The role of epithelial–mesenchymal transition programming in invasion and metastasis: a clinical perspective

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Abstract: Epithelial–mesenchymal transition (EMT) is involved in normal developmental cellular processes, but it may also be co-opted by a subset of cancer cells, to enable them to invade and form metastases at distant sites. Several gene transcription factors regulate EMT, including Snail1, Snail2, Zeb1, Zeb2, and Twist; ongoing studies continue to identify and elucidate other drivers. Specific micro ribonucleic acids (RNAs) have also been found to regulate EMT, including the microRNA-200 (miR-200) family, which targets Zeb1/Zeb2. Cancer “stem cells” – with the ability to self-renew and to regenerate all the cell types within the tumor – have been found to express EMT markers, further implicating both cancer stem cells and EMT with metastasis. Microenvironmental cues, including transforming growth factor-β, can direct EMT tumor metastasis, such as by regulating miR-200 expression. In human tumors, EMT markers and regulators may be expressed in a subset of tumor cells, such as in cells at the invasive front or tumor–microenvironment interface, though certain subtypes of cancer can show widespread mesenchymal-like features. In terms of therapeutic targeting of EMT in patients, potential areas of exploration could include targeting the cancer stem cell subpopulation, as well as microRNA-based therapeutics that reintroduce miR-200. This review will examine evidence for a role of EMT in invasion and metastasis, with the focus being on studies in lung and breast cancers. We also carry out analyses of publicly-available gene expression profiling datasets in order to show how EMT-associated genes appear coordinately expressed across human tumor specimens.

Keywords: EMT, epithelial–mesenchymal transition, tumor microenvironment, miR-200, cancer stem cells

Introduction

Metastasis is the primary cause of death in cancer patients.1 Tumor invasion and metastasis, whereby cancer cells escape from the primary tumor mass and colonize at distant sites, involve multiple steps, including localized invasion, intravasation, transport to other organs, extravasation, formation of micrometastasis, and colonization.2 Epithelial–mesenchymal transition (EMT), a process by which epithelial cells acquire characteristics of mesenchymal cells, is largely thought to play an important role in invasion and metastasis.2 EMT is a natural process involved, for example, with development and wound healing, and it may be co-opted by at least a subset of tumor cells that acquire the ability to invade and metastasize.

This review will examine evidence for a role of EMT in invasion and metastasis, with the focus being on studies in lung and breast cancers. We will consider this topic, from both the perspective of experimental studies and the perspective of analyses of human tumor specimens. In addition, we will make use of molecular profiling datasets.
of breast and lung tumors, which are available in the public domain, in order to assess how EMT-associated genes may appear coordinately expressed across human tumors.

**Epithelial–mesenchymal transition (EMT)**

During EMT, epithelial cells lose their cell polarity and molecular expression, enabling cell–cell adhesion, and they gain migratory and invasive properties; numerous other cellular changes may also be associated with cells undergoing EMT. Many processes involving EMT occur during embryogenesis; for example, neural crest cells undergo EMT in order to migrate away from the neural tube and to differentiate into bone, smooth muscle, peripheral neurons and glia, as well as melanocytes. During wound healing, epithelial cells differentiate into myofibroblasts that rebuild the extracellular matrix and facilitate wound contraction. When wound healing processes go awry in certain contexts, excess fibrous connective tissue can lead to organ fibrosis.

The molecular players involved in EMT, include specific markers that distinguish an epithelial cell from a mesenchymal cell, as well as regulators that can drive a cell towards a mesenchymal or epithelial state. Cells undergoing EMT typically show both an increase in protein abundances of vimentin, N-cadherin, fibronectin, integrin αvβ6, and a decrease in E-cadherin, desmoplakin, cytokeratins, and occludin. Several transcriptional suppressor families regulate EMT, including the zinc-finger proteins Snail1 and Snail2, the two-handed zinc-finger ΔEF1 family factors (ΔEF1/Zeb1 and SIP1/Zeb2), and the basic helix–loop–helix factors, Twist and E12/E47. Evidence also suggests that signals derived from the cellular microenvironment can regulate EMT, such as through cell–cell contacts mediated by families of transmembrane receptors and ligands expressed on adjacent cells.

Over the last few years, posttranscriptional regulation of EMT has become an emerging paradigm. Recently, specific micro ribonucleic acids (RNAs) (miRNAs or miRs) – small, noncoding RNAs that posttranscriptionally regulate gene expression – have been found to regulate EMT, the most notable example being the regulation of Zeb1 and Zeb2 by the microRNA-200 (miR-200) family, where loss of miR-200 leads to EMT. Other miRNAs that regulate Zeb1/2 include miR-205 and miR-192/215, and miRNAs that regulate Snail1 or Snail2 include miR-1, miR-29b, miR-30c, miR-34, and miR-203. Other EMT-associated genes involved in protein translation include: Y-box binding protein-1 (YB-1), which directly activates cap-independent translation of messenger RNAs (mRNAs) encoding Snail1 and other transcription factors related to EMT, and heterogeneous nuclear ribonucleoprotein E1 (hnRNP E1), which binds a transforming growth factor-β (TGFβ)–activated translation element in the transcripts of EMT genes DAB2 (disabled-2) and ILEI (interleukin-like EMT inducer). Moreover, EMT initiates widespread changes in alternative splicing of gene transcripts, in large part through down-regulation of epithelial splicing regulatory proteins 1 and 2 (ESRP1 and ESRP2).

**Role of EMT in tumor invasion and metastasis**

Since many normal cellular processes may be co-opted by cancer cells to their own advantage, there is much evidence that EMT aids tumor invasion and metastasis. Conceptually, this would seem plausible, as EMT would enable tumor epithelial cells to lose their cell polarity and cell–cell adhesive interactions and junctions, allowing the cells to escape from the primary tumor. Mesenchymal-like cancer cells could more effectively invade surrounding tissues and migrate to distant sites in ways that can reflect cell migration during development. Recently, EMT has been associated with the subset of tumor cells believed to be highly tumorigenic, also referred to as “cancer stem cells,” which would fit with the notion of a small percentage of tumor cells having the potential for invasion and metastases.

Much of the evidence for an EMT role in invasion and metastasis can be found in experimental studies, where EMT can be readily induced, for example, in vitro in a variety of cancer or immortalized cell lines. In our own studies, we have made use of a mouse model of human lung adenocarcinoma, driven by mutant K-ras and Tp53, where the tumors metastasize to sites commonly involved in lung cancer patients, and where metastasis is driven entirely by repression of the miR-200 family. In our system as well as others, microenvironmental cues, including TGFβ, can direct tumor metastasis by regulating miR-200 expression. Studies in breast cancer and other cancers also show a similar role for miR-200. Using cell lines derived from our mouse model, we have been able to identify additional genes with roles in the miR-200 pathway or EMT, including MIR34A, Jagged 2 (JAG2), and VEGFR1.

There is increasing support for the hypothesis that most tumors contain a subpopulation of cells, often referred to as tumor-initiating cells or “cancer stem cells,” with the ability to self-renew and to regenerate all the cell types within the tumor. These cancer stem cells, which can be isolated...
from the bulk tumor cells using specific cell surface and other markers, have also been found to express EMT markers.\textsuperscript{24,25} Moreover, inducing EMT in immortalized human mammary epithelial cells (for example, by forced expression of Twist or Snail transcription factors), results in the cells also acquiring traits associated with stem cells, such as the expression of stem cell markers and an increased ability to form mammospheres.\textsuperscript{25} In addition, over-expression of miR-200c causes both normal mammary stem cells and cancer-associated stem cells to lose their defining characteristics.\textsuperscript{33} The above would indicate that the cancer EMT and cancer stem cell theories may coincide, where the subpopulation of tumor cells with the ability to form metastases do so, in part, through the use of processes associated with EMT.

**EMT as observed in human tumor specimens**

While experimental models can help establish cause-and-effect relationships between genes and pathways, studies of human tumor specimens are also needed in order to help ground experimental observations as being relevant in the setting of human patients. However, the manifestation of EMT in this setting may be contrary to the expectations of some, which has hindered widespread acceptance of the idea of cancer-associated EMT among clinicians in particular.\textsuperscript{12} For one thing, tumor metastases established at distant sites appear to be more epithelial than mesenchymal, suggesting to some that EMT has not occurred, though an alternative explanation is that cancer-associated EMT is a transient state, and that mesenchymal-like cells can revert to an epithelial state upon tumor formation.\textsuperscript{12} Another complication in human tumor studies of EMT is that EMT can appear manifested in only a subset of tumor cells,\textsuperscript{11} for example, at the tumor–host interface. Analyses that average molecular signals across all cells within the tumor may, therefore, miss patterns distinctive to only a subpopulation of cells.

In specific contexts, EMT-associated features can be observed in human tumors, such as in cells at the invasive front of cancers.\textsuperscript{34} In addition, specific subsets of human breast cancer show widespread mesenchymal features; these subsets include the metaplastic subtype,\textsuperscript{26} as well as the expression-based claudin-low subtype.\textsuperscript{24} Both metaplastic and claudin-low breast cancers also express markers of breast cancer stem cells.

EMT has also been related to therapy resistance in cancer, with both preclinical and clinical evidence. After neoadjuvant chemotherapy in breast cancer, the remaining tumor cells have been found to be enriched in stem cell and EMT markers,\textsuperscript{24,36} indicating that different therapies might be needed to target this population. Similarly, chemotherapy-treated lung tumors show enrichment for lung cancer stem cells (with CD133+ marker).\textsuperscript{37} Functional studies also provide evidence for EMT’s involvement in chemoresistance; for example, a recent study that inhibited ZEB1 in docetaxel-resistant human lung adenocarcinoma cells, thereby significantly enhancing their chemosensitivity.\textsuperscript{38} In another recent study, a panel of lung cancer cell lines was probed using a gene signature of EMT, and the cell lines that appeared more mesenchymal also showed greater resistance to epidermal growth factor receptor and PI3K/Akt pathway inhibitors.\textsuperscript{39}

Global molecular analyses of human tumors, including gene expression profiling, have added a tremendous amount

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**Figure 1** EMT-associated genes appear coordinately expressed across human tumor specimens of both breast and lung cancer.

**Notes:** Two gene expression profiling datasets are represented, (A) one being a “compendium” of published data on human breast cancers,\textsuperscript{6} and (B) the other being a compendium of data on human lung cancers.\textsuperscript{6–11} Using a panel of canonical EMT markers as shown (from the review article by Lee et al\textsuperscript{4}), we have “scored” each of the tumor profiles for “EMT-ness” (i.e., similarity to mesenchymal cells). Yellow denotes relatively high mRNA expression; blue indicates lower mRNA expression. For each dataset, a subset of tumors appears to be relatively more mesenchymal-like as compared to the rest of the tumors. Genes represented in the breast cancer dataset are limited to those featured on the U133A array platform.

**Abbreviations:** n, number; EMT, epithelial–mesenchymal transition; mRNA, messenger ribonucleic acid.
of data into the public domain. In this review, we have surveyed two sizeable datasets on breast and lung cancer to see whether EMT-associated genes appear coordinately expressed across tumor specimens. For breast cancer, we had previously assembled a compendium of nine separate gene expression array datasets (n = 1,340), and recently we have carried out the same for lung adenocarcinoma (n = 1,492, representing eleven datasets), thereby giving us robust sample numbers for correlation analyses. With these data, we do find that genes canonically involved in EMT are, in fact, coordinately expressed with respect to each other, with a subset of tumors in each case looking relatively more mesenchymal-like as compared to the rest of the tumors (Figure 1).

Data from The Cancer Genome Atlas include profiling of both microRNAs and mRNAs, and in these datasets (for both breast and lung cancer), we see that lower expression of miR-200 family members is coordinate with EMT overall (Figure 2), with miR-200 showing a strong anticorrelation with its target genes ZEB1 and ZEB2 in particular.

Another potential use of public expression data is to uncover previously unknown or unappreciated correlations involving EMT. To this end, we have “scored” each of the tumor profiles for “EMT-ness” (ie, similarity to mesenchymal cells) by applying the following equation to the normalized values,

\[ VIM + CDH2 + FOXC2 + SNAI1 + SNAI2 + TWIST1 + FN1 + ITGB6 + MMP2 + MMP3 + MMP9 + SOX10 + GCS − CDH1 − DSP − OCLN, \] (1)

using a list of markers provided in another review. Some of the strongest correlates of EMT, as found in both breast and lung cancer, are provided in Table 1; however, many more genes could be implicated by using less stringent statistical cutoffs than what might be used for fitting within the printed page; out of 12,000 genes represented in both breast and lung datasets, over 4,000 have at least a nominally significant correlation with EMT (P < 0.01, Pearson’s correlation) in both datasets. The genes most up or down with EMT (Table 1) include many that are related to the extracellular matrix or to cell adhesion. The genes most down with EMT (ie, high in cells that appear more epithelial-like) include grainyhead-like 2 (GRHL2), which was recently found to play a major role in the suppression of oncogenic EMT in breast cancer cells.

One might suggest that if EMT were involved in metastases, then tumors showing EMT-like characteristics ought to show worse outcomes; however, such a survival correlation may be difficult to observe, given the reasons noted above, such as the presence of tumor cell subpopulations. For most gene array datasets in particular, the stroma content of the tumor samples may also contribute to mesenchymal-associated patterns. Notably, we do not see robust survival correlations for EMT markers in our tumor compendium datasets. At the same time, however, low miR-200 levels have been found elsewhere to be part of a larger microRNA expression profile that predicts poor outcome in early-stage lung cancer patients.
### Top 25 genes correlated with mesenchymal phenotype

<table>
<thead>
<tr>
<th>Entrez ID</th>
<th>Symbol</th>
<th>Gene title</th>
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<tbody>
<tr>
<td>6591</td>
<td>SNAI2*</td>
<td>Snail homolog 2 (Drosophila)</td>
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<tr>
<td>5118</td>
<td>PCOLCE</td>
<td>Procollagen C-endopeptidase enhancer</td>
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<tr>
<td>1513</td>
<td>CTSK</td>
<td>Cathepsin K (pseudodystosis)</td>
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<tr>
<td>9945</td>
<td>GPT2</td>
<td>Glutamine-fructose-6-phosphate transaminase 2</td>
</tr>
<tr>
<td>4313</td>
<td>MMP2*</td>
<td>Matrix metalloproteinase 2 (gelatinase A, 72 kDa gelatinase, 72 kDa type 4 collagenase)</td>
</tr>
<tr>
<td>1290</td>
<td>COL5A2</td>
<td>Collagen, type V, alpha 2</td>
</tr>
<tr>
<td>1292</td>
<td>COL6A2</td>
<td>Collagen, type 6, alpha 2</td>
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<tr>
<td>23452</td>
<td>ANGPTL2</td>
<td>Angiopoietin-like 2</td>
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<tr>
<td>2619</td>
<td>GAS1</td>
<td>Growth arrest-specific 1</td>
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<td>1289</td>
<td>COL5A1</td>
<td>Collagen, type V, alpha 1</td>
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<tr>
<td>11117</td>
<td>EMLN1</td>
<td>Elastin microfibril interfacer 1</td>
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<tr>
<td>25903</td>
<td>OLML1</td>
<td>Olfactomedin-like 1</td>
</tr>
<tr>
<td>1293</td>
<td>COL6A3</td>
<td>Collagen, type 6, alpha 3</td>
</tr>
<tr>
<td>51339</td>
<td>DACT1</td>
<td>Dapper, antagonist of beta-catenin, homolog 1 (Xenopus laevis)</td>
</tr>
<tr>
<td>7070</td>
<td>THY1</td>
<td>Thy-1</td>
</tr>
<tr>
<td>7291</td>
<td>TWIST1*</td>
<td>Twist homolog 1 (acrospermophylyndactyly 3; Saethre–Chotzen</td>
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<tr>
<td>1281</td>
<td>COL3A1</td>
<td>Collagen, type 3, alpha 1 (Ehlers–Danlos syndrome type 4, autosomal dominant)</td>
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<td>3912</td>
<td>LAMB1</td>
<td>Laminin, beta 1</td>
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<td>2191</td>
<td>FAP</td>
<td>Fibroblast activation protein, alpha</td>
</tr>
<tr>
<td>5176</td>
<td>SERPINF1</td>
<td>Serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, Pigment epithelium derived factor), member 1</td>
</tr>
</tbody>
</table>

### Top 25 genes correlated with epithelial phenotype

<table>
<thead>
<tr>
<th>Entrez ID</th>
<th>Symbol</th>
<th>Gene title</th>
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</thead>
<tbody>
<tr>
<td>3875</td>
<td>KRT18</td>
<td>Keratin 18</td>
</tr>
<tr>
<td>10053</td>
<td>API1M2</td>
<td>Adaptor-related protein complex 1, mu 2 subunit</td>
</tr>
<tr>
<td>780</td>
<td>DDR1</td>
<td>Discoidin domain receptor family, member 1</td>
</tr>
<tr>
<td>51361</td>
<td>HOOK1</td>
<td>Hook homolog 1 (Drosophila)</td>
</tr>
<tr>
<td>29956</td>
<td>LASS2</td>
<td>LAG1 longevity assurance homolog 2 (Saccharomyces cerevisiae)</td>
</tr>
<tr>
<td>64284</td>
<td>RAB17</td>
<td>Rab17, member of the RAS oncogene family</td>
</tr>
<tr>
<td>10040</td>
<td>TOM1LI</td>
<td>Target of myb-like 1 (chicken)</td>
</tr>
<tr>
<td>378708</td>
<td>APITD1</td>
<td>Apoptosis-inducing, TAF9-like domain 1</td>
</tr>
<tr>
<td>2166</td>
<td>FAAH</td>
<td>Fatty acid amide hydrolase</td>
</tr>
<tr>
<td>999</td>
<td>CDH1*</td>
<td>Cadherin 1, type 1, E-cadherin (epithelial)</td>
</tr>
<tr>
<td>55204</td>
<td>GOLPH3L</td>
<td>Golgi phosphoprotein 3-like</td>
</tr>
<tr>
<td>440026</td>
<td>TMEM41B</td>
<td>Transmembrane protein 41B</td>
</tr>
<tr>
<td>55930</td>
<td>MYOSC</td>
<td>Myosin VC</td>
</tr>
<tr>
<td>10605</td>
<td>PAIP1</td>
<td>Poly(A) binding protein interacting protein 1</td>
</tr>
<tr>
<td>79170</td>
<td>ATAD4</td>
<td>ATPase family, AAA domain containing 4</td>
</tr>
<tr>
<td>9053</td>
<td>MAP7</td>
<td>Microtubule-associated protein 7</td>
</tr>
<tr>
<td>10140</td>
<td>TOB1</td>
<td>Transducer of ERBB2, 1</td>
</tr>
<tr>
<td>51181</td>
<td>DCKR</td>
<td>Dicarbonyl/L-xylulose reductase</td>
</tr>
<tr>
<td>79977</td>
<td>GRHL2</td>
<td>Grainyhead-like 2 (Drosophila)</td>
</tr>
</tbody>
</table>

(Continued)
Regarding survival correlations involving mesenchymal-like or stem cell-like breast cancer cells, results have been somewhat mixed,\textsuperscript{56} however, the claudin-low breast cancer subtype has been associated with worse outcome compared to other subtypes.\textsuperscript{57} Another potential issue with analysis of array data in particular, would be the fact that expression values in this case are not absolute but relative; in contrast, ERCC1 mRNA can be used to normalize QT-PCR (quantitative polymerase chain reaction) values, and RNA sequencing can provide measures of both absolute and relative abundances.

**Potential for targeting EMT in cancer therapy and management**

Molecular biology studies of EMT have shed light onto the processes of invasion and metastases, and the hope is that these basic biology findings can be eventually translated into new therapeutic approaches. Based on our discussion, one could see at least two potential areas of focus for targeting EMT: targeting the cancer stem cell subpopulation of the tumor, and miRNA-based therapeutics to reintroduce miR-200 (a master regulator of EMT) into tumor cells. Molecular pathways associated with EMT and stem cells in breast cancer, which have been suggested for targeting, include Notch, Wnt, and TGFβ.\textsuperscript{56} In addition, one study carried out a chemical screen for compounds showing selective toxicity for breast cancer stem cells, with top hits including salinomycin.\textsuperscript{58} The appeal for the use of miRNAs as therapeutics is that they are small and might be more easily delivered into the cell; current challenges, on the other hand, include getting sufficient quantities of the therapeutic agents into the tumor, while minimizing toxicity and off-target effects, though work in this area is ongoing.\textsuperscript{59}

While we are not aware of ongoing clinical trials that directly target or evaluate EMT status in patients, there are trials currently evaluating stem cell markers pre- and/or posttherapy, as related to treatment or outcome. In breast cancer, current Phase II trials evaluating the CD44 cell marker include NCT01193881\textsuperscript{60} (involving treatment of HER2+ cancers with lapatinib and trastuzumab) and NCT01372579\textsuperscript{61} (involving treatment of triple-negative cancers in the neoadjuvant setting with carboplatin and eribulin mesylate). There are also a number of trials evaluating therapies that target cancer stem cell-associated pathways, including Notch; these include NCT01193881,\textsuperscript{62} a trial in advanced nonsmall cell lung cancer involving gamma-secretase inhibitor RO4929097.

**Current research and future directions**

It would be most desirable for us to be able to translate, in the near term, our understanding of EMT biology into improved treatment approaches for cancer. However, at the same time, exploring the biology even further in order to catalogue and elucidate the key players and drivers of EMT would represent an investment that could have long-term rewards in improved targeting of metastasis in patients. The publicly-available molecular datasets, including those from The Cancer Genome Atlas, can be a tool in screening for novel EMT-associated genes – though only a fraction of EMT correlates may turn out to be key drivers, and these would need to be established using functional studies.

One avenue of research that can be further expanded upon is the role of the tumor microenvironment in initiating EMT
and metastasis. It should be stated that we favor a model where diverse microenvironmental cues, rather than acquired genetic alterations alone, contribute to a subset of tumor cells that eventually evade and metastasize.\textsuperscript{13} To this end, better experimental systems – over conventional two-dimensional cell cultures on plastic, or even over three-dimensional cultures in Matrigel\textsuperscript{TM} (BD Biosciences, San Jose, CA, USA) – are needed in order to better mimic elements of the tumor microenvironment. Recent examples of such model systems include synthetic polymer-based scaffolds,\textsuperscript{60} as well as ex vivo three-dimensional models using a natural matrix, in order to form a barrier between the endothelial and epithelial spaces, allowing lung cancer cell lines to be able to form lung nodules with intact vasculature.\textsuperscript{64}

Conclusion

Over time, we have learned a great deal about the elements involved in EMT, as well as those involved in invasion and metastases; individual findings, as made from both experimental and clinical studies, are coming together to give us a more complete picture. A number of diverse theories and observations surrounding cancer cell behavior can potentially fall under the umbrella of cancer-associated EMT, which may include aspects of cancer stem cells and tumor microenvironmental influences. More questions remain, including those pertaining to what the key drivers of EMT are (both within and outside of the cancer cell) over the natural course of the disease. Better experimental models are needed in order to study the role of the tumor microenvironment. Analysis of molecular data on human tumors, combined with results from experimental studies, can identify new or underappreciated players. There is also a need to better map out the extent of tumor heterogeneity and to characterize the distinct cancer cell subpopulations. We believe that by increasing our knowledge in the above areas, we can increase the potential for making an impact in the clinical setting.

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Disclosure

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