The role of aberrant DNMT3Bs in tumor progression

Rabia Hameed, Stacey L. Raimondi

Department of Biology, Elmhurst College, Elmhurst, Illinois 60126, United States

Correspondence: Stacey L. Raimondi
E-mail: raimondis@elmhurst.edu
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Cancer is among the most common causes of death in the United States, second only to heart disease, and is initiated by a loss of cell cycle control resulting from the deregulation of oncogenes and/or tumor suppressor genes. Epigenetic changes, such as DNA methylation or histone modifications, are common in cancer cells leading to altered gene regulation and tumor progression. Recently, studies have identified aberrant DNA methyltransferase (DNMT) transcripts in cancer cells but not normal cells. Specifically, aberrant transcripts of DNMT3B have been shown to have a role in tumor progression including the ΔDNMT3B variants as well as DNMT3B7. This review focuses on epigenetic changes caused by aberrant DNMT3Bs in cancer and their role in tumor progression or suppression.

Keywords: DNMT3B; methylation; cancer; tumor progression

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Introduction

Cancer is among the most common causes of death in the United States. In 2015 alone, approximately 1.6 million new cancer cases will be diagnosed, excluding in situ cases. Nearly 590,000 people are expected to die from cancer, close to 1,600 people per day [1]. Cancer encompasses many different diseases all caused by a loss of cell cycle control resulting from the deregulation of oncogene and/or tumor suppressor gene expression [2]. More specifically, cell cycle control can be altered by DNA sequence alterations or epigenetic changes, both of which lead to altered gene expression.

Epigenetic changes, which cause changes to gene expression without altering the DNA sequence, are a result of DNA methylation and/or histone modifications [3]. Cancer cells, specifically, are characterized by abnormal DNA methylation patterns [4]. In normal cells, transcriptionally inactive genes are generally characterized by hypermethylated DNA sequences while transcriptionally active genes are often hypomethylated [5]. In cancer cells, genes which are normally hypomethylated and transcriptionally active may become hypermethylated and, consequently, silenced [6]. Examples of genes affected in this manner are tumor suppressor genes. Furthermore, genes that are normally transcriptionally inactive and hypermethylated may become hypomethylated and active in cancer cells [7].

DNA methylation patterns are established by DNA methyltransferases (DNMTs). In eukaryotes, three main DNMTs have been identified to date: DNMT1, DNMT3A, and DNMT3B [8, 9]. DNMT1 is primarily known as a maintenance methyltransferase, one that copies methylation patterns post-replication, and secondarily as a de novo methyltransferase, one that establishes new methylation patterns. DNMT3A and DNMT3B are both de novo methyltransferases [10].

Recently, several aberrant DNMT3B transcripts have been identified in cancer cells. In this review, we discuss the role of aberrant DNMT3Bs as tumor promoters and tumor suppressors.
suppressors. We also note the potential for development of novel therapeutics in targeting aggressive forms of cancer as a result of aberrant DNMT research.

**Aberrant DNMT3Bs as Tumor Promoters**

ΔDNMT3B variants are found in NSCLC and correlate with promoter methylation of RASSF1A

One of the first aberrant DNMT3B transcripts identified in cancer cells was the ΔDNMT3B family \[11\]. ΔDNMT3Bs, a subfamily of DNMT3B, consist of 7 aberrant transcripts resulting from unconventional pre-mRNA splicing events and are the most prevalent DNMT3Bs found in human lung cancer\[12\]. In 2006, Wang and colleagues measured expression of seven ΔDNMT3B variants and analyzed them for correlation with promoter methylation of the tumor suppressor genes p16 and RASSF1A\[11\]. Approximately 80% of non-small cell lung cancers (NSCLC) analyzed indicated expression of ΔDNMT3B variants compared to 18% expression in normal lungs. Variants ΔDNMT3B1, ΔDNMT3B2, and ΔDNMT3B4 were present in the highest quantities in NSCLCs. Furthermore, p16 and RASSF1A promoter methylation was observed in percentages of 49% and 39% in NSCLCs, respectively, with the strongest correlation between ΔDNMT3B4 and RASSF1A \[11\].

In order to confirm the correlation between p16 and RASSF1A methylation and ΔDNMT3B variants, Wang and colleagues knocked down ΔDNMT3B4 and ΔDNMT3B2 expression via siRNA in the NSCLC cancer cell line H1299\[13\]. The knockout resulted in demethylation of the RASSF1A promoter region in H1299 cells. In a separate assay using pyrosequencing to evaluate H1299 cell DNA, Wang and colleagues were able to confirm a decrease in RASSF1A promoter methylation from 94% in control cells down to 33% in siRNA-transfected cells \[13\]. No alteration of p16 promoter methylation in either the control or siRNA-treated H1299 cells was observed. Overall, these data indicate a correlation between the presence of ΔDNMT3B2/4 and RASSF1A promoter methylation, but not p16 methylation in NSCLC, indicating that aberrant DNMTs may play a role in cancer progression \[13\].

**ΔDNMT3B7, an aberrant DNMT3B transcript, is found in virtually all cancer cell types**

There are several aberrant DNMT3B transcripts, including the shorter delta variants, ΔDNMT3B1-7, as well as the longer variants, ΔDNMT3B1-7. While attempting to identify and understand the expression pattern of ΔDNMT3B in cancer cells, Ostler and colleagues encountered an unexpected product via polymerase chain reaction of cDNA derived from several cancer cell lines; this product was named DNMT3B7 \[14\]. DNMT3B7 is an aberrant transcript that is the result of a retained 94 base pair sequence from intron 10 which merges with exon 11 to form an early stop codon and subsequently a truncated protein \[14\].

Upon the analysis of 25 cancer cell lines, it was noted that DNMT3B7 expression was observed in all but one cancer cell line. The widespread prevalence of this aberrant transcript across many different cancer cell lines suggests a role in cancer progression. Furthermore, Ostler and colleagues stably transfected this truncated protein, DNMT3B7, into 293 cells (human embryonic kidney cells) and observed changes in methylation patterns of CpG islands that paralleled expression patterns noted in cancer cell lines \[14\]. One gene specifically noted in this paper was E-cadherin (CDH1), hypermethylation of which is correlated to a more aggressive cancer phenotype\[15,16,17\]. Ostler and colleagues demonstrated that hypermethylation of the E-cadherin CpG island paralleled a 2.19-fold decrease in gene expression \[14\]. Taken together, these data suggest a possible correlation between DNMT3B7 and tumor progression.

**DNMT3B7 expression disrupts embryonic development and accelerates lymphomagenesis**

Although previous studies have indicated altered methylation patterns in cancer cells compared to normal cells, there is little understanding of the explicit mechanism(s) that leads to such altered methylation. In the study mentioned previously, Ostler and colleagues noted the presence of DNMT3B7 in several different cancer cell lines \[14\]. Shah and colleagues investigated this in vivo \[18\]. First, two lines of transgenic mice that express DNMT3B7 were constructed and embryonic developmental effects were observed. Clear developmental abnormalities were noted including craniofacial, cardiac, and immune defects \[18\]. These results highlight the importance of proper methylation and subsequent gene silencing and activation during embryogenesis.

Next, Shah and colleagues crossed DNMT3B7-expressing transgenic mice with Eμ-Myc transgenic mice which model aggressive B-cell lymphoma. Approximately 7% of Eμ-Myc transgenic mice develop mediastinal lymphomas while most develop peripheral lymphomas \[19\]. Interestingly, over 50% of the Eμ-Myc/DNMT3B7 transgenic mice displayed mediastinal lymphomas while incidence of peripheral lymphomas was approximately the same for both \[18\]. These results demonstrate DNMT3B7’s possible role in altering the process of tumorigenesis in Eμ-Myc/DNMT3B7 transgenic mice. Overall, the results of this study highlight changed phenotypes in in vivo models, specifically altered
methylated patterns and increased tumor progression, in the presence of \textit{DNMT3B7}.

\textit{DNMT3B7} promotes tumor progression in breast cancer cells

Previous research has shown higher expression of \textit{DNMT3B7} in the more aggressive breast cancer cell line, MDA-MB-231, compared to the less aggressive breast cancer cell line, MCF-7 \cite{14}. As breast cancer is second only to non-melanoma skin cancers in prevalence among women in the United States \cite{1}, Brambert and colleagues set out to further understand the role of \textit{DNMT3B7} in breast cancer cells. Using The Cancer Genome Atlas data portal, breast invasive carcinoma (BRCA) RNAseqV2 data were analyzed. Clinical data separated based on stage of breast cancer indicated increased prevalence of \textit{DNMT3B7} in stages II-IV compared to stage I. In addition, data separated by molecular subtype and analyzed in the BRCA clinical database indicated increased expression of \textit{DNMT3B7} in triple negative/basal-like and HER2 type patients compared to Luminal A and Luminal B subtypes \cite{20}. It is well documented that patients with triple negative/basal-like and HER2-like cancer diagnoses have an aggressive form of cancer that is difficult to treat compared to patients diagnosed with luminal breast cancer \cite{1}. While these results suggest a correlation between \textit{DNMT3B7} and a more aggressive breast cancer phenotype, Brambert and colleagues transfected \textit{DNMT3B7} into poorly aggressive breast cancer cell lines to validate this hypothesis.

\textit{DNMT3B7} transfection into the poorly aggressive breast cancer cell lines, MCF-7 and T-47D, resulted in phenotype alterations that parallel phenotypes characteristic of the more aggressive breast cancer cell line, MDA-MB-231 \cite{20}. More specifically, hypermethylation and downregulation of E-cadherin was observed in the transfected cell lines. Loss of E-cadherin expression is a well-known marker of tumor progression \cite{15,16,17}. \(\beta\)-catenin, a binding partner of E-cadherin, also displayed altered localization in transfected cells from the membrane to the nucleus. Upon movement to the nucleus, \(\beta\)-catenin is able to act as a transcription factor and promote cell proliferation \cite{21}. Taken together, these data indicate movement from a less aggressive cancer phenotype to a more aggressive phenotype in the presence of \textit{DNMT3B7}.

To further support this hypothesis, Brambert and colleagues noted increased cell proliferation, cell adhesion turnover, and anchorage-independent growth in cells transfected with \textit{DNMT3B7} - all indicators of a more aggressive phenotype \cite{20}. These results correlate well with previous data indicating higher expression of \textit{DNMT3B7} in aggressive MDA-MB-231 cells, compared to the poorly aggressive MCF-7 line \cite{14}. Overall, the novel results presented in this study indicate expression of \textit{DNMT3B7} in poorly aggressive breast cancer cell lines leads to a more aggressive phenotype, thus suggesting a role for \textit{DNMT3B7} in breast cancer tumor progression. However, in order to support these data, it is important to consider other clinical parameters as well.

\textit{DNMT3B7} expression correlates to age, race, \(ER\), \(PR\), and HER2 receptor status

The previous study indicated the presence of \textit{DNMT3B7} in more aggressive stages/subtypes of breast cancer, thus suggesting clinical significance and necessitating the study of different clinical parameters. In order to determine which clinical parameters \textit{DNMT3B7} expression alters in breast cancer patients, Mullin and colleagues utilized a bioinformatics approach \cite{22}. Data indicate increased \textit{DNMT3B7} expression in patients between ages 25-55 compared to patients aged 56-90 \cite{22}. Furthermore, \textit{DNMT3B7} expression does not correlate with menopausal status but it does correlate with race. African American women not only present with more aggressive breast tumors \cite{21}, but they also have higher \textit{DNMT3B7} expression prevalence \cite{22}. These data suggest another possible correlation between \textit{DNMT3B7} expression and an aggressive breast cancer phenotype.

\textit{DNMT3B7} expression was also correlated to a more aggressive receptor phenotype. Patients expressing \textit{DNMT3B7} presented down-regulation of both estrogen receptor (\(ER\)) and progesterone receptor (\(PR\)) and up-regulation of HER-2 receptors. Both \(ER\) and \(PR\) are normally tumor suppressors \cite{24} whose function can be inhibited via hypermethylation, and this is one of the mechanisms Mullin and colleagues propose by which \textit{DNMT3B7} may play a role in a more aggressive tumor phenotype \cite{23}. Furthermore, the HER family plays a key role in intracellular regulation that results in cell proliferation, growth, and survival \cite{25}. Thus, up-regulation of HER2 would result in a more aggressive cancer phenotype. Overall, these data suggest that \textit{DNMT3B7} expression plays a role in tumor progression in breast cancer. However, it is important to note that there are other studies suggesting an alternate role for \textit{DNMT3B7} in cancer.

\textbf{Aberrant \textit{DNMT3Bs} as Tumor Suppressors}

\textit{DNMT3B7} alters DNA methylation and suppresses tumor progression in neuroblastoma

Previous data have suggested the role of the aberrant transcript, \textit{DNMT3B7}, in altering methylation and gene
expression in cancer cells \[14, 20\]. In order to characterize the role of \(DNMT3B\) in human neuroblastoma, Ostler and colleagues examined DNA methylation, gene expression, and phenotype \[26\]. Interestingly, higher \(DNMT3B\) expression was found in differentiated, less aggressive, neuroblastomas compared to their undifferentiated, more aggressive, counterparts. Because of this, Ostler and colleagues hypothesized that \(DNMT3B\) expression correlates to a less aggressive clinical phenotype in neuroblastoma.

Compared to ganglioneuroblastoma tumors, \(DNMT3B\)-transfected cell lines in this study demonstrated low \(DNMT3B\) expression through the first week of cellular growth with subsequent increases in expression as cell growth decreased. Furthermore, murine xenograft models with and without induced \(DNMT3B\) expression were observed for tumor vascularity, cell proliferation, and apoptosis. Overall, there was a marked decrease in tumor growth as well as inhibition of angiogenesis in xenografts with \(DNMT3B\) expression. Furthermore, \(DNMT3B\)-expressing cells displayed higher levels of genomic methylation in \textit{in vitro} and \textit{in vivo} models \[26\]. Taken together, these data suggest a mechanism by which \(DNMT3B\) is acting as a tumor suppressor in human neuroblastoma.

**Conclusions**

While it appears that aberrant \(DNMT3\) transcripts predominantly play a role in tumor progression \[11-14, 18, 20, 22\], research in the previous study by Ostler and colleagues demonstrates the possibility of an aberrant \(DNMT3B\) transcript, \(DNMT3B\), acting as a tumor suppressor \[26\]. One possible explanation for this may involve the epithelial-to-mesenchymal transition (EMT). During EMT, epithelial cells undergo biochemical changes that result in a mesenchymal cell phenotype characterized by increased invasiveness, abnormally high resistance to apoptosis, and other phenotypes found in more aggressive cancer cells \[27\]. On the other hand, neuroblastomas undergo a mesenchymal-to-epithelial transition as they increase in aggressiveness changing from differentiated, mesenchymal cells to undifferentiated, epithelial-like cells. In the studies where aberrant \(DNMTs\) promote tumor progression, we observe higher levels of \(DNMTs\) in the aggressive, mesenchymal-type cell. In neuroblastomas, we also see higher levels of \(DNMT3B7\) in the mesenchymal-type cell which is now the less aggressive cell type. Taken together, these results suggest that aberrant \(DNMTs\) may have a role in EMT and/or MET and future studies will be performed to examine this hypothesis.

In conclusion, aberrant \(DNMT3B\)s play a role in tumor progression and suppression that appears to be cancer type specific. Specifically, research presented in this review suggests the role of aberrant \(DNMT3B\)s in altered gene expression and cancer phenotypes. Future research in the field may include the role of aberrant \(DNMT3B\)s in alteration of molecular pathways, such as the Ras-MAP kinase pathway. Furthermore, continued study of these transcripts may lead to a better understanding which may pave the way for novel therapeutics targeting aberrant \(DNMTs\) in cancer cells.

**Conflicting interests**

The authors have no conflict of interest to declare.

**References**


