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# Effects of enhancer of zeste homolog 2 and mucin 1 expressions on treatment response in breast cancer

Abdullah Evren Yetişir[1*], Semra Paydaş[2], Mahmut Büyükşimşek[1], Ali Oğul[1], Özge Yaprak[3], Suzan Zorludemir[3], Melek Ergin[3], İrem Kolsuz[4], Mehmet Mutlu Kidi[2]

## SUMMARY

**OBJECTIVE:** Breast cancer is the most common malignancy in women. In the treatment of these patients, pathological complete response is defined as the absence of invasive cancer in breast or lymph node tissue after the completion of neoadjuvant chemotherapy. In this study, we aimed to investigate the relationship of enhancer of zeste homolog 2 and mucin 1 expressions with pathological complete response in patients with breast cancer receiving neoadjuvant chemotherapy.

**METHODS:** A total of 151 patients were included in the study. Enhancer of zeste homolog 2 and mucin 1 expressions were evaluated in the biopsy materials pre-neoadjuvant chemotherapy and post-neoadjuvant chemotherapy surgical material, and their relationship with pathological complete response was investigated.

**RESULTS:** The pathological complete response rates were significantly higher among the hormone receptor-negative patients, those with a high Ki-67 score, and patients with HER2-positive. Higher pathological complete response rates were obtained from patients with enhancer of zeste homolog 2 expression positivity pre-neoadjuvant chemotherapy. In addition, after neoadjuvant chemotherapy, enhancer of zeste homolog 2 expression was found to be completely negative in materials with pathological complete response; that is, in breast tissues considered to be tumor-free. While there was no significant relationship between mucin 1 expression and pathological complete response pre-neoadjuvant chemotherapy, mucin 1 expression was determined to significantly differ between the tissues with and without pathological complete response among the surgical materials examined.

**CONCLUSION:** In our study investigating the relationship between enhancer of zeste homolog 2 and mucin 1 expression and pathological complete response in patients who received neoadjuvant chemotherapy, we found that enhancer of zeste homolog 2 expression could be used as a predictive marker for pathological complete response. However, mucin 1 expression was not associated with pathological complete response.

**KEYWORDS:** Breast neoplasms. Enhancer of zeste homolog 2 protein. MUC1 protein, human. Neoadjuvant chemotherapy.

## INTRODUCTION

Breast cancer is the most common malignancy in women and the second most common cause of cancer-related mortality[1,2]. As a systemic control regimen, chemotherapy has dramatically increased the rate of disease-free and overall survival. Chemotherapy can be administered before or after surgery. When chemotherapy is applied before surgery, it is called neoadjuvant chemotherapy (NAC)[3,4]. Pathological complete response (pCR) is defined as the absence of invasive cancer in breast or lymph node tissue after the completion of NAC[5]. Patients with this response to chemotherapy have a significantly lower risk of tumor recurrence than those with residual carcinoma[6]. Enhancer of zeste homolog 2 (EZH2) is a polycomb group protein involved in stem cell regeneration and carcinogenesis. In breast cancer, increased EZH2 expression is associated with tumor aggressiveness. EZH2 expression in the normal breast epithelium is accepted as an independent risk factor for the development of breast cancer, and, therefore, it has been suggested that this expression can be used in the risk classification of benign breast biopsies[7]. Mucin 1 (MUC1) is a transmembrane protein normally expressed at low levels on the apical surfaces of epithelial cells, including the pancreas, breast, lung, and gastrointestinal tract. It has been shown to be associated with metastasis and invasion in many cancer types. It has also been reported that the overexpression of MUC1 is associated with a poor prognosis in breast cancer[8]. However, there are insufficient data on the relationship of EZH2 and MUC1 expressions with pCR in patients with breast cancer receiving NAC. Therefore, in this study, we aimed to investigate the relationship of EZH2 and MUC1 expressions with pCR in patients with breast cancer receiving NAC.

[1]Adana City Education and Research Hospital, Department of Medical Oncology – Adana, Turkey.
[2]Çukurova Üniversitesi, Faculty of Medicine, Department of Oncology – Adana, Turkey.
[3]Çukurova Üniversitesi, Faculty of Medicine, Department of Pathology – Adana, Turkey.
[4]Çukurova Üniversitesi, Faculty of Medicine, Department of Internal Medicine – Adana, Turkey.
*Corresponding author: evrenyetisir@hotmail.com
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## METHODS

In this study, 175 patients who received NAC and had fully accessible data were evaluated. Eligibility criteria were 18–70 years of age, stage 2 or 3 breast cancer, and noninflammatory invasive ductal carcinoma subtype. Patients who did not have laboratory test results, pre-NAC biopsy results, post-NAC surgical material pathology reports, or preparations were excluded from the study. After NAC, all the patients underwent breast-conserving surgery or modified radical mastectomy. A total of 151 patients were determined to meet the inclusion criteria.

The biopsy and surgical materials of the cases included in the study group were fixed in 10% formaldehyde. From the prepared paraffin blocks, 5-micron sections were obtained. EZH2 expression was determined with the EZH2 Mouse Monoclonal Antibody (415M-15, Cell Marque) and INI-1 (MRQ-27) Mouse Monoclonal Antibody (272M-15, Cell Marque) using the ultraView Universal DAB Detection Kit (Ventana, 760-500) on the Ventana BenchMark XT automated immunohistochemistry stainer. The results were evaluated using a light microscope (Nikon Eclipse E200) by a pathologist. Nuclear staining >1% of tumor cells was considered positive (Figure 1A).

The ab15481 antibody (Abcam, USA) was used for MUC1 expression. MUC1 was scored as 0 (no staining), 1+ (weak staining), 2+ (moderate staining), and 3+ (strong staining). While a score of 0 was evaluated as negative, scores 1+, 2+, and 3+ were considered to indicate positivity (Figure 1B). EZH2 and MUC1 expressions were evaluated in the biopsy materials pre-NAC and post-NAC surgical materials, and their relationship with pCR was investigated.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the local ethics committee of the university (approval number: date: June 10, 2016, meeting no: 54, decision no: 27).

The Statistical Package for the Social Sciences (SPSS) v. 23.0 software package was used for the statistical analysis of the data. Categorical measurements were summarized as numbers and percentages, and continuous measurements as mean and standard deviation values (median and minimum–maximum where appropriate). The Shapiro-Wilk test was used to determine whether the parameters in the study showed a normal distribution. The chi-square and Fisher's exact tests were used to compare categorical data. The Student's t-test was used for normally distributed parameters and the Mann-Whitney U test for non-normally distributed parameters. The statistical significance level was 0.05 in all tests.

## RESULTS

While pCR was achieved in 57 of the 151 patients included in the study, this response was not observed in the remaining 94 patients. There was no statistically significant difference between the patients with and without pCR in terms of age, grade, and menopausal status. However, a statistically significant difference was detected between the patients with and without pCR in relation to estrogen receptor (ER) and progesterone receptor (PR) status and percentages, and Ki-67 level. HER2 status also significantly differed between these two groups. Finally, there was no statistically significant difference in the rates of patients with T and N stages between the pCR and non-pCR groups (Table 1).

Enhancer of zeste homolog 2 expression significantly differed between the groups with and without pCR based on the examination of biopsy and surgical materials. While MUC1 expression did not statistically significantly differ between these two groups in the examination of biopsy materials, there was a statistical significance difference MUC1 expression among the surgical materials (Table 2).

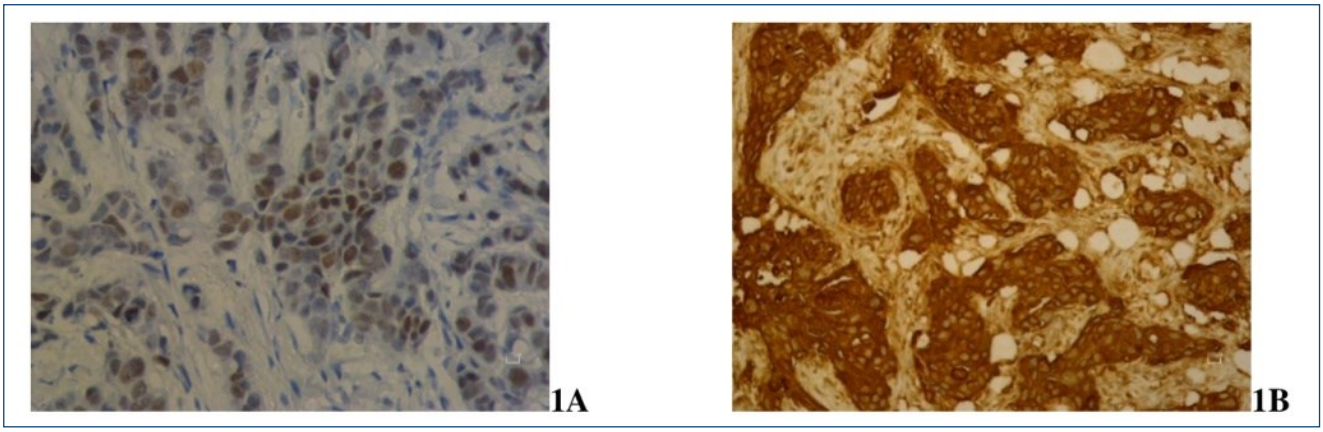


**Figure 1.** **(A)** Enhancer of zeste homolog 2 nuclear staining (400´). **(B)** Mucin 1 membranous and cytoplasmic staining (400´).

**Table 1.** Association between clinicopathological factors and pathological complete response (n=151).

| | Patients with pCR (n=57) | Patients without pCR (n=94) | p-value |
|---|---|---|---|
| Age (mean – years) | 52.8±11.7 | 50.9±10.5 | 0.292[a] |
| | 53 (28–76) | 51 (28–70) | |
| Grade | | | |
| II | 27 (49.1) | 49 (53.3) | 0.624[c] |
| III | 28 (50.9) | 43 (46.7) | |
| Menopausal status | | | |
| Premenopausal | 22 (38.6) | 42 (44.7) | 0.463[c] |
| Postmenopausal | 35 (61.4) | 52 (55.3) | |
| ER status | | | |
| Positive | 27 (47.4) | 83 (88.3) | **<0.001**[*c] |
| Negative | 30 (52.6) | 11 (11.7) | |
| ER level (% mean) | 30.7±39.3 | 65.2±37.5 | **<0.001**[**b] |
| | 0 (0–90) | 90 (0–100) | |
| PR status | | | |
| Positive | 17 (29.8) | 71 (75.5) | **<0.001**[**c] |
| Negative | 40 (70.2) | 23 (24.5) | |
| PR level (% mean) | 16.1±30.1<br>0 (0–90) | 36.5±36.0<br>20 (0–95) | **<0.001**[**b] |
| Ki-67 level (mean) | 52.1±25.7 | 28.7±18.5 | **<0.001**[**b] |
| | 60 (10–90) | 22.5 (3–80) | |
| HER2 status | | | |
| 0–I | 12 | 36 | **0.001**[c] |
| II | 12 (3 FISH +) | 32 (2 FISH +) | |
| III | 33 | 26 | |
| T stage | | | |
| T1 | 8 (14.0) | 7 (7.4) | 0.120[c] |
| T2 | 30 (52.6) | 38 (40.4) | |
| T3 | 3 (5.3) | 11 (11.7) | |
| T4 | 16 (28.1) | 38 (40.4) | |
| N stage | | | |
| 0 | 5 (8.8) | 4 (4.3) | 0.421[c] |
| 1 | 7 (12.3) | 19 (20.2) | |
| 2 | 33 (57.9) | 49 (52.1) | |
| 3 | 12 (21.1) | 22 (23.4) | |

*p<0.05, **p<0.001. [a]Student's t-test. [b]Mann-Whitney U test. [c]Chi-square and Fisher's exact tests. pCR: pathological complete response; ER: estrogen receptor; PR: progesterone receptor; FISH +: FISH positive. Fisher's exact test was used if the expected minimum was <5 according to the chi-square test. Statistically significant p-values are shown in bold (p<0.05).

## DISCUSSION

In breast cancer, which is the second most common cause of cancer-related mortality, it has been shown that patients with pCR have a longer disease-free and overall survival than those with residual cancer. Recent studies have identified pCR as the primary goal in predicting disease-free and overall survival times in NAC[9,10].

While it is very important to predict which patients will achieve pCR with NAC, this treatment is not completely

**Table 2.** Association between enhancer of zeste homolog 2 and mucin 1 expressions and pathological complete response (n=151).

| | Patients with pCR (n=57) | Patients without pCR (n=94) | p-value |
|---|---|---|---|
| EZH2 – biopsy | | | |
| Positive | 46 (88.5) | 51 (62.2 | **0.001****[a] |
| Negative | 6 (11.5) | 31 (37.8) | |
| MUC1 – biopsy | | | |
| Positive | 49 (94.2) | 78 (95.1) | 0.821[a] |
| Negative | 3 (5.8) | 4 (4.9) | |
| EZH2 – surgical | | | |
| Positive | – | 10 (11.8) | **0.013***[a] |
| Negative | 49 (100.0) | 75 (88.2) | |
| MUC1 – surgical | | | |
| Positive | 43 (87.8) | 88 (100.0) | **0.002****[a] |
| Negative | 6 (12.2) | – | |

*p<0.05, **p<0.001. [a]Chi-square and Fisher's exact tests. pCR: pathological complete response; EZH2: enhancer of zeste homolog 2; MUC1: mucin 1. Fisher's exact test was used if the expected minimum was <5 according to the chi-square test. Statistically significant p-values are shown in bold (p<0.05).

risk-free. Although the prediction of patients that will achieve longer disease-free and overall survival after NAC facilitates patient management, NAC may increase the ipsilateral tumor recurrence rate compared with adjuvant therapy. In addition, the existence of healthcare access barriers and socioeconomic inequalities are the main reasons for late-stage diagnosis in developing countries and delaying surgery may result in decreased overall survival[11,12].

Previous studies have explored many factors to predict pCR after NAC. Compared with luminal A tumors, HER2 overexpression and triple-negative subtypes are reported to be more sensitive to NAC[13]. High Ki-67 expression and lack of ER and PR expressions are associated with higher pCR[14,15]. A meta-analysis of 36 studies evaluating the pCR rate in patients with breast cancer with different Ki-67 labeling indices who received NAC showed that those with a high Ki-67 index had a significantly higher pCR rate[16]. Gomes da Cunha et al. examined the relationship between the Residual Cancer Burden (RCB) index and overall and disease-free survival in women undergoing NAC. It was found statistically significant that the RCB 0 subgroup had a better prognosis (pCR) than RCB 1, 2 and 3[17]. In a study evaluating pCR status according to HER2 status, 51 of 413 samples were HER2-positive and 287 were HER2-negative, while HER2 results of 75 patients could not be reached. In 94 (14.3%) of these patients, pCR was obtained from breast tissue and lymph nodes. pCR was found to be three times more common in HER2-positive patients (23.5%) than in HER2-negative patients (7%)[18]. In our study, consistent with the literature, higher rates of pCR were obtained from the patients with hormone receptor negativity, high Ki-67 score, and HER2 expression positivity.

The functions of EZH2 in cell proliferation, apoptosis, and aging have been previously described[19]. EZH2 dysregulation is highly tumorigenic and has been observed in various cancers where EZH2 acts as an oncogene or a tumor suppressor[20]. In a meta-analysis evaluating 11 studies (2,330 patients in total), 1,052 EZH2-positive and 1,278 EZH2-negative patients were examined. It was determined that EZH2 overexpression was significantly associated with ER and PR negativity, HER2 positivity, invasive ductal cancer, race, high histological grade, and triple-negative status, resulting in a poor overall survival rate. The authors concluded that EZH2 could be used as a prognostic marker in breast cancer[21].

In a study investigating the effect of MUC1 expression on treatment response and survival in patients with breast cancer receiving NAC, it was stated that MUC1, which could be detected at mRNA and protein levels, was frequently expressed in breast cancer. High MUC1 protein and mRNA expressions were associated with a lower probability of pCR and longer patient survival. Thus, MUC1 expression was suggested to be an independent predictor of treatment response and survival after NAC[22].

Predicting patients who will achieve pCR after NAC is very important for patient management, which increases the need for predictive markers of NAC response. In our study investigating the relationship of EZH2 and MUC1 expression with pCR in patients receiving NAC, we found that EZH2 expression could be used as a predictive marker

of pCR. Higher pCR rates were obtained from the patients with EZH2 expression positivity pre-NAC, while EZH2 expression was completely negative in materials with pCR after NAC. MUC1 expression was not associated with pCR, but there was a statistically significant difference in MUC1 expression between the tissues with and without pCR based on the examination of the surgical materials. Due to the small number of patients, we were not able to explore the relationship of pCR separately with each breast cancer subgroup and not examining more parameters, which can be considered a limitation of our study. The strengths of our study, i.e., the promising results of EZH2 and the insignificance of MUC1, may lead to further studies.

## CONCLUSION

Enhancer of zeste homolog 2 is a good predictive marker of pCR in patients receiving NAC. Further studies are needed to validate the use of EZH2 expression in the prediction of pCR in patients receiving NAC.

## ETHICAL APPROVAL

The study was approved by the local ethics committee of the university (approval number: 10.06.2016-54).

## AUTHORS' CONTRIBUTIONS

**AEY:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft. **SP:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. **MB:** Conceptualization, Data curation, Formal Analysis, Methodology. **AO:** Data curation, Formal Analysis, Investigation, Resources, Software. **ÖY:** Conceptualization, Resources, Software, Visualization. **SZ:** Conceptualization, Formal Analysis, Supervision, Validation, Visualization. **ME:** Formal Analysis, Supervision, Validation, Visualization. **İK:** Data curation, Methodology, Resources, Software, Writing – review & editing. **MMK:** Data curation, Investigation, Resources, Software.

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