A Review on EZH2 and its Epigenetic Association with Breast Cancer

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Abstract: Enhancer of zeste homolog2 (EZH2), first identified as homolog of the Drosophila enhancer of zeste gene, is histone H3 lysine methyltransferase (H3K27me3), a component of polycomb group proteins (PRC2) that represses the gene expression by modifying the histones epigenetically, thereby silencing developmental regulatory elements in stem as well as cancer cells leading to repression of early differentiation marker genes. Although the mechanistic approach of involving EZH2 to cancer progression has not yet been clearly deciphered, its invasiveness and metastatic potential has been revealed by significant elevation of its expression in normal breast cancer cells after commencement of which a pre-cancerous state was found in morphologically normal breast cancer cells. The tissue microarray analysis of breast carcinomas has shown that EZH2 to be intimately associated with markers of tumor cell proliferation as well as with aggressive diseases. Till now, no demethylating agents have been recommended for treatment of patients, but an in-vitro study using 3-deazaneplanocin, which reduces histone modifications through methylation by reducing the levels of EZH2, has shown a significant reduction in cell proliferation in breast cancer cells. This further signifies the role of EZH2 as a transcriptional repressor. By analyzing methylation profiles of different subtypes of breast cancers like basal-like, luminal A & B, roles of EZH2 have been established in the development of breast cancers. Crosstalk of EZH2 with other silencing/regulating factors like histone deacetylases and miRNAs, have to be considered for evaluating for progression of cell proliferation in different cancer cells including breast cancer.

Keywords: Breast cancer, tumorigenesis, apoptosis, epigenetic, methylation.

INTRODUCTION

Breast cancer is the most prevalent and the most frequently diagnosed cancer in U.S. women, excluding skin cancers. Girl children born today have about 1 in 8 chance of developing breast cancer at some point during their life which comprises almost one third of all types of cancer which do happens in females. Although due to early detection and improved treatments, mortality rates caused by breast cancer have been continuously decreased since 1990, it still remains the second leading cause of cancer deaths in U.S. women between the ages of 40 and 55, which is exceeded only by deaths due to lung cancer [1]. It is projected that, about an estimated 226,870 new cases of invasive breast cancer will be diagnosed in U.S. women in the year 2012. In addition to invasive breast cancer, 63,300 new cases of carcinoma in situ (CIS) are also likely to be diagnosed. In the current year, about 39,510 U.S. women may die because of breast cancer [1].

Based on gene expression profiles and several microarray studies, breast tumors have been divided into five molecular subtypes- basal-like, luminal A, luminal B, human epidermal growth factor receptor 2 (EGFR2) and normal-like. All subtypes have been suggested to originate from different precursor cells and follow different progression pathways [2]. The most widely practiced grading system for diagnosis and prognosis of breast tumors is Nottingham grading system. The same is based on three parameters: extent of tubule formation, mitosis rate, and nuclear pleomorphism. According to this system, breast cancer tumors have been divided into three grades: a) well differentiated, b) moderately differentiated and c) poorly differentiated [3]. Poorly differentiated breast tumors are the most aggressive type of breast cancer which is highly invasive and metastatic being enriched with epithelial-mesenchymal transition (EMT) markers and characterized by deregulated signaling pathways. The other characteristic features include high proliferation rate as well as least differentiation markers. Although breast cancer is being investigated from different angles, including a lot of emphasis on various estrogen receptors, in recent year epigenetic investigation has been given lot of emphasis to address the issues of several aspects of breast cancer including tamoxifen resistance in ER/PR positive breast tumors.

Epigenetics is described as the branch of science which is accepted as a way to make in-depth studies of regulation of gene expression. It is also accepted as a widely preferred mode of investigation to unravel the mechanism of breast tumor metastasis in a situation like no heritable changes are detected. Significant advances have been made in recent years in the field of epigenetics and now it has been understood that specific regions of the genome can be

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activated/silenced by the processes like acetylation/ deacetylation/phosphorylation/methylation/ubiquitination/ SUMOylation/ADP ribosylation and nucleosome remodeling through various covalent and non-covalent modifications of chromatin.

In the above context, co-regulators have been given extraordinary emphasis to find out the possible causes of breast cancer tumorigenesis. One of the co-regulators which we are exploring in this review is polycomb group protein Enhancer of Zeste Homolog 2 (EZH2). EZH2 has been found to be a transcriptional co-repressor. Its involvement in a variety of human malignancies suggests that EZH2 can be a promising diagnostic biomarker of various carcinomas [4] including aggressive breast carcinoma. Analysis, expression and regulation of EZH2 alone will not reveal a clear picture *in vivo*, as crosstalk of all the chromatin modifiers finally leads to gene activation/deactivation. Significant association of EZH2 with cell cycle regulators like Human Epidermal Growth Factor Receptor2 (EGFR2), and p53 has been successfully established. In the above context, the polycomb protein complex to which EZH2 belongs is first reviewed which is followed by exploration of its extent of involvement in important signaling pathways and thus in tumorigenesis.

Polycomb Group Proteins

Polycomb group (PcG) proteins which were first discovered in fruit flies belong to a family of proteins possessing the ability to modify chromatin through various modifications like methylation and ubiquitination. Besides transcriptional silencing, these proteins play key roles in developmental patterning, X-chromosome inactivation and stem cell maintenance. PcG proteins function through two distinct protein complexes termed Polycomb repressive complex 1 (PRC1) and Polycomb-repressive complex 2 (PRC2) as shown in Figure **1**.

The composition of PRC1 complexes is more variable, consisting of several proteins like chromobox homolog 2 (CBX2), which binds H3K27me3;ring finger protein 1A (RING1A), or RING1B, which catalyzes ubiquitination; and polycomb ring finger oncogene (BMI1) or polycomb group ring finger 6/Mel18(PCGF2), which modulates ubiquitination [5]. The subunits of PRC1: BMI-1, RING1, and HPC2 are strongly expressed in invasive neoplastic cells of breast cancer [6]. PRC2 consists of four core subunits, enhancer of zeste homolog 2 (EZH2), embryonic ectoderm development (EED), suppressor of zeste 12 (SUZ12) and retinoblastoma binding protein4/7(also known as RbAp 48/46) Figure **1**.

During gene silencing processes mediated by PcG proteins, methylation of H3K27 by PRC2 is followed by ubiquitination of H2A at K119 by PRC1, which is then followed by HDAC inhibition thereby finally resulting in chromatin condensation and transcriptional repression [5].

EZH2 is the catalytic core-subunit of PRC2 which consists of a SET domain responsible for trimethylation of histone3 (H3K27me). H3K27 trimethylation blocks the sites of other transcriptional activating factors, resulting in gene repression independent of promoter. The EZH2 subunit lacks its enzyme function on its own; instead, it attains its histone methyltransferase activity only when combined with other two non- catalytic partners, EED and SUZ12. The effects of EZH2 mediated methylation of a pair of lysine amino acid residues at the 4^{th} and 9^{th} position at the N-terminal of H3, has been studied. Methylation of lysine 9 induces chromatin compaction and silences the expression of gene whereas methylation of lysine4 promotes chromatin decondensation and thus gene activation.

Association of EZH2 Mediated Methylation with Aggressive Breast Cancer

EZH2 was first shown to be associated with aggressive prostate cancer [7]. Association of EZH2 with breast cancer was first reported in 2003 where it was shown that the expression of EZH2 was increased in malignant tumors and that it promoted anchorage-independence and invasive growth *in vitro*, affecting the proliferation rate to a lesser extent [8].

E-cadherin, is an essential molecule which play important role in the establishment of homotypic adhesion junctions [9]. Normal epithelial cells are characterized by a strong and well-established network of cell-to-cell contacts that take cares of proper development and the functionality of epithelial structures. These tight cellular contacts restrict the ability of epithelial cells to move or migrate thus preventing from cell-invasion. In cancerous cells, specifically aggressive ones epithelial cells lose this restriction and proceeds to cell-invasion leading to metastasis.

E-cadherin has been experimentally shown to be downregulated during tumorigenesis [10-12]. EZH2 has been demonstrated to mediate transcriptional silencing

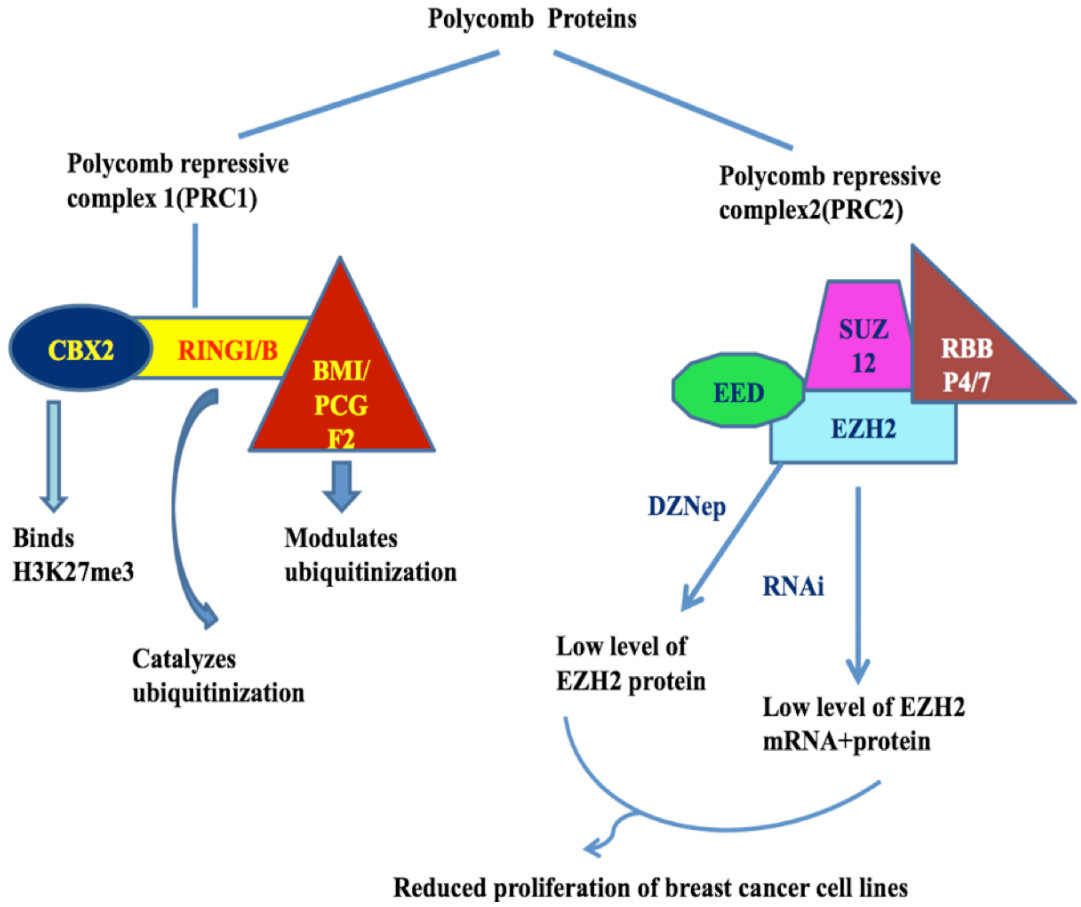

Figure 1: Polycomb group protein complexes: Polycomb group proteins function in two complexes PRC1 and PRC2. PRC1 is composed of three subunits namely CBX2, RINGI/B, and BMI/PCGF2 each with distinct role in chromatin modification. PRC2 consists of EED, SUZ12, EZH2, and RBBP4/7. Knockdown of EZH2 by RNA interference technique or by treatment with its inhibitor DZNep, leads to decreased proliferation rate in breast cancer cell lines demonstrating its potential role in aggressive breast cancer.

of E-cadherin by trimethylation of H3 lysine 27 which further gives strength to the possibility of significant association of EZH2 with aggressive breast cancer [13].

Recently in a study, epigenetic repression of a tumor suppressor gene kruppel-like factor (KLF) has been evidenced to be mediated by EZH2 through direct transcriptional repression [14]. Basically EZH2 has been found to be an important cell cycle regulator holding critical position in different cell cycle phases, as in S-phase entry and G2-M transition. The significant involvement of EZH2 in cell proliferation and thus in tumorigenesis is suggested on the basis of a study of human breast cancer using Ki-67 staining. The proliferation rate was figured out to be 21% in EZH2-positive tumors, whereas 6% in EZH2-negative cases [4]. Expression of markers of the basal epithelial phenotype of breast cancer, such as Cytokeratin5/6 (CK 5/6) and P-cadherin has also been shown to be strongly associated with EZH2.

Using immnunohistochemistry (IHC), EZH2 expression was investigated in a) BRCA1 mutant carriers who have increased risk of developing cancer and b) histological normal breast epithelial cells. Increased EZH2 expression was detected in the former. It has been reported that the breast cancer subtypes, express different methylation profiles [15]. Using ANOVA, variations in methylation frequencies in different breast cancer molecular subtypes, luminal A, luminal B, human epidermal growth factor receptor 2 (EGFR2) and normal-like was analyzed in various genes through CpG islands analysis. The methylation frequency of these CpGs was significantly different among the molecular subtypes [2]. These CpG islands were in particular found to be more frequently methylated in lumB subtype and less methylated in basal-like. To identify the differentially methylated genes, S-adenosine-methionine (SAM) analysis was done where it was found that the genes that were frequently methylated in lumB subtype are often

unmethylated among basal-like tumors, and the genes methylated in the basal-like group were more often unmethylated in the lumA subtype [16]. Basal-like tumors displayed significant increased expression levels of EZH2 compared with the other tumor subtypes which further signifies the role of methylation mediated by EZH2 in different tumor subtypes.

Activated p53 suppresses EZH2 gene expression by repressing its promoter. The repression of EZH2 promoter by p53 has been shown to depend on p53 transcriptional target p21 through RB/E2F pathway inactivation [17].

Regulation of tumor angiogenesis by EZH2 has also been studied. Using luciferase assay, EZH2 level has been shown to be increased by vascular endothelial growth factor (VEGF) in the tumor vasculature which was later confirmed when increase in EZH2 promoter activity was blocked with the VEGFR2 specific antibody DC101 [18]. EZH2, in turn, contributes to tumor angiogenesis by inactivating the anti-angiogenic factor, vasohibin1 (vash1) through methylation. Using bioinformatics tools, EZH2 has also been identified as a potential target of miR-26a. Overexpression of miR-26a suppressed the activity of a luciferase reporter construct fused with the Ezh23′-UTR in myogenesis and decreased Ezh2 mRNA expression [19].

EZH2 and Stem Cell Maintenance

Increased EZH2 expression in breast tumor initiating cells (BTICs) has been evidenced to be linked to decreased amount of critical DNA damage repair proteins such as RAD51 [20]. Decreased RAD51 expression leads to significant increase in spontaneous chromosomal break and chromosome instability, which can possibly lead to oncogenic translocation further contributing towards erratic behavior of cells in aggressive breast cancer.

Essential role of EZH2 in stem cell maintenance has also been studied in cancers other than breast cancer justifying its possible and important contribution towards cancerous cell promotion. Strong impairment of glioblastoma multiforme (GBM) cancer stem cell self-renewal *in vitro* and tumor-initiating capacity *in vivo* has been shown experimentally by targeted inhibition/ knockdown of EZH2 either by DZNep, the S-adenosylhomocysteine hydrolase inhibitor or short hairpin RNA (shRNA) [21]. Establishment of Ezh2-knockout embryonic stem cells has been experienced to be hard as Ezh2-deficient blastocysts displayed a reduced and impaired growth potential in a study focused to unravel the essential role for Ezh2 during early mouse development [22].

Recently haematopoietic stem cells have also been used to study the possible mechanism for EZH2-mediated chromatin modification leading to cancerous cell state. In the study, stem cell-specific genes of haematopoietic stem cells such as Evi-1 and Ntrk3 have been found to be regulated by EZH2-mediated methylation [23]. In an attempt to establish the significant role of EZH2 in differentiation, proliferation and maintenance, of neural stem cell (NSCs), the expression of Ezh2 was investigated in NSCs isolated from 14^{th} day embryonic mice *in vitro*. Expression of EZH2 was found to be higher in proliferating NSCs whereas reduced expression was seen in differentiating neural stem cells [24]. Also, EZH2 mediated dynamic repression of developmental pathways is thought to be responsible for maintaining murine embryonic stem cell pluripotency and plasticity [25].

Decreased EZH2 level has been found to activate the expression of phosphatidylinositol-4-phosphate 5-kinase (PIP5K1C) to evoke intracellular Ca^{2+} signaling. The high level Ca^{2+} is responsible for differentiation of human mesenchymal stem cells (hMSCs) into functional neuron lineage. Thus induction of neuronal differentiation is reported to be enhanced by lower EZH2 expression through Ca^{2+} signaling [26]. Strong involvement of EZH2 in stem cell differentiation and maintenance makes it more important among other chromatin regulators for in-depth study targeted to aggressive breast cancer specifically.

ROLE OF EZH2 IN VARIOUS CELL SIGNALING PATHWAYS

Over the past few years, emphasis has been on EZH2 expression pattern analysis in cells under various cell cycle phases and signaling pathways. Interestingly, all the findings are consistent with the significant role of EZH2 in cellular differentiation inhibition and cell cycle progression leading to tumorigenesis. EZH2 has been found to be involved in cell cycle regulation *via* pathways like Wnt/β-catenin, Ras, NF-KB, β-adrenergic signaling, BMP and Notch signaling.

Wnt/β-catenin

The Wnt/β-catenin pathway acts by regulating proteolysis of β–catenin, a multifunctional protein playing important role in gene regulation. Although in cancers, mutations of the gene encoding -β catenin i.e.

CTNNB1, and scaffold proteins APC & AXIN encoding genes have been the most probable significant routes to Wnt signaling dysfunction, recently experiments has targeted epigenetic modifications, which are thought to play important role in regulation of this signaling pathway. In liver cancer, studies have shown EZH2 regulating the repression of the Wnt antagonist DACT3 histone modification unlike of which other Wnt inhibitors (e.g. SFRP) has been shown to be silenced by DNA methylation [27] as shown in Figure **2**.

β-Adrenergic Receptor Signaling

Polymorphism of the gene encoding adrenergic receptor beta 2(ADRB2), a G protein-Coupled Receptor, plays important role in β-adrenergic signaling pathway. It has also been found to be associated with increased risk of breast and colorectal cancer. In this signaling pathway, ADRB2 elevates the intracellular level of cyclic AMP (cAMP), which in turn controls a wide range of cellular processes *via* different signaling pathways, like PIP3. In particular, activation of cAMP-Rap1 by ADRB2 has been shown to regulate cell adhesion and cellular transformation. Although the role of ADRB2 in prostate cancer progression has been studied, its role especially in the context of Polycomb regulation in breast cancer has yet not been investigated. ADRB2 is a direct target of EZH2 which establishes its role for β-adrenergic signaling in prostate cancer [28] and possibly in aggressive breast progression Figure **2**.

NOTCH SIGNALING

One of the most widely used signaling pathways in animal development is carried through the notch receptor protein. It has a general role in cell fate determination and lateral inhibition. At the same time, it regulates pattern formation during the development & renewal of tissues Figure **2**. EZH2 inhibits skeletal muscle differentiation epigenetically through Notch signaling. The silencing cascade in muscle differentiation is thought to be initiated by tumor necrosis factor (TNF), an important myogenic regulator,

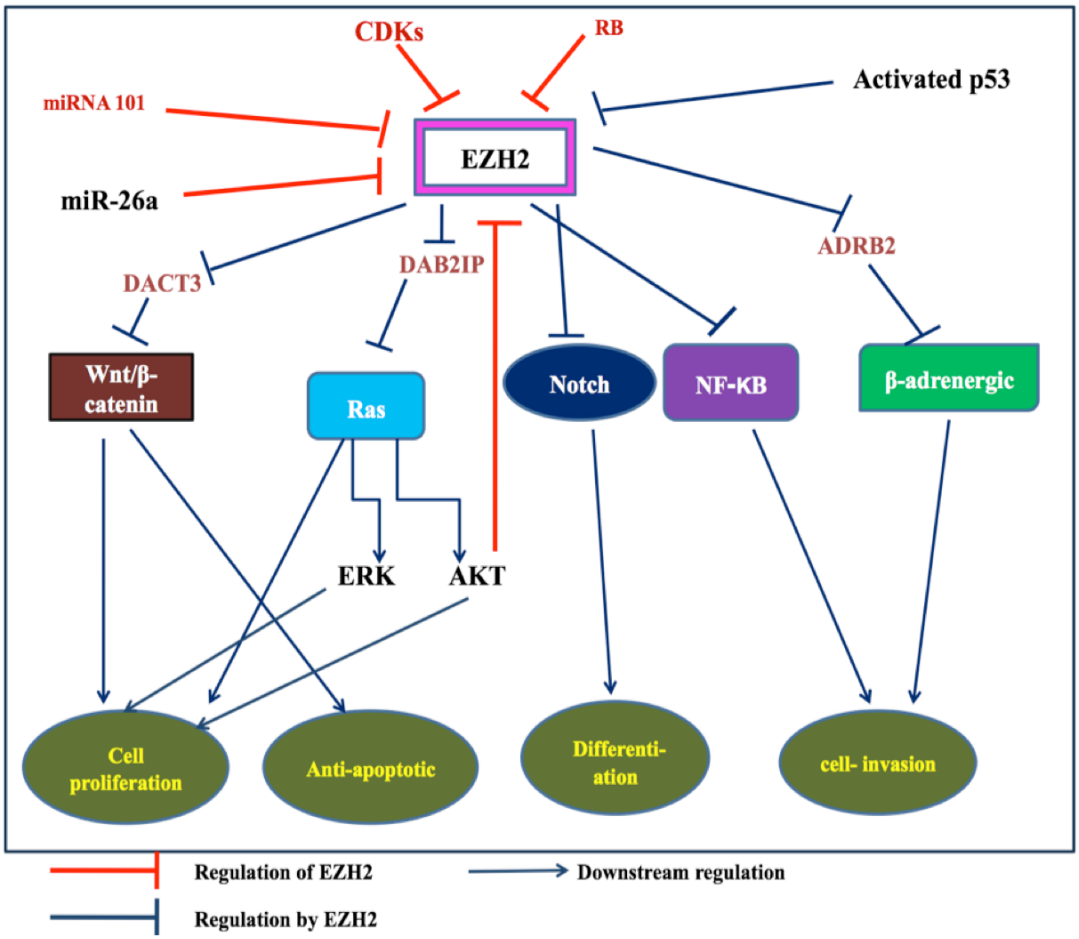

Figure 2: Regulation of EZH2 and its involvement in different cell signaling pathways and tumorigenesis: EZH2 is involved in various signaling pathways like Wnt/β-catenin, Ras, Notch, NF-κB and β-adrenergic deregulation of which leads to tumorigenesis. EZH2 is itself regulated by protein kinase like Protein kinase B, microRNAs like miRNA-101 and miRNA-26a.

which stimulates the recruitment of EZH2 for initial repression *via* H3K27me3, followed by DNA methylation carried by DNMT3b [29]. Since significant role of Notch signaling pathway in cell arrest and apoptosis in liver cancer has been shown by a group of scientists [30] its association with EZH2 to contribute significantly towards aggressiveness of breast cancer can be a promising area of findings.

Ras Signaling

The Ras signaling pathway plays key role in processes like cell proliferation or differentiation, both of which require changes in gene expression. Activated Ras activates several distinct effectors, such as the serine–threonine kinase raf-1, phosphoinositide 3′-kinase (PI3-K), ERK and AKT. These downstream effectors activate several distinct signaling cascades, leading to either activation of certain genes, such as 1) those encoding growth factors transforming growth factor-α, 2) vascular endothelial growth factor (VEGF), or 3) changes in actin cytoskeleton by activating Rho family G proteins. Unlike normal Ras, oncogenic Ras mutant proteins remain constitutively in the active GTP-bound form and continuously activate the downstream effectors which can lead to tumorigenesis. The above significant role of Ras pathway in development, points out the importance of epigenetic regulators in tumorigenesis. Recently, DAB2IP has been identified as tumor suppressor in prostate tumorigenesis which functions as negative regulator of Ras signaling pathway through its effectors ERK and AKT (Figure **2**). Involvement of EZH2 in this pathway has been studied [29]. Experimentally it has been demonstrated that DAB2IP loss activates NF-kB pathway leading to prostate cancer invasion. Where DAB2IP has been shown to substantially suppress Ras and its effector pathways, EZH2 has been demonstrated to activate Ras, ERK, AKT and NF-kB pathways by epigenetically silencing DAB2IP [27, 28, 30].

CROSSTALK OF EZH2 MEDIATED HISTONE METHYLATION WITH OTHER CHROMATIN MODIFICATIONS

Acetylation

The neutralization of the basic charge of the histone tails by acetylation (carried out by HATs) is responsible for the reduction in the affinity of the histones for DNA and also the interaction between nucleosome and other regulatory molecules which leads to activation of respective genes. In an attempt to understand the underlying modifications during embryo implantation, lower level of expression of EZH2 and thus trimethylation of differentiation markers PRL and IGFBP1 has been evidenced to be associated with higher level of acetylation of the same lysine residue [31, 32]. It has been proposed that the repressive chromatin results from hypermethylation and deacetylation of histone. This restricts differentiation process in comparison to which transcriptionally permissive chromatin is proven to be associated with acetylation and low level of methylation. The crosstalk between histone acetylation and methylation indicates the possibility of regulation of EZH2 through acetylation /deacetylation Figure **3**.

Phosphorylation

Phosphorylation of histone3 (H3) and of the linker histone by protein kinase possibly JIL-1, has been shown to be associated with formation of stable metaphasic chromosomes [33]. Phosphorylation at Ser 10 of H3 gained considerable attention when this modification was suspected to be associated with chromosome compaction Figure **3**. and segregation during mitosis and meiosis. Protein kinase JIL-1, which has some similarity with members of the human mitogen- and stress-activated protein kinase (MSK) family has been shown to phosphorylate histone H3 at Ser 10 *in vivo*. Aberrant histone phosphorylation has been shown to be associated with Alzheimer's disease [34]. Till date, no co-relation has been shown between histone phosphorylation and methylation by EZH2, but since histone phosphorylation leads to gene activation opposite to EZH2 methylation gene repression, there can be some regulatory pathway governing EZH2 regulation through histone phosphorylation. The methylation pattern can be compared with phosphorylation to get some insight of aberrant gene expression in cancers like breast cancer.

Ubiquitination

RING1B, a core component of Polycomb group protein (PRC1) is the first identified ubiquitin ligase (E3) responsible for mono ubiquitination of H2A at lysine 119 [35]. Loss of RING1B significantly decreased H2A monoubiquitination. RING1A and BMI1 the other two RING domains in the PRC1 complex, strongly stimulate the E3 ubiquitin ligase activity of RING1B. Although limited information is available for the relation of histone ubiquitination and EZH2 mediated histone methylation, it is the crosstalk of all the chromatin remodelers which leads to gene activation/deactivation as depicted in Figure **3**.

Deacetylation

Removal of acetyl groups from histone tails, opposite to histone acetyl transferase (HAT) activity is carried out by multiprotein complexes called HDACs such as Sin3 and NuRD. Both Sin3 and NuRD contain the proteins that bind to methylated DNA. There is strong evidence that PRC2 and possibly EZH2 interacts with HDACs in transcriptional silencing, resulting in tumor suppressor loss [36].

Nucleosome Remodeling

It is an ATP dependent modification that weakens the contact between the nucleosome and the DNA with which it is associated. In nucleosome remodeling, three types of changes can occur: a) Remodeling- it involves a change in nucleosome structure without any change in its position such as doubling in size of nucleosome and also an increased DNase sensitivity of the attached DNA. b) Sliding or cis-displacement: where physical movement of the nucleosome takes place along the DNA. c) Transfer or trans-displacement: here transfer of nucleosome takes place to a second DNA molecule or to a non-adjacent part of the same molecule. One of the various proteins responsible for nucleosome remodeling is Swi/Snf, made up of at least eleven proteins. Linker histone H1 plays a key role in nucleosome remodeling. EZH2 has been demonstrated to be involved in methylation of histone variants [37, 38]. Regulation of EZH2 through H1 methylation/ demethylation can be screened for investigating erratic methylation pattern in aggressive breast cancer.

DNA Methylation

Chemical modifications of chromatin by the event of methylation are probably the most widely studied epigenetic modification till date. Two types of methylation activity has been characterized 1) Maintenance methylation (by DNA methyl transferase1 or DNMT1)- where methyl groups are added to the newly synthesized strand of DNA at positions opposite to methylated sites on the parent strand. This retains the methylation pattern of the parent strand and 2) De-novo Methylation (by DNMT3a and DNMT3b) - where methyl groups are added at totally new sites so as to modify the methylation pattern in a specified region of the genome thus playing important role in embryonic development. Methylation of genes primarily occurs in the CpG rich islands of promoters. DNA methylation by DNMTs is controlled by EZH2. Physical interaction of EZH2 with DNA methyltransferases has been evidenced by analyzing the DNA methylation pattern on over-expression and knockdown of EZH2 [39]. Erratic behavior of cells in aggressive breast cancer can be investigated in terms of methylation (both histone and DNA) of various tumor suppressor genes and/or oncogenes. Since DNA methylation has proved its significant role in development, stability of chromatin structure, and gene expression maintenance; this association suggests the control of PcG protein EZH2 over CpG methylation and its involvement in the maintenance of function which all the three DNMTs possess. It may be a possible mechanism of epigenetic memory in which an “epigenetic module” consisting of both EZH2 and DNMTs allow co-ordinate and heritable transmission of silenced epigenetic states through DNA replication [39].

Methylation and Gene Repression

Abnormality in DNA and Histone methylation pattern of oncogenes and tumor suppressor genes leads to neoplastic transformation and malignant phenotypes. Aberration in gene methylation pattern can occur due to i) infidelity of DNMT1, ii) non-specific methylation by DNMT3a and DNMT3b, iii) faulty repair mechanism of aberrantly methylated DNA or iv) histone modification by histone methyltransferases like EZH2 all resulting in chromatin modification. DNA methylation has been shown to be involved in genome imprinting and X-inactivation. Both of these phenomena provide strong relation of methylation with genome silencing and thus possibly with aggressive breast cancer.

Regulation of EZH2

EZH2 regulates gene expression on the virtue of its methyltransferase activity but regulation of EZH2 has been studied to be self regulated by the tumor suppressor gene RB as well as microRNA-101 and miRNA-26a [40, 41] as depicted in Figure **3**. A serine/threonine-specific protein kinase Akt or Protein Kinase B (PKB) regulates EZH2 by phosphorylating it at Ser 21 thus inhibiting its methyltransferase activity [42]. Studies report the crucial interaction of histone deacetylases (HDACs) with PRC2 complex and EZH2 mediated gene silencing by treatment of cells with HDAC inhibitor Trichostatin A (TSA). An oncogene–tumor suppressor cascade drives metastatic prostate cancer by coordinately activating Ras and nuclear factor-kB [43].

Despite the presence of strong supporting evidence for benefits of targeting EZH2 inhibition, no therapies

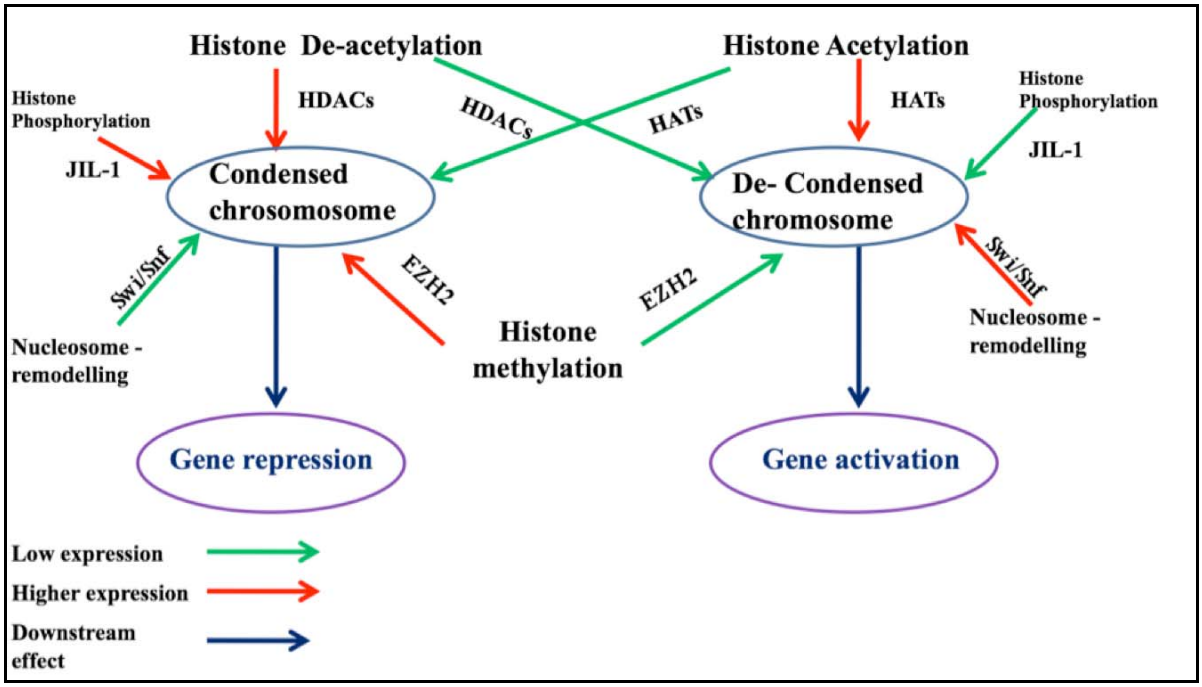

Figure 3: Correlation of EZH2 expression with expression of other chromatin modifiers leading to epigenetic regulation: Higher EZH2 expression is accompanied by lower expression of HATs and Swi/Snf and higher expression of HDACs and JIL-1. Lower EZH2 expression is accompanied by higher expression of HATs and Swi/Snf and lower expression of HDACs and JIL-1.

against EZH2 is clinically practiced. Some trail practices done for inhibiting EZH2 has used the derivative of nephanocin-A, a naturally occurring antibiotic called 3-deazaneplanocin (DZNep), which inhibits S-adenosyl homocysteine hydrolase. This results in accumulation of S-adenosylhomocysteine, an inhibitor of methylation [39]. Also RNAi mediated knock down of EZH2 has been compared to DZNep treatment both resulting in loss of H3K27 methylation but in slightly different fashion. In breast cancer cell lines, significant effect upon cell cycle, cell differentiation, proliferation, and apoptosis has been shown to occur upon treatment with DZNep/HDAC inhibitors. Protein kinases Akt and CDK may also be involved in EZH2 inhibition of by phosphorylation. Correlation of all chromatin modifiers with histone methylation mediated by EZH2 also provides the opportunity of analyzing EZH2 expression in de-regulated aggressive cancerous cells in relation to other modifications to get a clearer picture of its regulation.

EZH2 Mutation

In lymphoma, it has been reported that mutation in EZH2 at amino acid Y641 (within SET domain), results in its reduced ability to carry out first methylation at H3. At the same time, EZH2 becomes catalytically more efficient for subsequent methylation reaction [39]. In contrast to lymphoma, where mutation at single amino acid residue results in gain of function, in myeloid neoplasm mutations has been shown to result in various myelodysplastic syndromes, comprising mis-sense, nonsense and premature termination of translation. There is no report of mutation in PRC2 in subunits other than within SET domain. Three CDK phosphorylation motifs, one at Thr350 and others at Thr421 & Thr492 was found in EZH2 sequence. Sixty percent reduction was observed in phosphorylation upon mutation at Thr350 as compared to 30% or no reduction upon mutation at Thr421 & Thr492 [39]. It strongly suggests Thr350 as the major site of phosphorylation and possible therapeutic target against methyltransferase activity of EZH2. Role of EZH2 mutation towards aggressiveness of breast cancer can be evaluated by inspecting mutation at other domains linked with its catalytic property.

CONCLUSION

Involvement of EZH2 in various important signaling pathways such as cell proliferation, differentiation & migration, and cell death makes it an important gene for study. Studies on EZH2 so far report the involvement of EZH2 in all signaling pathways but less work has been done to study the underlying actual molecular mechanism. Deregulation of EZH2 and thus

methylation pattern is reported in some cancer but little is known about its downstream effectors in breast cancer. Regulation of EZH2 by p53 and miRNAs suspects more possible areas for research. Significant involvement of EZH2 in various stem cell maintenance can also be explored further specifically in breast cancer. Targeting EZH2 in combination of important breast cancer deregulators may add some significant steps in combating breast carcinoma. Collectively, significant association of EZH2 in various cell processes opens more research opportunities in the still developing epigenetic field.

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This is to certify that this work is not currently under consideration elsewhere and all authors have seen and approved the submission of the manuscript for consideration of publication in Journal of Cancer Research Updates.

CONFILECT OF INTEREST

The authors have declared that No competing interest exist.

ABBREVIATION

Ezh2 = Enhancer of zeste homolog2

PRC2 = polycomb group proteins

EGFR2 = Epidermal growth factor receptor 2

EMT = Epithelial-Mesenchymal Transition

ER = Estrogen receptor

PR = Progesterone Receptor

HATs = Histone acetyltransferases

MSK = mitogen- and stress-activated protein kinase

MSL = male specific lethal

H2A = Histone 2A

DNMT1 = DNA methyl transferase1

KLF = kruppel-like factor

PcG = Polycomb group

CBX2 = chromobox homolog 2

RING1A = Ring finger protein 1A

EED = embryonic ectoderm development

SUZ12 = suppressor of zeste 12

SAM = S-adenosine-methionine

VEGF = vascular endothelial growth factor

vash1 = vasohibin1

DZNep = 3-deazaneplanocin

LDL = low-density lipoprotein

UTR = untranslated region

GDS = guanine dissociation stimulator

ADRB2 = adrenergic receptor beta 2

TSA = Trichostatin A

GBM = glioblastoma multiforme

NSCs = neural stem cell

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