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RESEARCH ARTICLE

Melanoma differentiation-associated gene-7/interleukin-24 as a potential prognostic biomarker and second primary malignancy indicator in head and neck squamous cell carcinoma patients

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Abstract The significance of melanoma differentiation-associated gene-7/interleukin-24 (MDA-7/IL-24) expression in head and neck squamous cell carcinoma (HNSCC) remains unclear. This study was designed to investigate and evaluate the clinical significance of MDA-7/IL-24 expression in HNSCC by detecting expression by immunostaining in 131 HNSCC specimens. The function of MDA-7/IL-24 was investigated by real-time polymerase chain reaction (PCR) and Western blot in Ad5.mda-7-infected HNSCC cell lines. Our results showed that MDA-7/IL-24 was mainly expressed in the cytoplasm of HNSCC cells. MDA-7/IL-24 high patients presented with a favorable postoperative prognosis compared with MDA-7/IL-24 low patients, and high expression of MDA-7/IL-24 was significantly correlated with a lower incidence of second primary malignancies (SPMs) in the head and neck regions. In vitro assays showed that high expression of MDA-7/IL-24 could upregulate the expression of the epithelial terminal differentiation markers cytokeratin (KRT) 1, KRT4, KRT13, phosphorylated endoplasmic reticulum stress protein (p)-EIF2a, and the apoptosis-related protein cleaved caspase-3. It also downregulated the epithelial proliferative markers KRT5, KRT14, Integrin β4, and anti-apoptosis protein Bcl-2, which might be partially involved in the underlying mechanisms of Ad.mda-7-mediated HNSCC differentiation and apoptosis. Our results indicate that MDA-7/IL-24 can be a prognostic biomarker and an indicator of second primary malignancies (SPM) in HNSCC.

Keywords MDA-7/IL-24 · Squamous cell carcinoma of the head and neck · Second primary malignancy · Biomarker · Prognosis

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy worldwide, and it accounts for about 90 % of all head and neck malignancies [1]. Because of surgical improvements, radiotherapy, and chemotherapy, the life expectancy of HNSCC patients has increased over the recent decades. However, patient survival rate, specifically the 5-year survival rate, remains at approximately 50 % [2]. Second primary malignancies (SPMs) and distant metastases are the main factors that contribute to the poor survival rate of these patients [3].

With the development of molecular studies, knowledge of genetic alterations that precede or impede the development of HNSCC provides a rational basis for therapy. During the past 10 years or more, melanoma differentiation-associated gene-7 (MDA-7) has garnered particular interest in cancer research [4]. MDA-7, also called interleukin (IL)-24, was originally identified by subtraction hybridization from a human melanoma cell line that was induced to differentiate [5]. Previous studies show that loss of MDA-7/IL-24 is accompanied by melanoma progression [6]. Therefore, MDA-7/IL-24 is

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considered a tumor suppressor. Overexpression of MDA-7/IL-24 by plasmid transfection or adenovirus-mediated infection can inhibit growth of a variety of cancer cells [7–12]. Currently, Ad.mda-7 (INGN 241) is a proven, safe treatment approach and provides a favorable tumoricidal effect in over 40 % of patients in a phase I study performed in advanced carcinomas and melanomas [13]. At present, adenovirus-mediated MDA-7/IL-24 gene therapy is under investigation by several clinical trials.

In HNSCC, the expression pattern and clinical significance of MDA-7/IL-24 remain largely unknown. In this study, we investigated and evaluated the clinical significance of MDA-7/IL-24 expression in 131 HNSCC clinical specimens by immunostaining and patient history analysis. In addition, via transfection, real-time polymerase chain reaction (PCR), and Western blotting assays, we investigated the role of MDA-7/IL-24 in epithelial differentiation, apoptosis, and underlying mechanisms in HNSCC cells. Our results demonstrate that MDA-7/IL-24 is not only a potential therapeutic target for treating HNSCC but it can also be used as a prognostic biomarker in HNSCC and an indicator of second primary malignancies (SPMs) occurring in the head and neck regions.

Materials and methods

Patients

This study was approved by the Medical Ethical Committee of the Peking University School and Hospital of Stomatology. A total of 131 patients with histologically diagnosed HNSCC who underwent complete tumor resection and nodal dissection without any preoperative therapy from June 1999 to September 2007 at the Department of Oral and Maxillofacial Surgery were identified. Patient inclusion criteria were as follows: (1) primary tumor without evidence of metastasis, (2) received no pretreatment, and (3) complete medical information and thorough follow-up data.

Treatment and follow-up care procedures

All patients were treated with standardized surgery, including radical tumor resection, neck dissection, and, if necessary, reconstruction of tissue defects [14]. A 15-mm safety region was used for the local excision of the primary site. The radiotherapy and follow-up strategies were performed according to the NCCA guidelines® (China edition).

The definition of SPM

For the second primary malignancies (SPM) definition, we used the strict application of modified Warren and Gates' criteria for SPM [15]. Briefly, SPM diagnosis must meet three standards: (1) each of the tumors must be confirmed histologically, (2) each of the tumors must be distinct, and (3) the possibility of one being a metastasis and recurrence must be ruled out. Based on these criteria and molecular studies, if the second cancer was SCC and developed in the same region as the primary cancer, it was only coded as an SPM if more than 60 months had passed since the primary diagnosis [16].

Immunohistochemistry

Immunohistochemistry (IHC) procedures for MDA-7/IL-24 expression involved using 3-μm-thick sections cut from formalin-fixed paraffin-embedded HNSCC tissue samples followed by standard immunostaining procedures. A rabbit polyclonal antibody (sc-22769; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used as the primary antibody for detection of MDA-7/IL-24 expression. The scoring system and cutoff value are described in detail in a previous study [17]. Briefly, the labeling index for MDA-7/IL-24 expression was defined as the intensity of staining (negative, weak, moderate, and strong scored as 0, 1, 2, and 3, respectively) multiplied by the percentage of positive-staining tumor cells. The proportion of positive-staining tumor cells was graded according to the percentage as follows: 0, none; 1, 1 to 50 %; and 2, 50 to 100 %. MDA-7/IL-24 expression was determined at high power fields. Two pathologists who were blinded to the results of the previous assessments verified the images using Image Pro-Plus software (Media Cybernetics, Inc.ver.6.0). A cutoff value was set as the median of the labeling index: a value ≥2 was considered high expression; conversely, a value <2 was considered low expression.

Cell culture

Three human HNSCC cell lines, Fadu (ATCC® HTB-43™; American Type Culture Collection, Manassas, VA, USA), WSU-HN6, and Tca83, were cultured in DMEM (Gibco, Grand Island, NY, USA) supplemented with 10 % fetal bovine serum (FBS; Gibco), 100 U/mL penicillin, and 100 μg/mL streptomycin and maintained in a humidified incubator at 37 °C with 5 % CO_2.

Adenovirus-mediated MDA-7/IL-24 infection assay

The cells in logarithmic growth phase (60–70 % confluence) were used for infection with replication-deficient, recombinant adenovirus 5 encoding enhanced green fluorescence protein (Ad5.EGFP) or replication-deficient Ad5.EGFP encoding MDA-7 (Ad5.mda-7) at a multiplicity of infection (MOI) of 200. At 24 h post-infection, the cells were harvested for total RNA extraction for real-time PCR assay. At 48 h post-infection, cells were lysed in RIPA buffer (50 mM Tris–

HCl [pH 7.4], 150 mM NaCl, 1 % NP-40, and 0.1 % SDS; Applygen Technology, Beijing, China) with protease inhibitors (Roche Applied Science, Penzberg, Germany). The protocol used has been previously described [18], and primary antibodies against MDA-7/IL-24 (R&D Systems Inc. Minneapolis, MN, USA), p-EIF2a, t-EIF2a, cleaved caspase-3 (Cell Signaling Technology, Danvers, MA, USA), Bcl-2, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, Santa Cruz Biotechnology, Inc.) were used. Experiments were performed in triplicate, and the results were obtained from three independent experiments.

Real-time PCR

Total RNA was extracted from cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) reversely transcribed into single-stranded cDNA using M-MLV reverse transcriptase (Promega, Madison, WI, USA) according to the manufacturer's protocols. Real-time PCR was performed in an ABI Prism 7000 Sequence Detection System (Applied Biosystems, Life Technologies, Warrington, UK) using SYBR green reagent (Roche, Indianapolis, IN, USA). The primers for each gene were as follows: cytokeratin (KRT) 1, KRT4, KRT5, KRT13, KRT14, integrin β4, and GAPDH (Table 1). The standard PCR conditions were 10 min at 95 °C, followed by 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. All reactions were performed in triplicate. The expression levels of the target transcripts in each sample were calculated using the comparative $2^{-\Delta\Delta Ct}$ method after normalization to GAPDH expression.

Western blot

Cells were harvested and lysed in RIPA buffer (Applygen) containing proteinase inhibitors and phosphatase inhibitors. After measuring protein concentration using the BCA kit (Thermo Fisher Scientific, Rockford, IL, USA), equal amounts of protein samples were separated by 12 % sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membranes by wet blotting. The membranes were blocked in 10 % non-fat dry milk for 1 h and probed with antibodies against MDA-7/IL-24 (dilution, 1:500), p-EIF2a (1:1,000), t-EIF2a (1:1,000), cleaved caspase-3 (Cell Signaling Technology, Danvers, MA, USA (1:1,000)), Bcl-2 (1:1,000), and GAPDH (1:1,000) separately at 4 °C overnight. After incubation with peroxidase-linked secondary antibodies for 1 h at room temperature, the enhanced chemiluminescent (ECL) reagent was used to visualize the immunoreactive proteins.

Statistical analysis

Patient characteristics were expressed as percentages or means ± standard deviation (SD). The baseline data of the two groups were compared using the χ^2 test, except age, which was compared using an independent sample t test. Real-time PCR and Western blotting results are expressed as means ± SD. All calculations and analyses were performed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). A P value <0.05 was considered statistically significant.

Results

Patient characteristics

In total, 131 patients were enrolled in this study. The clinical and pathological parameters of these patients are shown in Table 2. The follow-up cutoff date was April 1, 2013, and the median follow-up time was 73 months.

MDA-7/IL-24 expression in HNSCC

MDA-7/IL-24 expression was examined in 131 cases by immunohistochemistry. The results showed that MDA-7/IL-24 was diffusely expressed in the cytoplasm of the cancer cells (Fig. 1).

Correlation of MDA-7/IL-24 with clinic pathological parameters

Based on the MDA-7/IL-24 expression level cutoff described in the "Materials and methods," 62 cases were evaluated as

Table 1 Real-time PCR primers used in this study

Target gene	Target gene	Reverse primer
KRT1	ATATGGGGGTGGTTATGGTCC	GTGACTTGATTTGCTCCCTTTCT
KRT4	AGACAGCAGTGTGTCCGAG	ATGCTTTTGTTCCCCCTGAGG
KRT5	ATGTCTCGCCAGTCAAGTGTG	CTGCCTCCTCTAGTGCTGA
KRT13	CCAGGACGCCAAGATGATTG	GGTGGTGGTAACAGAGGCAC
KRT14	CATGAGTGTGGAAGCCGACAT	GCCTCTCAGGGCATTCATCTC
Integrin β4	TGTGTTCCAGGTGTTTGAGC	TTTCTCATCATTGCGGTTCA
GAPDH	ATGGGGAAGGTGAAGGTCG	GGGGTCATTGATGGCAACAATA

high expression and 69 cases as low. Next, we analyzed the relationship between MDA-7/IL-24 expression level and age, gender, site, nodal status (pN+stage), T-stage, growth pattern, smoking status, pathologic grade, and alcohol history. There was no correlation between MDA-7/IL-24 expression and any of the above parameters, except for pathologic grade. A statistical association was found between MDA-7/IL-24 expression and pathologic grade (P=0.026). MDA-7/IL-24 was highly expressed in the high pathologic grade HNSCC specimens (Fig. 1 and Table 2).

MDA-7/IL-24 expression is significantly correlated with HNSCC prognosis

During follow-up, 50.4 % of the 131 patients died: 4.6 % as a result of causes unrelated to cancer, 26.7 % patients developed local recurrence, and 20.6 % patients developed SPMs. The median survival time of the high and low MDA-7/IL-24 patients was 66.01±32.31 and 62.28±35.04, respectively. The disease-free survival (DFS) rate was 48 % (60/125), and the disease-specific survival (DSS) rate was 60 % (75/125).

Table 2 Association between MDA-7/IL-24 expression and clinical pathological parameters in 131 patients with HNSCC

Variable	Patients (n=131)		IL-24 expression (n=131)		P value
	No.	%	Low	High	
Age (years): means ± SD	58.8±12.3		59.5±11.4	58.1±13.4	0.513
Gender					
Male	80	61.1	40	40	0.477
Female	51	38.9	29	22	
Sites					
Tongue	52	39.7	25	27	0.821
Buccal	20	15.3	12	8	
Mandibular gingiva	19	14.5	11	8	
Floor of the mouth	16	12.2	7	9	
Maxillary gingiva	13	9.9	8	5	
Oropharynx	7	5.3	3	4	
Hard palate	4	3.1	3	1	
T stage					
T1	30	22.9	15	15	0.532
T2	59	45	28	31	
T3	12	9.2	7	5	
T4	30	22.9	19	11	
N status					
N−	60	45.8	35	25	0.201
N+	59	45	27	32	
Unknown	12	9.2	7	5	
Pathologic grade					
I	52	39.7	31	21	0.026*
II	67	51.1	36	31	
III	12	9.2	2	10	
Growth pattern					
Exophytic	41	31.3	22	19	0.881
Ulcerative	37	28.2	18	19	
Infiltrative	53	40.5	29	24	
Smoking history					
Smoker	69	52.7	36	33	>0.999
Non-smoker	62	47.3	33	29	
Alcohol history					
Drinker	49	37.4	26	23	>0.999
Nondrinker	82	62.6	43	39	

*P<0.05

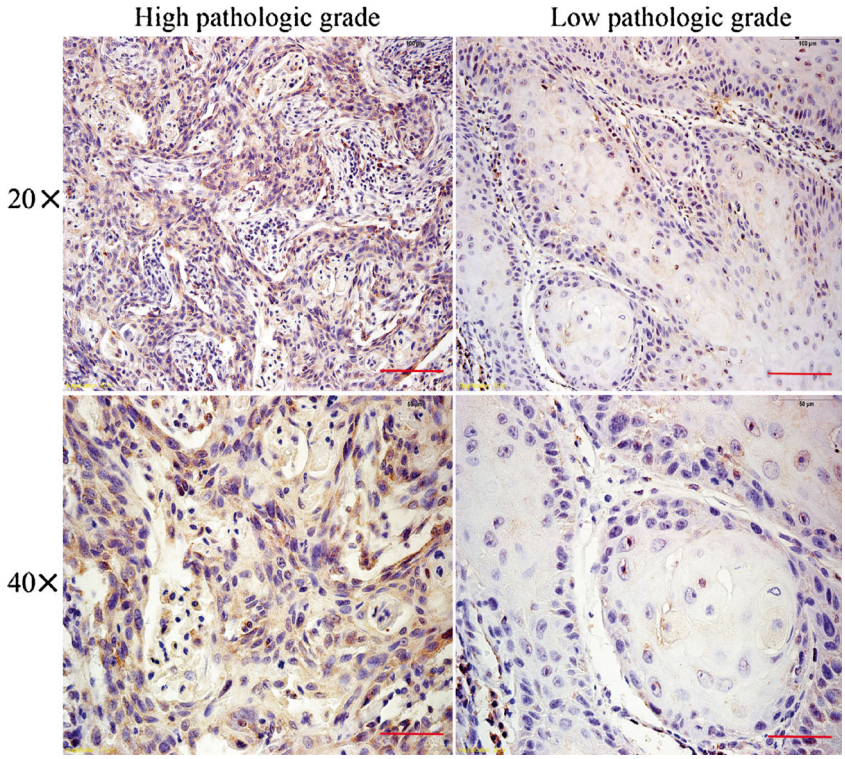

Fig. 1 Immunohistochemical analysis of MDA-7/IL-24 expression in representative high-grade case (pathologic grade III) and low-grade case (pathologic grade I) HNSCC samples. Original objective magnification, ×20 and ×40

With respect to MDA-7/IL-24 expression and prognosis, we found that the DFS rates were statistically different between the low and high MDA-7/IL-24 expression subgroups (31.9 versus 58.1 %, respectively, P=0.005; Fig. 2a). A similar result was obtained for the DSS rates for patients between the low and high subgroups (42 versus 71.0 %, respectively, P=0.012; Fig. 2b). Based on the above results, we concluded that high MDA-7/IL-24 expression was a predictor of good prognosis in HNSCC patients.

MDA-7/IL-24 expression is closely associated with SPM occurrence in the head and neck

Twenty-seven (20.6 %) patients developed a SPM, which were mostly located in the head and neck (51.8 %). The patients with low MDA-7/IL-24 expression had an increased risk of SPM in the head and neck compared with those with high MDA-7/IL-24 expression (19.0 versus 3.6 %, P=0.011). Although statistical analysis showed that the expression level of MDA-7/IL-24 was not correlated with SPM occurring outside the head and neck regions, the sample size was too small to draw a concrete conclusion (Table 3). There is no significant association between MDA-7/IL-24 expression and recurrence (low versus high expression: 30.4 versus 22.6 %, respectively; P=0.33). These results indicate that high MDA-7/IL-24 expression might be closely associated with SPM in HNSCC patients.

MDA-7/IL-24 as an epithelial differentiation regulator

To investigate why high expression of MDA-7/IL-24 is associated with good prognosis and low incidence of SPM in HNSCC patients, we generated an adenovirus-encoded MDA-7/IL-24 expression cassette to infect Tca83 and WSU-HN6 cell lines. Western bolt results indicate that MDA-7/IL-24 expression was highly expressed in the Ad5.mda-7 infection group (Fig. 3a, b). Real-time PCR results showed that compared with corresponding mock infection, MDA-7/IL-24 gene affected the status of WSU-HN6 cell terminal differentiation through upregulation of KRT1 (fold change, 2.17±0.15; P=0.026), KRT4 (2.02±0.14, P=0.022), and KRT13 (9.44±1.4, P=0.026) and downregulation of KRT5 (0.64±0.08, P<0.05), KRT14 (0.97±0.02, P=0.46), and integrin β4 (0.12±0.003, P=0.02). MDA-7/IL-24 gene changed the differentiation status of Tca83 cells through upregulation of KRT1 (fold change, 1.77±0.01; P<0.01), KRT4 (1.57±0.02, P<0.01), and KRT13 (1.68±0.04, P<0.01) and downregulation of KRT5 (0.82±0.002, P=0.02), KRT14 (0.86±0.0002, P=0.02), and integrin β4 (0.68±0.01, P=0.01) (Fig. 3c, d).

Molecular mechanisms of MDA-7/IL-24 as a tumor suppressor

To investigate the underlying mechanism of MDA-7/IL-24 as a tumor suppressor, we generated the same adenovirus

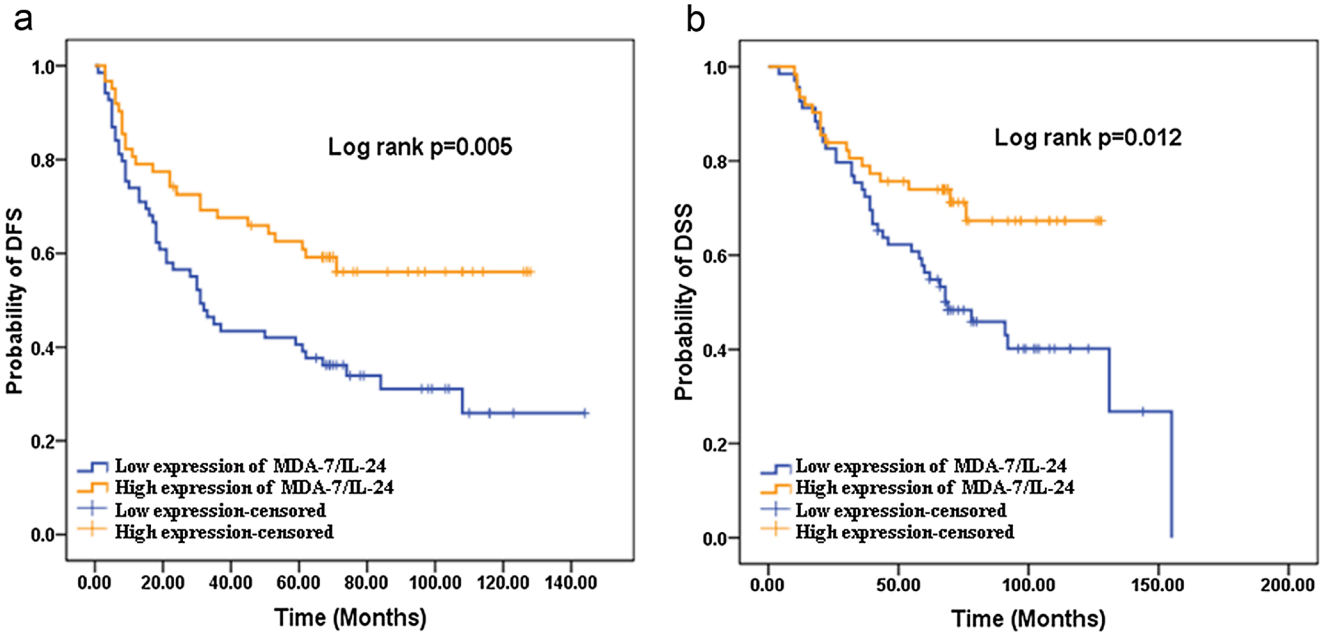

Fig. 2 Kaplan–Meier survival curves of MDA-7/IL-24 protein expression and prognosis in HNSCC patients. **a** MDA-7/IL-24 protein expression and disease-free survival (DFS). **b** MDA-7/IL-24 protein expression and disease-specific survival (DSS)

infection assay as above. The results showed that in these three HNSCC cell lines, overexpression of MDA-7/IL-24 markedly upregulated endoplasmic reticulum stress protein p-EIF2a and apoptosis-related protein cleaved caspase-3 expression, while downregulating the mitochondrion apoptosis-associated protein Bcl-2 compared with untreated controls (Fig. 4).

Discussion

For the first time, we assessed the clinical significance of MDA-7/IL-24 expression in HNSCC. Diffuse cytoplasmic immunostaining was observed in most MDA-7/IL-24 expression-positive cases, and we revealed that MDA-7/IL-24 expression was a significant factor predicting favorable postoperative prognosis in HNSCC.

Table 3 SPM that occurred in the head and neck regions correlated with the expression of MDA-7/IL-24

SPM	MDA-7/IL-24 expression		Sum	*P* value
	Low	High		
No	51	53	104	
Yes	18	9	27	0.131
HN	12	2	14	0.011*
Non-HN	6	7	13	>0.999

HN head and neck

**P* value 0.011 represents the statistical difference between the patients who did not have SPM and SPM that occurred in the head and neck regions

To date, there are two studies regarding the clinical significance of MDA-7/IL-24 expression. Researchers verified that downregulation of MDA-7/IL-24 expression in primary melanomas promoted invasion and metastasis, although it appeared that MDA-7/IL-24 expression had no effect on survival rate or factors associated with survival [6]. In non-small cell lung cancer (NSCLC), researchers showed that MDA-7/IL-24 status was only related with adenocarcinoma prognosis and was not related to SCC prognosis [17]. In our study, MDA-7/IL-24 was associated with HNSCC prognosis.

Moreover, we found that the expression of MDA-7/IL-24 was inversely related to the occurrence of SPMs in the head and neck regions. It is known that SPMs represent the leading long-term cause of mortality in HNSCC patients [19], and recent research shows that SPMs account for about one third of HNSCC deaths and triple the number of deaths resulting from distant metastases [3, 20]. Thus, more attention has been paid to SPMs, and in patients with HNSCC, the distribution of SPMs differs according to the primary cancer subsite. For instance, oral cavity cancer and oropharynx cancer have been reported by several studies to be most strongly associated with SPMs in the head and neck [21, 22]; we validated this finding. As for the effect of SPMs on prognosis, we assume that the prognostic value of this gene could be attributed to its correlation with SPMs.

SPMs are often explained by the concept of "field cancerization," which was first proposed by Slaughter et al. [23]. However, because of the time in which their study was performed, there was no molecular basis for this theory.

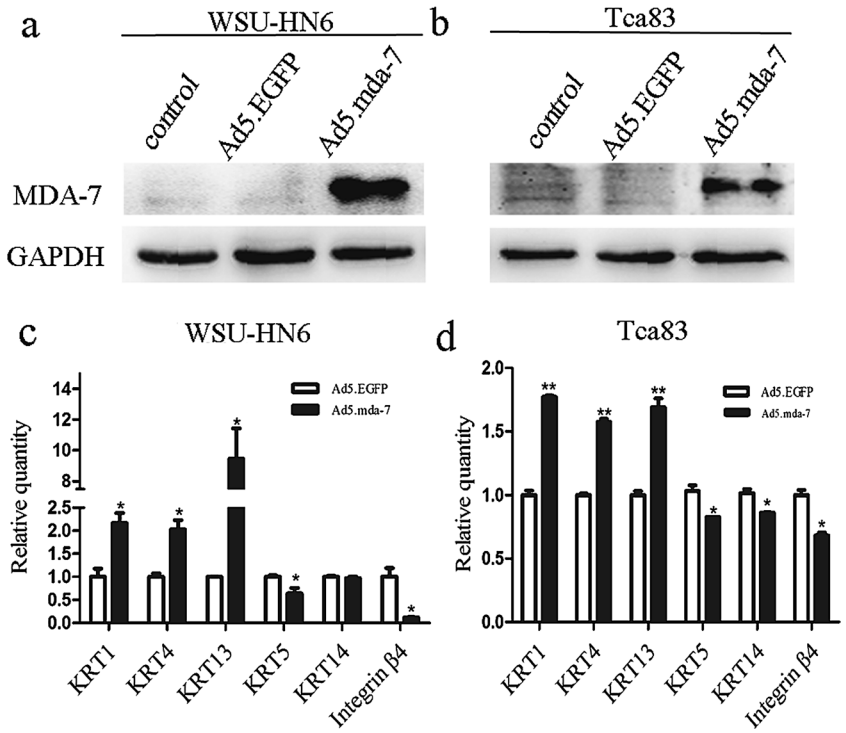

Fig. 3 MDA-7/IL-24 alters the expression pattern of epithelial differentiation markers. WSU-HN6 and Tca83 cells were infected with 200 multiplicity of infection of Ad5.EGFP or Ad5.mda-7 as described in the "Materials and Methods." At 24 h post-infection, the cells were harvested and subjected to real-time PCR to detect epithelial differentiation marker KRTs expression. At 48 h post-infection, the cells were harvested to detect MDA-7/IL-24 protein expression. **a**, **b** Western blot results showed that MDA-7/IL-24 was highly expressed after Ad5.mda-7 transfection compared with the corresponding blank controls or the Ad5.EGFP infection controls in WSU-HN6 and Tca83 cell lines. **c**, **d** MDA-7/IL-24 gene affected the status of WSU-HN6 and Tca83 cell terminal differentiation through upregulation of KRT1, KRT4, and KRT13 and downregulation of KRT5, KRT14, and integrin β4.$^{**}P<0.01$; $^{*}P<0.05$

Fortunately, in our study, we observed that the expression of MDA-7/IL-24 was associated with HNSCC pathologic grade. Importantly, MDA-7/IL-24 expression was not correlated with HNSCC recurrence, but correlated with SPM incidence in the head and neck regions in our study. Based on the above evidence, we hypothesize that MDA-7/IL-24 is a carcinogenesis- and development-related gene.

Next, we investigated the consequences of MDA-7/IL-24 in HNSCC using adenovirus-mediated MDA-7/IL-24 infection of HNSCC cell lines. Because keratin expression in tumors can be used as diagnostic tools [24] and evidence of their importance as prognostic markers becomes more ubiquitous [25], we tested keratin patterns in HNSCC cell lines following MDA-7/IL-24 overexpression. KRT1, KRT4, and

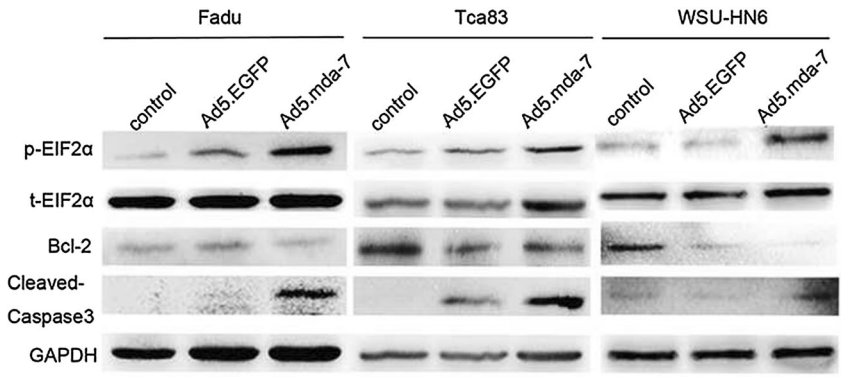

Fig. 4 Determination of p-EIF2a, cleaved caspase-3, and Bcl-2 protein levels in HNSCC cells as a consequence of infection with Ad5.mda-7. Cells were infected with 200 multiplicity of infection of Ad5.EGFP or Ad5.mda-7 as described in the "Materials and Methods." The results show that Ad5.mda-7 can induce cancer cell apoptosis, which explains the role of MDA-7/IL-24 as a tumor suppressor gene and explains its correlation with favorable prognostic in HNSCC

KRT13 are epithelial terminal differentiation biomarkers [26], while KRT5, KRT14 [27], and integrin β4 [28] are epithelial proliferative markers. MDA-7/IL-24 gene expression affected Tca83 and WSU-HN6 cell terminal differentiation through upregulation of KRT1, KRT4, and KRT13, and downregulation of KRT5, KRT14, and integrin β4, which provides further evidence of its prognostic role and as a SPM indicator in HNSCC.

In addition, overexpression of MDA-7/IL-24 through adenovirus-mediated MDA-7/IL-24 infection had a pro-apoptotic effect and, thus, exerted a tumor suppressor role in HNSCC cell lines, which verified its prognostic role in HNSCC. Previous data showed that Ad.mda-7 mainly induced an endoplasmic reticulum stress response (upregulation of the endoplasmic reticulum stress-associated protein p-EIF2α) and activated multiple apoptotic pathways (Bcl-2 family proteins converged on the mitochondrion and caspase-3-dependent signaling pathway), ultimately leading to a decrease in cell survival [29–32]. Therefore, to explain the underlying molecular mechanisms of the relationships between MDA-7/IL-24 and HNSCC, we investigated the proteins that play important roles in the regulation of p-EIF2α [33], Bcl-2 [34], and apoptosis-associated effector caspase protein (cleaved caspased-3) [29]. Our results clearly show that Ad5.mda-7 upregulated p-EIF2α and cleaved caspase-3 in the apoptotic cascade and downregulated Bcl-2 compared with the Ad5.EGFP infection group and blank control, which are consistent with reported data [30, 33, 34]. Thus, Ad5.mda-7 can induce cancer cell apoptosis, which explains the role of MDA-7/IL-24 as a tumor suppressor gene and supports its value as an indicator of favorable prognosis in HNSCC.

Our results showed high expression of MDA-7/IL-24 that predicts good prognosis, owing to its role in lower SPM occurrence, better terminal differentiation, and pro-apoptotic role in HNSCC. Also, high expression of this gene was associated with high pathologic grade of tumor. However, this does not mean that high-grade tumors have good prognosis. In other regions of the body, the differentiation status of cancer cells affects the prognosis of cancer patients. Conversely, in the head and neck regions, the prognosis of cancer patients is irrelevant to the differentiation stage of cancer cells because of the many vital organs located in head and neck regions. The limited space in these areas determines that the size, growth rate, and location of tumor are much more important than the differentiation status.

In conclusion, our results suggest that MDA-7/IL-24 can serve not only as a favorable prognostic biomarker but also as a novel indicator of SPMs in HNSCC. Our data provide new evidence to support the development of MDA-7-based therapy as a novel approach for HNSCC. Future studies should focus on the mechanisms related to the apoptotic effect of this cytokine and how to elevate MDA-7/IL-24 for HNSCC treatment.

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Conflicts of interest None

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