Expression and clinical significance of MAPK and EGFR in triple-negative breast cancer

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Abstract. To investigate the expression and clinical significance of mitogen-activated protein kinase (MAPK) and epidermal growth factor receptor (EGFR) in triple-negative breast cancer (TNBC), a total of 300 TNBC and 120 paired paracancerous tissues were examined. Immunohistochemistry was conducted to determine the expression levels of MAPK and EGFR, and the correlation between MAPK and EGFR expression was evaluated using Cramer's V test. The association between MAPK and EGFR expression, and various clinicopathological variables (such as lymph node metastasis, clinical stage, recurrence and metastasis) was also evaluated, using the χ^2 test. MAPK and EGFR expression levels in TNBC tissues were significantly higher than in the paired paracancerous tissues. Moreover, MAPK expression was associated with that of EGFR in TNBC tissues. The positive expression rates of MAPK and EGFR in patients with lymph node metastasis, advanced clinical stage, tumor recurrence and metastasis were higher than those without. Patients with positive expression of MAPK and EGFR in TNBC tissues had poorer prognoses and lower overall survival times than those without expression. In summary, the expression of MAPK and EGFR is closely associated with tumor invasion and the metastasis of TNBC, and may therefore be used as an indicator of poor prognosis in patients with TNBC.

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Abbreviations: MAPK, mitogen-activated protein kinase; EGFR, epidermal growth factor receptor; TNBC, triple-negative breast cancer; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2

Key words: TNBC, MAPK, EGFR, clinical significance

Introduction

Triple-negative breast cancer (TNBC) refers to a subtype in which the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER-2) are not expressed. TNBC exhibits high heterogeneity and invasiveness, and poor survival outcome, and is currently a key topic in the field of breast cancer research. Endocrine and targeted therapy for TNBC have not been successfully developed (1), and consequently, the most common treatment for TNBC is chemotherapy (2). However, long-term chemotherapy can lead to poor patient tolerance and side effects (3). Thus, the development of new targets for the treatment of TNBC is expected to further improve patient prognosis.

Kinase inhibitors have proven to be successful in improving the prognosis of TNBC (4). The expression of mitogen-activated protein kinases (MAPKs) is an independent risk factor for disease-free and overall survival in patients with TNBC (5). MAPKs play an important role in the development and progression of breast cancer (6), and consist of serine-threonine protein kinases that can be activated by various extracellular stimuli. They are also key components of numerous molecular signaling pathways, and play a central role in proliferation-related signaling pathways (7). MAPKs primarily regulate the functions of other proteins through phosphorylation, and play an important role in the occurrence, development and metastasis of multiple tumors (8). A total of three MAPK signaling pathways have been identified, among which the extracellular regulated kinase (ERK) and stress-activated protein kinase/c-Jun NH (2)-terminal kinase pathways are critically involved in the progression of TNBC (9).

Epidermal growth factor receptor (EGFR) is involved in various complex cellular signal transduction pathways (10). During tumor progression, EGFR can form homodimers or heterodimers by binding to ligands such as EGF, and subsequently activating multiple signal transduction pathways; these include the MAPK/ERK and phosphoinositide 3-kinase (PI3K)/Akt pathways, which promote tumor angiogenesis, proliferation, invasion and metastasis (11). Furthermore, EGFR plays a central role in cell proliferation and differentiation (12) and is closely associated with the growth of TNBC (11).

In the present study, the expression levels of MAPK and EGFR in TNBC tissues were investigated, and the relationship between their respective expression levels and certain clinicopathological features of patients with TNBC was further investigated.

Materials and methods

Patient samples. A total of 300 female patients with pathologically confirmed TNBC from the 3rd Affiliated Teaching Hospital of Xinjiang Medical University (Urumqi, China), were enrolled in the present study between January 2011 and December 2013. Their median age was 46.5 years old (range, 21-71 years old). The clinical data of the patients are exhibited in Table I. The inclusion criteria were as follows: i) The subjects exhibited no ER, PR or HER-2 expression; ii) subjects that were diagnosed with TNBC for the first time and received surgery for TNBC; and iii) the clinicopathological data were complete. The exclusion criteria were as follow: The subjects had carcinoma *in situ* or non-TNBC. Breast cancer tissues were collected during surgery. Paired breast para-cancerous tissues (n=120), obtained from the 300 enrolled patients with TNBC were selected as controls. Written informed consent was obtained from each patient and the present study was approved by the ethics review board of Xinjiang Medical University.

Immunohistochemistry. The expression levels of MAPK and EGFR were determined using immunohistochemical staining. The tissues were fixed with 10% neutral formalin for 24 h at room temperature, embedded in paraffin and cut into 4-μm sections. The tissue sections were then dewaxed using xylene, and rehydrated in using a graded alcohol series. Subsequently, the sections were incubated with 3% hydrogen peroxide for 10 min at room temperature to inhibit endogenous peroxidase activity. After blocking with 10% BSA at 37˚C for 40 min, the sections were incubated with primary antibodies against MAPK (1:200; cat. no. M-9692) and EGFR (1:100; cat. no. ZM-0093; both Beijing Zhongshan Golden Bridge Biotechnology Co. Ltd.) at 37˚C for 90 min. After washing with PBS, secondary antibody anti-mouse IgG (cat. no. ZDR-5006, Beijing Zhongshan Golden Bridge Biotechnology Co. Ltd) was added and incubated for 20 min at room temperature. Finally, the sections were treated with DAB chromogenic reagent and counterstained with hematoxylin. The tumor tissues with positive expression of MAPK and EGFR were used as positive controls. PBS was used instead of the primary antibody as a negative control.

Evaluation of staining results. The staining results were evaluated by two individuals in a double-blinded manner. Concerning MAPK expression; cells exhibiting yellow or brown staining in the cytoplasm and the nucleus were considered to be positively stained. A total of five fields were randomly selected under high magnification (magnification, x200) using Olympus C-7070WZ light microscope (Olympus, Tokyo, Japan), and 100 cells per field were counted. The positive rate was the ratio of positively-stained cells to the total number of cells counted. The percentage of cells with positive staining corresponded with the following scores: 1, <25%; 2, 25-50%; 3, 50-75%; and 4, >75%. The staining intensity was evaluated as follows: 0, no staining; 1, light yellow; 2, brownish-yellow; and 3, tan. The degree of staining was calculated by multiplying the percentage of positive staining by the staining intensity. A total score of ≤3 points was defined as negative staining, and a total score >3 points was defined as positive staining.

EGFR results were based on the staining continuity of the cell membranes; the immunohistochemistry staining results were scored as follows: 0, no staining; 1, cell membrane staining was discontinuous and exhibited brown/tan staining >10%; 2, membrane staining was continuous with incomplete shape and exhibited brown/tan staining >10%; and 3, the membrane staining was continuous and with brown/tan staining >10%. The staining intensity was scored as follows: 1, light brown; 2, medium brown; and 3, dark brown. The degree of staining was calculated by adding the scores of the continuity of the cell membranes and the intensity of staining. A total score of <3 points was defined as negative staining, and a total score ≥3 points was defined as positive staining.

Follow-up. Follow-up was performed using hospital review and the telephone. The beginning of the follow-up period was defined as the date of surgery and follow-up occurred 5 years post-operation. December 1, 2018, or the date of patient death, was considered to be the deadline for follow-up. Overall survival time was defined as the time between first diagnosis and mortality of the patient. Local recurrence referred to recurrence of the tumor in the ipsilateral breast, chest wall or regional lymph nodes. Distant metastases were confirmed by clinical examination, imaging, and pathological diagnosis of tissue biopsy. The follow-up rate was 94.7%. Tumor-free survival was defined as the time from the start of surgery to the time of recurrence or metastasis.

Statistical analysis. The data were analyzed using SPSS 19.0 (IBM Corp.). The χ^2 test was used to analyze differences in the data between the two groups, and the correlation between MAPK and EGFR expression in TNBC tissues were analyzed using Cramer's V test. The relationship between MAPK and EGFR expression with lymph node metastasis, clinical stage, recurrence and metastasis was analyzed using the χ^2 test. Kaplan-Meier analysis was used for survival analysis and the log-rank test was used to examine differences in survival between groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Expression levels of MAPK and EGFR in TNBC tissues. Expression levels of MAPK and EGFR in TNBC tissues were detected using immunohistochemistry. The representative staining results are displayed in Fig. 1. As shown in Table II, the number of cases exhibiting positive expression of MAPK and EGFR in TNBC tissues was 118/300 (39.3%) and 136/300 (45.3%), respectively, compared with paratumor tissues. In 120 paired paracancerous tissues, the number of cases with positive expression of MAPK and EGFR were 20 (16.7%) and 26 (21.7%), respectively. The expression levels of both MAPK and EGFR in the cancerous tissues were significantly

Table I. Clinical data of patients with TNBC.

	MAPK				EGFR			
Group age, years	+	-	χ^2 value	P-value	+	-	χ^2 value	P-value
<40	41 (46.1)	48 (53.9)	2.405	0.12	46 (51.5)	43 (48.3)	2.060	0.150
≥40	77 (36.5)	134 (63.5)			90 (42.7)	121 (57.3)		
Ethnicity								
Han	65 (40.1)	97 (59.9)	0.439	0.803	72 (44.4)	90 (55.6)	0.130	0.94
Uighur	31 (36.5)	54 (63.5)			39 (45.9)	46 (54.1)		
Other	22 (41.5)	31 (58.5)			25 (47.2)	28 (52.8)		

MAPK, mitogen-activated protein kinase; EGFR, epidermal growth factor receptor; TNBC, triple negative breast cancer. The percentage of the patient population is indicated in brackets.

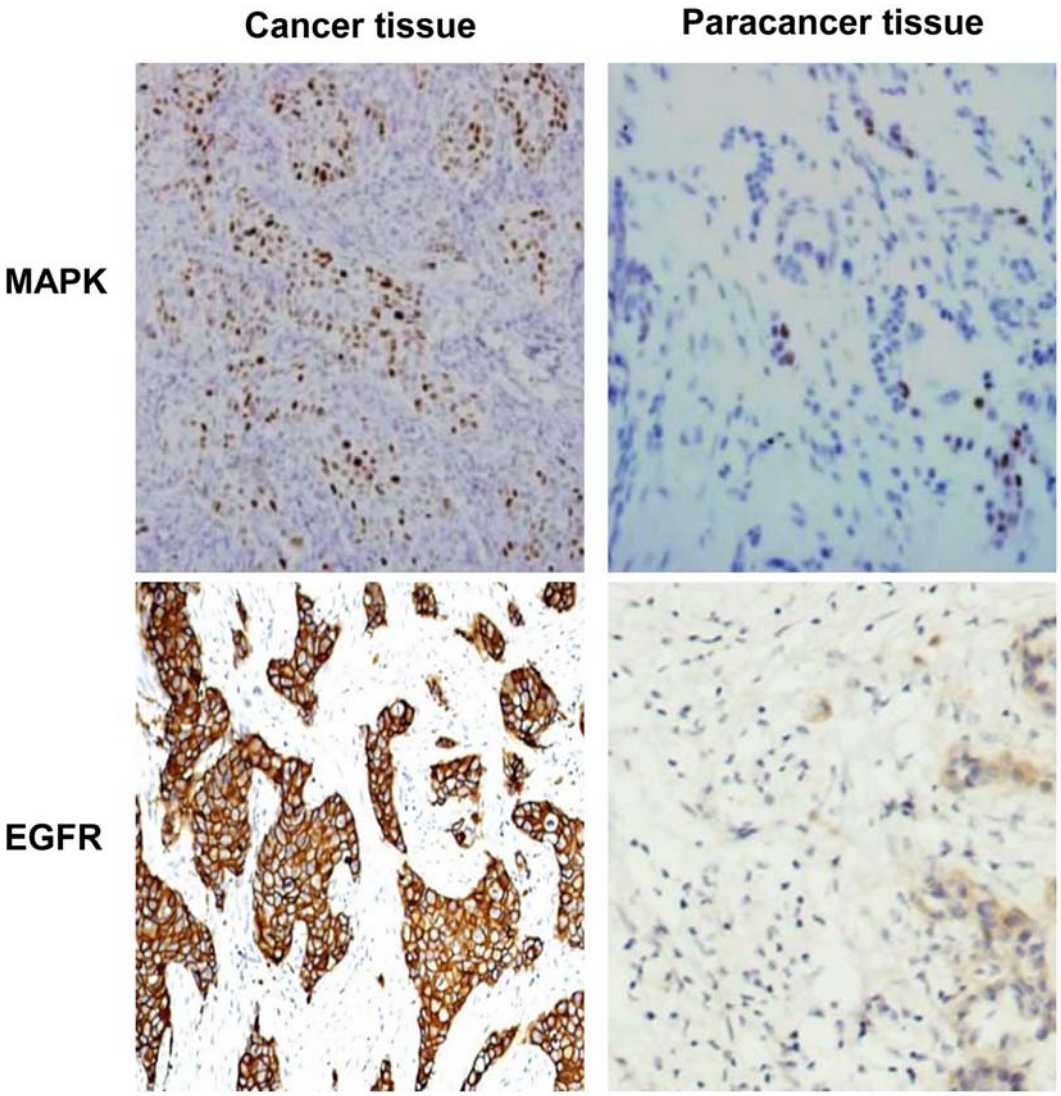

Figure 1. Expression of MAPK and EGFR detected by immunohistochemistry. The expression levels of MAPK and EGFR in cancerous tend paired paracancerous tissues were detected using immunohistochemistry. Magnification, x200. MAPK, mitogen-activated protein kinase; EGFR epidermal growth factor receptor.

higher, compared with paired paracancerous tissues (P<0.05). Clinical data analysis determined that there were no statistical differences in the expression levels between different ages or ethnic distributions (Table I). The results of the present

Table II. Expression of MAPK and EGFR in TNBC and paracancerous tissues.

	MAPK		EGFR	
Groups	+	-	+	-
Cancer tissues (%)	118 (39.3)	182 (60.7)	136 (45.3)	164 (54.7)
Paracancer tissues (%)	20 (16.7)	100 (83.3)	26 (21.7)	94 (78.3)
χ^2 value	19.962		20.262	
P-value	<0.01		<0.01	

MAPK, mitogen-activated protein kinase; EGFR, epidermal growth factor receptor; TNBC, triple negative breast cancer.

Table III. Association between MAPK and EGFR expression in TNBC tissues.

	MAPK				
	Positive	Negative	Total	*C*-value	P-value
EGFR					
Positive	90	46	136		
Negative 2	8	136	164	0.500	<0.01
Total	118	182	300		

MAPK, mitogen-activated protein kinase; EGFR, epidermal growth factor receptor; TNBC, triple negative breast cancer.

study indicate that MAPK and EGFR expression levels are increased in TNBC tissues, potentially implicating them in the occurrence and progression of TNBC.

Correlation between MAPK and EGFR expression levels in TNBC. To determine the degree of correlation between the expression levels of MAPK and EGFR in TNBC tissues, Cramer's V test was performed. As shown in Table III, there was a high degree of correlation between MAPK and EGFR expression in TNBC tissues (C=0.500, P<0.01). This result indicates that MAPK and EGFR are co-expressed in TNBC.

Association between MAPK and EGFR expression, and the clinicopathological features of patients with TNBC. The association between MAPK and EGFR expression levels with TNBC clinicopathological features, such as lymph node metastasis, clinical stage, recurrence and metastasis, was evaluated using the χ^2 test. In patients with lymph node metastasis, the positive expression levels of MAPK and EGFR were 48.7 and 55.6%, respectively (P<0.05; Table IV), significantly higher than those of patients without lymph node metastasis. Similarly, an advanced clinical stage was associated with higher positive expression levels of both MAPK and EGFR (Table IV). At the end of follow-up, 85/300 patients (28.3%) experienced recurrence and metastasis. Of these, 46/85 (54.1%) exhibited positive MAPK expression and 52/85 (61.2%) exhibited positive EGFR expression. In the 215 cases without recurrence or metastasis, the positive expression level of MAPK was 33.5% (72/215), and that of EGFR was 39.1% (84/215); these differences were statistically significant. The results demonstrate that there is a significant association between MAPK and EGFR expression and lymph node metastasis, clinical stage, recurrence and metastasis in patients with TNBC.

Association between MAPK and EGFR expression levels, and patient survival time. To understand the effect of MAPK and EGFR expression on the prognosis of TNBC patients, 5-year tumor-free survival analysis was performed on the enrolled patients. The survival curves of 85 patients with recurrence and metastasis were constructed using Kaplan-Meier analysis and the log-rank test. Compared with patients without MAPK expression, MAPK-positive patients exhibited a log-rank value of 11.427, where P<0.01 (Fig. 2A). Compared with EGFR-negative patients, those with positive EGFR expression indicated a log-rank value of 11.864 and P<0.01 (Fig. 2B). This indicates that the prognosis of patients with positive MAPK and EGFR expression was poor, compared with expression-negative individuals. The results of the present study indicate that the patients with positive MAPK and EGFR expression demonstrated shorter survival times than those negative for MAPK and EGFR expression.

Discussion

TNBC is a subtype of breast cancer (13) that exhibits different clinicopathological features and prognosis compared with non-TNBC (9). In patients with TNBC, high expression levels of EGFR induce the activation of the MAPK signal-transduction pathway, stimulating the proliferation of malignant cells and causing the hormone-independent proliferation of TNBC cells (14). Therefore, the effect of EGFR on MAPK expression, and the effect of both proteins on the prognosis of patients with TNBC, requires further elucidation.

The MAPK signaling pathway is an important signal transduction pathway involved in the development of TNBC (15,16). MAPKs play a central role in the expression of ER, PR and HER-2, and are closely related to the invasion, metastasis and prognosis of TNBC (17). Higher MAPK activity is associated with shorter survival time in patients with TNBC, and may therefore be an indicator of a poor prognosis (15). Upregulation or continuous activation

Table IV. Association between MAPK and EGFR expression, lymph node metastasis, and recurrence and metastasis in patients with TNBC.

			MAPK				EGFR			
		Patients, n	Relative positive	Relative negative	χ^2 value	P-value	Relative positive	Relative negative	χ^2 value	P-value
Lymph node metastasis	Yes	117	57 (48.7)	60 (51.3)	7.08	0.008	65 (55.6)	52 (44.4)	7.05	0.008
	No	183	61 (33.3)	122 (66.7)			73 (39.9)	110 (60.1)		
Clinical stage	I	82	24 (29.3)	58 (70.7)	10.9	0.004	22 (26.8)	60 (73.2)	16.9	0.001
	II	140	51 (36.4)	89 (63.6)			60 (42.9)	80 (57.1)		
	III	78	42 (53.8)	36 (46.2)			46 (59.0)	32 (41.0)		
Recurrence and metastasis	Yes	85	46 (54.1)	39 (45.9)	10.86	0.001	52 (61.2)	33 (38.8)	12.01	0.001
	No	215	72 (33.5)	143 (66.5)			84 (39.1)	131 (60.9)		

MAPK, mitogen-activated protein kinase; EGFR, epidermal growth factor receptor; TNBC, triple negative breast cancer. The percentage of the patient population is indicated in brackets.

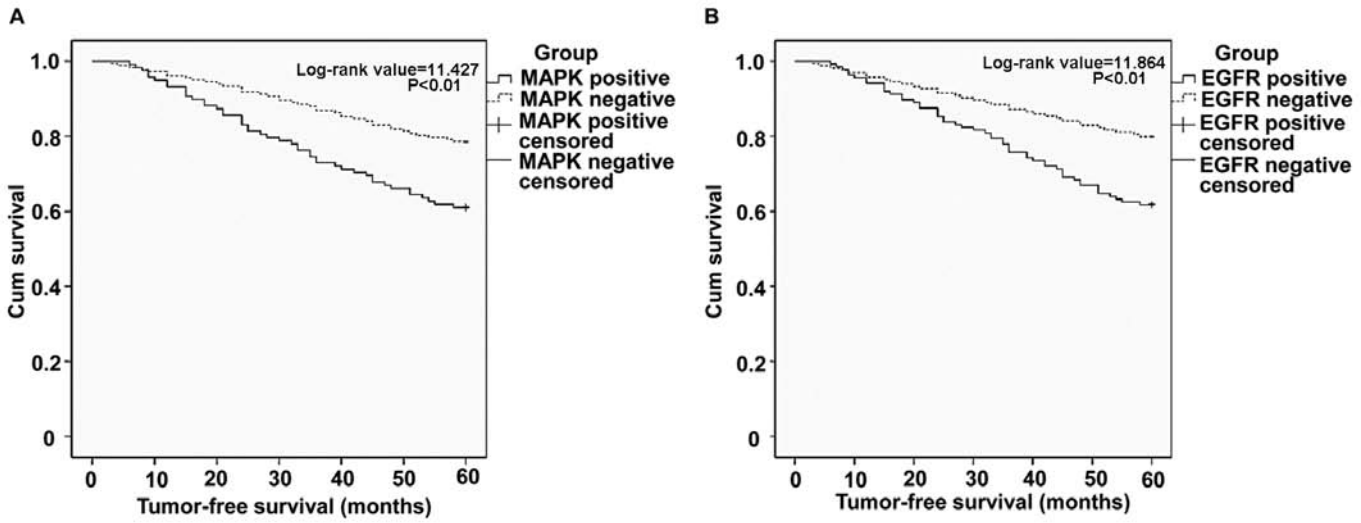

Figure 2. Relationship between MAPK and EGFR expression and survival rate in TNBC. Kaplan-Meier analysis was used for survival analysis and the log-rank test was used to analyze the differences in survival rate. Survival of patients with and without (A) MAPK and/or (B) EGFR expression. MAPK, mitogen-activated protein kinase; EGFR epidermal growth factor receptor; TNBC, triple negative breast cancer.

of EGFR causes the activation of MAPKs (18). During the progression of TNBC, EGFR forms homodimers or heterodimers by binding to its ligands (including EGF), and activating multiple signaling pathways (such as PI3K/Akt or MAPK/ERK) that promote tumor-cell proliferation, invasion and migration (19). In the present study, the positive expression rates of MAPK and EGFR in TNBC tissues were 39.3 and 45.3%, respectively, similar to previous studies [45%; (20)]. Correlation analysis determined that MAPK was significantly associated with EGFR expression, suggesting that MAPK and EGFR may act synergistically to promote TNBC progression.

Lymph node metastasis and TNM staging are important for evaluating clinical prognosis in patients with TNBC (21), and can provide a basis for the selection of treatment options (22). The present study determined that in patients with TNBC, both MAPK and EGFR expression were significantly associated with both lymph node metastasis and clinical stage. It has also been discovered that patients with high levels of MAPK and EGFR expression are more prone to early recurrence, metastasis and poor prognosis (23-25). However, two studies elucidated that there was no significant correlation between MAPK expression and lymph node metastasis (6,20), which was not consistent with the results of the present study. This may be attributable to study size. Further analysis in the present study determined that the expression levels of MAPK and EGFR were associated with recurrence and metastasis. Positive expression of MAPK and EGFR in TNBC patients with recurrence and metastasis was significantly higher than in those without. Furthermore,

patients with high expression levels of MAPK and EGFR had a poorer prognosis and lower survival rates than those with low expression, consistent with previous studies (26,27). Moreover, the proliferation of TNBC cells may be inhibited by targeting the MAPK pathway, thus achieving therapeutic effects (17,28,29). Taken together, the results of the present study indicate that MAPK and EGFR play a primary role in TNBC development, and may be considered as independent prognostic indicators and new targets for the treatment of TNBC. Thus, the different statuses of TNBC patients should be considered, and individualized treatments should be employed to further improve prognosis.

The present study had certain limitations; firstly, it was a retrospective study analyzing a small sample population. Secondly, immunohistochemistry experiments were performed using antibodies with broad MAPK recognition, instead of antibodies specific to certain members of the MAPK family.

In conclusion, MAPK and EGFR expression levels were increased in TNBC patients (compared with paratumor tissues), and were associated with lymph node metastasis, advanced clinical stage, recurrence and metastasis, and poor survival rate. In the future, MAPK and EGFR may be used as independent prognostic indicators and novel targets for the treatment of TNBC, thus individualized treatment plans should be devised according to the different statuses of patients with TNBC, with the aim of further improving prognosis.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

Experimental conception and design was conducted by WJ and LY. Data acquisition and analysis was performed by WJ, XW, CZ, LX and LY. WJ and XW drafted the manuscript, and all authors read and approved the final manuscript.

Ethics approval and consent to participate

Informed consent was obtained from all patients and the study was approved by the ethics review board of Xinjiang Medical University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Corbex M, Bouzbid S, Traverse-Glehen A, Aouras H, McKay-Chopin S, Carreira C, Lankar A, Tommasino M and Gheit T: Prevalence of papillomaviruses, polyomaviruses, and herpesviruses in triple-negative and inflammatory breast tumors from algeria compared with other types of breast cancer tumors. PLoS One 9: e114559, 2014.
2. Collignon J, Lousberg L, Schroeder H and Jerusalem G: Triple-negative breast cancer: Treatment challenges and solutions. Breast Cancer (Dove Med Press) 8: 93-107, 2016.
3. Maia AR, de Man J, Boon U, Janssen A, Song JY, Omerzu M, Sterrenburg JG, Prinsen MB, Willemsen-Seegers N, de Roos JA, *et al*: Inhibition of the spindle assembly checkpoint kinase TTK enhances the efficacy of docetaxel in a triple-negative breast cancer model. Ann Oncol 26: 2180-2192, 2015.
4. Sikov WM, Berry DA, Perou CM, Singh B, Cirrincione CT, Tolaney SM, Kuzma CS, Pluard TJ, Somlo G, Port ER, *et al*: Impact of the addition of carboplatin and/or bevacizumab to neoadjuvant once-per-week paclitaxel followed by dose-dense doxorubicin and cyclophosphamide on pathologic complete response rates in stage II to III triple-negative breast cancer: CALGB 40603 (Alliance). J Clin Oncol 33: 13-21, 2015.
5. Ali AM, Ansari JA, El-Aziz NMA, Abozeed WN, Warith AMA, Alsaleh K and Nabholtz JM: Triple negative breast cancer: A tale of two decades. Anticancer Agents Med Chem 17: 491-499, 2017.
6. Qi MS and Elion EA: MAP kinase pathways. J Cell Sci 18: 3569-3572, 2015.
7. Zhang Y, Wei L, Yu J, Li G, Zhang X, Wang A, He Y, Li H and Yin D: Targeting of the β6 gene to suppress degradation of ECM via inactivation of the MAPK pathway in breast adenocarcinoma cells. Oncol Rep 32: 1787-1795, 2014.
8. Cotrim CZ, Fabris V, Doria ML, Lindberg K, Gustafsson JÅ, Amado F, Lanari C and Helguero LA: Estrogen receptor beta growth-inhibitory effects are repressed through activation of MAPK and PI3K signalling in mammary epithelial and breast cancer cells. Oncogene 32: 2390-2402, 2013.
9. Borah N, Gunawardana S, Torres H, McDonnell S and Van Slambrouck S: 5,6,7,3',4',5'-Hexamethoxyflavone inhibits growth of triple-negative breast cancer cells via suppression of MAPK and Akt signaling pathways and arresting cell cycle. Int J Oncol 51: 1685-1693, 2017.
10. Nalwoga H, Arnes JB, Wabinga H and Akslen LA: Expression of EGFR and c-kit is associated with the basal-like phenotype in breast carcinomas of African women. APMIS 116: 515-525, 2008.
11. Normanno N, Campiglio M, Maiello MR, De Luca A, Mancino M, Gallo M, D'Alessio A and Menard S: Breast cancer cells with acquired resistance to the EGFR tyrosine kinase inhibitor gefitinib show persistent activation of MAPK signaling. Breast Cancer Res Treat 112: 25-33, 2008.
12. Abdelrahman AE, Rashed HE, Abdelgawad M and Abdelhamid MI: Prognostic impact of EGFR and cytokeratin 5/6 immunohistochemical expression in triple-negative breast cancer. Ann Diagn Pathol 28: 43-53, 2017.
13. Shi Y, Yang F, Huang D and Guan X: Androgen blockade based clinical trials landscape in triple negative breast cancer. Biochim Biophys Acta Rev Cancer 1870: 283-290, 2018.
14. Kim S, You D, Jeong Y, Yu J, Kim SW, Nam SJ and Lee JE: Berberine down-regulates IL-8 expression through inhibition of the EGFR/MEK/ERK pathway in triple-negative breast cancer cells. Phytomedicine 50: 43-49, 2018.
15. Gholami S, Chen CH, Gao S, Lou E, Fujisawa S, Carson J, Nnoli JE, Chou TC, Bromberg J and Fong Y: Role of MAPK in oncolytic herpes viral therapy in triple-negative breast cancer. Cancer Gene Ther 21: 283-289, 2014.
16. Giltnane JM and Balko JM: Rationale for targeting the Ras/MAPK pathway in triple-negative breast cancer. Discov Med 17: 275-283, 2014.
17. Zhao M, Howard EW, Parris AB, Guo Z, Zhao Q and Yang X: Alcohol promotes migration and invasion of triple-negative breast cancer cells through activation of p38 MAPK and JNK. Mol Carcinog 56: 849-862, 2017.

18. Oh AS, Lorant LA, Holloway JN, Miller DL, Kern FG and El-Ashry D: Hyperactivation of MAPK induces loss of ERalpha expression in breast cancer cells. Mol Endocrinol 15: 1344-1359, 2001.
19. Majorini MT, Manenti G, Mano M, De Cecco L, Conti A, Pinciroli P, Fontanella E, Tagliabue E, Chiodoni C, Colombo MP, *et al*: cIAP1 regulates the EGFR/Snai2 axis in triple-negative breast cancer cells. Cell Death Differ 25: 2147-2164, 2018.
20. Jing W: Expression and significance of MAPK protein in breast cancer. Journal of Ningxia Medical College 36: 743-745, 2014 (In Chinese).
21. Zenzola V, Cabezas-Quintario MA, Arguelles M, Pérez-Fernández E, Izarzugaza Y, Correa A and García-Foncillas J: Prognostic value of Ki-67 according to age in patients with triple-negative breast cancer. Clin Transl Oncol 20: 1448-1454, 2018.
22. Walsh EM, Shalaby A, O'Loughlin M, Keane N, Webber MJ, Kerin MJ, Keane MM, Glynn SA and Callagy GM: Outcome for triple negative breast cancer in a retrospective cohort with an emphasis on response to platinum-based neoadjuvant therapy. Breast Cancer Res Treat 174: 1-13, 2019.
23. Evans MK, Brown MC, Geradts J, Bao X, Robinson TJ, Jolly MK, Vermeulen PB, Palmer GM, Gromeier M, Levine H, *et al*: XIAP regulation by MNK links MAPK and NFκB signaling to determine an aggressive breast cancer phenotype. Cancer Res 78: 1726-1738, 2018.
24. Rakha EA, El-Sayed ME, Green AR, Lee AH, Robertson JF and Ellis IO: Prognostic markers in triple-negative breast cancer. Cancer 109: 25-32, 2007.
25. Shah SP, Roth A, Goya R, Oloumi A, Ha G, Zhao Y, Turashvili G, Ding J, Tse K, Haffari G, *et al*: The clonal and mutational evolution spectrum of primary triple-negative breast cancers. Nature 486: 395-399, 2012.
26. Liu W, Zhang L, Shi J, Liu Y, Zhou L, Hou K, Qu X and Teng Y: Clinical significance of pAkt and pErk1/2 expression in early-stage breast cancer patients treated with anthracycline-based adjuvant chemotherapy. Oncol Lett 9: 1707-1714, 2015.
27. Ono H, Sowa Y, Horinaka M, Iizumi Y, Watanabe M, Morita M, Nishimoto E, Taguchi T and Sakai T: The histone deacetylase inhibitor OBP-801 and eribulin synergistically inhibit the growth of triple-negative breast cancer cells with the suppression of survivin, Bcl-xL, and the MAPK pathway. Breast Cancer Res Treat 171: 43-52, 2018.
28. Uehara N, Kanematsu S, Miki H, Yoshizawa K and Tsubura A: Requirement of p38 MAPK for a cell-death pathway triggered by vorinostat in MDA-MB-231 human breast cancer cells. Cancer Lett 315: 112-121, 2012.
29. Peng B, He R, Xu Q, Yang Y, Hu Q, Hou H, Liu X and Li J: Ginsenoside 20(S)-protopanaxadiol inhibits triple-negative breast cancer metastasis in vivo by targeting EGFR-mediated MAPK pathway. Pharmacol Res 142: 1-13, 2019.