A STUDY ON THE HISTONE METHYLTRANSFERASE ENHANCER OF ZESTE HOMOLOG 2 EXPRESSION IN BREAST CARCINOMA

Pathology

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ABSTRACT

The over expression of EZH2 in Breast Carcinoma. Compare EZH2 expression in different immunophenotypes of breast carcinoma(basal, luminal, triple-negative). The study EZH2 expression level in association to staging and grading of breast carcinoma Patients attending the Dept of Pathology, Sri Krishna Medical Collage Muzaffarpur, Bihar with epithelial breast tumor. We found that EZH2 in Breast Carcinoma was significantly associated with grade of the tumour. EZH2 was significantly associated with stage of the tumour. It was found that EZH2 was significantly correlated with ki67 in Breast Carcinoma patients. Triple negative Breast Carcinoma patient was significantly associated with high grade of EZH2 tumour.

KEYWORDS

EZH2 expression, Breast Carcinoma, Staging & Grading

INTRODUCTION

Brief account of the present knowledge or understanding: Breast Carcinoma comprises an extraordinarily diverse group of diseases in terms of histology and clinical behaviour and can be classified according to immunophenotypic features. ER-positive breast carcinoma is defined as >1% of tumor cells being positive for ER, and usually these are also positive for PR. HER-2 positive carcinoma shows either protein expression detected by IHC or HER-2 gene amplification by FISH. Triple negative breast carcinoma is defined as combined negativity for ER, PR, HER-2 which constitutes about 20% of breast cancer. High expression of a certain marker, EZH2 is related to unfavourable outcome to tamoxifen in metastatic breast cancer and is an independent predictor of survival in women with breast cancer. EZH2 is a Polycomb Group protein, transcriptional repressor involved in cellular memory, which is homologous to Drosophila Enhancer of Zeste and involved in gene silencing. There is a functional link between dysregulated cellular memory, transcriptional repression and neoplastic transformation. EZH2 has enzymatic activity and functions as histone H3 methyltransferase. PCG protein belong to atleast two multimeric complexes PRC-1 and EED-EZH2. EED protein interacts directly with type 1 histone deacetylases in mammalian cells and in Drosophila. EED/EZH2 complex also methylate H3-K9 and K27 with a strong predilection for the latter. Methylation of both is thought to be involved in targeting the PRC1 complex to specific genetic target loci. EED-EZH2 recruits type-1 HISTONE DEACETYLASE. Activity is abrogated in presence of HISTONE DEACETYLASE inhibitor TSA and SAHA.

Through our gene expression profiling studies, we identified EZH2 as being over expressed in metastatic prostate cancer¹. In clinically localized prostate cancer, EZH2 was found to be predictive of poor outcome postprostatectomy (i.e., biochemical recurrence or metastasis). EZH2 is a Polycomb Group (PcG) protein homologous to Drosophila Enhancer of Zeste and involved in gene silencing². PcG proteins are presumed to function in controlling the transcriptional memory of a cell². Dysregulation of this gene silencing machinery can lead to cancer³. In the context of prostate cancer, we provided evidence that EZH2 functions as a transcriptional repressor, and inhibition of EZH2 blocks prostate cell growth.

Enhancer of zeste homolog 2 (EZH2) is an evolutionary conserved gene identified in many species, sharing similar structural motifs and domains. As a histone methyltransferase, EZH2 has been identified as a catalytic subunit of PRC2 for tri-methylation of histone H3 at Lys 27 (H3K27me3) by SET domain in its C-terminus, which silences targeted genes and is involved in various biological functions (e.g. cell cycle, cell proliferation, cell differentiation, etc.). The role of EZH2 in cancer progression was also considered and unraveled. EZH2 is aberrantly over expressed in various malignant tumors, such as prostate cancer, breast cancer, and ovarian cancer. EZH2 mediates H3K27me3 and functions as a critical factor in promoting tumor

growth and metastasis in many malignant tumor models. In addition, EZH2 gain or loss of function mutations have also been discovered in various cancer³. Accumulating studies indicate that inhibition of EZH2 by small molecular inhibitors or gene knockdown results in reduced cancer cell growth and tumor formation⁴. Beyond playing its role in a PCR2-dependent manner as a histone modifier, EZH2 also acts in a PCR2 and histone independent manner in cancer. For example, EZH2 can methylate non-histone protein STAT3 in glioblastoma, and participate in androgen receptor-associated complexes in castration-resistant prostate cancer (CRPC) as a co-activator⁵. The diverse functions of EZH2 in cancer derive from its genetic, transcriptional, post-transcriptional and post-translational regulation in different circumstances and different types of cancer⁴.

To study the over expression of EZH2 in Breast Carcinoma.

To study EZH2 expression level in association to staging and grading of breast carcinoma

MATERIALS AND METHODS:

DEFINITION OF POPULATION-

Patients attending the Dept of Pathology, Sri Krishna Medical Collage Muzaffarpur, Bihar with epithelial breast tumor.

INCLUSION CRITERIA-

- I. Epithelial breast tumor with known Estrogen, Progesterone, Her2neu receptor expression.
- II. Type of mastectomy whether modified or radical to be decided by the surgeon.

EXCLUSION CRITERIA-

- I. Benign breast lesion
- II. Mastectomy due to cosmetic reasons.
- III. History of trauma.
- IV. History of any other stromal tumor.
- V. Any history of previous chemo or radiotherapy for other type of cancer.
- VI. History of neo-adjuvant chemotherapy or radiotherapy in breast carcinoma tissue specimens.

RESULT AND ANALYSIS

In our study, 6(14.6%) patients were 31-40 years old, 17(41.5%) patients were 41-50 years old, 9(22.0%) patients were 51-60 years old and 9(22.0%) patient were 61-70 years old. In our study, 22(53.7%) patients had Left side and 19(46.3%) patient had Right side. In our study, 1(2.4%) patients had CARCINOMA WITH MEDULLARY FEATURES, 37(90.2%) patients had IDC NOS, 1(2.4%) patients had LC NOS and 2(4.9%) patient had MC. In our study, 6(14.6%) patients had T1N0M0, 2(4.9%) patients had T1N1M0, 3(7.3%) patients had T1N2M0, 5(12.2%) patients had T2N0M0, 3(7.3%) patients had T2N2M0, 8(19.5%) patients had T3N0M0, 4(9.8%) patients had T3N1M0 3(7.3%) patients had T3N2M0, 2(4.9%) patients had

T4aN1M0, 2(4.9%) patients had T4AN2M0, 1(2.4%) patients had T4AN2M0 and 2(4.9%) patient had T4BN2M0. In our study, 6(14.6%) patients had stage I 15(36.6%) patients had stage II and 20(48.8%) patient had stage III. In our study, 6(14.6%) patients had grade I, 16(39.0%) patients had grade II and 19(46.3%) patient had grade III. In our study, 18(43.9%) patients had ER NEG and 23(56.1%) patient had ER POS. In our study, 19(46.3%) patients had PR NEG and 22(53.7%) patient had PR POS.

Neves Filho EH et al ⁶(2020) found that EZH2 is an important epigenetic regulator, but its role in diffuse large B-cell lymphoma (DLBCL) pathogenesis and its relationship with MYC, BCL2, and TP53 expression, chromosomal rearrangements, and clinical features are still poorly understood. 9(22.0%) patients had A, 10(24.4%) patient had BH-, 4(9.8%) patient had BH+, 6(14.6%) patient had NLH+ and 12(29.3%) patient had TN. therapeutic response to R-CHOP in a series of 139 DLBCL cases. EZH2 positivity was associated with MYC and TP53 expression ($p = 0.0002$ and $p = 0.0000$, respectively) and to high proliferative index (Ki67>70%, $p = 0.0082$). TP53 immunodetection showed associations with EZH2 expression in the germinal center (GC) DLBCL. EZH2 expression had no impact on therapeutic efficacy in R-CHOP-treated patients.

Boostani F et al ⁷(2018) found that the overexpression and mutation status of EZH2 affect cancer progression and patient outcome in several human tumors. the mean EZH2 % (mean $\pm$ s.d.) of patients was 51.2195 $\pm$ 25.0195. Specimens were analyzed immunohistochemically for EZH2, estrogen receptor, progesterone receptor, Ki-67, P53, and human epidermal growth factor receptor 2 (HER2) expressions. Regression analysis was performed to calculate hazard ratios (HRs) and 95% CIs. Kaplan-Meier and Cox regression models were used to estimate the overall survival (OS) and disease-free survival (DFS). EZH2 expression did not have any significant effect on the OS, although the mean OS in high EZH2 expression was shorter than for those with low EZH2 expression (655 vs 787 days; log-rank $P=0.336$). Guo S et al ⁸(2016) found that enhancer of zest homolog 2 (EZH2), a histone 3 methyltransferase, is associated with aggressive behavior of many tumors and is a promising target of molecular therapy. 27(65.9%) patients had HER 2 NEU NEG and 14(34.1%) patient had HER 2 NEU POS. Of these cases, 138 (61.1 %) were defined as EZH2-overexpressing with a multiplicative score > 3. EZH2 expression was inversely related to the status of ER and PR (Chi-square, $p < 0.001$). The percentage of EZH2 overexpression in ER-positive breast carcinoma with a high proliferative index or HER2-positive cases were 61.2 and 74 %, respectively. In summary, triple-negative breast carcinoma showed the highest overexpression of EZH2. EZH2 overexpression is associated aggressive pathologic features including high nuclear grade, high proliferative index, and positivity of HER2 of breast carcinoma.

We showed in our study, 27(65.9%) patients had HER 2 NEU NEG and 14(34.1%) patient had HER 2 NEU POS. In our study, 9(22.0%) patients had A, 10(24.4%) patient had BH-, 4(9.8%) patient had BH+, 6(14.6%) patient had NLH+ and 12(29.3%) patient had TN. In our study, 1(12.5%) patients had F19, 1(12.5%) patient had 14 & <=20 => 2, 1(12.5%) patient had >20% => 3, 1(12.5%) patient had A=LUMINAL A, 1(12.5%) patient had BH+=LUMINAL B(HER 2 NEU POS), 1(12.5%) patient had BH-=LUMINAL B(HER 2 NEU NEG), 1(12.5%) patient had Ki 67 <=14% =>1, 1(12.5%) patient had NLH+=NON LUMINAL HER 2 NEU POSITIVE and 1(12.5%) patient had TN=TRIPLE NEGATIVE. In above table showed that the mean Age (mean $\pm$ s.d.) of patients was 51.2683 $\pm$ 9.3676. In above table showed that the mean Age in years (mean $\pm$ s.d.) of patients was 2.5122 $\pm$ 1.0030. In above table showed that the mean Ki 67 (mean $\pm$ s.d.) of patients was 40.4878 $\pm$ 22.4668. In above table showed that the mean GRADE Ki 67 (mean $\pm$ s.d.) of patients was 2.4878 $\pm$.8100. In above table showed that the mean EZH2 % (mean $\pm$ s.d.) of patients was 51.2195 $\pm$ 25.0195. In above table showed that the mean GRADE EZH2 (mean $\pm$ s.d.) of patients was 2.2683 $\pm$.7754.

We found in Group-1, 1(12.5%) patients were 31-40 years old, 3(37.5%) patients were 41-50 years old, 3(37.5%) patients were 51-60 years old and 1(12.5%) patients were 61-70years old. In Group-2, 1(7.1%) patients were 31-40 years old, 5(35.7%) patients were 41-50 years old, 4(28.6%) patients were 51-60 years old and 4(28.6%) patients were 61-70years old. In Group-3, 4(21.1%) patients were 31-40 years old, 9(47.4%) patients were 41-50 years old, 2(10.5%) patients were 51-60 years old and 4(21.1%) patients were 61-70years

old. Association of Age in years vs group was not statistically significant ($p=0.6360$). In Group-1, 5(62.5%) patients were Left side and 3(37.5%) patients were Right side. In Group-2, 6(42.9%) patients were Left side and 8(57.1%) patients were Right side. In Group-3, 11(57.9%) patients were Left side and 8(42.1%) patients were Right side. Association of SIDE vs group was not statistically significant ($p=0.5929$). In Group-1, 8(100.0%) patients had IDC NOS. In Group-2, 13(92.9%) patients had IDC NOS and 1(7.1%) patients had LC NOS. In Group-3, 1(5.3%) patients had CARCINOMA WITH MEDULLARY FEATURES, 16(84.2%) patients had IDC NOS and 2(10.5%) patients were MC. Association of histopathological types vs group was not statistically significant ($p=0.4726$).

Our study showed that in Group-1, 4(50.0%) patients had T1N0M0, 1(12.5%) patients had T1N1M0, 2(25.0%) patients had T1N2M0 and 1(12.5%) patients had T2N0M0. In Group-2, 1(7.1%) patients had T1N0M0, 1(7.1%) patients had T1N1M0, 1(7.1%) patients had T1N2M0, 4(28.6%) patients had T2N0M0, 3(21.4%) patients had T2N2M0, 1(7.1%) patients had T3N0M0 and 3(21.4%) patients had T3N1M0. In Group-3, 1(5.3%) patients had T1N0M0, 7(36.8%) patients had T3N0M0, 1(5.3%) patients had T3N1M0, 3(15.8%) patients had T3N2M0, 2(10.5%) patients had T4aN1M0, 2(10.5%) patients had T4AN2M0, 1(5.3%) patients had T4AN2M0 and 2(10.5%) patients had T4BN2M0. Association of TNM vs group was statistically significant ($p=0.0015$). In Group-1, 4(50.0%) patients had stage I, 2(25.0%) patients had stage II and 2(25.0%) patients had stage III. In Group-2, 1(7.1%) patients had stage I, 6(42.9%) patients had stage II and 7(50.0%) patients had stage III. In Group-3, 1(5.3%) patients had stage I, 7(36.8%) patients had stage II and 11(57.9%) patients had stage III. Association of stage vs group was statistically significant ($p=0.0370$). In Group-1, 4(50.0%) patients had grade I, 2(25.0%) patients had grade II and 2(25.0%) patients had grade III. In Group-2, 1(7.1%) patients had grade I, 6(42.9%) patients had grade II and 7(50.0%) patients had grade III. In Group-3, 1(5.3%) patients had grade I, 8(42.1%) patients had grade II and 10(52.6%) patients had grade III. Association of grade vs group was statistically significant ($p=0.0403$).

Yoo KH et al ⁹(2012) found that histone modifications are thought to control the regulation of genetic programs in normal physiology and cancer. Abnormal EZH2 expression has been associated with various cancers including breast cancer. Here, therediscuss the contribution of EZH2 and the PRC2 complex in controlling the H3K27 methylation status and subsequent consequences on genomic instability and the cell cycle in breast cancer cells. There also discuss distinct molecular mechanisms used by EZH2 to suppress BRCA1 functions.

Gong Y et al ¹⁰(2011) found that enhancer of zeste homolog 2 (EZH2), a member of polycomb group proteins, Univariate survival analysis indicated that patients who had EZH2-positive IBC had a significantly lower 5-year OS rate than patients who had EZH2-negative IBC ($P = .01$).

We found in Group-1, 2(25.0%) patients had NEG and 6(75.0%) patients had POS. In Group-2, 5(35.7%) patients had NEG and 9(64.3%) patients had POS. In Group-3, 11(57.9%) patients had NEG and 8(42.1%) patients had POS. Association of ER vs group was not statistically significant ($p=0.2174$). In Group-1, 2(25.0%) patients had NEG and 6(75.0%) patients had POS. In Group-2, 5(35.7%) patients had NEG and 9(64.3%) patients had POS. In Group-3, 12(63.2%) patients had NEG and 7(36.8%) patients had POS. Association of PR vs group was not statistically significant ($p=0.1187$). In Group-1, 5(62.5%) patients had NEG and 3(37.5%) patients had POS. In Group-2, 7(50.0%) patients had NEG and 7(50.0%) patients had POS. In Group-3, 15(78.9%) patients had NEG and 4(21.1%) patients had POS. Association of HER 2 NEU vs group was not statistically significant ($p=0.2172$). In Group-1, 3(37.5%) patients had A, 1(12.5%) patients had BH-, 2(25.0%) patients had BH+, 1(12.5%) patients had NLH+ and 1(12.5%) patients had TN. In Group-2, 5(35.7%) patients had A, 3(21.4%) patients had BH-, 1(7.1%) patients had BH+, 4(28.6%) patients had NLH+ and 1(7.1%) patients had TN. In Group-3, 1(5.3%) patients had A, 6(31.6%) patients had BH-, 1(5.3%) patients had BH+, 1(5.3%) patients had NLH+ and 10(52.6%) patients had TN. Association of IMMUNO vs group was statistically significant ($p=0.0254$). In Group-1, 1(50.0%) patients had Ki 67 <=14% =>1 and 1(50.0%) patients had TN=TRIPLE NEGATIVE. In Group-2, 2(25.0%) patients had >20% => 3 and 2(25.0%) patients had NLH+=NON LUMINAL HER 2 NEU POSITIVE. In Group-3, 1(25.0%) patients had >14 & <=20 => 2, 1(25.0%) patients had A=

LUMINAL A, 1(25.0%) patients had BH+=LUMINAL B(HER 2 NEU POS), 1(25.0%) patients had BH-=LUMINAL B(HER 2 NEU NEG. Association of F19 vs group was not statistically significant ($p=0.3134$).

CONCLUSION

We found that EZH2 in Breast Carcinoma was significantly associated with grade of the tumour. EZH2 was significantly associated with stage of the tumour. It was found that EZH2 was significantly correlated with ki67 in Breast Carcinoma patients. Triple negative Breast Carcinoma patient was significantly associated with high grade of EZH2 tumour.

Table: Distribution of ER, PR, STAGE and GRADE.

		Frequency	Percent
ER	NEG	18	43.9%
	POS	23	56.1%
	Total	41	100.0%
PR	NEG	19	46.3%
	POS	22	53.7%
	Total	41	100.0%
STAGE	I	6	14.6%
	II	15	36.6%
	III	20	48.8%
	Total	41	100.0%
GRADE	I	6	14.6%
	II	16	39.0%
	III	19	46.3%
	Total	41	100.0%

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