Prognostic significance of the expression of nuclear eukaryotic translation initiation factor 5A2 in human melanoma

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Abstract. Eukaryotic translation initiation factor 5A2 (EIF5A2) expression is upregulated in various cancers. The present authors previously demonstrated that cytoplasmic EIF5A2 expression increases with melanoma progression and inversely correlates with patient survival. Other studies have suggested that nuclear EIF5A2 may also play a role in oncogenesis. The present study used immunohistochemistry and tissue microarray with a large number of melanocytic lesions (n=459) and demonstrated that nuclear EIF5A2 expression was significantly upregulated between common acquired nevi, dysplastic nevi and primary melanomas, and between primary melanomas and metastatic melanomas. Nuclear EIF5A2 expression was inversely associated with overall and disease-specific 5-year survival rate for all (P<0.001) and primary (P=0.014 and P=0.015, respectively) melanoma patients. Nuclear EIF5A2 expression was directly associated with melanoma thickness (P=0.036) and American Joint Committee on Cancer staging (P<0.001), which suggests the possible role of nuclear EIF5A2 in melanoma cell invasion. Subsequently, the present study investigated the association between the expression of nuclear EIF5A2 and matrix metalloproteinase-2 (MMP-2), which is an important factor for promoting cancer cell invasion. Nuclear EIF5A2 and a strong MMP-2 expression were directly associated, and their concurrent expression was significantly associated with a poorer overall and disease-specific 5-year survival rate for all and primary melanoma patients. Nuclear and cytoplasmic EIF5A2 expression were also demonstrated to be significantly associated, and simultaneous expression of the two forms of EIF5A2 was significantly associated with poor overall and disease-specific 5-year survival rates for all and primary melanoma patients. Multivariate Cox regression analysis revealed that nuclear EIF5A2 expression alone and in combination with cytoplasmic EIF5A2 expression was an adverse independent prognostic factor for all and primary melanoma patients. In conclusion, the present study for the first time, to the best of our knowledge, demonstrated that nuclear EIF5A2 expression is an independent prognostic marker in melanoma, and revealed its role in melanoma progression and patient survival. Therefore, nuclear EIF5A2 may have the potential to serve as a therapeutic marker for melanoma.

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Introduction

Melanoma is the most lethal form of skin cancer that arises from melanocytes, and although it constitutes only ~4% of all skin cancers it is responsible for >80% of mortalities in patients with skin cancer (1). Since the 1950s, the incidence of melanoma has been rapidly increasing in non-Hispanic white populations worldwide (2). Although surgery may cure the majority of patients in early stages of melanoma, patients with distant metastasis have a poor prognosis with a median survival time of only 6-8 months, and <15% of patients with distant metastatic melanoma survive for 5 years (3). Since melanoma has an extremely high metastatic potential, it is crucial to discover factors that are involved in the progression and metastasis of melanoma in an attempt to identify an effective treatment regimen for this devastating disease.

Eukaryotic translation initiation factor (EIF) 5A is best known for its cytoplasmic role in translation regulation as a eukaryotic translation initiation factor (4,5). In mammals, it is encoded by two highly-associated genes EIF5A1 and EIF5A2. However, a number of studies have suggested that EIF5A1 may also be active in the nucleus, particularly in mammals (6-8). EIF5A1 has been revealed to take part in the nucleocytoplasmic transport of incompletely spliced and unspliced human immunodeficiency virus-1 mRNAs (6) that are translocated across the nuclear envelope via chromosomal maintenance 1 (CRM1), which is a member of the importin β family of transport receptors. The main role of CRM1 is to facilitate the translocation of rRNA, U snRNA and ribosomal subunits across the nuclear envelope (9). EIF5A1 has also been revealed to be capable of interacting and colocalizing with transport receptor exportin 4, which is another member of the importin β family (8). In addition, it has been suggested that EIF5A1 may recognize nitric oxide synthase 2 mRNA in the nucleus and facilitate its

transport to the cytoplasm in a CRM1 dependent-manner (10). Furthermore in *Saccharomyces cerevisiae,* EIF5A1 appears to be involved in mRNA degradation/turnover, which is a process highly associated with nucleocytoplasmic transport of mRNA (11,12). In summary, these data demonstrate a nuclear activity for EIF5A1 that is possibly associated with cellular mRNA metabolism.

EIF5A2, a phylogenetically conserved vertebrate variant of EIF5A1, was first reported to be highly expressed in testis and colorectal adenocarcinoma, and at moderate levels in the brain (13). EIF5A2 shares 83% amino acid identity with EIF5A1 (14) and has been associated with various oncogenic roles, including invasion and metastasis, and a poor prognosis in a variety of cancers, such as ovarian cancer (15), gastric adenocarcinoma (16), colorectal cancer (17,18), hepatocellular carcinoma (HCC) (19), ovarian cancer (20), non-small cell lung cancer (21) and bladder cancer (22,23). However, no studies have investigated the nuclear expression or activity of EIF5A2 in cancer, with the exception of Zender *et al* (24), who addressed the importance of nuclear EIF5A2 in HCC.

Previously, the present authors reported an increase in the cytoplasmic expression of EIF5A2 in melanoma and its role in melanoma progression and patient survival (25). The present study investigates, using immunohistochemistry and tissue microarray (TMA), the status of nuclear EIF5A2 expression in melanoma. The results revealed that nuclear EIF5A2 is an independent prognostic marker in melanoma, and its expression is significantly increased during melanoma progression. In addition, upregulation of nuclear EIF5A2 was determined to be associated with a significantly poorer 5-year survival rate for all and primary melanoma patients. Furthermore, simultaneous nuclear and cytoplasmic EIF5A2 expression, as well as concurrent nuclear EIF5A2 and matrix metalloproteinase-2 (MMP-2) expression, were associated with a poorer 5-year patient survival rate.

Materials and methods

Patient specimens. In total, 459 formalin-fixed, paraffin-embedded human tissues, consisting of 28 common acquired nevi, 49 dysplastic nevi, 242 primary melanomas and 140 metastatic melanomas, were used in the present study. The human skin tissues and the patients' data were acquired from the 1990-1998 archives of the Department of Pathology, Vancouver General Hospital (Vancouver, Canada), and their use was approved by the Clinical Research Ethics Board of the University of British Columbia (Vancouver, Canada; certificate number H09-01321), in accordance with the Declaration of Helsinki guidelines (26). Patient consent was not required under the Canadian law, since the present report is a retrospective study using anonymized data and several patients had already succumbed to the disease.

TMA construction and immunohistochemistry. TMA construction and immunohistochemical staining were performed as previously described (27,28). Briefly for immunohistochemical staining, the TMA slides were dewaxed by heating at 55˚C for 30 min followed by three 5 min washes with xylene. Subsequently, the samples were rehydrated by washing in 100, 95 and 80% ethanol, and distilled water for 5 min each. For antigen retrieval, the samples were heated for 30 min at 95˚C in 10 mmol/l sodium citrate (pH 6.0). The samples were incubated with 3% hydrogen peroxide in order to block endogenous peroxidase activity, and were next incubated for 30 min with universal blocking serum (Dako Canada ULC, Mississauga, ON, Canada) and then incubated with a primary rabbit anti-EIF5A2 antibody (dilution, 1:100; catalog no. E9781; Sigma-Aldrich, St. Louis, MO, USA) overnight at 4˚C. Subsequently, the slides were incubated for 30 min with a non-diluted biotin-labelled secondary antibody raised in swine (catalog no. K0690; Dako Canada ULC), and then incubated with streptavidin-peroxidase (Dako Canada ULC). Subsequently, the samples were developed with 3,3-diaminobenzidine [Vector Laboratories (Canada), Inc., Burlington, ON, Canada] and counterstained with hematoxylin. Dehydration of the sections was performed using a standard procedure and the slides were sealed with coverslips. For blocking experiments, the anti-EIF5A2 antibody was incubated with a 10 times concentration of its synthetic immunogenic peptide (dilution, 1:10; Biomatik Corporation, Cambridge, ON, Canada) at 4˚C the night prior to immunohistochemical staining.

Evaluation of immunostaining. Three independent observers, including one dermatopathologist, from the Departments of Dermatology and Skin Science or Pathology of the University of British Columbia, simultaneously evaluated and scored the nuclear EIF5A2 staining of the TMA, one core at a time, and a consensus score was reached. The intensity of nuclear EIF5A2 staining was scored as 0, 1+, 2+ and 3+ based on visual estimation. The percentage of nuclear EIF5A2-positive cells was scored as follows: 1, 0-25% cells stained; 2, 26-50% cells stained; 3, 51-75% cells stained; and 4, 76-100% cells stained. An immunoreactive score was used to determine the level of nuclear EIF5A2 staining by multiplying the scores of staining intensity and the percentage of positive cells. On the basis of the immunoreactive score, the nuclear EIF5A2 staining pattern was defined as 0, negative; and 1-12, positive (29,30).

Statistical analysis. Differences in demographics and clinicopathological characteristics and nuclear EIF5A2 expression between patients was evaluated by the χ^2 test. Survival time was calculated between the date of melanoma diagnosis and the date the patient succumbed to the disease or last follow-up. Kaplan-Meier analysis and log-rank test were used to assess the association between nuclear EIF5A2 expression and patient survival times. The Cox proportional hazards regression model was performed for univariate and multivariate survival analysis. $P<0.05$ was considered to indicate a statistically significant difference, and all tests were two-sided. SPSS version 16.0 software (SPSS, Inc., Chicago, IL, USA) was used for all analysis.

Results

Association between nuclear EIF5A2 expression and clinicopathologic characteristics of melanoma patients. A total of 713 melanoma patients were enrolled for TMA construction. Due to loss of biopsy cores, insufficient tumour cells present in the cores or loss to follow-up, 382 melanoma (primary melanoma, 242 cases; metastatic melanoma, 140 cases) and 77 cases of nevi (common acquired nevi, 28 cases; dysplastic nevi, 49 cases) were evaluated for nuclear EIF5A2 staining.

Table I. Nuclear EIF5A2 staining and clinicopathological characteristics of patients with melanoma.

Characteristics	Total, n	Nuclear EIF5A2 staining		χ^2 value	P-value
		Positive, n (%)	Negative, n (%)		
All melanoma patients	382	242 (63.4)	140 (36.6)		
Age, years				0.533	0.465
≤60	198	122 (61.6)	76 (38.4)		
>60	184	120 (65.2)	64 (34.8)		
Gender				0.040	0.841
Male	229	146 (63.8)	83 (36.2)		
Female	153	96 (62.7)	57 (37.3)		
AJCC stage				44.611	<0.001[a]
I	127	56 (44.1)	71 (55.9)		
II	115	67 (58.3)	48 (41.7)		
III	54	51 (94.4)	3 (5.6)		
IV	86	68 (79.1)	18 (20.9)		
Primary melanoma patients	242	123 (50.8)	119 (49.2)		
Age, years				1.349	0.245
≤61	123	58 (47.2)	65 (52.8)		
>61	119	65 (54.6)	54 (45.4)		
Gender				1.413	0.235
Male	133	63 (47.4)	70 (52.6)		
Female	109	60 (55.0)	49 (45.0)		
Tumor thickness, mm				4.398	0.036
≤2	134	60 (44.8)	74 (55.2)		
>2	108	63 (58.3)	45 (41.7)		
Ulceration				0.705	0.401
Absent	194	96 (49.5)	98 (50.5)		
Present	48	27 (56.2)	21 (43.8)		
Subtype				2.843	0.416
Lentigo maligna	37	21 (56.8)	16 (43.2)		
Superficial spreading	89	39 (43.8)	50 (56.2)		
Nodular	41	22 (53.7)	19 (46.3)		
Unspecified	75	41 (54.7)	34 (45.3)		
Tumor site[b]				0.029	0.865
Sun-protected	188	95 (50.5)	93 (49.5)		
Sun-exposed	54	28 (51.9)	26 (48.1)		
Metastatic melanoma patients	140	119 (85.0)	21 (15.0)		
Age, years				0.248	0.618
≤59	73	61 (83.6)	12 (16.4)		
>59	67	58 (86.6)	9 (13.4)		
Gender				0.510	0.475
Male	96	83 (86.5)	13 (13.5)		
Female	44	36 (81.8)	8 (18.2)		

[a]Comparison between AJCC stage I and II, and III and IV using the χ^2 test. [b]Sun-protected sites include the trunk, arm, leg and feet; sun-exposed sites include the head and neck. EIF5A2, eukaryotic translation initiation factor 5A2; AJCC, American Joint Committee on Cancer.

A synthetic immunogenic peptide against anti-EIF5A2 antibody was used in order to validate the specificity of the antibody used for staining, and the results revealed that this peptide considerably blocked nuclear and cytoplasmic EIF5A2 staining (Fig. 1) (25). The clinical features of the melanoma patients are listed in Table I.

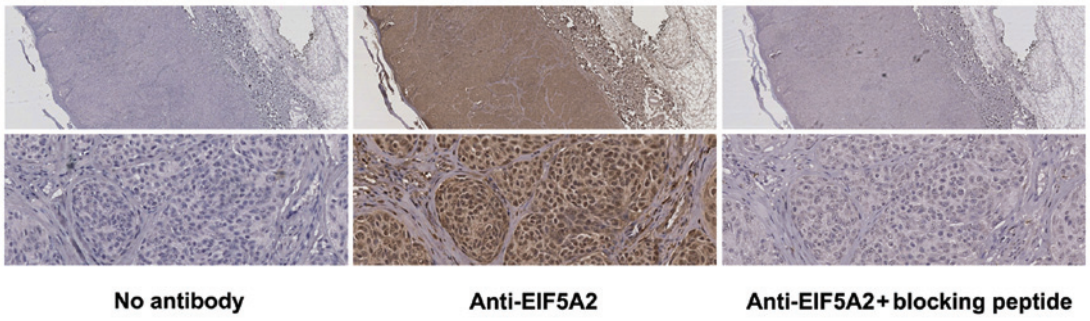

Figure 1. Synthetic immunogenic peptide against anti-EIF5A2 antibody considerably blocked EIF5A2 staining. For the blocking experiment, anti-EIF5A2 antibody and 10 times concentration of its immunogenic peptide (dilution, 1:10) were incubated together at 4˚C the night before immunohistochemical staining. Top panel, magnification x40; bottom panel, magnification x100. EIF5A2, eukaryotic translation initiation factor 5A2. Modified from Khosravi *et al* (25).

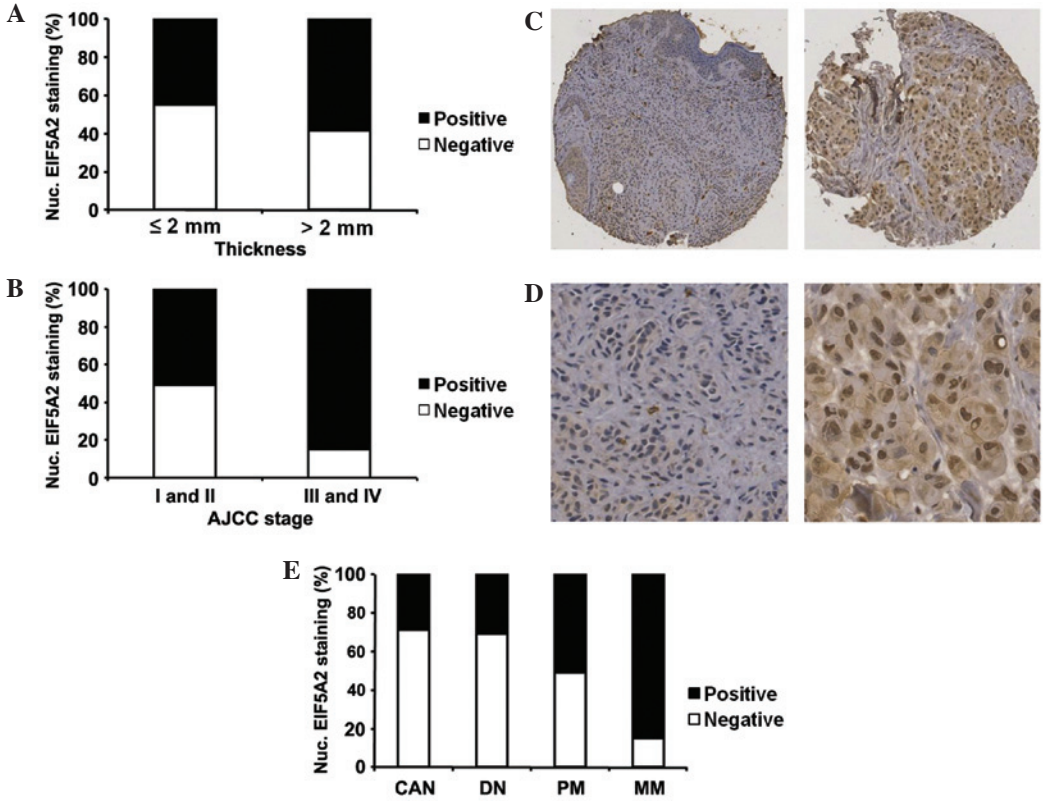

Figure 2. Association between nuclear EIF5A2 expression and tumor thickness, AJCC stage and various stages of melanoma progression. (A) Nuclear EIF5A2 expression was significantly higher in melanoma patients with tumour thickness >2 mm compared with melanoma patients with tumour thickness ≤2 mm (P=0.036; χ^2 test). (B) Nuclear EIF5A2 expression was significantly higher in advanced stage melanomas (AJCC stages III and IV) compared with early stage melanomas (AJCC stages I and II) (P<0.001; χ^2 test). (C and D) Representative images of nuclear EIF5A2 immunohistochemical staining in melanocytic lesions at (C) x100 and (D) x400 magnification. Left panel, negative nuclear EIF5A2 staining; right panel, positive nuclear EIF5A2 staining. (E) Nuclear EIF5A2 expression was increased in MM compared with CAN, DN, and PM (P<0.001; χ^2 test). Nuclear EIF5A2 expression was also increased in PM compared with CAN and DN (P=0.010 and P=0.026, respectively; χ^2 test). EIF5A2, eukaryotic translation initiation factor 5A2; AJCC, American Joint Committee on Cancer; CAN, common acquired nevi; DN, dysplastic nevi; PM, primary melanoma; MM, metastatic melanoma; Nuc., nuclear.

Thickness is one feature that is an extremely important prognostic marker for primary melanoma patients (31). The present analysis demonstrated that in primary melanoma patients, positive nuclear EIF5A2 expression was exhibited by 58% of melanoma patients with a tumour thickness of >2 mm, compared with 45% of melanoma patients with a tumour thickness of ≤2 mm (P=0.036; Table I; Fig. 2A). This suggests that in primary melanoma, nuclear EIF5A2 expression may be induced during the transition between thin and thick melanoma. In all melanoma patients, the expression of nuclear EIF5A2 was detected in 85% of advanced stage melanomas [American Joint Committee on Cancer (AJCC) stages III and IV] compared with 51% of early stage melanomas (AJCC stages I and II) (P<0.001; Table I; Fig. 2B) (32). The association between positive nuclear EIF5A2 expression and melanoma thickness and AJCC stages may be an indication of the involvement of nuclear EIF5A2 expression in melanoma cell invasion.

Nuclear EIF5A2 expression increases with melanoma progression. To study the alterations in the expression of

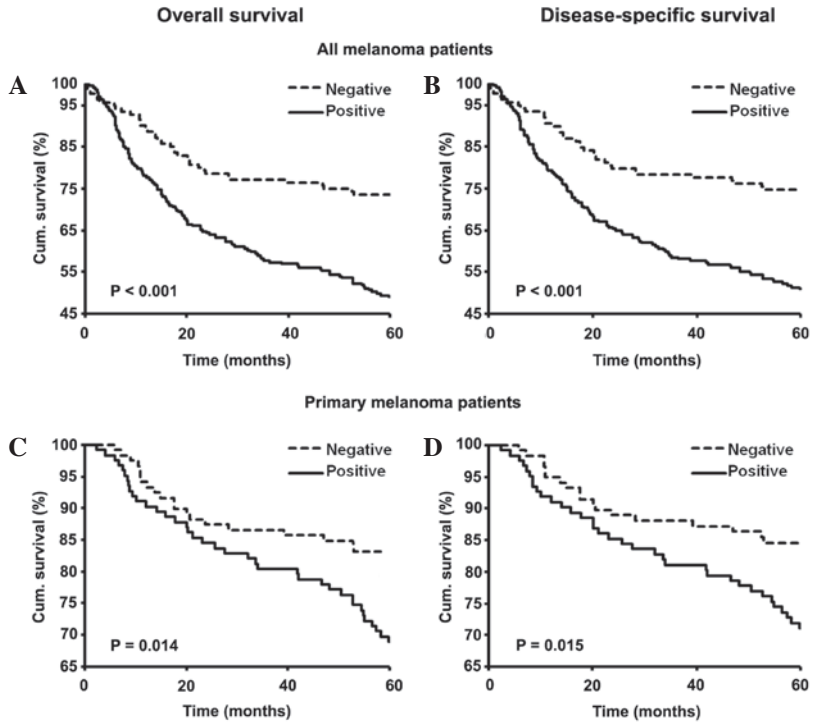

Figure 3. Kaplan-Meier analysis for the association between nuclear EIF5A2 expression and 5-year survival rates of melanoma patients. (A-D) Nuclear EIF5A2 expression was associated with a poorer overall and disease-specific 5-year survival rate in (A and B) all melanoma patients (P<0.001, log-rank test) and (C and D) primary melanoma patients (P=0.014 and P=0.015, respectively, log-rank test). EIF5A2, eukaryotic translation initiation factor 5A2; Cum., cumulative.

nuclear EIF5A2 with melanoma progression, immunohistochemical staining was performed on TMA slides and samples were categorized into negative and positive EIF5A2 staining groups (Fig. 2C and D). Positive nuclear EIF5A2 staining was observed in 29% of common acquired nevi, 31% of dysplastic nevi, 51% of primary melanomas and 85% of metastatic melanomas. Consequently, nuclear EIF5A2 expression was observed to be significantly higher in primary melanomas compared with dysplastic nevi and common acquired nevi (P=0.010 and P=0.026, respectively; Fig. 2E) and in metastatic melanomas compared with primary melanomas, dysplastic nevi and common acquired nevi (P<0.001; Fig. 2E). This suggests the role of nuclear EIF5A2 in the transformation between nevus and malignant tumors and the development of melanoma metastasis. No difference in nuclear EIF5A2 expression was observed between common acquired nevi and dysplastic nevi (P=0.852; Fig. 2E).

Nuclear EIF5A2 expression is positively associated with poor patient survival. The present study evaluated the association between nuclear EIF5A2 expression and the 5-year survival rate of primary and metastatic melanoma patients by constructing Kaplan-Meier survival curves. Overall and disease-specific 5-year survival rates were poorer for all (P<0.001; Fig. 3A and B) and primary (P=0.014 and P=0.015, respectively; Fig. 3C and D) melanoma patients with positive staining for nuclear EIF5A2 compared with patients with negative staining. The results from the Kaplan-Meier survival analysis were further supported by univariate Cox proportional hazard regression analysis, which indicated that nuclear EIF5A2 expression was a significant prognostic factor for the overall and disease-specific 5-year survival rates of all melanoma patients [hazards ratio (HR), 2.26 and 2.27; 95% confidence interval (CI), 1.57-3.27 and 1.56-3.31; P<0.001; Table II] and primary melanoma patients (HR, 1.95 and 2.00; 95% CI, 1.14-3.36 and 1.13-3.53; P=0.015 and P=0.017; Table II).

Nuclear EIF5A2 is an independent prognostic marker for melanoma patients. Results from multivariate Cox regression analysis revealed that nuclear EIF5A2 was an adverse independent prognostic marker for overall and disease-specific 5-year survival rates of all melanoma patients (HR, 1.78 and 1.77; 95% CI, 1.22-2.60 and 1.20-2.62; P=0.003 and P=0.004; Table III) and primary melanoma patients (HR, 1.78 and 1.90; 95% CI, 1.02-3.12 and 1.06-3.43; P=0.043 and P=0.032; Table III). For the analysis, gender, age, AJCC and EIF5A2 expression were included for all melanoma patients, and gender, age, tumor thickness, ulceration status, tumor site, histological subtype and EIF5A2 expression were included for primary melanoma patients.

Concurrent cytoplasmic and nuclear EIF5A2 expression is correlated with a poorer 5-year survival rate for all and primary melanoma patients. Previously, the present authors investigated the expression of cytoplasmic EIF5A2 in melanoma using TMA and revealed that cytoplasmic EIF5A2 expression is a prognostic marker for melanoma patients (25). The present study analyzed the association between cytoplasmic and nuclear expression of EIF5A2 using the 382 melanoma cases, and the results revealed that there was a direct association between the positive staining of cytoplasmic and nuclear EIF5A2 (P<0.001; Fig. 4A). To further examine this association

Table II. Univariate Cox regression analysis on 5-year overall and disease-specific survival rates of 382 total melanoma and 242 primary melanoma patients.

		Overall survival				Disease-specific survival			
Characteristics	Total, n (%)	Mortalities, n	Mortality rate, %	HR (95% CI)	P-value[a]	Mortalities, n	Mortality rate, %	HR (95% CI)	P-value[a]
All melanoma patients	382 (100.0)	160				152			
Nuclear EIF5A2 expression									
Negative	140 (36.6)	37	26.4	1.00	<0.001	35	25.0	1.00	<0.001
Positive	242 (63.4)	123	50.8	2.26 (1.57-3.27)		117	48.3	2.27 (1.56-3.31)	
Age, years									
≤60	198 (51.8)	79	39.9	1.00	0.426	77	38.9	1.00	0.648
>60	184 (48.2)	81	44.0	1.13 (0.83-1.55)		75	40.1	1.08 (0.78-1.48)	
Gender									
Male	229 (60.0)	99	43.2	1.00	0.616	93	40.1	1.00	0.755
Female	153 (40.0)	61	39.9	0.92 (0.67-1.27)		59	38.6	0.95 (0.68-1.32)	
AJCC stage									
I-II	242 (63.4)	58	24.0	1.00	<0.001	53	22.0	1.00	<0.001
III-IV	140 (36.6)	102	72.9	5.07 (3.66-7.03)		99	70.7	5.37 (3.84-7.53)	
Primary melanoma patients	242 (63.4)	58				53			
Nuclear EIF5A2 expression									
Negative	119 (49.2)	20	16.8	1.00	0.015	18	15.1	1.00	0.017
Positive	123 (50.8)	38	30.9	1.95 (1.14-3.36)		35	28.5	2.00 (1.13-3.53)	
Age, years									
≤61	123 (50.8)	19	15.4	1.00	0.002	19	15.4	1.00	0.010
>61	119 (49.2)	39	32.8	2.41 (1.39-4.17)		34	28.6	2.10 (1.20-3.69)	
Gender									
Male	133 (55.0)	31	23.3	1.00	0.749	28	21.1	1.00	0.733
Female	109 (45.0)	27	24.8	1.07 (0.64-1.80)		25	22.9	1.10 (0.64-1.88)	
Ulceration									
Absent	194 (80.2)	30	15.5	1.00	<0.001	26	13.4	1.00	<0.001
Present	48 (19.8)	28	58.3	5.38 (3.20-9.03)		27	56.3	6.00 (3.49-10.31)	
Tumor thickness, mm									
≤2	134 (55.4)	11	8.2	1.00	<0.001	9	6.7	1.00	<0.001
>2	108 (44.6)	47	43.5	6.71 (3.48-12.9)		44	40.1	7.69 (3.75-15.78)	

Table II. Continued.

Characteristics	Total, n	Overall survival				Disease-specific survival			
		Mortalities, n	Mortality rate, %	HR (95% CI)	P-value[a]	Mortalities, n	Mortality rate, %	HR (95% CI)	P-value[a]
Tumor site[b]									
Sun-protected	188 (77.7)	45	23.9	1.00	0.892	43	22.9	1.00	0.460
Sun-exposed	54 (22.3)	13	24.1	0.96 (0.52-1.78)		10	18.5	0.77 (0.39-1.54)	
Subtype									
Others	153 (63.2)	42	27.5	1.00	0.129	38	24.8	1.00	0.177
Superficial spreading	89 (36.8)	16	18.0	1.56 (0.88-2.78)		15	16.9	1.51 (0.83-2.74)	

[a]Log rank test. [b]Sun-protected sites include trunk, arm, leg and feet; sun-exposed sites include head and neck. EIF5A2, eukaryotic translation initiation factor 5A2; AJCC, American Joint Committee on Cancer; CI, confidence interval; HR, hazard ratio.

and its effect on patient survival, the melanoma samples were classified into three groups according to their staining as follows: Category 1, negative cytoplasmic and nuclear EIF5A2 staining; category 2, negative cytoplasmic and positive nuclear EIF5A2 or positive cytoplasmic and negative nuclear EIF5A2 staining; category 3, positive cytoplasmic and nuclear EIF5A2 staining. Based on the results from Kaplan-Meier survival analysis, overall and disease-specific 5-year survival rates for all (P<0.001; Fig. 4B and C) and primary (P=0.002; Fig. 4D and E) melanoma patients were poorest for category 3 patients, best for category 1 patients and intermediate for category 2 patients. Furthermore, multivariate Cox regression analysis demonstrated that the simultaneous positive expression of cytoplasmic and nuclear EIF5A2 (category 3) was an independent prognostic factor for overall and disease-specific 5-year survival for all (HR, 1.87 and 1.87; 95% CI, 1.31-2.68 and 1.28-2.67; P=0.001 and P=0.001; Table IV) and primary (HR, 2.01 and 2.11; 95% CI, 1.15-3.51 and 1.17-3.78; P=0.014 and P=0.013; Table IV) melanoma patients.

Simultaneous expression of nuclear EIF5A2 and MMP-2 is associated with poorer 5-year survival rates for all and primary melanoma patients. Results from the TMA study suggested the involvement of nuclear EIF5A2 for developing melanoma invasion, metastasis and poor patient survival. Since cell invasion is one of the hallmarks of cancer that may lead to increased metastasis and poor patient survival, the association between the expression of nuclear EIF5A2 and MMP-2, which is an important factor in the promotion of cancer cell invasion, was evaluated (33). A previous TMA study by the same group indicated that MMP-2 expression was a prognostic marker for melanoma (34). As a result, the present study examined the association between the expression of nuclear EIF5A2 and MMP-2 using the same 369 melanoma cases that had been previously analyzed in the aforementioned TMA study (34). The result revealed that positive staining of nuclear EIF5A2 was directly associated with strong MMP-2 expression (P=0.015; Fig. 5A). To more extensively study the association between MMP-2 and nuclear EIF5A2 expression and their effects on patient survival, the samples were classified into three groups based on their staining as follows: Category 1, negative nuclear EIF5A2 expression and negative-moderate MMP-2 expression; category 2, negative nuclear EIF5A2 expression and strong MMP-2 expression or positive nuclear EIF5A2 expression and negative-moderate MMP-2 expression; category 3, positive EIF5A2 expression and strong MMP-2 expression. Kaplan-Meier survival analyses revealed that overall and disease-specific 5-year survival outcomes for all (P<0.001; Fig. 5B and C) and primary melanoma (P<0.001 and P=0.001, respectively; Fig. 5D and E) patients were most favorable for category 1, least favorable for category 3 and moderate for patients in category 2.

Discussion

Recently, the present authors demonstrated that there was an increase in cytoplasmic EIF5A2 expression with melanoma progression (25); therefore, additional investigation of the nuclear expression of EIF5A2 using TMA data consisting of 459 melanocytic lesions was undertaken. The present study

Table III. Multivariate Cox regression analysis indicating that nuclear EIF5A2 expression is an adverse independent prognostic marker for 5-year survival rates of melanoma patients.

	Overall survival					Disease-specific survival				
Characteristics	β	SE	HR	95% CI	P-value	β	SE	HR	95% CI	P-value
All melanoma (n=382)										
Nuclear EIF5A2 (neg vs. pos)	0.576	0.194	1.778	1.22-2.60	0.003	0.575	0.199	1.777	1.20-2.62	0.004
Age (≤60 vs. >60 years)	0.099	0.159	1.104	0.81-1.51	0.534	0.050	0.163	1.052	0.76-1.45	0.758
Gender (male vs. female)	0.168	0.169	1.183	0.85-1.65	0.319	0.206	0.173	1.229	0.88-1.72	0.232
AJCC stage (1 + 2 + 3 vs. 4)	1.174	0.173	3.236	2.31-4.54	<0.001	1.215	0.177	3.372	2.38-4.77	<0.001
Primary melanoma (n=242)										
Nuclear EIF5A2 (neg vs. pos)	0.578	0.286	1.783	1.02-3.12	0.043	0.643	0.300	1.902	1.06-3.43	0.032
Age (≤61 vs. >61 years)	0.269	0.298	1.309	0.73-2.35	0.367	0.080	0.307	1.083	0.59-1.98	0.795
Gender (male vs. female)	-0.107	0.276	0.899	0.52-1.55	0.699	-0.110	0.288	0.896	0.51-1.58	0.704
Ulceration (absent vs. present)	1.134	0.297	3.109	1.74-5.57	<0.001	1.269	0.310	3.559	1.94-6.54	<0.001
Thickness (≤2 vs. >2 mm)	1.410	0.362	4.095	2.01-8.33	<0.001	1.563	0.394	4.772	2.20-10.34	<0.001
Site (sun-protected vs. exposed)	-0.335	0.322	0.715	0.38-1.34	0.298	-0.556	0.358	0.573	0.28-1.16	0.120
Subtype (superficial vs. others)	0.052	0.301	1.054	0.58-1.90	0.862	0.012	0.313	1.012	0.55-1.87	0.969

EIF5A2, eukaryotic translation initiation factor 5A2; AJCC, American Joint Committee on Cancer; pos, positive; neg, negative; β, regression coefficient; SE, standard error of β; HR, hazard ratio; CI, confidence interval; superficial, superficial spreading.

Table IV. Multivariate Cox regression analysis indicating that the simultaneous expression of cytoplasmic and nuclear EIF5A2 is an adverse independent prognostic marker for 5-year survival rates of melanoma patients.

	Overall survival					Disease-specific survival				
Characteristics	β	SE	HR	95% CI	P-value	β	SE	HR	95% CI	P-value
All melanoma (n=382)										
EIF5A2 (cat. 1 + 2 vs. 3)	0.626	0.184	1.871	1.31-2.68	0.001	0.614	0.188	1.848	1.28-2.67	0.001
Age (≤60 vs. >60 years)	0.112	0.159	1.118	0.82-1.53	0.483	0.063	0.163	1.065	0.77-1.47	0.698
Gender (male vs. female)	0.195	0.170	1.216	0.87-1.70	0.251	0.234	0.174	1.264	0.90-1.78	0.178
AJCC stage (1 + 2 + 3 vs. 4)	1.169	0.174	3.220	2.29-4.52	<0.001	1.214	0.178	3.365	2.38-4.77	<0.001
Primary melanoma (n=242)										
EIF5A2 (cat. 1 + 2 vs. 3)	0.699	0.284	2.012	1.15-3.51	0.014	0.745	0.299	2.107	1.17-3.78	0.013
Age (≤61 vs. >61 years)	0.328	0.299	1.388	0.77-2.50	0.273	0.132	0.309	1.141	0.62-2.09	0.670
Gender (male vs. female)	-0.128	0.277	0.880	0.51-1.51	0.644	-0.125	0.289	0.882	0.50-1.55	0.665
Ulceration (absent vs. present)	1.107	0.296	3.026	1.69-5.41	<0.001	1.245	0.310	3.472	1.89-6.37	<0.001
Thickness (≤2 vs. >2 mm)	1.355	0.363	3.876	1.90-7.89	<0.001	1.505	0.395	4.506	2.08-9.77	<0.001
Site (sun-protected vs. exposed)	-0.352	0.320	0.704	0.38-1.32	0.272	-0.573	0.356	0.564	0.28-1.13	0.108
Subtype (superficial vs. others)	0.054	0.301	1.056	0.59-1.90	0.857	0.015	0.312	1.015	0.55-1.87	0.962

Category 1, negative cytoplasmic and nuclear EIF5A2 staining; category 2, negative cytoplasmic and positive nuclear EIF5A2 staining or positive cytoplasmic and negative nuclear EIF5A2 staining; category 3, positive cytoplasmic and nuclear EIF5A2 staining. EIF5A2, eukaryotic translation initiation factor 5A2; AJCC, American Joint Committee on Cancer; cat., category; β, regression coefficient; SE, standard error of β; HR, hazard ratio; CI, confidence interval; Nuc., nuclear; Cyt., cytoplasmic; superficial, superficial spreading.

revealed that an increase in nuclear EIF5A2 expression was significantly associated with melanoma progression, thickness and AJCC stage. Nuclear EIF5A2 expression was significantly associated with poorer overall and disease-specific 5-year survival rates of all and primary melanoma patients. This may be a result of the direct association identified by the present study between nuclear EIF5A2 expression and melanoma thickness and AJCC stage, which is also consistent with the hypothesis that EIF5A2 is an oncogene in various cancers (35). Notably, nuclear expression of EIF5A2

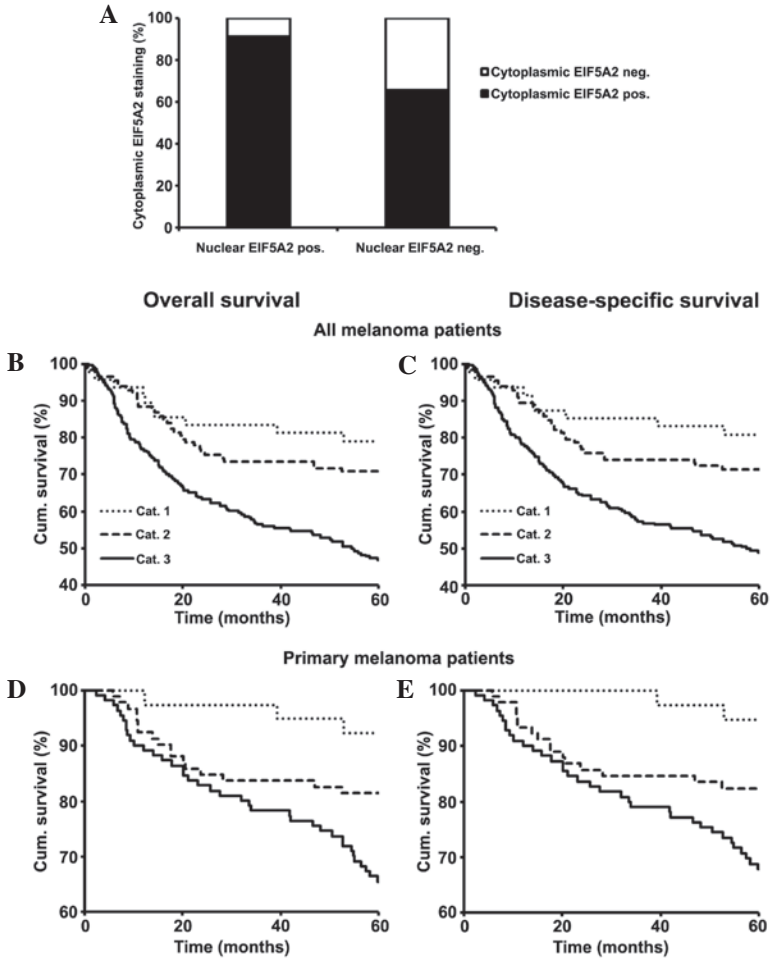

Figure 4. Simultaneous nuclear and cytoplasmic EIF5A2 expression is associated with a poorer 5-year survival rate for melanoma patients. (A) Nuclear EIF5A2 expression directly associates with cytoplasmic EIF5A2 expression in human melanoma (n=382; P<0.001; χ^2 test). (B-D) Simultaneous negative expression of nuclear and cytoplasmic EIF5A2 (cat. 1) was significantly associated with a better overall and disease-specific 5-year survival outcome compared with negative nuclear EIF5A2 expression and positive cytoplasmic EIF5A2 expression or positive nuclear EIF5A2 expression and negative cytoplasmic EIF5A2 expression (cat. 2), or positive nuclear and cytoplasmic EIF5A2 expression (cat. 3) in (B and C) all melanoma patients (overall and disease-specific 5-year survival, P<0.001; log rank test) and (D and E) primary melanoma patients (overall and disease-specific 5-year survival, P=0.002; log rank test). EIF5A2, eukaryotic translation initiation factor 5A2; neg, negative; pos, positive; cat., category; Cum. cumulative.

and a combination of nuclear and cytoplasmic expression of EIF5A2 were identified as independent prognostic markers for overall and disease specific 5-year survival of all and primary melanoma patients.

Furthermore, the present study observed a direct association between nuclear EIF5A2 expression and a strong expression of MMP-2, which may demonstrate why nuclear EIF5A2 expression is directly correlated with melanoma thickness and a poorer 5-year survival rate for melanoma patients. In addition, simultaneous negative-moderate MMP-2 expression and loss of nuclear EIF5A2 expression (category 3) was demonstrated to be associated with an improved 5-year survival rate compared with either strong MMP-2 expression and loss of nuclear EIF5A2 expression or positive expression of nuclear EIF5A2 and negative-moderate expression of MMP-2 (category 2). This may possibly be a rational for dual therapeutic targeting of nuclear EIF5A2 and MMP-2 in melanoma patients; however, this requires additional study. The importance of MMP-2 in melanoma has also been demonstrated by other studies. MT1-MMP was demonstrated to increase melanoma cell invasion and motility by activating its target MMP-2 (36). Another study identified that expression of activated MMP-2 in a melanoma xenograft model is associated with increased malignancy, highlighting the role of MMP-2 in melanoma invasion and metastasis (37).

The present study also compared the nuclear and cytoplasmic EIF5A2 expression in the same cases of melanocytic lesions and revealed a significant association between positive staining of nuclear and cytoplasmic EIF5A2. However, this association is not perfect, which may indicate the differential regulation of nuclear and cytoplasmic EIF5A2 expression in melanoma. Notably, the present results revealed that simultaneous expression of nuclear and cytoplasmic EIF5A2 (category 3) was associated with a poorer survival outcome compared with the expression of only one form of EIF5A2 (category 2), which was associated with an intermediate survival outcome. This suggests that oncogenic properties of nuclear and cytoplasmic EIF5A2 may at least partly differ from each other in melanoma. Therefore, concurrent expression of the two forms (category 3) may have synergistic or additive effects on melanoma progression,

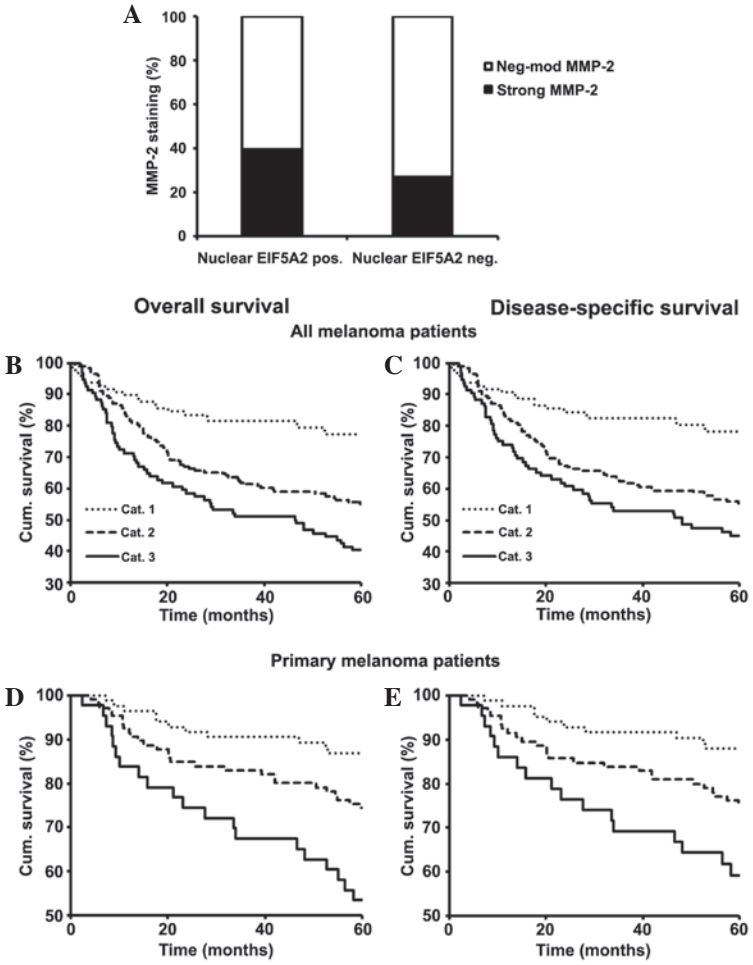

Figure 5. Simultaneous nuclear EIF5A2 expression and strong MMP-2 expression are associated with a poorer 5-year survival rate in melanoma patients. (A) Nuclear EIF5A2 expression was directly associated with strong MMP-2 expression in human melanoma (n=369; P=0.015; χ^2 test). (B-D) Simultaneous negative nuclear EIF5A2 expression and negative-moderate MMP-2 expression (cat. 1) was significantly associated with a better overall and disease-specific 5-year survival outcome compared with negative nuclear EIF5A2 and strong MMP-2 expression or positive nuclear EIF5A2 and negative-moderate MMP-2 expression (cat. 2), or positive nuclear EIF5A2 and strong MMP-2 expression (cat. 3) in (B and C) all melanoma patients (overall and disease-specific 5-year survival, P<0.001; log rank test) and (D and E) primary melanoma patients (overall 5-year survival, P<0.001; disease-specific 5-year survival, P=0.001; log rank test). EIF5A2, eukaryotic translation initiation factor 5A2; MMP-2, matrix metalloproteinase-2; cat., category.

metastasis and patient survival. Previous *in vitro* results by the present authors, indicated that EIF5A2 promotes melanoma cell invasion partly via increasing the activity of MMP-2 (25). However, further investigation is required to determine which form of EIF5A2 functions this way, and to what extent it is responsible for this role.

To the best of our knowledge, the present study is the first to report the nuclear expression of EIF5A2 and its importance in melanoma. However, the presence of EIF5A2 in the nuclei of HCC cells has been previously demonstrated (24). Exportin 4 (Xpo4) is a tumour suppressor that belongs to the importin-β family of nuclear transporters, and EIF5A is known to be a substrate of Xpo4 (8,38). Knockdown of Xpo4 in murine hepatoma cells leads to nuclear accumulation of EIF5A1 and EIF5A2, which significantly increases the *in vitro* proliferation of murine liver progenitor cells (24). In a human HCC cell line, XPO4 inactivation was revealed to contribute to tumour maintenance. In the same study, EIF5A2 was required for efficient proliferation of cells lacking XPO4, suggesting the importance of nuclear accumulation of EIF5A2 in mediating the oncogenic effects associated with XPO4 loss (24). A similar phenomenon is observed when an increase or decrease in the nuclear accumulation of β-catenin and FOXO affects tumorigenesis as a result of deregulation of the WNT and AKT signalling pathways, respectively (39). In addition, nuclear expression of EIF5A2 has also been observed in the human bladder carcinoma 5637 cell line (23).

In conclusion, the present study examined the expression profile of nuclear EIF5A2 and revealed that nuclear EIF5A2 expression is increased during melanoma progression and is associated with a poor 5-year survival rate in all melanoma and primary melanoma patients. In addition, the present study identified that nuclear EIF5A2 was an independent prognostic factor for the 5-year survival of all and primary melanoma patients, suggesting that nuclear EIF5A2 may be a potential target candidate for melanoma therapy. Simultaneous expression of cytoplasmic and nuclear EIF5A2 was associated with a poor survival outcome as well. Similar to HCC, the presence of nuclear and cytoplasmic EIF5A2 may be a reason for the existence of a shuttling mechanism between the cytoplasm and nucleus in melanoma. Additional investigation is required to further determine the mechanisms

behind the subcellular localization of EIF5A2, the biological functions of nuclear EIF5A2 and its role in the tumorigenesis of melanoma.

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References

1. Miller AJ and Mihm MC Jr: Melanoma. N Engl J Med 355: 51-65, 2006.
2. Linos E, Swetter SM, Cockburn MG, Colditz GA and Clarke CA: Increasing burden of melanoma in the United States. J Invest Dermatol 129: 1666-1674, 2009.
3. Cummins DL, Cummins JM, Pantle H, Silverman MA, Leonard AL and Chanmugam A: Cutaneous malignant melanoma. Mayo Clin Proc 81: 500-507, 2006.
4. Saini P, Eyler DE, Green R and Dever TE: Hypusine-containing protein eIF5A promotes translation elongation. Nature 459: 118-121, 2009.
5. Patel PH, Costa-Mattioli M, Schulze KL and Bellen HJ: The Drosophila deoxyhypusine hydroxylase homologue nero and its target eIF5A are required for cell growth and the regulation of autophagy. J Cell Biol 185: 1181-1194, 2009.
6. Hauber I, Bevec D, Heukeshoven J, Krätzer F, Horn F, Choidas A, Harrer T and Hauber J: Identification of cellular deoxyhypusine synthase as a novel target for antiretroviral therapy. J Clin Invest 115: 76-85, 2005.
7. Kruse M, Rosorius O, Kratzer F, Bevec D, Kuhnt C, Steinkasserer A, Schuler G and Hauber J: Inhibition of CD83 cell surface expression during dendritic cell maturation by interference with nuclear export of CD83 mRNA. J Exp Med 191: 1581-1590, 2000.
8. Lipowsky G, Bischoff FR, Schwarzmaier P, Kraft R, Kostka S, Hartmann E, Kutay U and Görlich D: Exportin 4: A mediator of a novel nuclear export pathway in higher eukaryotes. EMBO J 19: 4362-4371, 2000.
9. Hutten S and Kehlenbach RH: CRM1-mediated nuclear export: To the pore and beyond. Trends Cell Biol 17: 193-201, 2007.
10. Maier B, Ogihara T, Trace AP, Tersey SA, Robbins RD, Chakrabarti SK, Nunemaker CS, Stull ND, Taylor CA, Thompson JE, *et al*: The unique hypusine modification of eIF5A promotes islet beta cell inflammation and dysfunction in mice. J Clin Invest 120: 2156-2170, 2010.
11. Zuk D and Jacobson A: A single amino acid substitution in yeast eIF-5A results in mRNA stabilization. EMBO J 17: 2914-2925, 1998.
12. Schrader R, Young C, Kozian D, Hoffmann R and Lottspeich F: Temperature-sensitive eIF5A mutant accumulates transcripts targeted to the nonsense-mediated decay pathway. J Biol Chem 281: 35336-35346, 2006.
13. Jenkins ZA, Hååg PG and Johansson HE: Human eIF5A2 on chromosome 3q25-q27 is a phylogenetically conserved vertebrate variant of eukaryotic translation initiation factor 5A with tissue-specific expression. Genomics 71: 101-109, 2001.
14. Clement PM, Henderson CA, Jenkins ZA, Smit-McBride Z, Wolff EC, Hershey JW, Park MH and Johansson HE: Identification and characterization of eukaryotic initiation factor 5A-2. Eur J Biochem 270: 4254-4263, 2003.
15. Guan XY, Fung JM, Ma NF, Lau SH, Tai LS, Xie D, Zhang Y, Hu L, Wu QL, Fang Y and Sham JS: Oncogenic role of eIF-5A2 in the development of ovarian cancer. Cancer Res 64: 4197-4200, 2004.
16. Marchet A, Mocellin S, Belluco C, Ambrosi A, DeMarchi F, Mammano E, Digito M, Leon A, D'Arrigo A, Lise M and Nitti D: Gene expression profile of primary gastric cancer: Towards the prediction of lymph node status. Ann Surg Oncol 14: 1058-1064, 2007.
17. Xie D, Ma NF, Pan ZZ, Wu HX, Liu YD, Wu GQ, Kung HF and Guan XY: Overexpression of EIF-5A2 is associated with metastasis of human colorectal carcinoma. Hum Pathol 39: 80-86, 2008.
18. Zhu W, Cai MY, Tong ZT, Dong SS, Mai SJ, Liao YJ, Bian XW, Lin MC, Kung HF, Zeng YX, *et al*: Overexpression of EIF5A2 promotes colorectal carcinoma cell aggressiveness by upregulating MTA1 through C-myc to induce epithelial-mesenchymaltransition. Gut 61: 562-575, 2012.
19. Tang DJ, Dong SS, Ma NF, Xie D, Chen L, Fu L, Lau SH, Li Y, Li Y and Guan XY: Overexpression of eukaryotic initiation factor 5A2 enhances cell motility and promotes tumor metastasis in hepatocellular carcinoma. Hepatology 51: 1255-1263, 2010.
20. Yang GF, Xie D, Liu JH, Luo JH, Li LJ, Hua WF, Wu HM, Kung HF, Zeng YX and Guan XY: Expression and amplification of eIF-5A2 in human epithelial ovarian tumors and overexpression of EIF-5A2 is a new independent predictor of outcome in patients with ovarian carcinoma. Gynecol Oncol 112: 314-318, 2009.
21. He LR, Zhao HY, Li BK, Liu YH, Liu MZ, Guan XY, Bian XW, Zeng YX and Xie D: Overexpression of eIF5A-2 is an adverse prognostic marker of survival in stage I non-small cell lung cancer patients. Int J Cancer 129: 143-150, 2011.
22. Chen W, Luo JH, Hua WF, Zhou FJ, Lin MC, Kung HF, Zeng YX, Guan XY and Xie D: Overexpression of EIF-5A2 is an independent predictor of outcome in patients of urothelial carcinoma of the bladder treated with radical cystectomy. Cancer Epidemiol Biomarkers Prev 18: 400-408, 2009.
23. Wei JH, Cao JZ, Zhang D, Liao B, Zhong WM, Lu J, Zhao HW, Zhang JX, Tong ZT, Fan S, *et al*: EIF5A2 predicts outcome in localised invasive bladder cancer and promotes bladder cancer cell aggressiveness in vitro and in vivo. Br J Cancer 110: 1767-1777, 2014.
24. Zender L, Xue W, Zuber J, Semighini CP, Krasnitz A, Ma B, Zender P, Kubicka S, Luk JM, Schirmacher P, *et al*: An oncogenomics-based in vivo RNAi screen identifies tumor suppressors in liver cancer. Cell 135: 852-864, 2008.
25. Khosravi S, Wong RP, Ardekani GS, Zhang G, Martinka M, Ong CJ and Li G: Role of EIF5A2, a downstream target of Akt, in promoting melanoma cell invasion. Br J Cancer 110: 399-408, 2014.
26. World Medical Association: World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. JAMA 310: 2191-2194, 2013.
27. Dai DL, Martinka M and Li G: Prognostic significance of activated Akt expression in melanoma: A clinicopathologic study of 292 cases. J Clin Oncol 23: 1473-1482, 2005.
28. Zhang Z, Chen G, Cheng Y, Martinka M and Li G: Prognostic significance of RUNX3 expression in human melanoma. Cancer 117: 2719-2727, 2011.
29. Chen G, Cheng Y, Tang Y, Martinka M and Li G: Role of Tip60 in human melanoma cell migration, metastasis, and patient survival. J Invest Dermatol 132: 2632-2641, 2012.
30. Jafarnejad SM, Ardekani GS, Ghaffari M, Martinka M and Li G: Sox4-mediated Dicer expression is critical for suppression of melanoma cell invasion. Oncogene 32: 2131-2139, 2013.
31. Soong SJ, Shaw HM, Balch CM, McCarthy WH, Urist MM and Lee JY: Predicting survival and recurrence in localized melanoma: A multivariate approach. World J Surg 16: 191-195, 1992.
32. Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Atkins MB, Byrd DR, Buzaid AC, Cochran AJ, Coit DG, Ding S, *et al*: Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol 27: 6199-6206, 2009.
33. Gialeli C, Theocharis AD and Karamanos NK: Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting. FEBS J 278: 16-27, 2011.

34. Rotte A, Martinka M and Li G: MMP2 expression is a prognostic marker for primary melanoma patients. Cell Oncol (Dordr) 35: 207-216, 2012.
35. Wang FW, Guan XY and Xie D: Roles of eukaryotic initiation factor 5A2 in human cancer. Int J Biol Sci 9: 1013-1020, 2013.
36. Shaverdashvili K, Wong P, Ma J, Zhang K, Osman I and Bedogni B: MT1-MMP modulates melanoma cell dissemination and metastasis through activation of MMP2 and RAC1. Pigment Cell Melanoma Res 27: 287-296, 2014.
37. Hofmann UB, Westphal JR, Waas ET, Zendman AJ, Cornelissen IM, Ruiter DJ and van Muijen GN: Matrix metalloproteinases in human melanoma cell lines and xenografts: Increased expression of activated matrix metalloproteinase-2 (MMP-2) correlates with melanoma progression. Br J Cancer 81: 774-782, 1999.
38. Kurisaki A, Kurisaki K, Kowanetz M, Sugino H, Yoneda Y, Heldin CH and Moustakas A: The mechanism of nuclear export of Smad3 involves exportin 4 and Ran. Mol Cell Biol 26: 1318-1332, 2006.
39. Kau TR, Way JC and Silver PA: Nuclear transport and cancer: From mechanism to intervention. Nat Rev Cancer 4: 106-117, 2004.