Oncology

Bi-phasic expression of Heterochromatin Protein 1 (HP1) during breast cancer progression: Potential roles of HP1 and chromatin structure in tumorigenesis

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Epigenetics in cancer prognosis and therapy is gaining recognition in recent years. Breast cancer is a genetic disease harboring numerous genetic mutations, including tumor suppressor BRCA1 and BRCA2 mutations. However, the functions of BRCA1 in cancer cells are also altered by non-genetic mechanisms, including DNA methylation and chromatin structure. Therefore, identification of epigenetic markers for breast cancer is very important for early diagnosis and effective therapy. This review focuses on recent findings on the roles of Heterochromatin protein 1 (HP1) in BRCA1 functions and breast cancer progression. We previously showed that BRCA1 function and breast cancer progression are frequently associated with HP1 expression level and potentially with chromatin structure. Herein, we suggest that bi-phasic expression of HP1 during breast cancer progression indicates dual roles of HP1 in tumorigenesis. Exploiting differential HP1 expression in tumors could lead to effective cancer therapy. Re-setting the chromatin structure may be a critical step for high-efficiency cancer therapy for many breast cancer patients. *Journal of Nature and Science, 1(7):e127, 2015.*

Epigenetics | HP1 | breast cancer | BRCA1 | PARP inhibitor | biomarker

Introduction

Breast cancer is a genetic disease that is caused by the accumulation of various genetic mutations. *BRCA1, BRCA2, p53, PTEN, HER2* and other genes are frequently mutated in breast cancer patients (1). Especially BRCA1 and BRCA2 mutations are associated with the risk of breast and ovarian cancers (2, 3). Women with inherited mutations in BRCA1/2 have a high risk of developing breast cancer (~80% by the age of 70), ovarian cancer (~30 - 40%), and several other types of cancer during their lifetimes. BRCA1 and BRCA2 mutations are found in 20 ~ 25 % of hereditary breast cancer patients and in 5 ~ 10 % of all breast cancer patients. Patients with germline *BRCA1* and *BRCA2* mutations tend to develop cancer at younger ages and have higher chance of acquiring aggressive types of breast cancer.

BRCA1 has diverse molecular functions, including transcription regulation and cell cycle control. Notably, BRCA1 is critical for maintaining genomic integrity in response to DNA damage (4). BRCA1 promotes homologous recombination (HR) repair and induces G2/M cell cycle arrest during DNA damage response. Thus, mutations of *BRCA1* gene often lead to a dramatic increase of genomic instability and tumorigenesis. In addition to genetic mutations of *BRCA1* gene, other mechanisms including epigenetic alterations in breast cancer cells can lead to impairment of tumor suppressor function of BRCA1. This so-called BRCAness is represented by loss of HR repair activity without BRCA1 mutations. BRCAness is frequently observed in many cancer cells including breast and ovarian cancer. BRCAness includes the loss of BRCA1 expression by aberrant DNA methylation of the *BRCA1* promoter (5, 6). BRCAness may also be caused by the altered expression of chromatin factors including Heterochromatin protein 1 (HP1). Previously, we demonstrated that deficiency of HP1 may lead to loss of HR repair and defective cell cycle checkpoint control in human cell lines (7). We also showed that HP1 expression level is aberrantly lost or elevated in breast cancer samples (8).

Here, we outline recent findings on the expression and potential roles of HP1 in BRCA1 functions and breast cancer progression. We also suggest that epigenetic therapy or other HP1-targeting therapy may be required for many breast cancer patients.

1) BRCA1 function regulated by HP1 and chromatin structure

Eukaryotic chromatin is composed of nucleosome, consisting of 146 base pairs of DNA wrapped around a histone octamer core. Chromatin is not homogeneous in nucleus, but has euchromatin and heterochromatin regions (9). Euchromatin is a less condensed and more transcriptionally active region, whereas heterochromatin is a highly condensed and transcriptionally silent region. Euchromatin and heterochromatin have distinct epigenetic codes including specific histone modifications and DNA methylation. Euchromatin is usually associated with high levels of histone H3/H4 acetylation (H3/H4Ac) and H3K4 methylation (H3K4Me). Heterochromatin is characterized by low level of histone acetylation, high level of H3K9 methylation (H3K9Me), DNA CpG methylation and association with HP1. Euchromatin and heterochromatin structures may have important roles for BRCA1 functions during DNA damage response. Interestingly, DNA damage-induced BRCA1 foci are mostly associated with heterochromatin structure, suggesting potential significance of chromatin structure for BRCA1 functions (10). BRCA1 is also responsible for maintaining heterochromatin structure by regulating histone H2A ubiquitylation (11). We previously demonstrated that a heterochromatin associated factor, HP1, is required for HR repair and cell cycle checkpoint functions in cells (7). In other words, HP1 deficiency causes BRCAness phenotype in mammalian cells. The importance of heterochromatin structure for BRCA1 function was further demonstrated by other groups (12). Two repressive chromatin components, the macrohistone variant macroH2A1 and H3K9 methyltransferase and the tumor suppressor PRDM2 are also required for BRCA1 function including HR repair (12). Furthermore, a histone modification marker for heterochromatin, H3K9 methylation by Suv39h1, is also involved in BRCA1 and HP1 loading on chromatin and in proper DNA damage response (DDR) (13, 14). However, it is still not clear how HP1 and heterochromatin structure contribute for BRCA1 functions. BRCA1 functions during DNA damage response may be modulated through the direct interaction of HP1 and BRCA1. Otherwise, BRCA1 functions may be indirectly regulated by the interaction of HP1 with other DDR factors like BARD1 (13, 15).

2) Bi-phasic expression of HP1 during breast cancer progression: an epigenetic marker for cancer prognosis

Many molecular alterations including genetic mutations and epigenetic changes are accumulated during cancer progression. Since each cancer samples including breast cancer have heterogeneous nature, it is important to classify cancer subtypes by using prognostic biomarkers (16-18). Prognostic factors that are frequently used in breast cancer are age, tumor size, status of lymph nodes, histological types of the tumor, pathological grade, and hormone receptor status. In addition, the most common molecular markers for breast cancers include BRCA1/2, HER2/Neu, estrogen receptor (ER), progesterone receptor (PR), p53, Ki-67 and others.

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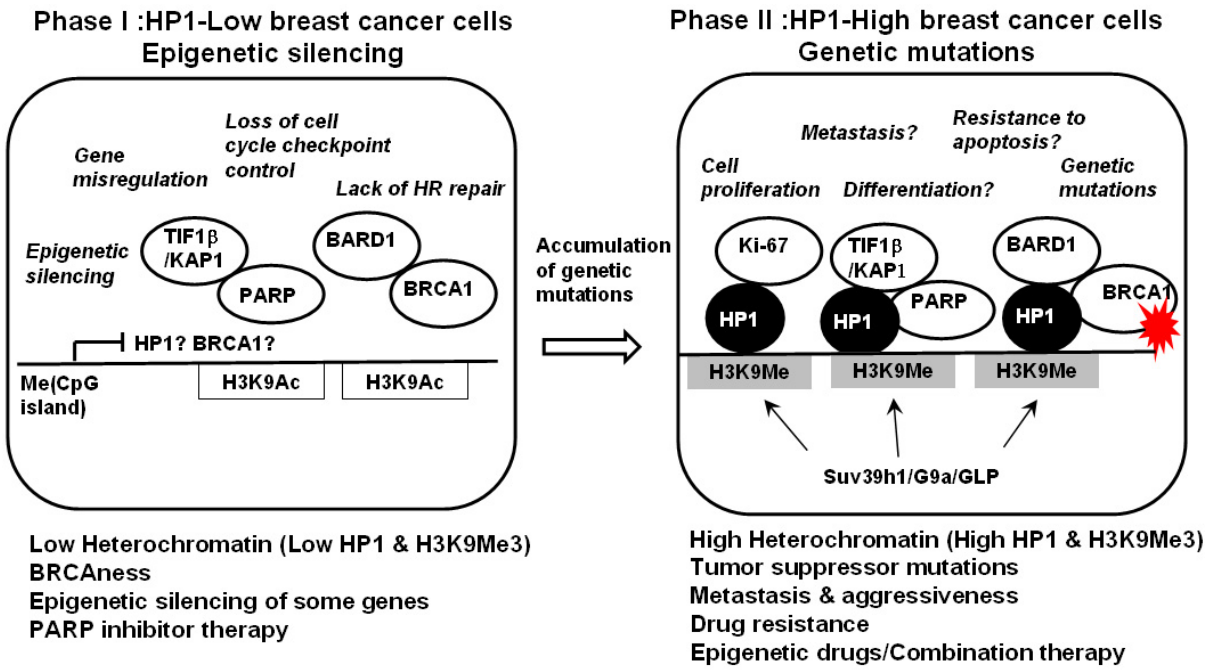

Figure 1. Bi-phasic expression of HP1 during breast cancer progression and potential roles of HP1 in tumorigenesis. HP1 expression is frequently lost or elevated during breast cancer progression. Loss of HP1 expression may occur during initial stages of breast cancer development (Phase I). HP1 deficiency and other epigenetic silencing (like hypermethylation at CpG islands) lead to BRCAness and the impairment of HR repair. Genetic mutations may be accumulated at this stage. PARP inhibitor therapy is effective for breast cancer group with low HP1 expression. However, HP1 expression may be elevated during late stage of breast cancer (Phase II). Protein complex of HP1 and Ki-67 or TIF1β/KAP1/TRIM28 may be increased at this late stage, supporting aggressive cancer cell growth. Some protein complexes including HP1/BRCA1 are not functional due to genetic mutations. Cancer cells at this stage may be more resistant to apoptosis and more prone to cancer metastasis. Re-setting the chromatin structure by epigenetic drugs may be required for effectively curing many breast cancer patients including HP1-high group.

Epigenetic alterations in cancer include global and local changes in DNA modifications, histone modifications, chromatin remodeling, non-coding RNA expression and others. These epigenetic alterations may represent an early sign of breast cancer (19, 20). Therefore, identification of epigenetic alterations during tumorigenesis is critical for early cancer prognosis. Although many epigenetic alterations were reported in breast cancer, few epigenetic factors are currently used for breast cancer prognosis.

Several groups analyzed the expression level of HP1, a well-known epigenetic factor, in cancer cells (21). Especially, expression level of HP1α is either decreased or increased in breast cancer samples (22, 23). However, the implications of aberrant expression of HP1α in tumorigenesis are not clear yet. Recently we analyzed the expression levels of three HP1 subtypes in breast cancer samples by a combined analysis of previously published microarray data and immunohistochemistry (8). Expression levels of all three HP1 subtypes are frequently and similarly altered in breast cancer samples. We classified the breast cancer samples into two groups according to HP1 expression level; HP1-High and HP1-Low groups (8). Interestingly, all three HP1 expression levels were positively correlated with Ki-67 level among prognostic markers. Since Ki-67 is a well known cell proliferation marker (24), HP1-high group may indicate actively growing breast cancer group. Furthermore, our microarray analysis of breast cancer samples showed that HP1β-high group patients are more associated with poorly differentiated breast cancer grade, aggressive cancer types and lower chances of survival (both OS (overall survival) and PFS (Progression free survival)).

Our analysis of breast cancer samples shows the bi-phasic expression of HP1 during breast cancer development (Fig. 1). Probably, HP1 expression is probably lost or reduced during early stage of breast cancer (Phase I). It is possible that epigenetic silencing may be responsible for loss of HP1 expression at Phase I. Epigenetic silencing including DNA hypermethylation around CpG islands may lead to loss of HP1 expression and to the impairment of BRCA1 functions. While global CpG methylation is usually decreased during tumorigenesis, it is well established that local hypermethylation including CpG island methylation may result in silencing of important tumor suppressor genes like BRCA1 in cancer cells (25, 26). Many genetic mutations are probably accumulated at this stage, because of loss of HR repair and cell cycle checkpoint control.

HP1 expression levels are somehow elevated during late stage of breast cancer progression (Phase II). High HP1 expression forms a complex with Ki-67 (27, 28) and HP1/Ki-67 complex induces the proliferation of cancer cells. Other protein complexes including HP1 and TIF1β/KAP1/TRIM28 may further promote tumorigenesis of cancer cells at this stage (29). HP1 may form a protein complex with BRCA1 harboring genetic mutations. However, this HP1/BRCA1 mutant complex may not be functional and cannot prevent genomic instability in cancer cells. Genetic mutations, including mutations on tumor suppressor genes, may lead to conversion of cancer cells to more aggressive types of cells. These show that bi-phasic expression of HP1 in breast cancer cells may have important implications in breast cancer progression. HP1 may have multiple roles, at least dual roles, during breast cancer progression (Fig. 1).

3) PARP inhibitor therapy for HP1-low breast cancer patients

Previously, we suggest that PARP inhibitor therapy may be effective for breast cancer patients with low HP1 expression (8). Poly(ADP-ribose) polymerase (PARP) is a target of the specific drugs for killing breast cancer cells with BRCA1/2 mutations (30, 31). Since PARP family members are involved in the repair of DNA single strand breaks, PARP inhibitor specifically induces apoptosis of cancer cells with BRCA1/2 mutations. Normal cells can survive from PARP inhibition because their homologous recombination (HR) repair mechanism can handle DNA breaks generated from PARP inhibition and DNA replications. We previously showed that breast cancer cells deficient of HP1 expression are hypersensitive to PARP inhibitor treatment (8). In addition to *BRCA1/2* mutation carriers, breast cancer patients with low/no HP1 expression can also benefit from PARP inhibitor therapy (Fig. 1). Thus, low HP1

expression, especially low HP1β, can be applied as a BRCAness marker and may be a predictive marker for PARP inhibitor therapy. This therapeutic value of PARP inhibitor for HP1-low group patients should be further verified clinically in future.

However, cancer therapy for HP1- high group may be more challenging because this group has larger amounts of compact heterochromatin structure and is usually associated with more aggressive cancer. HP1-high group cancer cells are probably more resistant to chemotherapy and also to radiotherapy. To overcome resistance of cancer cells to drugs, re-setting the chromatin structure may be required for effective cancer therapy (Fig. 1). Epigenetic drugs including H3K9 methylation inhibitors can be applied for remodeling heterochromatin of cancer cells.

4) Re-setting the chromatin structure for breast cancer therapy

Epigenetic drugs are being tested for cancer therapy at clinical and pre-clinical level (32). Several epigenetic therapies were already approved by the FDA, and many more therapeutics is also being tested. Different from genetic mutations, epigenetic changes can usually be controlled by drug treatment. These epigenetic drugs have promising therapeutic potential by restoring aberrant epigenetic alterations in cancer. For instance, epigenetic silencing of BRCA1 can be overcome by epigenetic drug treatment in some breast cancer cells. Therefore, epigenetic therapy may also be an attractive option for cancer therapy, because aberrant alterations of histone modifications, DNA methylation and HP1 expression are frequently associated with breast cancer. These epigenetic alterations in cancer cells often lead to silencing of tumor suppressor genes and to increase of genomic instability (20, 32, 33).

Since HP1 interacts with chromatin through specific binding to dimethylated or trimethylated histone H3 K9 residues (H3K9Me2 or Me3), G9a/GLP (G9a like protein) and Suv39h1 may be the target enzymes for controlling HP1 binding and chromatin structure. Several chemical inhibitors for G9a/GLP and Suv39h1 are already available and these drugs act by either blocking cofactor S-Adenosylmethionine (SAM) binding sites or competitive inhibiting for a substrate histone H3. For example, chaetocin (for Suv39h1) and BIX-01338 (for G9a) were previously identified through random screening as SAM binding inhibitors (34). BIX-01294 and UNC0638, substrate-competitive compounds, can also reduce histone H3 K9 methylation level by inhibiting G9a and GLP (35, 36). BRD4770 can reduce H3K9 methylation level without inducing apoptosis (37).

In addition to H3K9 methylation inhibitors, drugs for histone acetylation or DNA methylation level in cancer cells may re-set the abnormal chromatin structure in cancer cells. Many histone deacetylase (HDAC) inhibitors were developed and tested for cancer therapy (20, 32). These include vorinostat (SAHA), romidepsin (FK228), MS-275 (entinostat), LBH-589 (panobinostat) and other HDAC inhibitors. Most of HDAC inhibitors enhance the global histone acetylation level and decrease the heterochromatin content in cancer cells. Similarly, DNMT inhibitors like 5-Azacytidine may also reduce the global DNA CpG methylation level and heterochromatin level in cancer cells (25, 26). Conceivably, we suggest that epigenetic drugs including H3K9 methylation inhibitors, HDAC inhibitors, DNMT1 inhibitor may be effective for re-setting compact chromatin structure of HP1-high cancer cells. Furthermore, successful cancer therapy may require combination therapy of epigenetic drugs and other chemotherapy agents like cytotoxic agents, hormone therapeutics, and others. Novel combinations of drugs may be helpful for effectively removing cancer cells with minimizing side effects on normal cells. Thus, analysis of epigenetic markers and application of epigenetic therapy for breast cancer patients may be an important part of personalized cancer therapy.

5) Developing novel cancer therapy targeting for HP1

In addition to epigenetic drugs, other cancer therapeutics directly targeting HP1 may be helpful for cancer therapy. There are three subtypes of HP1 in human cells and each HP1 subtypes have common and also distinct functions in cells (38). Interestingly, HP1α and HP1β are mostly associated with heterochromatin, and HP1γ associates more with euchromatin. These suggest that each subtype of HP1 may have different roles in transcription regulation and other subtype-specific functions. However, all three subtypes of HP1 show surprisingly similar behavior during DNA repair and cancer progression (7, 8). Depletion of each HP1 subtypes similarly reduces BRCA1 foci formation and impairs G2/M cell cycle checkpoint control and homologous recombination (HR) repair (7). Furthermore, expression levels of three HP1 subtypes are very similar in most of breast cancer samples (about 75.6 %) (8). The respective expression level of all three HP1 subtypes shows a positive correlation with Ki-67 level in cancer cells. This suggests that elevated level of three subtypes of HP1 may have similar effects on cancer progression and growth. Thus, therapeutic drugs targeting common characteristics of HP1 subtypes may need to be developed in future. Since HP1 form a protein complex with Ki-67, the interaction of HP1 and Ki-67 can be an important target for cancer therapy (27, 28). Although the roles of HP1 and Ki-67 complex were not well defined, this HP1/Ki-67 complex may be important for heterochromatin formation, mitosis and cell proliferation. Therefore, inhibition of HP1/Ki-67 complex formation by drugs may impair the growth of breast cancer cells and others.

Therapeutic values of PARP inhibitors for cancer cells with high HP1 expression also need further investigation. The direct interaction of HP1 and poly(ADP-ribose) polymerase-1 and -2 (Parp-1 and parp-2) and poly(ADP-ribosyl)ation of HP1 were previously demonstrated (39). Poly(ADP-ribosyl)ation of HP1 may modulate the interaction with TIF1β/KAP1/TRIM28, hetero-chromatin structure formation and cell differentiation (14, 29). Therefore, it is necessary to revisit the importance of complex of HP1 with TIF1β/KAP1/TRIM28 and the poly(ADP-ribosyl)ation of HP1 during cancer progression. Since HP1 and TIF1β/KAP1/TRIM28 are critical factors involved in DNA damage response and cell proliferation, the interaction of HP1 with TIF1β/KAP1/TRIM28 may have crucial roles during tumorigenesis (29). Therefore, destabilizing HP1 complex with TIF1β/KAP1/TRIM28 by PARP inhibitors may be helpful for removing cancer cells.

Furthermore, HP1 proteins are modified by various posttranslational modifications including phosphorylation, acetylation, methylation, sumoylation, ubiquitination and others (40). Since the functions of HP1 are modulated by diverse protein modifications, the intervention on these modifications on HP1 may be an attractive therapeutic target for cancer therapy.

HP1 in cancer cells may also have unidentified cytoplasmic functions to promote the tumorigenesis. Previously, we showed that HP1 expression is mostly detected in cytoplasm in some cancer samples (8), suggesting potential cytoplasmic functions of HP1 in cancer cells.

There are still many unexplored functions of HP1 in cancer progression (Fig. 1). For example, the roles of HP1 in cancer stem cells and cancer metastasis were not well established yet. HP1 targeted drugs or other epigenetic drugs can be applied for preventing metastasis and for removing breast cancer stem cells.

Taken together, we summarized the recent progress on the roles of HP1 in breast cancer cells. Cancer cells probably control the expression of HP1 and chromatin structure to avoid cell defense mechanisms like DNA repair, cell cycle control, apoptosis and others. Cancer cells may even utilize HP1 overexpression to promote active cell growth. Considering bi-phasic expression and potential roles of HP1 in cancer cells, different cancer therapy should be applied depending on HP1 expression level. Further elucidations of HP1 functions during carcinogenesis may also provide new therapy methods for breast cancer and other diseases in future.

Conclusions

HP1 (Heterochroromatin protein 1), an important epigenetic factor, has diverse roles not only in gene regulation, but also in DNA repair and others. Interestingly, breast cancer cells show bi-phasic expression of HP1 during cancer growth (Phase I and Phase II). The altered HP1 expression in cancer may indicate potentially important

roles of HP1 for breast cancer growth. Low HP1 expression at Phase I may contribute to BRCAness by impairing HR repair and cell cycle checkpoint control. High HP1 expression at Phase II may promote cancer cell growth together with Ki-67, TIF1β/KAP1/TRIM28 and others. We speculate that epigenetic alterations may have main roles in Phase I and accumulated genetic mutations may have key roles in Phase II. However, epigenetic alterations and genetic mutations are not separate events in cancer. Probably, these two mechanisms may cooperatively promote active cancer growth, metastasis, and apoptosis avoidance during cancer progression. We suggest that altered expression of HP1 plays critical roles in transition between Phase I and Phase II of breast cancer progression. Furthermore, different levels of HP1 in cancers may be responsible for different responses to various cancer therapy agents including cytotoxic agents. PARP inhibitor therapy can be applied for breast cancer patients with low HP1 expression. However, cancer therapy for HP1 -high group may be much more challenging. We suggest that re-setting chromatin structure by epigenetic drugs is crucial for some cancer patients, since HP1 is mostly associated with heterochromatin structure. Otherwise, novel cancer therapy directly targeting for HP1 should be developed for breast cancer patients showing high HP1 expression. For instance, disruption of protein complex of HP1 and Ki-67, TIF1β/KAP1/TRIM28 and others may be required for inhibition of breast cancer at this stage. In future, molecular roles of HP1 at each phase of breast cancer should be further characterized. Probably different cancer therapy strategy should be developed for each phase of breast cancer cells. It is interesting to check whether epigenetic drugs can recover the normal expression level of HP1 in cells. It is also important to test whether epigenetic drugs can re-set and make cancer cells more susceptible to various therapeutics. Further identification of epigenetic biomarkers for each type of cancers and at each stage will provide valuable tools for designing targeted and personalized cancer therapy in future.

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