Ann Dermatol Vol. 33, No. 5, 2021
pISSN 1013-9087 · eISSN 2005-3894
https://doi.org/10.5021/ad.2021.33.5.432

ORIGINAL ARTICLE

Prognostic Significance of Epidermal Growth Factor Receptor Expression in Distant Metastatic Melanoma from Primary Cutaneous Melanoma

Keon Hee Lee*, Hyun Yi Suh*, Mi Woo Lee, Woo Jin Lee, Sung Eun Chang

Department of Dermatology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Background: Epidermal growth factor receptor (EGFR) is overexpressed in many cancers. However, EGFR expression in melanoma and its role are conflicting. **Objective:** This study aimed to evaluate EGFR expression in distant metastatic melanoma and analyze its relationship with histologic and clinical characteristics and survival. **Methods:** Diagnostic tissues from 55 cases of distant metastatic melanoma was evaluated by immunohistochemistry for EGFR expression. Clinicopathologic features and survival outcomes were analyzed according to EGFR expression. **Results:** The positive EGFR expression in distant metastatic melanoma was significantly correlated with the absence of ulceration. The EGFR expression in distant metastatic melanoma was significantly associated with poor survival, under the conditions of male sex and primary cutaneous melanoma without ulceration or Breslow thickness ≤4.0 mm. This study bears limitations of a retrospective study in a single institution. **Conclusion:** EGFR immunostaining had predictive values for survival outcome. The EGFR expression in distant metastatic melanoma in male, no ulcer, or Breslow thickness ≤4.0 mm appeared to be involved in disease progression. **(Ann Dermatol 33(5) 432~439, 2021)**

-Keywords-
ErbB receptors, Melanoma, Neoplasm metastasis

INTRODUCTION

Epidermal growth factor receptor (EGFR) is one of the transmembrane type I receptor tyrosine kinases, which belongs to the ErbB/HER protein family[1]. It is a major chemoattractant for invading cancer cells and is widely known to stimulate cell proliferation, angiogenesis, differentiation, migration, survival, and adhesion by downstream complex signaling pathways[2]. When ligand binds to the EGFR, the receptor undergoes dimerization, which results in receptor tyrosine kinase autophosphorylation, channelling of predominant mitogenic signals, and various signaling pathway activation, including the most significant Ras/Raf/MEK/extracellular signal-regulated kinase (ERK) pathway[1,3]. The protein kinase cascade from RAF to MEK to ERK provides opportunities for feedback regulation and signal amplification[4]. Signal transduction participates in regulation of cell proliferation, prevents apoptosis and promotes cell invasion, initiates actin polymerization, and begins microfilament reorganization, and these processes are essential for cell migration[3]. The actin-rich adhesive structures secrete proteases that digest extracellular matrix (ECM) elements, making the path for cancer cells to migrate through the surrounding microenvironment[5]. It generates a pro-

Received June 10, 2020, Revised February 9, 2021, Accepted for publication February 22, 2021

*These authors have equally contributed to the article.

Corresponding author: Woo Jin Lee, Department of Dermatology, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Korea. Tel: 82-2-3010-1663, Fax: 82-2-486-7831, E-mail: uucm79@hanmail.net
ORCID: https://orcid.org/0000-0002-0549-464X

Sung Eun Chang, Department of Dermatology, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Korea. Tel: 82-2-3010-3467, Fax: 82-2-486-7831, E-mail: csesnumd@gmail.com
ORCID: https://orcid.org/0000-0003-4225-0414

trusive force that allows cancer cells to form invadopodia, penetrate through ECM, and metastasize[6].

Deregulation of these cascades by activated EGFR is implicated in oncogenesis, because EGFR is a critical protein for the proliferation of cells[7]. EGFR is often overexpressed and dysregulated in several human malignancies, including breast cancer, epithelial gastrointestinal malignancies, gliomas, non-small cell lung cancer, colorectal cancer, and head and neck squamous cell carcinoma; and its overexpression is associated with tumor progression and poor outcome prognosis[8]. Nowadays, EGFR is used as a target antigen for specific anticancer therapies in many malignancies[9]. Correspondingly, blocking the EGFR signaling pathway is well known to suppress growth and metastasis of certain types of lung cancer[10].

Melanoma was one of the first cancers wherein EGFR protein expression was suggested as a probable metastatic marker[11]. Melanoma expresses several receptor tyrosine kinases, including EGFR family members; however, their roles have been controversial[12]. Many authors thought that EGFR expression could be indicative of cell maturation or of cellular proliferative capacity[13]. EGFR can be expressed in melanoma cells but not in normal melanocytes. The increasing EGFR protein expression turned out that it was linked with the progression of melanocytic lesions in most studies[14]. EGFR overexpression often occurs in the advanced stage of melanoma[15]. De Wit et al.[16] identified significant differential EGFR expression at various stages of melanocyte tumor progression involving 61% of dysplastic nevus, 89% of primary cutaneous melanoma, and 91% of metastatic melanoma. In addition, the staining intensity was stronger in malignant lesions than in benign lesions. Furthermore, Real et al.[17] found EGFR (+) only in undifferentiated melanoma cells and EGFR (−) in differentiated cell lines. However, reported results about the correlation between EGFR expression and prognosis in malignant melanoma are rather inconsistent, and there is no clear evidence about prognostic and diagnostic relevance[18]. Several studies reported about low EGFR expression in human melanocytes and melanoma[11]. Another study showed that EGFR expression did not correlate with the proliferation in melanoma cell panel. EGFR expression in the tissues also had no significant association with the proliferative state of cells[17].

Although little data exists to guide management of metastatic melanoma, the current first-line standard treatments are PD-1 blockade (nivolumab, pembrolizumab) with or without CTLA-4 blockade (ipilimumab). In addition, BRAF inhibition (vemurafenib, dabrafenib, encorafenib) combined with MEK inhibition (cobimetinib, trametinib, binimetinib) are recommended for BRAF V600-mutated metastatic melanoma[19].

There is a lack of prior researches on the correlation between EGFR expression and metastatic melanoma, and the same goes for the controversial role of EGFR in melanoma. Thus, this study aims to evaluate EGFR expression in distant metastatic melanoma and analyze its relationship with histopathologic and clinical characteristics, as well as survival outcomes.

Table 1. Clinicopathologic features of patients with metastatic melanoma

Clinicopathological feature	Number (%)	Mean (SD)
Sex		
Male	30 (54.5)	
Female	25 (45.5)	
Age at time of diagnosis (yr)		60.0 (11.7)
EGFR		
Negative	34 (61.8)	
Positive	21 (38.2)	
BRAF		
Negative	47 (85.5)	
Positive	8 (14.5)	
Location of primary melanoma		
Acral	24 (43.6)	
Extremities	14 (25.5)	
Head and neck	7 (12.7)	
Trunk	7 (12.7)	
Inguinal area	3 (5.5)	
Ulceration		
Absent	43 (78.2)	
Present	12 (21.8)	
Breslow thickness (mm)		
≤4.0	18 (32.7)	
>4.0	37 (67.3)	
Clark level		
3	8 (14.5)	
4	26 (47.3)	
5	21 (38.2)	
Sentinel lymph node involvement		
Negative	8 (22.9)	
Positive	27 (77.1)	
Location of metastasis		
Skin	38 (33.3)	
Lung	26 (22.8)	
Brain	14 (12.3)	
Liver	11 (9.6)	
Bone	11 (9.6)	
Digestive system	9 (7.9)	
Spine	5 (4.4)	
Follow-up duration (mo)		38.3 (31.1)
Mortality		
Dead	45 (81.8)	
Alive	10 (18.2)	

SD: standard deviation, EGFR: epidermal growth factor receptor.

MATERIALS AND METHODS

The database of the Asan Medical Center in Korea was investigated for cutaneous melanoma patients with distant metastasis confirmed through biopsy between January 2000 and December 2017 after receiving approval from the Institutional Review Board of the Asan Medical Center (no. 2018-1248). Patients with only in-transit, satellite, or microsatellite metastases were excluded. In-transit metastases are formally classified when these lesions are located between the primary site of the tumor and the locoregional lymph nodes[20].

Variables of interest

Age at diagnosis, sex, primary site of melanoma, ulceration, lymph node involvement and location of distant metastasis, follow-up results, and survival rate were collected from medical records and clinical photographs as clinical data. The researchers analyzed H&E stain sections of the primary cutaneous melanoma tissue, reviewing the Breslow thickness and Clark's levels, as well as two intervening sections for EGFR and BRAF stains as pathologic data. Overall survival (OS) data were calculated from the date of initial diagnosis to the date of death because of any cause, or the patient's last follow-up examination.

Statistical analysis

Clinicopathologic comparison between the EGFR (+) and EGFR (−) groups was performed using statistical methods of analysis. Chi-square test or Fisher's exact test was used when the dependent variable was categorical, depending on whether the number of cells whose expected frequency was less than 5 is less than 25% of the total. When the dependent variable was continuous, the independent t-test or Mann–Whitney U test was used, depending on whether normality was satisfied. Survival analysis was conducted using the Kaplan–Meier method, and the differences in survival between the subgroups were compared. All analyses were performed using Window SPSS ver. 21.0 (IBM Corp., Armonk, NY, USA). *p*-values of <0.05 were considered to indicate statistical significance.

RESULTS

Clinicopathologic features of patients with distant metastatic melanoma

A total of 55 cases of distant metastatic melanoma from primary cutaneous melanoma were included in this retrospective review between January 2000 and December 2017. The demographic data and clinical features of these cases are summarized in Table 1. Among the patients, 30 (54.5%) were male, and 25 (45.5%) were female. The mean age at the time of diagnosis was 60.0 years old. EGFR immunohistochemical stain of metastatic melanoma tissues was positive in 21 (38.2%) patients (Fig. 1). BRAF immunohistochemical stain was positive in 8 (14.5%)

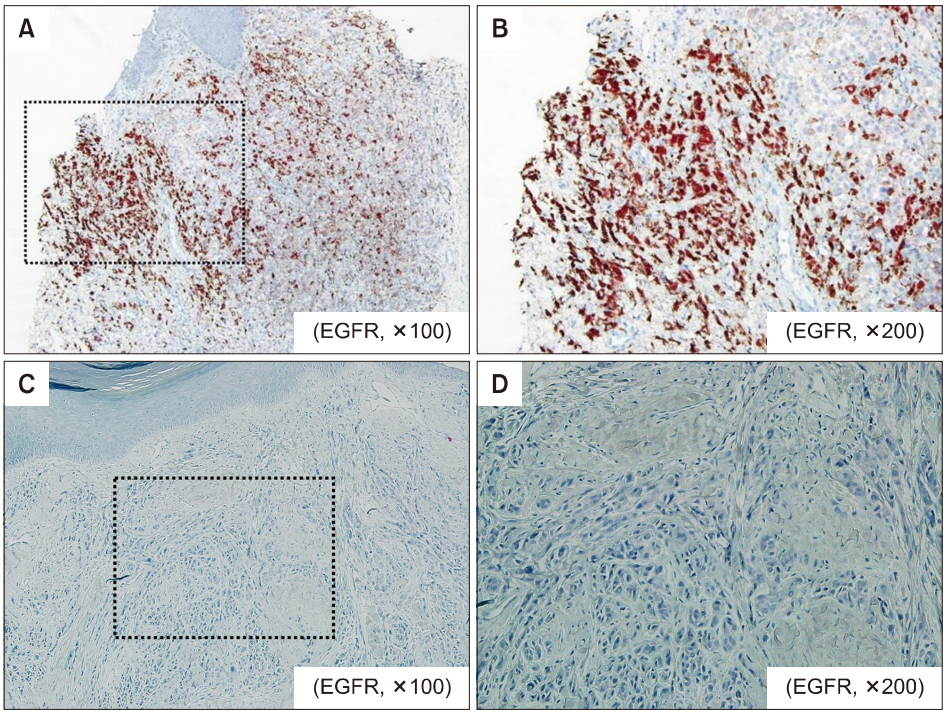

Fig. 1. Epidermal growth factor receptor (EGFR) immunostaining in melanoma sections. (A, B) EGFR protein is strongly expressed on the melanoma section of the labia minora of a 63-year-old female patient. (C,D) EGFR protein is not expressed on the melanoma section of the left 2nd toe of a 61-year-old female patient. Original magnification: (A) ×100, (B) ×200, (C) ×100, (D) ×200.

patients. The frequency of primary cutaneous melanoma location was highest in the acral area (n=24, 43.6%), followed by the extremities (n=14, 25.5%), trunk area (n=7, 12.7%), head and neck area (n=7, 12.7%), and inguinal area (n=3, 5.5%) (Table 1). This is the result in line with the previous study revealing cutaneous melanoma in Korea is most common in acral area[21]. Furthermore, 12 cases (21.8%) had ulcerations on the cutaneous lesion. The Breslow thickness of 37 cases (67.3%) was >4.0 mm, which indicates T4 stage in the American Joint Committee on Cancer (AJCC) melanoma staging system. In the aspect of Clark's level of invasion, 47 cases (85.5%) were level 4 or 5. A total of 35 patients had sentinel lymph node (SLN) biopsy, of which 77.1% showed positive melanoma involvement in the SLN. Moreover, 38 cases (33.3%) had metastasis in different skin areas from the primary site, and the most common location of metastasis, except for the skin, was lungs (n=26, 22.8%) (Table 1). The average follow-up time of the patients was 38.3 months. In summary, a total of 45 patients (81.8%) died during the observation period.

Table 2. Epidermal growth factor receptor (EGFR) expression and clinicopathologic parameters

Variable	EGFR		*p*-value
	Negative (n=34)	Positive (n=21)	
Sex			0.389†
Female	17	8	
Male	17	13	
Age (yr)	59.3±12.0	61.1±11.3	0.583‖
BRAF			0.236§
Negative	31	16	
Positive	3	5	
Location of primary melanoma			0.554†
Acral	17	7	
Extremities	9	5	
Head and neck	4	3	
Trunk	3	4	
Inguinal area	1	2	
Ulceration			0.019*§
Absent	23	20	
Present	11	1	
Breslow thickness (mm)	6.79±3.17	6.00±2.92	0.064†
≤4.0	8	10	0.291‡
>4.0	26	11	
Clark level			0.243†
3	3	5	
4	16	10	
5	15	6	
Sentinel lymph node involvement			1.000§
Negative	6	2	
Positive	20	7	
Follow-up duration (mo)	40.3±30.7	35.0±27.1	0.549‖
Mortality			0.287§
Dead	26	19	
Alive	8	2	

Values are presented as number only or mean±standard deviation. Statistically significant (*$p<0.05$). †Chi square test; ‡Mann-Whitney U test; §Fisher's exact test; ‖Independent t-test.

Association between EGFR expression and clinicopathologic variables

The clinicopathologic variables of distant metastatic melanoma were stratified depending on the expression of EGFR in tumor tissues to assess their associations (Table 2). There were significant correlations between EGFR expression and absence of ulceration ($p=0.019$). Among 21 patients with EGFR (+) metastatic melanoma, only one patient had a skin lesion with ulceration. The BRAF (+) rate was higher in the EGFR (+) group compared with the EGFR (−) group, but there was no statistically significant difference ($p=0.236$). The proportion of patients with Breslow thickness >4.0 mm of melanoma was lower in the EGFR (+) group than in the EGFR (−) group; however, no statistical significance was found ($p=0.064$). Any

Table 3. Epidermal growth factor receptor (EGFR) expression and location of metastasis

Variable	EGFR		*p*-value
	Negative (%) (n=34)	Positive (%) (n=21)	
Skin			0.768†
No	11 (32.4)	6 (28.6)	
Yes	23 (67.6)	15 (71.4)	
Lung			0.088†
No	21 (61.8)	8 (38.1)	
Yes	13 (38.2)	13 (61.9)	
Brain			0.091†
No	28 (82.4)	13 (61.9)	
Yes	6 (17.6)	8 (38.1)	
Liver			0.731‡
No	28 (82.4)	16 (76.2)	
Yes	6 (17.6)	5 (23.8)	
Bone			1.000‡
No	27 (79.4)	17 (81.0)	
Yes	7 (20.6)	4 (19.0)	
Digestive system			0.719‡
No	29 (85.3)	17 (81.0)	
Yes	5 (14.7)	4 (19.0)	
Spine			1.000‡
No	31 (91.2)	19 (90.5)	
Yes	3 (8.8)	2 (9.5)	

Values are presented as number (%). †Chi square test; ‡Fisher's exact test.

other significant association with EGFR expression was not found in the other factors, including SLN involvement. A comparison between EGFR expression and metastatic location was also not statistically meaningful, although the ratio of brain metastasis in thee EGFR (+) group was slightly higher than that in the EGFR (−) group ($p=0.091$) (Table 3).

Survival outcomes in distant metastatic melanoma patients

The EGFR (+) group showed a shorter average follow-up duration than the EGFR (−) group as shown in Table 2, but it was not statistically significant ($p=0.549$). The mortality rate observed in the EGFR (+) group was higher compared with the EGFR (−) group, but there was no statistical significance either ($p=0.287$). The researchers also performed multivariate analysis with EGFR expression, sex, age, ulceration, Breslow thickness, SLN involvement, and BRAF expression as independent variables to consider all variables affecting follow-up duration. However, no statistically significant variable can be obtained from the multiple regression analysis.

The median OS of all the patients with metastatic melanoma obtained using Kaplan–Meier survival analysis was 31.2 months (95% confidence interval [CI], 18.3～44.1 months). The median OS of the EGFR (+) group was 22.2 (95% CI, 18.3～36.1) months, and it was shorter than that of the EGFR (−) group, whose median OS was 37.0 months (95% CI, 31.8～42.2). However, the researchers could not find a significant difference between the cumulative survival rates of both groups ($p=0.076$).

The OS data in this research are graphed in Fig. 2. The blue lines represent the EGFR (−) group, and the green lines represent the EGFR (+) group. At first, EGFR expression had no meaningful predictive value for the OS in patients with metastatic melanoma (Fig. 2A; $p=0.076$). When analyzed by subgroups, EGFR expression on meta-

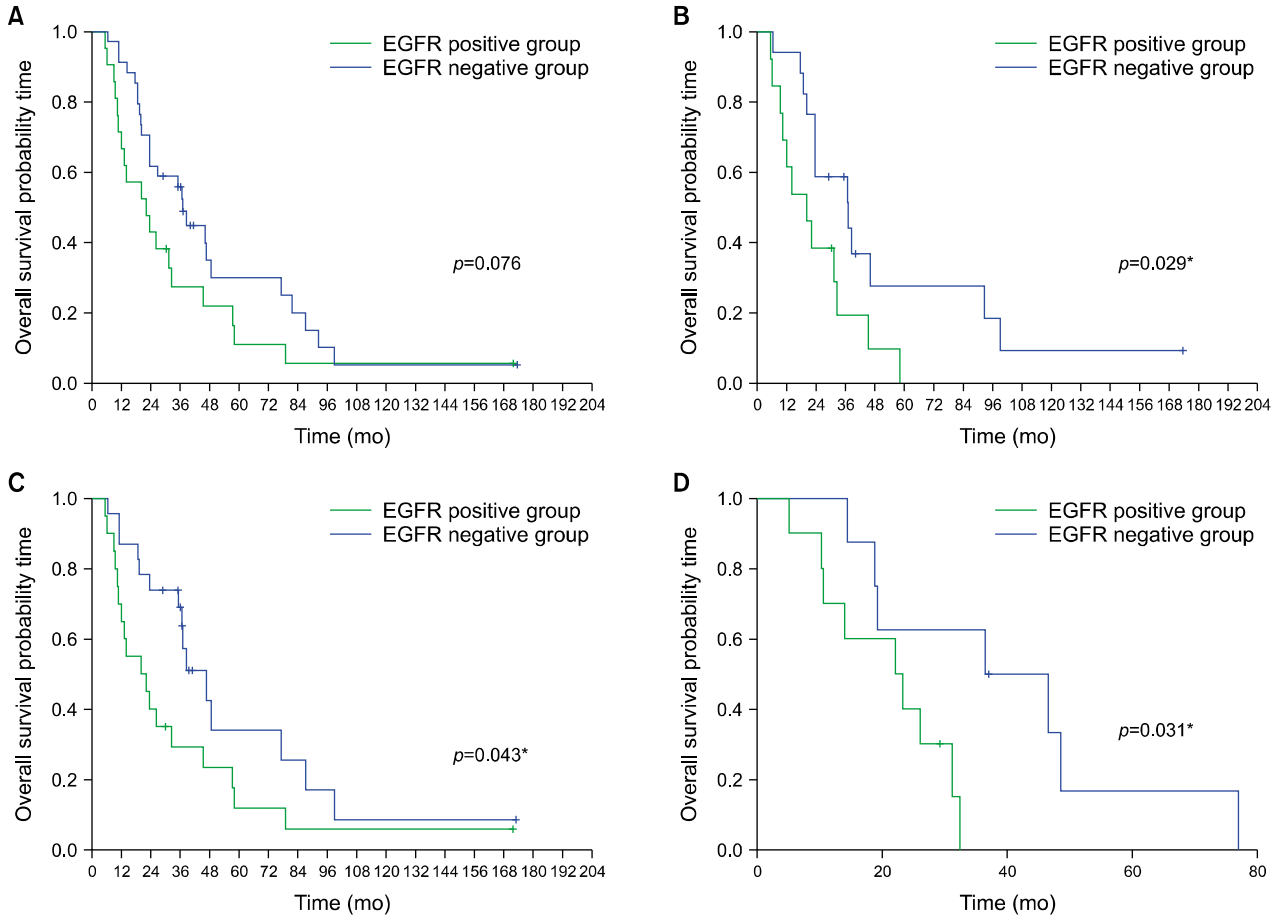

Fig. 2. Kaplan–Meier survival analysis in metastatic melanoma for comparison according to epidermal growth factor receptor (EGFR) expression. (A) Overall survival of all cases of metastatic melanoma showed no difference between the EGFR positive group and EGFR negative group. Overall survival outcomes in metastatic melanoma between the EGFR positive group and EGFR negative group were significantly different under the condition of (B) male, (C) without ulcer, or (D) Breslow thickness ≤4.0 mm. *Statistically significance ($p<0.05$).

static melanoma in case of male (Fig. 2B; n=30, $p=0.029$), cutaneous melanoma without ulceration (Fig. 2C; n=43, $p=0.043$), or Breslow thickness ≤4.0 mm (Fig. 2D; n=18, $p=0.031$) was significantly associated with poor prognosis based on the survival data. In other subgroups, including female, cutaneous melanoma with ulcer, or Breslow thickness >4.0 mm, no significant difference were found concerning the survival outcomes depending on whether EGFR expression was present or not.

DISCUSSION

In a previous study, *EGFR* gene amplification has been found in primary cutaneous melanoma, and it was associated with poor prognosis[22]. Rákosy et al.[11] also studied with 81 samples of cutaneous melanoma about *EGFR* gene copy number index. They found that tumors with a higher gene copy number formed metastasis within 5 years after diagnosis, and high-level gene amplification that is usually the whole gain of chromosome 7 was only observed in metastatic tumors. Generally, melanoma patients with highly amplified or extra *EGFR* gene copies showed poor outcomes with higher invasion capacity. *EGFR* gene copy number alternation was associated with elevated EGFR mRNA expression. However there was no strong correlation between gene copy number alteration and EGFR protein expression level. In this regard, the researchers conducted a study to investigate the relationship between EGFR protein expression and clinical data of patients with metastatic melanoma from primary cutaneous melanoma, which has the poorest prognosis and rare treatment options.

When comparing our research results about EGFR expression and clinicopathologic parameters with a previous study[23], EGFR protein expression was not less frequently observed in metastatic melanoma patients with a positive SNL (Table 2). Nevertheless, there was no statistically significant correlation between SNL involvement and EGFR expression.

In terms of metastasis, when EGFR is expressed in melanoma, there was a tendency to show metastasis at a higher rate in the lungs (1.62 times, $p=0.088$) and the brain (2.16 times, $p=0.091$) compared with the event when EGFR expression was negative, although it was not statistically significant (Table 3). In the 8th edition of the AJCC melanoma staging system, M1a is defined as distant metastasis to skin, soft tissues, and/or non-regional lymph nodes. M1b is also defined as distant metastasis to the lungs with or without M1a sites. If there is a metastasis to the central nervous system, it is unconditionally defined as M1d in order for the poor prognosis[24]. From this point of view, the tendency that the proportion of lung metastasis corresponding to M1b and brain metastasis corresponding to M1d may be higher in the EGFR (+) group, suggesting that there may be a possibility of affecting patient survival in metastatic melanoma. When comparing the mortality among the metastatic melanoma patients in our study, it was higher in the the EGFR (+) group, but there was no statistical significance either (Table 2). The median OS was 31.2 months (95% CI, 18.3～44.1) in 55 metastatic melanoma patients. To further analyze survival outcome in more detail, there was no significant difference in OS according to EGFR expression ($p=0.076$) in the Kaplan–Meier survival analysis. It was not consistent with the results of the past study about 44 nodular melanoma patients, which documented that EGFR overexpression showed a correlation with shorter OS[18]. Our results showed also slightly shorter median OS in the EGFR (+) group.

When analyzing metastatic melanoma patients per subgroup, the OS of the EGFR (+) group was found to be statistically significantly shorter under the condition of male ($p=0.029$), without ulceration of primary cutaneous melanoma lesion ($p=0.043$), or Breslow thickness ≤4.0 mm of primary cutaneous melanoma lesion ($p=0.031$; Fig. 2). On another note, statistically significant results were not obtained in subgroups of female, with ulceration, or Breslow thickness >4.0 mm.

There was no statistically significant difference in the sex ratio ($p=0.389$) of metastatic melanoma patients according to EGFR expression (Table 2). In this regard, the EGFR expression of male metastatic melanoma patients may be considered as a factor influencing prognosis. Moreover, there was one study on sex differences and EGFR expression in melanoma patients, which reported the higher rate of deletion and low level *EGFR* gene amplification in male patients[11].

Generally, ulceration is known as the third most powerful predictor of survival outcome in melanoma[25]. When analyzing ulceration in primary cutaneous melanoma with metastasis and EGFR expression, ulceration is rarely accompanied in the EGFR (+) group compared with the EGFR (−) group ($p=0.019$; Table 2). This is in conflict with the findings of previous researches that the presence of ulceration in melanoma has a higher *EGFR* gene copy number index, and the high EGFR expression level of nodular melanoma significantly presented ulceration more often[11,18] Furthermore, metastatic melanoma without ulceration showed significant poorer prognosis when accompanied by EGFR expression in our data ($p=0.043$; Fig. 2C). This might be due to the rapid exacerbation of metastasis when EGFR is expressed even before primary lesion ulceration is observed by the naked eye, encouraging pa-

tients to seek medical attention.

Although there was no statistical significance, the proportion of Breslow thickness ≤4.0 mm was higher in the EGFR (+) group ($p=0.064$; Table 2). Based on the AJCC 8th edition, if the Breslow thickness is >4.0 mm, it is the T4 stage. This results are consistent with the negative correlation between membrane EGFR expression and melanoma thickness in the prior study[18]. However, there was also a study that reported that the *EGFR* gene copy number index was higher when the Breslow thickness was > 4.0 mm[11]. In our study, when the Breslow thickness was ≤4.0 mm, the prognosis of the EGFR (+) group was found to be worse ($p=0.031$; Fig. 2D). It can be inferred that, as in ulceration, melanoma metastasizes rapidly due to EGFR expression and develops the systemic spreading function early before the primary cutaneous lesion becomes severe.

Looking at the data of the patients, no significant relationship was observed between EGFR and BRAF expression findings in metastatic melanoma ($p=0.236$, Table 2). The ratio of EGFR positivity (38.2%) was higher than that of BRAF positivity (14.5%) in the samples of metastatic melanoma tissue. The difference in survival rate depending on EGFR expression did not show any statistical significance regardless of BRAF mutation in the Kaplan–Meier survival analysis.

In conclusion, EGFR expression in distant metastatic melanoma had predictive values for survival outcomes under the certain conditions. EGFR expression in distant metastatic melanoma in male, without ulceration, or Breslow thickness ≤4.0 mm of the primary cutaneous melanoma lesion may be involved in the progression of the disease.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

FUNDING SOURCE

None.

DATA SHARING STATEMENT

Research data are not shared.

ORCID

Keon Hee Lee, https://orcid.org/0000-0001-8288-1275
Hyun Yi Suh, https://orcid.org/0000-0001-8820-0880
Mi Woo Lee, https://orcid.org/0000-0003-4669-9454
Woo Jin Lee, https://orcid.org/0000-0002-0549-464X
Sung Eun Chang, https://orcid.org/0000-0003-4225-0414

REFERENCES

1. Hynes NE, MacDonald G. ErbB receptors and signaling pathways in cancer. Curr Opin Cell Biol 2009;21:177-184.
2. Seshacharyulu P, Ponnusamy MP, Haridas D, Jain M, Ganti AK, Batra SK. Targeting the EGFR signaling pathway in cancer therapy. Expert Opin Ther Targets 2012;16:15-31.
3. Di Domenico M, Giordano A. Signal transduction growth factors: the effective governance of transcription and cellular adhesion in cancer invasion. Oncotarget 2017;8:36869-36884.
4. Avruch J, Khokhlatchev A, Kyriakis JM, Luo Z, Tzivion G, Vavvas D, et al. Ras activation of the Raf kinase: tyrosine kinase recruitment of the MAP kinase cascade. Recent Prog Horm Res 2001;56:127-155.
5. Yamaguchi H. Pathological roles of invadopodia in cancer invasion and metastasis. Eur J Cell Biol 2012;91:902-907.
6. Mader CC, Oser M, Magalhaes MA, Bravo-Cordero JJ, Condeelis J, Koleske AJ, et al. An EGFR-Src-Arg-cortactin pathway mediates functional maturation of invadopodia and breast cancer cell invasion. Cancer Res 2011;71:1730-1741.
7. Feigin ME, Muthuswamy SK. ErbB receptors and cell polarity: new pathways and paradigms for understanding cell migration and invasion. Exp Cell Res 2009;315:707-716.
8. Nicholson RI, Gee JM, Harper ME. EGFR and cancer prognosis. Eur J Cancer 2001;37 Suppl 4:S9-S15.
9. Motoyama AB, Hynes NE, Lane HA. The efficacy of ErbB receptor-targeted anticancer therapeutics is influenced by the availability of epidermal growth factor-related peptides. Cancer Res 2002;62:3151-3158.
10. Liu X, Huang YG, Jin CG, Zhou YC, Chen XQ, Li J, et al. MicroRNA-370 inhibits the growth and metastasis of lung cancer by down-regulating epidermal growth factor receptor expression. Oncotarget 2017;8:88139-88151.
11. Rákosy Z, Vízkeleti L, Ecsedi S, Vokó Z, Bégány A, Barok M, et al. EGFR gene copy number alterations in primary cutaneous malignant melanomas are associated with poor prognosis. Int J Cancer 2007;121:1729-1737.
12. Czyz M. Fibroblast growth factor receptor signaling in skin cancers. Cells 2019;8:540.
13. Yamada T, Takagi M, Shioda S. Evaluation of epidermal growth factor receptor in squamous cell carcinoma of the oral cavity. Oral Surg Oral Med Oral Pathol 1992;73:67-70.
14. Ellis DL, King LE Jr, Nanney LB. Increased epidermal growth factor receptors in melanocytic lesions. J Am Acad Dermatol 1992;27:539-546.
15. Kovacs E, Zorn JA, Huang Y, Barros T, Kuriyan J. A structural perspective on the regulation of the epidermal growth factor receptor. Annu Rev Biochem 2015;84:739-764.
16. De Wit PE, Moretti S, Koenders PG, Weterman MA, Van Muijen GN, Gianotti B, et al. Increasing epidermal growth factor receptor expression in human melanocytic tumor progression. J Invest Dermatol 1992;99:168-173.
17. Real FX, Rettig WJ, Chesa PG, Melamed MR, Old LJ,

Mendelsohn J. Expression of epidermal growth factor receptor in human cultured cells and tissues: relationship to cell lineage and stage of differentiation. Cancer Res 1986;46:4726-4731.
18. Katunarić M, Jurišić D, Petković M, Grahovac M, Grahovac B, Zamolo G. EGFR and cyclin D1 in nodular melanoma: correlation with pathohistological parameters and overall survival. Melanoma Res 2014;24:584-591.
19. Ascierto PA, McArthur GA, Dréno B, Atkinson V, Liszkay G, Di Giacomo AM, et al. Cobimetinib combined with vemurafenib in advanced BRAF(V600)-mutant melanoma (coBRIM): updated efficacy results from a randomised, double-blind, phase 3 trial. Lancet Oncol 2016;17:1248-1260.
20. Testori A, Ribero S, Bataille V. Diagnosis and treatment of in-transit melanoma metastases. Eur J Surg Oncol 2017;43:544-560.
21. Lim Y, Lee J, Lee DY. Is the survival rate for acral melanoma actually worse than other cutaneous melanomas? J Dermatol 2020;47:251-256.
22. Giard DJ, Aaronson SA, Todaro GJ, Arnstein P, Kersey JH, Dosik H, et al. In vitro cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. J Natl Cancer Inst 1973;51:1417-1423.
23. Boone B, Jacobs K, Ferdinande L, Taildeman J, Lambert J, Peeters M, et al. EGFR in melanoma: clinical significance and potential therapeutic target. J Cutan Pathol 2011;38:492-502.
24. Keung EZ, Gershenwald JE. The eighth edition American Joint Committee on Cancer (AJCC) melanoma staging system: implications for melanoma treatment and care. Expert Rev Anticancer Ther 2018;18:775-784.
25. Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Atkins MB, Byrd DR, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol 2009;27:6199-6206.