

Nomogram Incorporating the WNT/ β -Catenin Signaling Pathway for Predicting the Survival of Cutaneous Melanoma

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Background: Accurate prediction of the survival of cutaneous melanoma (CM) permits the selection of the optimal treatment. Currently, the TNM stage has limitations in predicting the survival of CM. There is evidence that the WNT/ β -catenin signaling pathway has the potential to predict the CM prognosis. However, it still needs further investigation.

Objective: This study aims to establish a nomogram incorporating the WNT/ β -catenin signaling pathway to improve the predicted accuracy of the overall survival (OS) of CM.

Methods: Two hundred and eighty CM patients were recruited and followed up. The clinicopathological characteristics and the key genes of the WNT/ β -catenin signaling pathway (VEGF, β -catenin, and DKK1) were chosen as potential variables associated with the OS. In the training cohort (n = 190), a nomogram was built to estimate the 1-, 3-, and 5-year OS, and its discriminations and calibrations were valid by the verification cohort (n = 90). The predicted accuracies of the nomogram with or without the Wnt/ β -catenin pathway and TNM stage were compared.

Results: A nomogram integrating independent risk factors (ulceration, lymph node metastasis, distant metastasis, Breslow thickness, dermal mitoses, β -catenin, VEGF, and DKK1), which were evaluated by a multivariate analysis, was constructed to predict the 1-, 3-, and 5-year OS of CM patients. Good discrimination and calibration were obtained regardless of the training or validation datasets. The nomogram incorporating the Wnt/ β -catenin signaling pathway showed the highest accuracy [area under the curve (AUC)=0.914, 0.852, 0.785] compared with the nomogram without the Wnt/ β -catenin signaling pathway (AUC=0.693, 0.640, 0.615) and the TNM stage (AUC=0.726, 0.693, 0.673).

Conclusion: The prognostic value of the established nomogram incorporating the WNT/ β -catenin signaling pathway was better than it without WNT/ β -catenin signaling pathway and TNM stage, which might be beneficial in the development of optimal treatment options.

Keywords: cutaneous melanoma, WNT/ β -catenin signaling pathway, TNM, overall survival, nomogram

Introduction

Cutaneous melanoma (CM) is a highly aggressive malignant tumor with increasing morbidity and mortality rates worldwide.¹ The use of the American Joint Committee on Cancer (AJCC) staging, which usually determines the prognosis of CM patient based on tumor thickness, ulceration, mitotic rate, lymph-node metastasis, and distant metastasis, has limitations in predicting the survival of CM since the survival outcomes vary widely even within the same stage.²⁻⁵ It has been

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hypothesized that the prognostic prediction of CM will be improved by adding measurements of molecular features to the current staging. New biomarkers to address the inconsistencies of an imperfect staging are needed.⁶ Evidence from melanocyte development indicate the possibility that WNT/ β -catenin signaling orchestrates melanoma progression by regulating cell proliferation and invasion and modulating the immune microenvironment.^{7,8} An increasing level of nuclear β -catenin has been shown to play a critical role in melanoma during disease progression.^{9,10} In actively proliferating melanoma cells, nuclear β -catenin triggers the expression of microphthalmia-associated transcription factor that in turn activates the transcription of several downstream genes including VEGF.^{11–13} Besides, aberrant expression of WNT/ β -catenin antagonists (eg, DKKs) is common in melanoma and is associated with elevated β -catenin level.¹⁴ Hence, it is not surprising that the WNT/ β -catenin signaling pathway may play an important role in the initiation and progression of CM.^{15–17} Growing research has shown that key genes of the WNT/ β -catenin signaling pathway (β -catenin, VEGF, and DKK1) have the potential to act as new biomarkers to predict the prognosis of CM patients.^{18–21} However, an interstudy variability exists despite extensive studies.²²

This study aims to establish a nomogram associated with the clinicopathological characteristics and the WNT/ β -catenin signaling pathway and assess whether the incorporation of the WNT/ β -catenin signaling pathway increased the accuracy in the prediction of the CM prognosis.

Patients and Methods

Population

This prospective study was conducted following the declaration of Helsinki. All procedures were approved by the ethical committee of North China University of Science and Technology Affiliated Hospital. Written informed consents were obtained from all participants.

A total of 280 CM patients (53.03±16.82 years) who underwent complete surgical resection between January 2010 and December 2014 were recruited in this study. The pathologic diagnoses of CM were evaluated from postsurgical pathology. None of the patients received preoperative chemotherapy or radiation therapy. Patients with other malignancies and incomplete follow-up data were excluded.

Collection of Clinical and Pathological Indicators

Drawing on the literature and the available evidence, the following clinical and pathological characteristics were chosen as potential variables. The continuous variables were transformed into dichotomous variables at median values. Clinical characteristics including gender, age (<55 and \geq 55 years), surgical resection range, lymph node metastasis, distant metastasis, dermal mitoses (<2/mm² and \geq 2/mm²),²³ history of misdiagnosis, and post-operative interferon alfa-2b therapy. Pathological characteristics including Breslow thickness (<1 mm, 1–2 mm, and >2 mm),²⁴ ulceration, Clark level (I–III vs IV–V),²⁵ histologic subtype, and TNM stage (AJCC, seventh ed.).²⁶

Immunohistochemistry

Formalin-fixed paraffin-embedded tissue specimens were first deparaffinized and rehydrated with xylene and graded alcohol, and then incubated in boiled citrate buffer (pH=6.0) for optimal antigen retrieval. Primary antibody of VEGF (Cat No. TA802346, Zhongshan Golden Bridge Biotechnology, China), β -catenin (Cat No. ZM-0442, Zhongshan Golden Bridge Biotechnology, China), and DKK1 (21112-1-AP, Proteintech, Wuhan, China) at 1:200 dilution was used to incubate the specimens overnight at 4°C. After rinsing with phosphate-buffered solution, the secondary antibody (Zhongshan Golden Bridge Biotechnology, Beijing, China) labeled with streptavidin-biotin-peroxidase reagent was used to incubate the slides for 2 h at room temperature and the 3,3'-diaminobenzidine solution (Zhongshan Golden Bridge Biotechnology, Beijing, China) was applied for antigen visualization. Finally, the slides were counterstained with hematoxylin (Zhongshan Golden Bridge Biotechnology, Beijing, China).

The slides were scanned by Olympus Dot-slide microscope digital system and evaluated in 10 randomly selected fields in the core of the tumor. The results of immunohistochemistry were evaluated by two senior pathologists to select the densest tumor area. The expressions of β -catenin, VEGF, and DKK1 were scored based on the percentage of

Table I Criteria for Immunohistochemistry Score

Variables	1 Point	2 Points	3 Points
β -catenin	<1/3	1/3–2/3	>2/3
VEGF	<10%	10–50%	>50%
DKK1	<25%	25–50%	>50%

Abbreviations: VEGF, vascular endothelial growth factor, DKK1, Dickkopf 1.

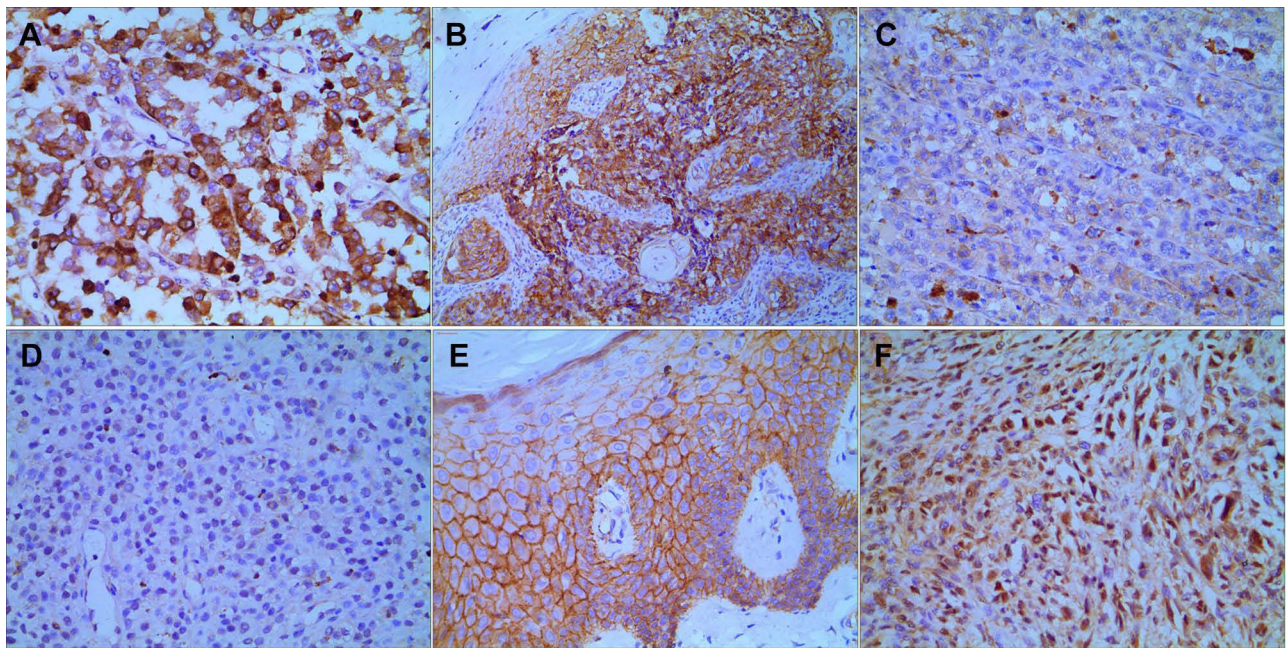


Figure 1 Immunohistochemical staining of CM and control tissue. In the CM tissue, positive VEGF expression (A), positive β -catenin expression in the cytoplasm and nuclei (B), and negative DKK1 expression (C) were displayed. In the control tissue, negative VEGF expression (D), positive β -catenin expression in the cell membrane (E), and positive DKK1 expression (F) were displayed. Positive cells exhibited brown cytoplasmic staining (at 400 \times magnification).

Abbreviations: CM, cutaneous melanoma; VEGF, Vascular Endothelial Growth Factor; DKK1, Dickkopf 1.

immunohistochemical staining in tumor cells (Table 1). Examples of different staining intensities are shown in Figure 1.

Study Protocol

The CM patients were followed up every 3 months for the first 3 years and then every 6 months for the fourth and fifth years. Overall survival (OS) rates were analyzed from the date of operation to the date of death or last follow-up. The recruited patients were randomly divided into two cohorts [(training cohort (n = 190) and verification cohort (n = 90)]. In the training cohort, univariate and multivariate analyses were used to determine the independent prognosis factors in CM patients. A nomogram was built based on the independent prognosis factors to estimate the 1-, 3-, and 5-year OS. The accuracies of the nomogram with or without the Wnt/ β -catenin pathway and TNM stage in predicting the 1-, 3-, and 5-year OS were compared.

Statistical Analysis

The follow-up was analyzed by Kaplan–Meier (KM) method. Log rank tests were used to determine univariate prognostic factors and those with $P < 0.05$ were entered into a multivariate Cox regression model, which estimated the hazard ratio (HR) and 95% confidence interval (CI) for

parameters associated with the prognosis of CM. The nomogram was verified internally and externally in the training and validation cohorts. The discrimination of the nomogram was evaluated by the area under the curve (AUC) of the receiver operating characteristic (ROC) curve, while the calibration was evaluated by the calibration curve and Hosmer–Lemeshow (HL) test. The AUCs of ROC were used for the comparison of the accuracies of different prediction models.

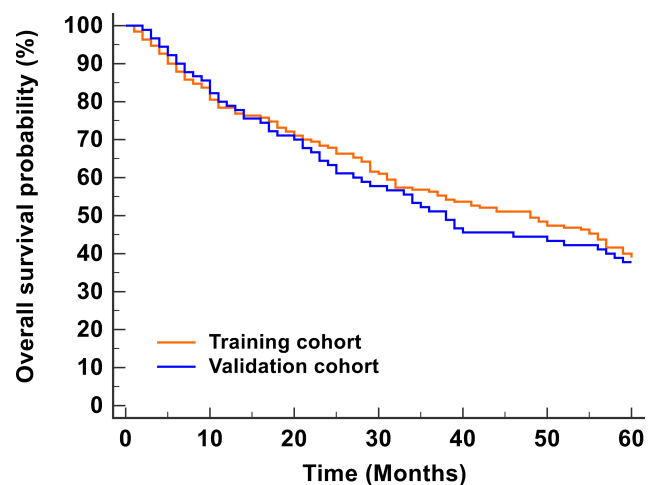


Figure 2 KM analysis for the OS probability of CM patients. The 1-, 3-, 5-year OS rates in the training cohort (78.4%, 56.3%, 43.9%) and the validation cohort (77.8%, 51.6%, 41.0%) were similar.

Abbreviations: KM, Kaplan-Meier; OS, overall survival; CM, cutaneous melanoma.

IBM SPSS Statistics for Windows version 22.0 (IBM Corp., Armonk, NY, United States), R package in R version 3.6.2. and MedCalc Statistical Software version 22.0.1 (MedCalc Software Ltd, Ostend, Belgium) were used to analyse the data.

Results

Characteristics and OS of CM Patients

There is no obvious difference in the data between the training and validation cohorts, which demonstrated that it was

reasonable to be used as the training and validation datasets. No significant difference was observed in the 1-, 3-, and 5-year OS rates between the training (78.4%, 56.3%, 43.9%) and validation datasets (77.8%, 51.6%, 41.0%) (Figure 2).

Independent Prognostic Factors

In the training cohort, the presence of ulceration, lymph node metastasis, distant metastasis, high levels of Breslow thickness, Clark levels IV–V, dermal

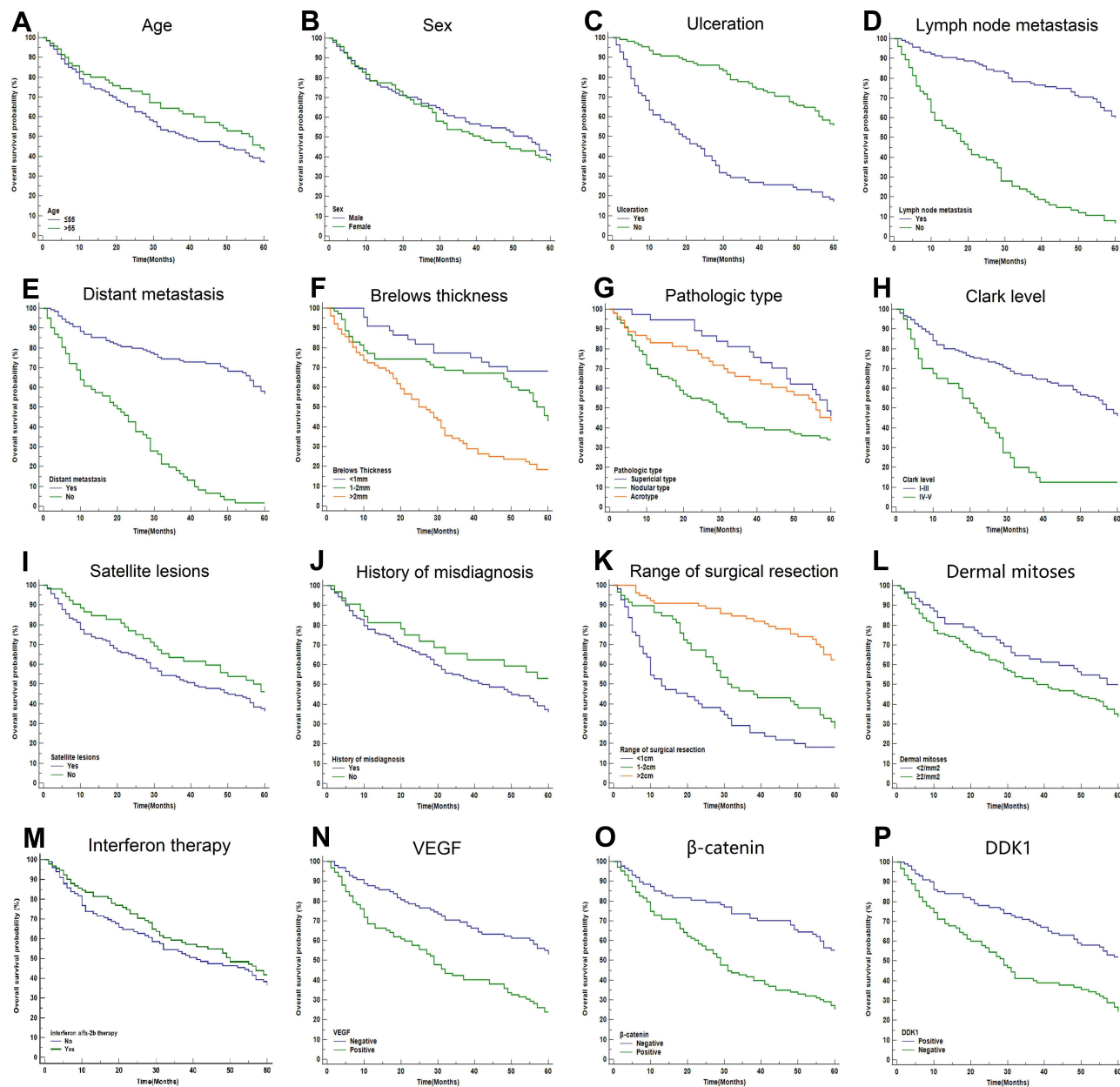


Figure 3 KM analyses of indicators in predicting the prognosis of CM patients within 5 years. KM curves at different classifications of age (A), sex (B), ulceration (C), lymph node metastasis (D), distant metastasis (E), Breslow thickness (F), pathologic type (G), Clark level (H), satellite lesions (I), history of misdiagnosis (J), range of surgical resection (K), dermal mitoses (L), interferon therapy (M), VEGF (N), β -catenin (O), and DKK1 (P) were shown. It revealed that ulceration, distant metastasis, lymph node metastasis, Breslow thickness, Clark levels, dermal mitoses, β -catenin, VEGF, and DKK1 were associated with the OS.

Abbreviations: KM, Kaplan-Meier; CM, cutaneous melanoma; VEGF, vascular endothelial growth factor; DKK1, Dickkopf 1; OS: overall survival.

mitoses $\geq 2/\text{mm}^2$, positive staining of β -catenin, VEGF, and negative staining of DKK1 were associated with shorter OS (all $P < 0.05$) (Figure 3 and Table 2). Multivariate Cox analysis showed that ulceration, lymph node metastasis, distant metastasis, Breslow thickness, dermal mitoses, β -catenin, VEGF, and

DKK1 were independently associated with OS of CM patients (Figure 4).

Nomogram Establishment and Validation

A nomogram integrating these independent risk factors was constructed to predict the 1-, 3-, and 5-year OS of

Table 2 Association of Survival with Clinical and Pathological Parameters in CM Patients Within 5 Years

Variables		N (%)	1-Year OS Rate	3-Year OS Rate	5-Year OS Rate	χ^2	P
Age	≥ 55	120 (63.2%)	76.8%	55.3%	40.0%	1.232	0.281
	< 55	70 (36.8%)	81.4%	64.3%	42.9%		
Sex	Male	97 (51.1%)	78.4%	56.3%	38.9%	0.223	0.638
	Female	93 (48.9%)	83.1%	63.6%	45.5%		
Ulceration	Yes	108 (56.8%)	61.0%	28.0%	17.1%	49.541	< 0.001
	No	82 (43.2%)	91.7%	77.8%	55.6%		
Lymph node metastasis	Yes	75 (39.5%)	58.7%	22.7%	6.7%	90.293	< 0.001
	No	115 (60.5%)	91.3%	78.3%	60%		
Distant metastasis	Yes	61 (32.1%)	60.7%	18.0%	0%	95.581	< 0.001
	No	129 (67.9%)	86.8%	74.4%	56.6%		
Breslow thickness	$< 1\text{mm}$	44 (23.2%)	90.9%	77.3%	68.2%	29.240	< 0.001
	1–2mm	74 (38.9%)	83.8%	70.6%	44.1%		
	$> 2\text{mm}$	72 (37.9%)	66.7%	32.1%	38.9%		
Pathologic type	Superficial type	100 (52.6%)	94.6%	81.1%	45.9%	7.013	0.132
	Nodular type	37 (19.5%)	70.0%	42.0%	34.0%		
	Acrotype	53 (27.9%)	78.4%	55.3%	38.9%		
Clark level	I–IV	150 (77.3%)	83.1%	67.6%	46.6%	30.550	< 0.001
	V	40 (20.6%)	61.9%	16.7%	0%		
Satellite lesions	Yes	88 (46.3%)	75.4%	53.6%	36.2%	2.201	0.143
	No	102 (52.6%)	86.5%	63.5%	46.2%		
Dermal mitoses	$< 1/\text{mm}^2$	45 (23.68%)	88.9%	73.3%	66.7%	14.625	< 0.001
	$\geq 1/\text{mm}^2$	145 (77.32%)	75.2%	51.0%	30.3%		
History of misdiagnosis	Yes	32 (16.8%)	77.8%	54.4%	36.1%	2.613	0.109
	No	158 (83.2%)	81.3%	65.6%	53.1%		
Range of surgical resection	$< 1\text{cm}$	55 (28.9%)	48.3%	26.7%	16.7%	46.224	< 0.001
	1–2cm	58 (30.6%)	86.2%	46.6%	27.6%		
	$> 2\text{cm}$	77 (40.5%)	97.2%	88.9%	66.7%		
Interferon therapy	Yes	91 (47.9%)	83.5%	59.3%	41.8%	0.890	0.345
	No	99 (52.1%)	73.7%	53.5%	36.4%		
VEGF	Positive	121 (63.7%)	67.2%	36.2%	15.5%	70.741	< 0.001
	Negative	69 (36.3%)	95.9%	87.8%	75.7%		
β -catenin	Positive	103 (54.2%)	62.4%	28.7%	11.9%	87.230	< 0.001
	Negative	87 (45.8%)	95.5%	85.4%	69.7%		
DKK1	Positive	69 (36.3%)	90.9%	78.5%	58.6%	26.593	< 0.001
	Negative	121 (63.7%)	63.8%	37.7%	18.8%		

Abbreviations: CM, cutaneous melanoma; VEGF, vascular endothelial growth factor; DKK1, Dickkopf 1.

Hazard Ratio Plot

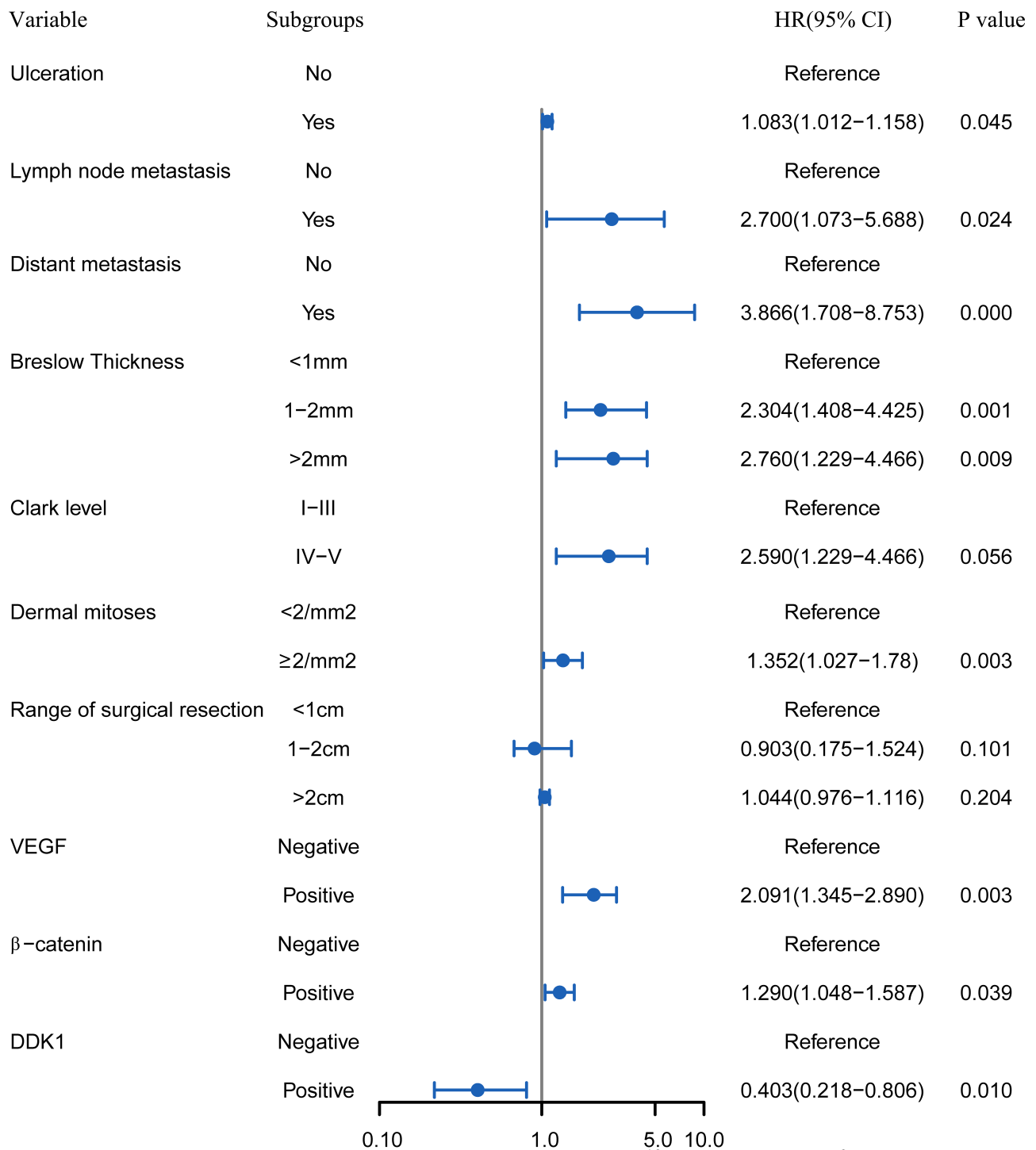


Figure 4 Multivariate COX regression analysis of independent indicators in predicting the prognosis of CM patients. **Abbreviations:** CM, cutaneous melanoma; VEGF, vascular endothelial growth factor; DKK1, Dickkopf 1; HR, hazard ratio; 95% CI, 95% confidence interval.

CM patients (Figure 5). It assigned a score to each variable (based on HR of COX regression) and estimated OS by the sum of each score.

Good discriminations of 1-, 3-, and 5-year OS were demonstrated according to the AUC values (0.914, 0.852, 0.785; all >0.75) (Figure 6A–C) in the internal validation. The calibration plots indicated that the 1-, 3-, and 5-year OS rates predicted by the nomogram were consistent with the actual prognosis (Figure 6D–F). Good calibrations were found because of no significant difference in the HL test ($P = 0.424, 0.477, 0.358$). For the external validation, the ROC curves and calibration plots indicated good discriminations and calibrations as well (Figure 6G–I; AUC= 0.904, 0.840, 0.773) (Figure 6J–L; HL test, $P = 0.278, 0.347, 0.473$).

Accuracy Comparison of Different Prediction Models

The nomogram incorporating the Wnt/ β -catenin signaling pathway showed the highest accuracy in predicting the 1-, 3-, and 5-year OS (AUC=0.914, 0.852, 0.785) compared with the nomogram without the Wnt/ β -catenin signaling pathway (AUC=0.693, 0.640, 0.615) and the TNM stage (AUC=0.726, 0.693, 0.673). No obvious difference was observed between the accuracy of the nomogram without the Wnt/ β -catenin signaling pathway and the TNM stage (Figure 7).

Discussion

Accurate prediction of the prognosis is critical to the selection of the optimal treatment and improving the

survival of CM patients. In this study, the multivariate COX regression revealed that except for the components of TNM staging (Breslow thickness, ulceration, dermal mitoses, lymph-node metastasis, and distant metastasis), the key genes of the WNT/ β -catenin signaling pathway (β -catenin, VEGF, and DKK1) were associated with the survival of CM. The developed nomogram incorporating the WNT/ β -catenin signaling pathway showed good discrimination and calibration through internal and external validation. Its prognostic value was better than the nomogram without WNT/ β -catenin signaling pathway and TNM staging system.

The understanding of the prognostic factors of CM is improving with the refinements of diagnostic techniques and treatment strategies. This updated understanding can in turn promote the diagnosis and treatment of CM. For example, the T1 stage was perfected based on mitosis in the seventh edition AJCC, while the threshold of T1a and T1b stage was improved regarding tumor thickness = 0.8 mm and the presence of ulceration in the eighth edition AJCC.²⁷ It suggests that pathologists should pay more attention to tumor thickness and the presence of epidermal defects, and clinicians should focus on the symptoms of ulcer. These examples indicate that the research on the prognosis of CM is constantly evolving and complementary to clinical diagnosis and treatment.

VEGF and DKK1 are downstream molecules of the Wnt/ β -catenin signaling pathway. Research of VEGF were earlier, and Endostar, a potent endogenous antiangiogenic factor, has entered clinical application for melanoma and

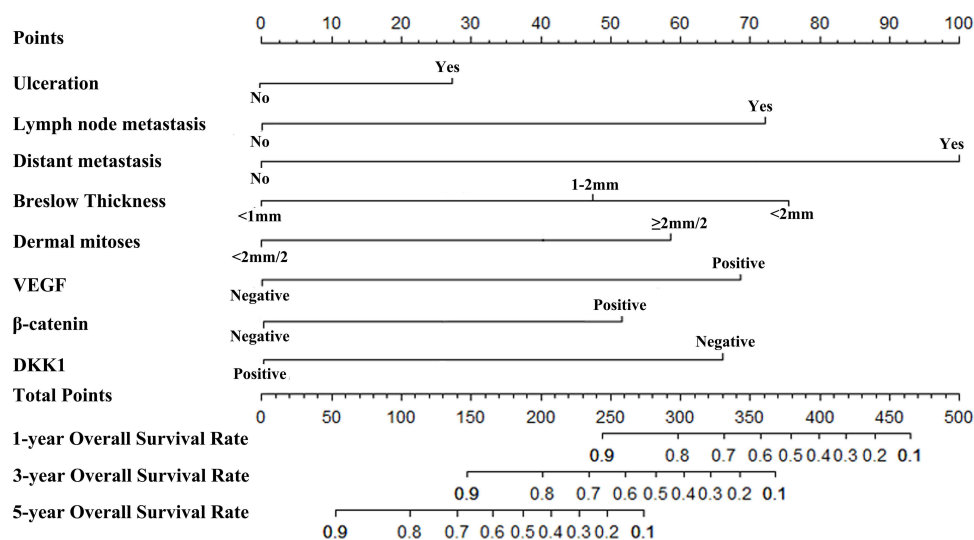


Figure 5 Nomogram predicting the prognosis of CM patients.

Abbreviations: CM, cutaneous melanoma; VEGF, vascular endothelial growth factor; DKK1, Dickkopf 1; HR, hazard ratio; 95% CI, 95% confidence interval.

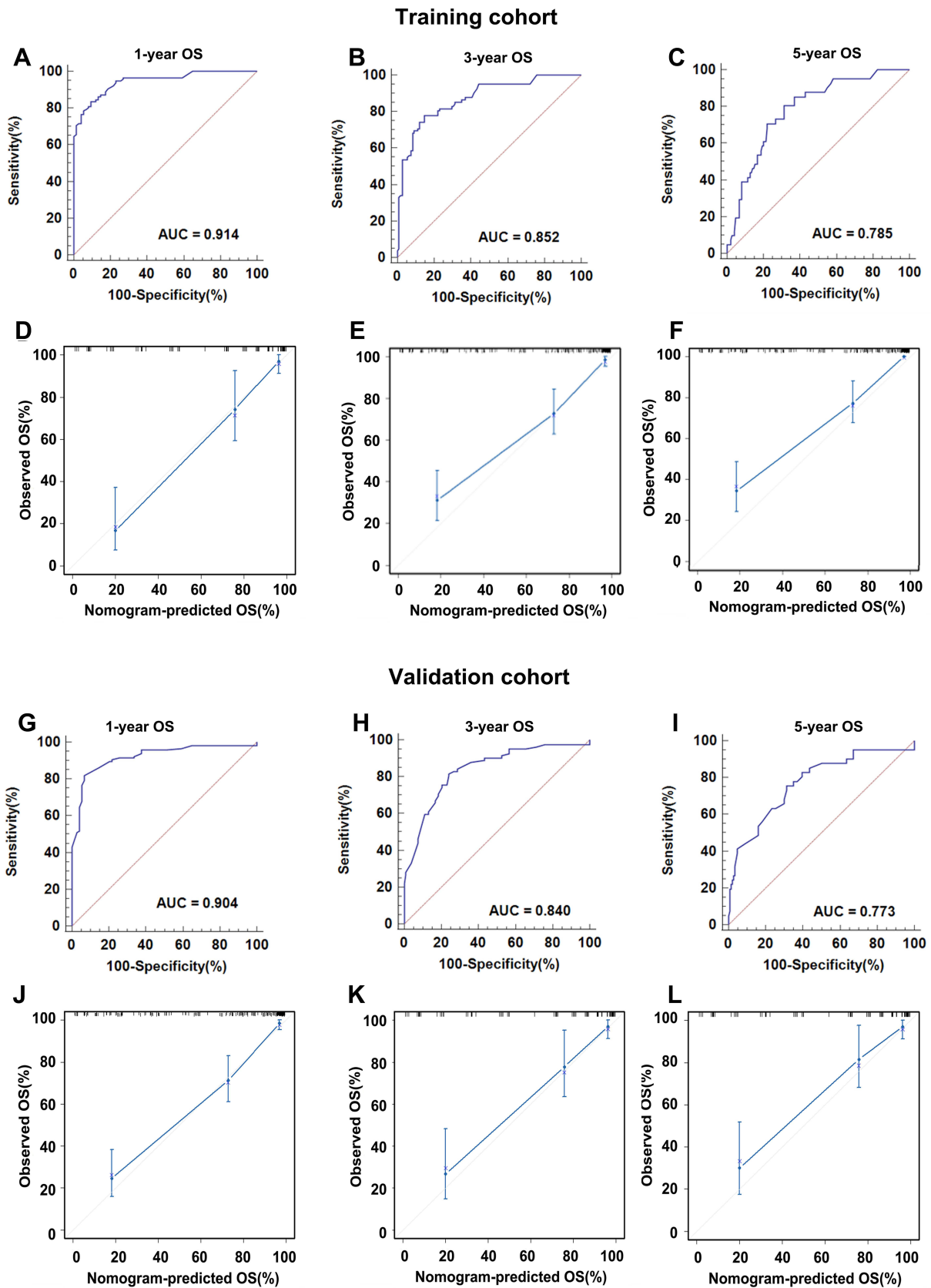


Figure 6 Internal and external validation of the nomogram predicting the OS of CM patients. The AUCs in the training cohort and validation cohort were 0.914, 0.852, 0.785 (A–C) and 0.904, 0.840, 0.773 (G–I), respectively, indicating good discrimination. The calibration plots in the training and validation datasets were shown in (D–F) and (J–L). Both indicated that the nomogram-predicted prognosis compared well with the actual prognosis.

Abbreviations: OS, overall survival; CM, cutaneous melanoma; AUC, area under the curve.

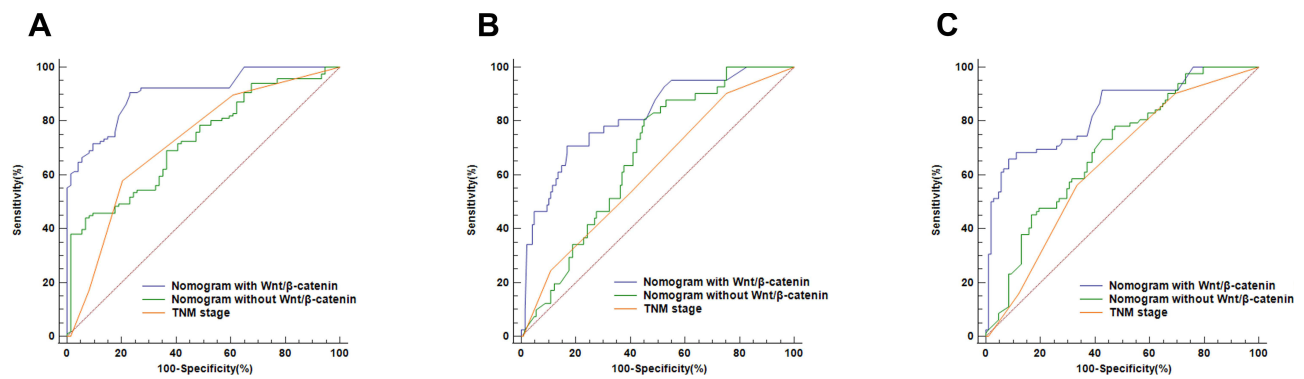


Figure 7 ROC curves of different models predicting the 1-, 3-, and 5- year OS of CM patient (A–C). The AUCs of the nomogram with the Wnt/ β -catenin signaling pathway (0.914, 0.852, 0.785) were highest compared with the nomogram without the Wnt/ β -catenin signaling pathway (0.693, 0.640, 0.615) and the TNM stage (0.726, 0.693, 0.673). **Abbreviations:** ROC, receiver operating characteristic, OS, overall survival, CM, cutaneous melanoma, AUC, area under the curve, TNM, Tumor-Node-Metastasis.

achieved encouraging results,^{28,29} while the research on β -catenin or DKK1 genes are still in the stage of mechanism exploration. Survival analyses in the present study showed that VEGF, β -catenin, and DKK1 could affect the prognosis of CMM. Further multivariate analysis demonstrated that β -catenin, VEGF, and DKK1 expression levels were independent factors of the CM prognosis although their use is not currently mandated in CM reporting. Most studies demonstrated that the ectopic expression of β -catenin in the cytoplasm and nucleus activates the transcription of downstream genes to promote tumor cell differentiation, proliferation, and metastasis.^{30,31} DKK1 blocks the nuclear translocation of β -catenin, downregulation of its downstream targets VEGF resulting in reducing perivascular coverage in melanoma tumors.^{32–34} Although our results revealed that the Wnt/ β -catenin signaling pathway was valuable prognostic markers for CM patients, they were unfeasible for predicting the prognosis of CM independently. Part of the reason for this is that the Wnt/ β -catenin signaling pathway has also been reported to be upregulated in cutaneous inflammation and benign cutaneous neoplasm.^{35,36}

Despite the strong evidence-based predictive capacity of the current AJCC melanoma staging system, it has long been observed that the prediction of CM prognosis remains a difficult one to perform accurately and consistently.^{37,38} Therefore, accurate prediction of survival for CM patients would be difficult only to rely on TNM stage. In the present study, we established a nomogram based on conventional clinical characteristics, which have been recognized as prognostic factors for CM,^{39–41} and the WNT/ β -catenin signaling pathway. Its accuracy was significantly improved compared to the TNM stage and the

nomogram without the Wnt/ β -catenin signaling pathway. It suggested that the nomogram could assist physicians in performing individualized survival predictions, which would facilitate the selection of better treatment options and surveillance strategies. For example, if the overall score of a CM patient was about 259 [Breslow thickness 1–2 mm (48), ulceration (28), no lymph node metastasis (0), and distant metastasis (0), dermal mitosis $<2/\text{mm}^2$ (0), positive VEGF (68), positive β -catenin (50), and negative DKK1 (65)], it indicated that the 1-, 3-, and 5-year OS rates were about 88%, 60%, and 15%, respectively. For these stage IIA patients, surveillance strategies could be intensified, or adjuvant interferon alfa-2b therapy could be considered, because most of them will not receive adjuvant therapy according to the current AJCC stage.

There are some limitations in this study. First, the TNM stage for the present study was based on the seventh AJCC staging manual. The most recent revision of the AJCC staging manual (eighth edition) was released in 2016 and implemented in 2018. With the revision of staging criteria, the accuracy in predicting the prognosis of CM may be improved. Second, potential selection bias might be inevitable in our single-center study with a limited sample size. Third, we could not exclude the biases from genetic susceptibility and specific genetic polymorphisms, considering that about 5–12% of melanomas are hereditary.³⁸ Therefore, we plan to conduct a larger prospective study according to the eighth AJCC melanoma staging system to improve the constructed nomogram.

In conclusion, the established nomogram incorporating the WNT/ β -catenin signaling pathway might be useful in predicting OS of CM patients. It delivered a pragmatic model, which might be useful in helping clinicians to choose, with greater accuracy, the best clinical therapy for CM patients.

Ethical Approval

Ethical approval for the study was obtained from the ethics committee of North China University of Science and Technology Affiliated Hospital.

Informed Consent

Written informed consent was obtained from all patients.

Acknowledgments

The authors wish to thank all the study participants, research staff, and students who participated in this work.

Author Contributions

Yu-xin Zhou and Xin Wang contributed equally and are co-first authors.

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Approval of the manuscript: all authors.

All authors contributed to data analysis, drafting or revising the article, gave final approval for the version to be published, agreed to the submitted journal, and agree to be accountable for all aspects of the work.

Funding

Science and Technology Planning Project of Hebei Province (NO. 112761175) Science and Technology Planning Project of Tangshan city (NO. 12140209A-30).

Disclosure

The authors declare that they have no conflicts of interest.

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