12.33 ± 14.43</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>107.00 ± 5.66</td>
<td>42.00 ± ND</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>254.00 ± ND</td>
<td>140.67 ± 24.42</td>
<td>0.55</td>
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<tr>
<td></td>
<td>Prostate</td>
<td>187.67 ± 45.79</td>
<td>116.50 ± 65.76</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
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<td>133 ± 15.87</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>Brain (oligodendroglioma)</td>
<td>103.00 ± 56.95</td>
<td>78.00 ± 41.01</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>Brain (ependymoma)</td>
<td>103.00 ± 56.95</td>
<td>89.64 ± 30.66</td>
<td>0.87</td>
</tr>
</tbody>
</table>

NOTE: SAGE Genie Digital Northern analysis from the CGAP (52) was used to assess antizyme inhibitor and antizyme mRNA expression levels in normal versus cancerous tissues from a variety of organs. The unique sequence TCTTTCACCC from the 3’ untranslated region of antizyme inhibitor mRNA was used for analysis, and the sequence TTGTAATCGT was used for antizyme. The number of times the gene-specific sequence was detected out of every 200,000 tags was determined and then compared with the observed level in normal tissue. Values are given as average ± SD.

Abbreviation: ND, not determined.

and prostate, although there is significantly more variation in these datasets.

Expression of antizyme inhibitor and antizyme in various cancers can also be explored using SAGE Genie Digital Northern analysis provided through the National Cancer Institute’s Cancer Genome Anatomy Project (CGAP; ref. 52). As summarized in Table 1, antizyme inhibitor levels were substantially elevated in cancers of the prostate, brain (ependymoma), breast, and liver. Moderate increases were seen in cancers of the lung and pancreas. Antizyme inhibitor levels also may be increased in tissues from the stomach and kidney, although data from normal tissues were not available for comparison. Although antizyme expression is regulated primarily at the level of translation, decreased antizyme mRNA transcription may result in decreased levels of antizyme protein and, consequently, increased tumorigenesis. As shown in Table 1, antizyme transcript levels seemed to be decreased in tumors of the bone marrow, muscle, and lung, and moderately decreased in tumors of the liver and prostate.

Although gene expression data imply changes in the antizyme inhibitor/antizyme ratio in many human cancers, these data cannot be directly applied to protein levels, especially for antizyme, which is subject to posttranscriptional regulation. Confirmation of these results by immunohistochemical staining in tumor tissue microarrays would not only validate the electronic gene expression analysis but also allow us to better understand whether misregulation of the antizyme/antizyme inhibitor pathway is a common feature of numerous tumor types or is more specific to certain cancers.

Functions of antizyme and antizyme inhibitor outside of the polyamine pathway

Intracellularly, the ratio of antizyme inhibitor to antizyme is critically important for determining whether conditions favor cell growth repression (high antizyme) or growth activation (high antizyme inhibitor). Thus far, we have primarily focused on how changes in the level of antizyme inhibitor or antizyme affect ODC and polyamine levels; however, both antizyme and antizyme inhibitor have been shown to have additional functions outside of the polyamine pathway, which are discussed in detail below. This finding is particularly intriguing, because the therapeutic potential of targeting antizyme and antizyme inhibitor may be influenced directly by these additional functions.

Antizyme-mediated degradation of cyclin D1 and other proteins

The realization that antizyme has roles other than regulating polyamine levels came with the observation that antizyme can degrade proteins other than ODC. The first demonstration of this degradation was the finding that antizyme can degrade cyclin D1 in an ubiquitin-independent reaction, although cyclin D1 can also be degraded through the ubiquitin-mediated pathway. Antizyme was found not only to immunoprecipitate with cyclin D1 but also to mediate cyclin D1 degradation in an in vitro system using purified proteasomes (53). Because cyclin D1 activity is required for cell cycle passage, this effect provides a novel polyamine-independent mechanism for the growth repression observed in numerous cell lines upon antizyme
overexpression. Since this initial report, antizyme has been found to regulate degradation of numerous other proteins involved in cell cycle regulation, including Smad 1 (54), Aurora A (55), and Mps1 (56, 57), a protein that regulates centrosome duplication. The effect of antizyme and antizyme inhibitor on centrosomes is discussed in detail in the following section. It is likely that additional targets of antizyme-mediated degradation remain to be identified. It is also possible that antizyme binds to ODC with higher affinity than it does to cyclin D1 or other cell cycle mediators. Precise measurement of the binding constants between antizyme and its interacting proteins, for example, by surface plasmon resonance, would greatly enhance our understanding of the relative importance of these interactions within the cell. Furthermore, under conditions in which antizyme levels are elevated, degradation of proteins other than ODC may exert a greater cellular effect.

**Antizyme and antizyme inhibitor function at centrosomes**

In addition to cytoplasmic and nuclear sites, antizyme and antizyme inhibitor localize, in part, to centrosomes (58). These results suggest that centrosomal antizyme and antizyme inhibitor may have an important role in regulating normal centriole duplication. This role was confirmed by studies in which antizyme overexpression caused a decrease in the number of cells with excess centrosomes in a variety of cell types, whereas antizyme inhibitor overexpression stimulated excess centrosome duplication (58). Centrosome amplification is a common feature of numerous solid tumors and seems to be an early event in tumorigenesis. These results suggest that centrosome duplication may be mediated in part by antizyme. This effect provides a novel mechanism whereby changes in the antizyme inhibitor/antizyme axis affect additional compounds of tumor behavior. Thus far, antizyme has been reported to be involved in degradation of Aurora A and Mps1, 2 kinases that promote centrosome duplication. It is likely that antizyme and antizyme inhibitor are involved in directly regulating ubiquitin-independent degradation of multiple proteins that regulate centrosome duplication and that this takes place directly at the centrosome. It is possible that altered polyamine levels affect centrosome duplication, but this has not yet been shown.

**Antizyme and DNA methylation**

In addition to direct effects of antizyme on cell cycle progression, antizyme has also been suggested to act as a tumor suppressor by altering DNA methylation. Ectopic expression of antizyme in the hamster oral keratinocyte cell line HCPC-1 led to a reduction in ODCl activity and demethylation of 5-methyl cytosines at CGCG sites (59). This intriguing result suggests that antizyme overexpression could lead to reactivation of cellular genes that had been previously silenced by hypermethylation during cancer development. Later mechanistic studies showed that antizyme overexpression was directly associated with hypomethylation of CpG islands in genomic DNA and histone H3 lysine 9 dimethylation (H3K9me2; ref. 60).

Furthermore, the protein levels of both DNA methyltransferase 3B and the histone H3K9me-specific methyltransferase G9a were decreased upon antizyme expression, providing additional evidence for a direct link between changes in antizyme levels and methylation status. Whether the observed effect of antizyme on gene methylation could be exploited for the therapeutic reactivation of silenced genes in tumors remains to be determined, but it has intriguing implications for future cancer therapy.

**Nonpolyamine functions of antizyme inhibitor**

Antizyme inhibitor has also been shown to have functions beyond antizyme binding and regulating antizyme functions. When the antizyme-binding region of antizyme inhibitor was deleted, overexpression of the mutant protein (AZIAZ) was still able to enhance cell proliferation, although not to the same extent as overexpression of wild-type antizyme inhibitor (61). This finding suggests that antizyme inhibitor has antizyme-independent functions, perhaps mediated by preventing degradation of cyclin D1 or other cell cycle regulators. Antizyme inhibitor also partially localizes to the centrosome in a variety of mammalian cells as described above, and overexpression of antizyme inhibitor can induce centriole amplification. Whether antizyme inhibitor directly promotes centrosome duplication by interfering with antizyme-mediated functions or through some other mechanism has yet to be fully explored.

**Can altering the antizyme inhibitor–to–antizyme balance promote tumor growth?**

From the experiments described above, it is clear that alteration of the antizyme inhibitor/antizyme ratio within the cell could have a direct effect not only on rates of cell proliferation but also on centrosome duplication and gene methylation. Alterations in the antizyme inhibitor/antizyme ratio can also affect the uptake of extracellular polyamines. Because antizyme inhibitor inhibits antizyme, increased antizyme inhibitor expression would be expected to result in increased ODC activity and increased polyamine uptake. These effects are expected to be somewhat transitory, however, because increased polyamine uptake will induce expression of antizyme, which will inhibit polyamine transport. The ratio of antizyme/antizyme inhibitor within the cell will determine the precise effect on polyamine transport following an increase in the antizyme inhibitor level (62). Numerous experiments thus far have shown that constitutive overexpression of antizyme inhibitor results in increased polyamine uptake and increased accumulation of polyamine analogs (36, 63). This increase may mimic the situations in some human cancers. Measurement of polyamine transport and accumulation of polyamine analogs following inducible expression of antizyme or antizyme inhibitor would clarify the short- and long-term effects of these proteins on polyamine transport.

The results described in this review argue that changes in cell proliferation following an increase in the antizyme inhibitor level would lead to cell transformation and increased tumor growth, via decreased antizyme-mediated...
Could we target antizyme inhibitor for cancer therapy?

On the basis of its critical role in regulating cell growth and transformation, there has been long-term interest in targeting ODC as a tumor treatment, and several ODC inhibitors are now in clinical trials (45, 66–68). Despite dramatic effects in cell culture systems, early monotherapy trials with DFMO were largely unsuccessful, perhaps because of the ability of cells to enhance polyamine uptake to bypass the effect of DFMO treatment. It is possible that future combinations of DFMO with either polyamine transport inhibitors or cytotoxic drugs would provide more effective therapeutic treatment for many cancer types.

Considerable recent progress has been made in targeting the polyamine pathway in neuroblastoma (69). One frequent event in neuroblastoma development is Myc-N amplification (70), and ODC is known to be a downstream target of Myc. Tumor microarray analysis of 88 neuroblastoma tumors showed that elevated ODC mRNA levels were associated with several indicators of good prognosis (71). In contrast, elevated levels of antizyme-2 were associated with several indicators of good prognosis (71). Treatment of neuroblastoma cells with DFMO induces G1 cell cycle arrest (72), and DFMO treatment impairs development of neuroblastoma in E74-Myc transgenic mice (73). On the basis of these results, an ongoing phase I clinical trial is being conducted to determine the efficacy of DFMO-etoposide in neuroblastoma (NCT01059071).

We believe that targeting antizyme inhibitor for cancer therapy may actually prove to be a better strategy than targeting ODC, because such a treatment would affect cell proliferation not only through the polyamine pathway but also through additional cell cycle mediators. As previously described, one of the limitations associated with using DFMO to inhibit ODC is that DFMO does not prevent cells from taking up polyamines from the microenvironment. It is possible that inhibiting antizyme inhibitor would have this same limitation; therefore, a combination of antizyme inhibitor inhibition and an agent to block polyamine uptake may have more therapeutic effects than antizyme inhibitor inhibition alone. Similar studies have already shown that combining DFMO treatment with polyamine transport inhibitors can deplete intracellular polyamine pools in cultured cells and can effectively inhibit tumor growth in the K6/ODC mouse model of squamous cell carcinoma (74). The pathway involved in polyamine uptake in mammalian cells has not yet been fully characterized, and identifying the proteins involved could have a significant impact on the design of current and future therapeutics (75).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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