Detection of Long Non-Coding RNA in Archival Tissue: Correlation with Polycomb Protein Expression in Primary and Metastatic Breast Carcinoma

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Abstract

A major function of long non-coding RNAs (lncRNAs) is regulating gene expression through changes in chromatin state. Experimental evidence suggests that in cancer, they can influence Polycomb Repressive Complexes (PRC) to retarget to an occupancy pattern resembling that of the embryonic state. We have previously demonstrated that the expression level of IncRNA in the HOX locus, including HOTAIR, is a predictor of breast cancer metastasis. In this current project, RNA in situ hybridization of probes to three different lncRNAs (HOTAIR, ncnFoxA1, and ncnFoxD4), as well as a immunohistochemical staining of EZH2, is undertaken in formalin-fixed paraffin-embedded breast cancer tissues in a high throughput tissue microarray format to correlate expression with clinicopathologic features. Though overall EZH2 and HOTAIR expression levels were highly correlated, the subset of cases with strong HOTAIR expression correlated with ER and PR positivity, while the subset of cases with strong EZH2 expression correlated with an increased proliferation rate, ER and PR negativity, HER2 underexpression, and triple negativity. Co-expression of HOTAIR and EZH2 trended with a worse outcome. In matched primary and metastatic cancers, both HOTAIR and EZH2 had increased expression in the metastatic carcinomas. This is the first study to show that RNA in situ hybridization of formalin fixed paraffin-embedded clinical material can be used to measure levels of long non-coding RNAs. This approach offers a method to make observations on lncRNAs that may influence the cancer epigenome in a tissue-based technique.

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Introduction

One aspect of tumor development involves the alteration of gene expression patterns due to epigenetic changes. The Polycomb group (PcG) proteins work in multiprotein complexes called Polycomb Repressive Complexes (PRCs) that repress transcription of gene expression by modification of chromatin. PcG proteins bind and repress promoters of genes that encode proteins with key roles in cell fate determination and in embryonic development. During cell fate determination, PcG proteins are displaced and recruited to different subsets of target genes. In cancer, PcG target genes are frequently epigenetically silenced by DNA methylation [1–3]. This silencing may be due to the high expression of PcG proteins in cancer [4].

EZH2, the human homolog of the Drosophila protein Enhancer of Zeste, is a PcG protein in the PRC2 complex [5]. EZH2 is amplified and highly expressed in many cancers including melanoma, endometrial, prostate, and breast carcinoma [6–10]. In breast carcinoma, EZH2 protein levels have been found to be strongly associated with poor clinical outcomes [8]. Klee et al. [8] identified EZH2 to be overexpressed in invasive and metastatic breast cancer compared to normal breast epithelial cells. In addition, they found that EZH2 levels significantly correlated with negative ER status, negative PR status, and lymph node status, but not Her2 overexpression. Later, groups found that EZH2 was also significantly associated with tumor cell proliferation [6,11].

Long non-coding RNA (lncRNA) may be one of the regulators of PcG proteins. LncRNAs are RNAs that are longer than 200 nucleotides in length and do not code for proteins, although they can interact with proteins. They are thought to have diverse functions including imprinting, X chromosome inactivation, chromatin remodeling, transcription regulation, cell cycle control, and possibly be candidate oncogenes and tumor suppressors [12–14]. Over 8,100 lncRNAs have been cataloged to date [15]. Experimental evidence suggests that in cancer, they can influence PRCs to retarget to an occupancy pattern resembling that of the embryonic state. Approximately 20% of all human lncRNA have been observed to bind to the PRC2 complex, leading to the proposal that lncRNA guide PcG proteins to their target genes [16].

In a recent paper by Gupta et al. [17], lncRNA in the HOX loci were found to become dysregulated during breast cancer progression. This study identified a distinct set of HOX lncRNA to be overexpressed in primary tumors and very frequently overexpressed in metastases. One such lncRNA, HOTAIR, was increased in primary tumors and metastases, and its expression
level in primary tumors was a predictor of eventual metastasis and
death. HOTAIR had previously been shown to recruit PcG proteins to
chromatin through interaction with the PRC2 complex [18]. Overexpression of HOTAIR induced localization of PRC2
subunit EZH2 onto many genes; this PRC2 occupancy pattern
more resembled the embryonic state [17].

In this study, we measured the expression of lncRNAs in
formalin-fixed paraffin-embedded (FFPE) tissues by in situ
hybridization to understand how lncRNA expression is correlated
with clinical features. We use RNA in situ hybridization probes of
HOTAIR and two other HOX locus lncRNAs (ncHoxA1 and
ncHoxD4), which were identified in the Gupta et al. paper [17] to
be co-expressed in metastatic breast carcinomas, to see if HOX
locus lncRNA expression and EZH2 protein expression correlate
with clinicopathologic features. Lastly, using matched primary and
metastatic breast carcinomas we determine if HOTAIR and EZH2
have increased expression in metastatic versus primary breast
carcinoma.

Materials and Methods

LncRNA Probes

Probes of 400 to 500 nucleotides were created based upon
unique non-conserved sequences and constructed as previously
described [17]. In brief, multiple antisense probes targeting
different parts of each of the lncRNA sequences were developed
based upon predictions of the lncRNA secondary structures.
Sequences that had high evolutionary conservation were avoided, as
they may be preferentially involved in tertiary RNA structures that
could be difficult to hybridize to in a FFPE environment. In
addition, sense stranded probes (opposite strand to the targeting
antisense probe) were constructed for each lncRNA to evaluate for
non-specific hybridization. The sense and antisense RNA probes
labeled with Digoxigenin (DIG) were generated by PCR
amplification of a T7 promotor which was incorporated into the
primers. Per manufacturer’s protocol (Roche Diagnostics), a DIG
RNA labeling kit and T7 polymerase performed in vitro
transcription. The primers used to construct these probes are as
follows: HOTAIR Anti Sense Forward: gcagtggggaaactcntgc, HOTAIR Anti Sense Reverse: CTAATACGACTCTA-
TAGGGGcttgggttaaggcctgc, ncHoxA1-53 Anti Sense Forward: agttcggaggaggaaggac, ncHoxA1-53 Anti Sense Reverse: CTA-
TAGGACTCTATAGGGaaaggctactgaag, nc-HoxD4-27 Anti Sense Forward: ttagatgagggctcaacg, nc-HoxD4-27 Anti Sense Reverse: CTAATACGACTCTATAGGGgcctcgctgt-
tatttca.

RNA in situ Hybridization

The RNA in situ hybridization was performed as previously
described [17]. Hybridization included sense or antisense ribop-
robes at 200 ng/ml dilutions. The stains were then scored by eye
by authors (KC and RW), on a two- or three-tiered scoring system,
using the following criteria for the two-tiered system: 0 = negative;
1 = equivocal/interpretable; 2 = positive; and for the three-
tiered system: 0 = negative; 1 = equivocal/interpretable; 2 = weak positive; 3 = strong positive.

EZH2 Antibody

The primary EZH2 antibody used was BD Transduction
Laboratories, clone 11, at a 1:25 titration. The immunohisto-
chemical reactions were visualized using Vector Elite ABC kit (BD
Transduction Laboratories). The intensity of staining was inter-
preted by histopathologic evaluation by the primary author (KC),
using the following criteria: 0 = negative; 1 = equivocal/interpretable; 2 = weak positive; 3 = strong positive.

Breast Tissue Microarrays

Breast tumors were collected and studied using Health
Insurance Portability and Accountability Act (HIPAA)-compliant
Stanford University Medical Center institutional review board
(IRB) approval. The Stanford IRB waived the need for written
consent from the participants due to the use of archival tissue. This
study is reported according to the Recommendations for Tumor
Marker Prognostic Studies (REMARK) criteria [19]. Two tissue
microarrays comprised of formalin-fixed paraffin embedded tissues
were used: the first microarray (TA-221) contained 283 primary
breast carcinomas and control specimens [20,21], and the second
microarray (TA-248) contained 110 metastases from the primary
breast cancers on the first array as well as control specimens [22].
Of the 110 metastases, 36 different cases were represented (some
cases had multiple metastases). For each of the primary breast
carcinomas, clinicopathologic features including metastasis, hor-
mone status (ER, PR, and HER2/neu), and Ki67 proliferation
index had previously been identified (Table 1). Not all cases had all
data available. Statistics were performed using a two-tailed Fisher
exact test and Chi-squared test. Serial sections of 4 μm were cut from
the tissue microarray block and used for in situ hybridization and
immunohistochemical analysis.

Outcome data for the 243 of the 283 breast cancer cases
represented in TA-221 was obtained through the Stanford Cancer
Registry. The median follow-up for living patients is 1897 days (62
months), with a range of 325 to 3456 days. GraphPad Prism was
used to compute Kaplan–Meier survival curves. The log-rank test
for trend p-value was calculated to assess the probability that there
was a trend in survival scores across the groups. Overall survival
(OS) was defined by death from any cause.

Results

LncRNA Scoring

In order to determine if lncRNA can be measured in formalin-
fixed paraffin embedded tissue, probes of 400 to 500 nucleotides
based upon unique lncRNA sequences to HOTAIR, ncHoxA1, and
ncHoxD4 were created. Using RNA in situ hybridization, these
probes were hybridized to the breast carcinoma tissue microarrays.
Figure 1 depicts representative staining of four breast lesions with
these in situ hybridization probes. The RNA probes stained
predominantly as dot-like patterns in the cytoplasm surrounding
the nucleus, as well as scattered dots within the nucleus. This
pattern is similar to previous experiments that we have performed
and reported in Gupta et al. in which we validated the use of these

| Feature     | N     |
|-------------|-------|
| Metastases  | 109/261 (42%) |
| ER+         | 194/236 (82%) |
| PR+         | 170/236 (72%) |
| Her2>2.2 Her2<1.8 | 19/222 (8.6%) |
| Ki67>10% Ki67≤10% | 134/237 (57%) |

Table 1. Clinicopathologic features of primary breast carcinoma from breast tissue microarray.

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probes [17] (please see Supplementary Figure 7 in Gupta et al. [17]). In that journal article, we transduced HOTAIR into breast cancer cell line MDA-MB-231 which allowed stable overexpression of HOTAIR greater than 1000 fold. A cell line with no HOTAIR expression was also used as a control. These cell lines were implanted into mice and allowed to form lung metastases, which were then formalin-fixed and embedded into paraffin. RNA in situ hybridization of HOTAIR in the HOTAIR over-expressing cell line demonstrated nuclear and cytoplasmic staining that was specific to only those cells that were transduced; HOTAIR was not identified in the surrounding tissues or in the tissues that were transduced with the HOTAIR non-expressing cell line.

On the breast carcinoma tissue microarrays, the probes were scored on a two or three-tiered scale: ncHoxA1 and ncHoxD1 were scored negative versus positive; HOTAIR, which demonstrated a wider dynamic range of staining intensity, was scored negative versus weakly positive versus strongly positive. Of the 283 lesions on the primary breast carcinoma tissue microarray, unequivocal scores were obtained in 221/283 (78%) HOTAIR, 118/283 (42%) ncHoxA1, and 142/283 (50%) ncHoxD4 carcinomas. ncHoxA1 and ncHoxD4 had the same expression score in the same tissue sample 92.66% of the time ($p<0.0001$). HOTAIR and ncHoxD4 had the same score in the same tissue 68.4% of the time ($p<0.0004$). HOTAIR and ncHoxA1 had the same score in the same tissue 53.1% of the time.

EZH2 Scoring

EZH2 is a Polycomb group protein known to interact with lncRNA, including HOTAIR [17]. In order to determine if all three lncRNA HOTAIR, ncHoxA1, and ncHoxD4 co-express with EZH2, and to help determine the roles of both the lncRNA and Polycomb protein in breast carcinoma, EZH2 protein immunohistochemical staining was performed on the breast carcinoma microarrays. The EZH2 antibody staining was scored on a threetiered scale similar to HOTAIR. Of the 283 lesions on the primary breast carcinoma tissue microarray, unequivocal scores were obtained in 218/283 (77%). EZH2 had the same expression score

![Figure 1. Expression levels in four different breast lesions.](image)

Four different breast lesions, in rows, are stained each for hematoxylin and eosin (H&E), and RNA in situ probes for HOTAIR, ncHoxA1, and ncHoxD4, in columns. Expression levels of ncHoxA1, and ncHoxD4 are depicted as negative (−) and positive (+). For HOTAIR, expression levels are depicted as negative (−), weakly positive (+), and strongly positive (++). doi:10.1371/journal.pone.0047998.g001
In the same tissue sample as ncHoxA1 40.4% of the time, ncHoxD4 47.7% of the time, and HOTAIR 75.7% the time ($p<0.0001$).

**Clinicopathologic Correlation**

Outcomes of breast cancer depend on a number of variables, including the presence of metastasis, hormone receptor status, and proliferation rate. In the breast carcinoma tissue microarray employed, each primary breast carcinoma had known clinicopathologic data including metastasis, ER status, PR status, HER2/neu status, and K67 for proliferation index. Though EZH2 levels are known to significantly correlate with negative ER status, negative PR status, lymph node status, and tumor cell proliferation [6,8,11], and HOTAIR levels correlate with eventual metastasis and death [17], other clinicopathologic correlations between HOTAIR and the other two lncRNAs (ncHoxA1 and ncHoxD4) are unknown. Using the known clinicopathologic data from the breast carcinoma microarray, these correlations can be determined. As depicted in Table 2, strongly positive HOTAIR expression significantly correlated with ER positivity ($p=0.026$) and PR positivity ($p=0.0004$). Under the usual proliferation rate divisions of $\leq 10\%$ and $>10\%$, HOTAIR did not significantly correlate with proliferation index. ncHoxA1 did not significantly correlate with any clinical data, but expression was higher with a higher proliferation index ($p=0.222$). Likewise, ncHoxD4 did not significantly correlate with any clinical data, but had higher expression with PR positivity ($p=0.288$). However, when ncHoxA1 and ncHoxD4 had correlating expression levels, the proliferation rate was more often increased ($p=0.043$), especially when they both had increased expression ($p=0.052$). Alone, lncRNA and EZH2 expression levels did not significantly correlate with metastasis.

Strongly positive EZH2 was confirmed to correlate with ER negativity ($p<0.0001$), PR negativity ($p<0.0001$), and proliferation index ($p<0.0001$). In addition, strongly positive EZH2 scores correlated with the lack of Her2 overexpression ($p=0.004$) and triple negative breast carcinomas ($p=0.0014$).

**Primary Versus Metastatic Carcinoma**

Though both HOTAIR lncRNA and EZH2 protein have been found to be overexpressed in both primary and metastatic breast carcinomas [8,17], their expression in matched primary and metastatic cancers has yet to be explored. As formalin-fixed paraffin embedded tissue can now be employed to measure lncRNA qualitatively, matched primary and metastatic breast carcinomas can more easily be identified. A metastatic breast carcinoma tissue microarray containing 110 metastatic lesions matched to primary breast carcinomas on the first breast cancer microarray was used to determine the expression of both HOTAIR and EZH2 in the matched pairs. On the metastatic breast carcinoma tissue microarray, unequivocal scores were obtained in 81/110 (74%) HOTAIR-stained lesions, and 101/110 (92%) EZH2-stained lesions. For HOTAIR, 11 metastases were negative, 49 were weakly positive, and 22 were strongly positive; in reference, on the primary breast carcinoma array, 40 carcinomas were negative, 131 were weakly positive, and 50 were strongly positive. Fifty four (54) pairs were present in which both the primary and the metastatic carcinoma had unequivocal scores. When pairing the primary breast carcinoma with its metastatic focus/foci, there was a significant enrichment for increased HOTAIR expression over equivalent or decreased expression, with nine (9) pairs having decreased HOTAIR expression at the metastatic focus (17%), 17 pairs having equivalent expression (31%), and 28 pairs having increased expression (52%) ($p=0.0064$). Figure 2, row 1, demonstrates one such carcinoma pair in which HOTAIR expression is increased in metastases compared to the primary carcinoma, while row 2 demonstrates a carcinoma with decreased HOTAIR expression in the metastases.

For EZH2, 4 metastases were negative, 59 were weakly positive, and 38 were strongly positive; in reference, on the primary breast carcinoma array, 24 carcinomas were negative, 159 were weakly positive, and 35 were strongly positive. Eighty-two (82) pairs were present in which both the primary and the metastatic carcinoma had unequivocal scores. When pairing the primary breast carcinoma with its metastatic focus/foci, there was a significant enrichment for equivalent or increased EZH2 expression versus decreased expression, with six (6) pairs having decreased EZH2 expression at the metastatic focus (7%), 43 pairs having equivalent expression (52%), and 33 pairs having increased expression (40%) ($p<0.0001$). Figure 2, row 4, demonstrates increased EZH2 staining in metastatic foci compared to their matched primary carcinoma, and row 3 demonstrates a carcinoma with decreased EZH2 expression in the metastatic foci.

**Overall Survival**

Gupta et al. [17] identified an interdependency between HOTAIR and EZH2 in promoting cancer invasiveness. In order to determine if expression of the lncRNA and protein together had an influence on patient outcome, the individual scores on the primary breast cancer tumor array were stratified into five groups as follows: both EZH2 and HOTAIR negative scores, one negative and one positive score, both EZH2 and HOTAIR weakly positive scores, one weak positive and one strong positive score, and both EZH2 and HOTAIR strongly positive scores. As outcome data was available for individuals who were sampled on this tissue microarray, a Kaplan Meier curve was constructed based on these five groups (Figure 3). Of the 243 cases with outcome data, ...
38 did not survive. Of these 243 cases, both HOTAIR and EZH2 expression levels were available in 159 individuals. As illustrated by Figure 3, strong EZH2 expression together with strong HOTAIR expression correlates with a trend toward worse survival (logrank test for trend p-value 0.0739), though only six samples are in this group.

**Discussion**

RNA in situ Hybridization on Formalin-fixed Paraffin Embedded Tissue

This is the first study to show that RNA in situ hybridization of formalin-fixed paraffin embedded (FFPE) clinical material can be used for qualitative measurements of long non-coding RNAs. In creating the lncRNA probes, multiple difficulties were broached. LncRNAs can derive function from forming secondary structures, including hairpins loops. These secondary structures can sterically hinder the ISH probe hybridization. We were able to anticipate the sequences that form secondary structures through detailed sequence analyses of the lncRNAs including phylogenetic analysis for conserved sequences. We avoided sequences that had high evolutionary conservation, as they may be preferentially involved in tertiary RNA structures that could be difficult to hybridize to in a FFPE environment. Using this approach we developed multiple probes that targeted different parts of the lncRNA sequence. In addition, lncRNAs are not only known to have decreased

![Figure 2. Expression of HOTAIR and EZH2 in primary versus metastatic carcinoma.](image-url)
expression levels compared to protein-coding genes, but also to have more tissue specificity in their expression patterns [15], so we had to develop a probe that would be able to identify low expression levels. The specificity of the probe for HOTAIR was verified in our prior report [17], in which a breast cancer cell line MDA-MB-231 was transduced with HOTAIR. The cell line was implanted into mice and allowed to form lung metastases, which were then fixed in formalin and embedded into paraffin. RNA in situ hybridization of HOTAIR in the resulting FFPE tissue demonstrated staining in only those cells that were transduced.

RNA probes for ncHoxA1, ncHoxD4, and HOTAIR were created and found to create a dot-like pattern in the cytoplasm surrounding the nucleus upon in situ hybridization (Figure 1). Interestingly, lncRNA in situ hybridization did not specifically localize in the nucleus, whereas EZH2 immunohistochemistry did. This lncRNA staining pattern is similar to the nuclear and cytoplasmic staining pattern in our previous report in which HOTAIR was transduced into breast cancer cell line MDA-MB-231 [17]. Though this current project was not using cell lines, the finding of nuclear and cytoplasmic HOTAIR is consistent. Of note, single molecule RNA FISH against HOTAIR in primary lung and foot fibroblast cells showed both nuclear and cytoplasmic localization [16], and recent chromatin immunoprecipitation experiments have localized HOTAIR in conjunction with PRC2 complex on chromatin in cancer cells [14]. Interestingly, the latter study [14] showed that efficient detection of chromatin-localized RNA required nonreversible glutaraldehyde crosslinking, but was lost with reversible formaldehyde crosslinking. Thus, the current FFPE in situ hybridization experiments may have insufficient resolution or efficiency to strongly detect the nuclear lncRNA signal.

Long non-coding RNA functions are still being determined, and many methods to help explore their roles rely on fresh tissue which is limited in supply. Formalin-fixed paraffin embedded (FFPE) tissue is used worldwide for tissue storage, preserves tissue architecture, and is kept indefinitely, so long-term clinical follow-up is possible. Thus, FFPE is a valuable resource for investigating not just lncRNA but other RNA. In addition, any cancer, not just breast cancer, can be studied using in situ hybridization probes for lncRNAs. RNA in situ hybridization of FFPE tissue offers a method to make observations on lncRNAs that may influence the cancer epigenome in a tissue-based technique.

LncRNA and Polycomb Proteins

From prior studies [18], HOTAIR is known to recruit PcG proteins to chromatin through its interaction with the PRC2 complex, and, specifically, in the Gupta et al. paper [17], HOTAIR overexpression induces localization of PRC2 subunit EZH2 onto many genes in breast carcinoma. In this study, using in situ hybridization to evaluate HOTAIR lncRNA expression and immunohistochemistry to evaluate protein expression of EZH2, we confirm that EZH2 and HOTAIR are coexpressed in breast cancer, as they have the same expression score 75.7% of the time (p<0.0001). Thus, in breast carcinoma, there is increased HOTAIR expression and increased EZH2 expression. Although EZH2 did not have significant correlating expression scores with either ncHoxA1 or ncHoxD4, HOTAIR and ncHoxD4 did have the same score 68.4% of the time (p<0.0004). Lastly, ncHoxA1 and ncHoxD4 had the same expression score 82.6% of the time (p<0.0001). Correlating scores between these lncRNAs in cancer suggest that, in some way, they may be acting in concert to modify the epigenome. To illustrate this idea, alone, ncHoxA1 or ncHoxD4 expression did not significantly correlate with proliferation index. However, when these two lncRNAs had correlating expression levels, the proliferation rate was more often increased (p = 0.043), especially when they both had increased expression (p = 0.052). Thus, ncHoxA1 and ncHoxD4 may act in parallel pathways or jointly to bring retarget PcG to genes.

Clinicopathologic Correlations

The current study provides evidence that expression levels of lncRNA do trend with some clinicopathologic data, such that increased ncHoxA1 trends with proliferation rate, and ncHoxD4 trends with positive PR receptor status. In addition, the strong relationship between EZH2 and proliferation index in carcinoma is reconfirmed. However, it is interesting that strongly positive HOTAIR expression significantly correlated with ER and PR positivity, whereas strongly positive EZH2 significantly correlated with ER and PR negativity, even though HOTAIR and EZH2 had correlating expression levels. HOTAIR has been identified to interact with EZH2 and SUZ12 components of the PRC2 complex and to target the complex to silence transcription of genes at the HOXD locus [18]. In addition, HOTAIR has also been found to induce the localization of PRC2 subunits on 854 genes [17]. Thus, so far, the functions of HOTAIR depend on its interaction with PRC2. The PRC2 complex, however, is found to bind other lncRNAs besides HOTAIR; Khalil et al. [16] and Zhao et al. [23] identified thousands of lncRNAs, of which 24% associate with PRC2. Hence EZH2 in PRC2 has a much larger genomic range than that of HOTAIR. EZH2 has also been hypothesized to have neoplastic properties which are independent of the PRC2 complex [24]. In one such paper, Shi et al. [25] identified EZH2 to activate transcription in genes which are targets of estrogen and Wnt signaling pathways; this activity of EZH2 was not related to PRC2 complex activity. Thus, the broader outreach of EZH2 may allow it to better activate or influence genes involved in negative estrogen receptor expression breast carcinoma. HOTAIR may just...
be limited to its role in silencing the HOXD locus genes or the specific 654 genes, which could possibly lead to estrogen receptor positivity.

In this paper, we confirm that EZH2 expression correlated with ER and PR negativity. Unlike previous reports [5], we were able to find a significant association between the lack of HER2 overexpression \( p=0.004 \) and triple negative breast carcinomas \( p=0.004 \). However, this finding is not too surprising, since EZH2 has been recently directly linked to BRCA1. In Gonzalez et al. [26], invasive ER-negative breast carcinomas showed EZH2 overexpression and BRCA1 downregulation. Downregulation of EZH2 expression in breast cancer cells led to decreased proliferation, delayed cell-cycle transition, and upregulation of the BRCA1 protein. In addition, a BRCA1 knockout rescued the effects of EZH2. Since most BRCA1-related breast carcinomas (heritable mutations or sporadic defects) are frequently triple negative cancers [reviewed in Diaz et al. [27]] with BRCA1 protein downregulation, EZH2 is most likely upregulated in these carcinomas.

Metastatic Breast Carcinoma and Survival

Though Gupta et al. [17] identified increased HOTAIR expression in a cross-sectional study of primary and metastatic breast carcinoma, and Kleer et al. [8] identified high levels of EZH2 in breast cancer metastases, this is the first study to look at matched primary and metastatic breast cancers and correlate EZH2 protein and HOTAIR expressions in archival material. In the first part of this study, neither HOTAIR expression nor EZH2 expression correlated with the clinicopathologic feature of metastases. However, upon comparing pairs of primary versus metastatic carcinoma, both HOTAIR and EZH2 expressions more often had equivalent (31% and 52%), respectively or increased expression (52% and 40%, respectively) in the metastases compared to the primary carcinoma. Thus, the data support increased expression of both EZH2 and HOTAIR in the metastatic cancers, compared to primary carcinoma.

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