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N6-methyladenosine RNA modification (m6A) is of prognostic value in HPV-dependent vulvar squamous cell carcinoma

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Abstract

Background: Vulvar squamous cell carcinoma (VSCC) is an uncommon gynecologic malignancy but with an increasing incidence in recent years. Etiologically, VSCC is classified into two subtypes: HPV-dependent and HPV-independent. Localized VSCC is treated surgically and/or with radiation therapy, but for advanced, metastatic or recurrent disease, therapeutic options are still limited.

N6-methyladenosine (m6A) is the most prevalent post-transcriptional messenger RNA (mRNA) modification and involved in many physiological processes. The group of m6A proteins can be further divided into:‚writers' (METTL3, METTL4, METTL14, WTAP, KIAA1429),‚erasers' (FTO, ALKBH5), and‚readers' (HNRNPA2B1, HNRNPC, YTHDC1, YTHDF1-3). Dysregulated m6A modification is implicated in carcinogenesis, progression, metastatic spread, and drug resistance across various cancer entities. Up to date, however, only little is known regarding the role of m6A in VSCC.

Methods: Here, we comprehensively investigated protein expression levels of a diverse set of m6A writers, readers and erasers by applying immunohistochemical staining in 126 patients with primary VSCC.

Results: In the entire study cohort, dominated by HPV-independent tumors, m6A protein expression was not associated with clinical outcome. However, we identified enhanced protein expression levels of the‚writers' METTL3, METTL14 and the‚reader'YTHDC1 as poor prognostic markers in the 23 patients with HPV-dependent VSCC.

Conclusion: Our study suggests dysregulated m6A modification in HPV-associated VSCC.

Keywords: m6A, N6-methyladenosine RNA modification, Vulvar squamous cell carcinoma, HPV, Biomarker

Background

Vulvar carcinoma is responsible for 3% of all gynecological malignancies worldwide and represents the fourth most common tumor of the female genital tract [1]. In the last decade, the incidence of human papillomavirus (HPV)-dependent and HPV-independent vulvar carcinoma has increased by more than 20%, likely driven by increased high-risk HPV exposure and a generally aging population [2–4].

Vulvar squamous cell carcinoma (VSCC) is the predominant histological subtype. VSCC can be further subclassified into two etiologic subtypes: (i) HPV-dependent VSCC [5, 6], accounting for 34% of invasive VSCC [7]; and (ii) HPV-independent VSCC arising on the basis of *lichen sclerosus and atrophicus* [8], a chronic vulvar dermatosis affecting mostly elderly patients. Of note, HPV-independent VSCC displays a worse overall prognosis than HPV-dependent VSCC [9].

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In localized disease, tumor excision with inguinofemoral sentinel lymphonodectomy and/or inguinofemoral systematic lymphonodectomy represents the therapeutic mainstay. Additional radiotherapy is applied in the presence of risk factors. With > 85% survival rates, the 5-year overall survival (OS) is excellent in localized disease [10]. However, for patients with locally advanced, metastatic or recurrent disease, there are only limited treatment strategies with an overall poor 5-year OS of only 15–50% [11]. Hence, there is an unmet need for new therapeutic options in this difficult-to-treat patient population [12]. In this context, a deeper understanding of the VSCC tumor biology, in particular for the respective etiologic subtypes, might pave the way to identify novel therapeutic approaches in VSCC.

N6-methyladenosine (m6A) is the most abundant messenger RNA (mRNA) modification. Briefly, three different enzyme groups are involved in m6A modification: (i) methylases ('writers'; METTL 3, METTL 4, METTL 14, WTAP, KIAA1429) that catalyze the transfer of S-adenosyl methionine groups to RNA adenine bases; (ii) demethylases ('erasers'; FTO, ALKBH5) that have the capacity to reverse the methylation process; and (iii) 'readers' (HNRNPA2B1, HNRNPC, YTHDC1, YTHDF1-3) that recognize m6A RNA modification and activate downstream regulatory pathways [13].

m6A modifications were previously identified to be involved in tumorigenesis, proliferation, angiogenesis and tumor immunity across diverse cancer entities [14–20]. This central role of m6A emphasizes its great potential in both diagnostic and therapeutic applicability [21]. Recently, we were able to provide evidence for m6A involvement in CC that bears etiological and tumor biological similarity to VSCC [22]. To the best of our knowledge, there is no data regarding m6A modification in VSCC. In this study, we thus comprehensively analyzed protein expression levels of a diverse set of m6A writers, readers and erasers by immunohistochemistry in a cohort of 126 VSCC patients to understand the effects of RNA modifications on tumorigenesis, especially with regard to the two etiologic subtypes.

Methods

Patients and specimens

The retrospective single-center study population included 126 patients with primary VSCC treated at the University Hospital Bonn between 2002 and 2017. The collection of tissue was within the framework of the Biobank initiative of the University Hospital Bonn. Tissue was obtained from biopsies or surgical specimens. All patients provided written informed consent prior to the collection of biomaterials. The study was approved by the Ethics Committee of the Medical Faculty of the University of Bonn (vote: 208/21).

Clinicopathological characteristics of the entire cohort, the HPV-independent and the HPV-dependent subcohorts, obtained from a clinical database, are presented in Table 1. The histopathological diagnosis was based on the World Health Organization (WHO) criteria. The 2010 revision of the International Federation of Gynecology and Obstetrics (FIGO) system was used to determine the tumor grade. The 7th TNM classification of the Union for International Cancer Control (UICC) allowed to determine the tumor stage.

Tissue microarray (TMA) construction

The TMA was generated from formalin-fixed paraffin (FFPE)-embedded VSCC tissue specimens. Hematoxylin and eosin (HE) stained sections were applied to identify representative tumor areas. Subsequently, a 1 mm core biopsy (0.785mm^2) was taken from the selected cancer areal and arranged in TMA blocks.

DNA extraction und HPV analysis

Tumor tissue was deparaffinized and macrodissected from unstained slides. The tumor tissue was then lysed with proteinase K overnight. DNA extraction from FFPE-embedded tissue was performed with the BioRobot M48 Robotic workstation and the corresponding MagAttract DNA Mini M48 Kit (Qiagen, Germany). Determination of HPV subtypes was performed applying the HPV Type 3.5 LCD-Array Kit (Chipron, Germany) according to the manufacturer's instructions as described previously [23]. With this assay the detection of 32 different HPV subtypes is possible (HPV types 06,11,16,18,31,33,35,39, 42,44,45,51,52,53,54,56, 58,59,61,62,66,67,68,70, 72,73,81,82,83,84,90 and 91).

Immunohistochemistry

Immunostaining of METTL3, METTL4, METTL14, WTAP, KIAA1429, FTO, ALKBH5, HNRNPA2B1, HNRNPC, YTHDC1, YTHDF1,YTHDF2, and YTHDF3 was performed on the TMAs using an automated staining system (BenchMark ULTRA; Ventana Medical Systems) which performed deparaffinization, pretreatment with cell conditioning buffer (CC1 buffer, pH8), and incubation with primary antibodies (FTO (1:50; Atlas Antibodies #HPA041086), ALKBH5 (1:200; Novus #NBP1-82,188), METTL3 (1:1000; Biorbyt #orb374082), METTL4 (1:40; Atlas Antibodies #HPA040061), METTL14 (1:100; Atlas Antibodies #HPA038002), WTAP (1:100; Atlas Antibodies #HPA010550), KIAA1429 (1:25; Atlas Antibodies #HPA031530), HNRNPC (1:25; Atlas Antibodies #HPA051075), HNRNPA2B1 (1:100; Atlas Antibodies #HPA001666), YTHDC1 (1:25; Atlas Antibodies

Table 1 Clinicopathological characteristics of the entire VSCC cohort, HPV-independent cohort, and HPV-dependent sub-cohorts. No HPV status was available for 24 patients. SD = standard deviation

Clinicopathological parameters	All (*N* = 126)	HPV-independent (*N* = 79)	HPV-dependent (*N* = 23)
Age (years)			
Mean (± SD)	64.1 ± 14.4	65.1 ± 14.0	57.5 ± 15.2
Min–max	25—93	33—93	25—84
Overall survival (months)			
Mean *(± SD)*	54.0 ± 42	58.8 ± 44.7	53.7 ± 39.5
Median	46.0	58.0	48.0
TNM classification			
T1	102 (81.0%)	65 (82.3%)	16 (69.6%)
T2	17 (13.5%)	12 (15.2%)	4 (17.4%)
T3	3 (2.4%)	1 (1.3%)	2 (8.7%)
Tx	4 (3.2%)	1 (1.3%)	1 (4.3%)
N0	48 (38.1%)	33 (41.8%)	4 (17.4%)
N1	10 (7.9%)	6 (7.6%)	4 (17.4%)
N2	21 (16.7%)	16 (20.3%)	4 (17.4%)
N3	1 (0.8%)	0	0
Nx	46 (36.5%)	24 (30.4%)	11 (47.8%)
Grading			
G1	11 (8.7%)	5 (6.3%)	2 (8.7%)
G2	82 (65.1%)	52 (65.8%)	16 (69.6%)
G3	29 (23.0%)	19 (24.1%)	4 (17.4%)
not determined	4 (3.2%)	3 (3.8%)	1 (4.3%)
HPV-subtypes			
16			18 (76%)
33			3 (12%)
33 + 16			3 (12%)

#HPA036462), YTHDF1 (1:10; Biorbyt #orb179018), YTHDF2 (1:200; Biorbyt #orb39199), YTHDF3 (1:200; Biorbyt #orb374095) at 4 °C overnight. Signal detection was performed with the UltraView DAB IHC Detection Kit (Ventana).

Immunostained cells were analyzed with an Olympus BX51 microscope and the Panoramic Viewer 3DHistech. Staining intensities were evaluated for all m6A proteins separately by MC and DJR. In case of discordance between these two investigators, TT was consulted as a board-certified gynecopathologist. In addition, random reviews of the staining intensities were conducted by TT. In detail, a four-tier scoring system was applied to categorize staining intensities (0: no staining, 1: low staining, 2: moderate staining, 3: high staining). Staining intensities were divided into two groups (low and high expression) based on the median protein expression in the entire study cohort.

Statistical analysis

Kaplan–Meier survival analyses and log-rank tests allowed to compare OS between the two groups (low vs. high expression) for each analyzed protein. Correlation analyses were performed applying the nonparametric Mann–Whitney U test. In addition, the two-sided Fisher's exact test was used for the evaluation of statistical significance; a significance threshold was considered at a p-value of < 0.05. We performed multiple hypotheses testing using the method of Benjamini and Hochberg and converted p-values to false discovery rate (FDR) q-values with a significance threshold of $q < 0.1$. Non-parametric Spearman's p correlation coefficients were calculated for co-expression analysis. Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS ®) version 28 (SPSS Inc., IBM Corp.) and the GraphPad Prism software (GraphPad software).

Results

Proteins of m6A are frequently expressed in VSCC

Across the cohort of 126 primary VSCC samples (Table 1) we identified protein expression of all distinct m6A writers, readers and erasers. The proteins involved in the different m6A functions were present in the different cell compartments reflecting the diversity of RNA metabolism. Writers were typically observed in the nucleus including METTL3, METTL14, WTAP and KIAA1429. Likewise, immunohistochemical analysis revealed a strong nuclear staining for the eraser FTO, and the two readers HNRNPC und HNRNPA2B1. In contrast, the readers YTHDF1, YTHDF2 and YTHDF3 as well as the writer METTL4 showed a strong cytoplasmic staining (Table 2).

Proteins of m6A are differently expressed in VSCC subtypes

Given the two etiologically distinct VSCC subgroups, namely HPV-dependent and HPV-independent VSCC, each subgroup was next examined separately. In the HPV-dependent subgroup of 23 patients, 76% of cases were positive for HPV type 16, 12% for HPV type 33, and 12% displayed a co-infection with both HPV types 16 and 33. The HPV-independent cohort comprised 79 patients. For 24 patients, HPV status was unknown (Table 1).

First, we analyzed m6A proteins for their different expression regarding to the VSCC subtypes. For most m6A proteins (10/13), we did not find a differential expression between the two etiologic subtypes (Fig. 1A-B, D-E, H-M). However, we observed differences for 3 proteins that were all significantly enriched in HPV-dependent VSCC: the writer METTL14 (63% vs 34% in HPV-independent VSCC; $p=0.049$, Fisher's exact test; Fig. 1C), and the erasers FTO (47% vs 13% in HPV-independent VSCC; $p=0.002$, Fisher's exact test; Fig. 1F), and ALKBH5 (79% vs 59% in HPV-independent VSCC; $p=0.040$, Fisher's exact test; Fig. 1G).

Proteins of m6A indicate poor outcome in HPV-dependent but not HPV-independent VSCC

In the entire cohort, none of m6A proteins analyzed was associated with OS (Table 2). Likewise, when focusing our analysis on HPV-independent VSCCs only, we also did not find an association with outcome (Table 2). However, when evaluating the subgroup of HPV-dependent VSCC, high expression levels of the writers METTL3 ($p=0.010$, $q=0.08$, log-rank test and Benjamini and Hochberg corrected log-rank test; Fig. 2A-C; Table 2), METTL 14 ($p=0.020$, $q=0.09$, Fig. 2D-F) and the reader YTHDC1 ($p=0.012$, $q=0.08$, F ig. 2G-I) were significantly correlated with shorter OS. Increased expression of the writer METTL4 ($p=0.034$, Supplementary Fig. 1A-C) and the reader YTHDF2 ($p=0.040$, Supplementary Fig. 1D-F) were also associated with poor outcome but did not remain significant when correcting for multiple hypothesis testing at a significance threshold of $q<0.1$. Protein expression levels of METTL3, METTL4, METTL14, YTDHC1, and YTHDF2 were not associated with the clinicopathological parameters nodal stage and histomorphological grading in the entire study cohort and the two subgroups, respectively (Supplementary Table 1).

We found high positive Spearman's p correlation coefficients for the expression of the prognostic m6A proteins METTL3, METTL14, and YTDHC1, respectively in the HPV-dependent subgroup (Fig. 3). In 6/15 patients, high levels of the writers METTL3 and METTL14 were cooccurring (Spearman's $p=0.797$; two-sided t-test $p=<0.001$). Likewise, in 5/15 patients the writer METTL3 and the reader YTHDC1 (Spearman's $p=0.036$; two-sided t-test $p=0.872$) and in 7/15 patients the writer METTL14 and YTHDC1 (Spearman's $p=0.443$; two-sided t-test $p=0.034$) were at high levels. Of these 3 proteins, we identified METTL14 to be the protein that typically cooccurred with the others in contrast to METTL3 and YTHDC1 that gave additional information to the other two.

Discussion

In the present study, our analysis suggests that expression levels of the 'writers' METTL3 and METTL14 and the 'reader' YTHDC1 are involved in HPV-dependent VSCC tumorigenesis, but not HPV-independent tumor development. HPV is a small DNA virus that is usually transmitted sexually. Sexually active individuals carry a lifetime risk for HPV infection of around 80–90% [24]. It is estimated, that 5% of human cancers are caused by a persistent infection with high risk HPV types [25] including not only VSCC but also cervical, penile, and head and neck SCC [26]. HPV-dependent VSCC account for 30% of all VSCC cases and exhibit a more favorable prognosis compared to the HPV-independent VSCC subtype. Although HPV-dependent and HPV-independent VSCC represent etiologically different subtypes, both are treated equally in current clinical practice [27].

Previous research has shown that m6A modification is implicated in viral infection by modulating the interaction between the virus and the host. Thus, m6A can influence both, the susceptibility of the host cells to viral infection, and the replication of the virus in the host cell [28, 29]. There is only sparse known regarding m6A modification in the context of HPV infection and cancer. In cervical cancer (CC), that is predominantly caused by infection with high-risk HPV, there is broad scientific evidence, that abnormal m6A modification plays an essential role in tumor proliferation, angiogenesis and

Table 2 Summary of the analyzed m6A proteins as indicated and their correlation with overall survival (indicated as %alive) for the entire study cohort, HPV-independent, and HPV-dependent VSCC. The HPV-status was not available for 24 patients. Samples were grouped according to high and low expression based on the staining intensities. *p*-values for the group comparisons are based on log-rank tests (significance threshold $p < 0.5$). *q*-values are based on multiple hypotheses testing using the method of Benjamini and Hochberg with a significance threshold of $q < 0.1$

			All			HPV-independent			HPV-dependent			
Proteins	**Localisation**	**Staining intensity (low/high)**	**N (low/high)**	**%alive (low/high)**	***p*-value**	**N (low/high)**	**%alive (low/high)**	***p*-value**	**N (low/high)**	**%alive (low/high)**	***p*-value**	***q*-value**
Writer												
METTL3	nuclear	0–2 + /3 +	77/27	62.3/40.7	0.104	51/18	56.9/38.9	0.435	11/7	81.8/28.6	**0.010**	**0.08**
METTL4	cytoplasmatic	0–1 + /2–3 +	60/36	58.3/50.0	0.113	44/24	54.5/45.8	0.128	8/7	87.5/42.9	**0.034**	0.10
METTL14	nuclear	0–1 + /2–3 +	54/45	55.6/55.6	0.586	44/23	47.7/60.9	0.541	6/10	100/55.6	**0.020**	**0.09**
WTAP	nuclear	0–2 + /3 +	90/16	54.4/68.8	0.311	63/6	49.2/83.3	0.132	16/4	68.8/75.0	0.694	0.74
KIAA1429	nuclear	0–1 + /2–3 +	66/35	56.1/60.0	0.782	47/22	53.2/59.1	0.661	10/5	60.0/40.0	0.482	0.70
Eraser												
FTO	nuclear	0–2 + /3 +	83/21	55.4/76.2	0.113	61/9	54.1/66.7	0.361	10/9	50.0/77.8	0.257	0.48
ALKBH5	cytoplasmatic/ nuclear	0–1 + /2–3 +	44/63	50.0/65.1	0.231	35/35	45.7/62.9	0.161	4/15	50.0/66.7	0.699	0.74
Reader												
HNRNPA2B1	nuclear	0–2 + /3 +	85/23	57.6/73.9	0.429	62/10	54.8/70.0	0.602	17/2	58.8/100	0.297	0.48
HNRNPC	nuclear	0–1 + /2–3 +	54/48	51.9/62.5	0.162	34/35	50.0/54.3	0.518	11/7	63.6/71.4	0.736	0.74
YTHDC1	membraneous/ cytoplasmatic/ nuclear	0–2 + /3 +	78/23	61.5/43.5	0.111	58/12	58.6/41.7	0.496	12/7	83.3/28.6	**0.012**	**0.08**
YTHDF1	cytoplasmatic	0–2 + /3 +	70/26	57.1/50.0	0.893	52/15	55.8/33.3	0,409	10/4	60.0/75.0	0.551	0.72
YTHDF2	cytoplasmatic	0–1 + /2–3 +	31/73	58.1/54.8	0.422	21/48	52.4/52.1	0.665	5/13	100/46.2	**0.040**	0.10
YTHDF3	cytoplasmatic	0–1 + /2–3 +	45/51	55.6/58.8	0.725	28/37	50.0/56.8	0.554	6/7	66.7/57.1	0.084	0.18

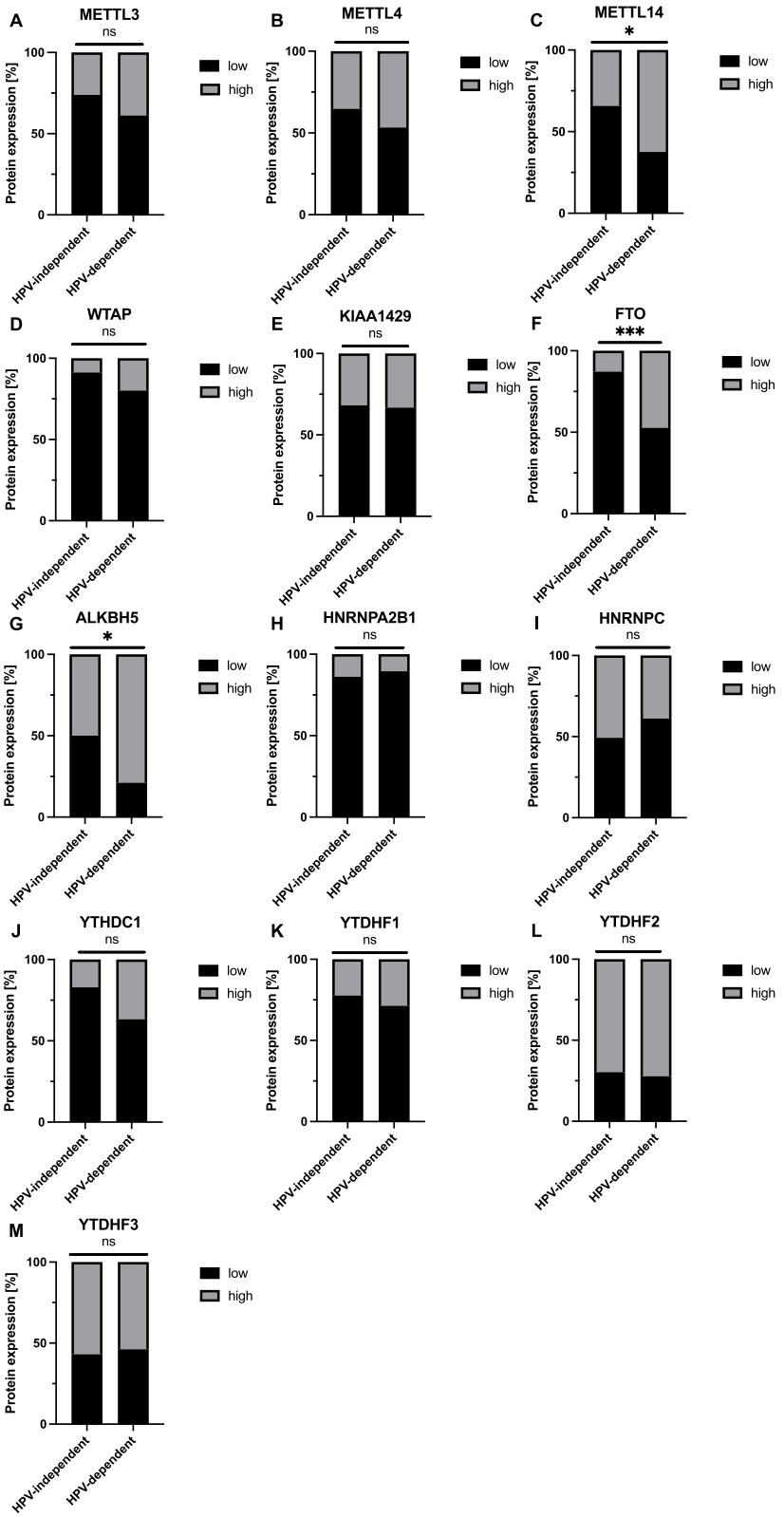

Fig. 1 Differential expression (high vs. low) of m6A protein depending on the VSCC subtype. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (Fisher's exact test)

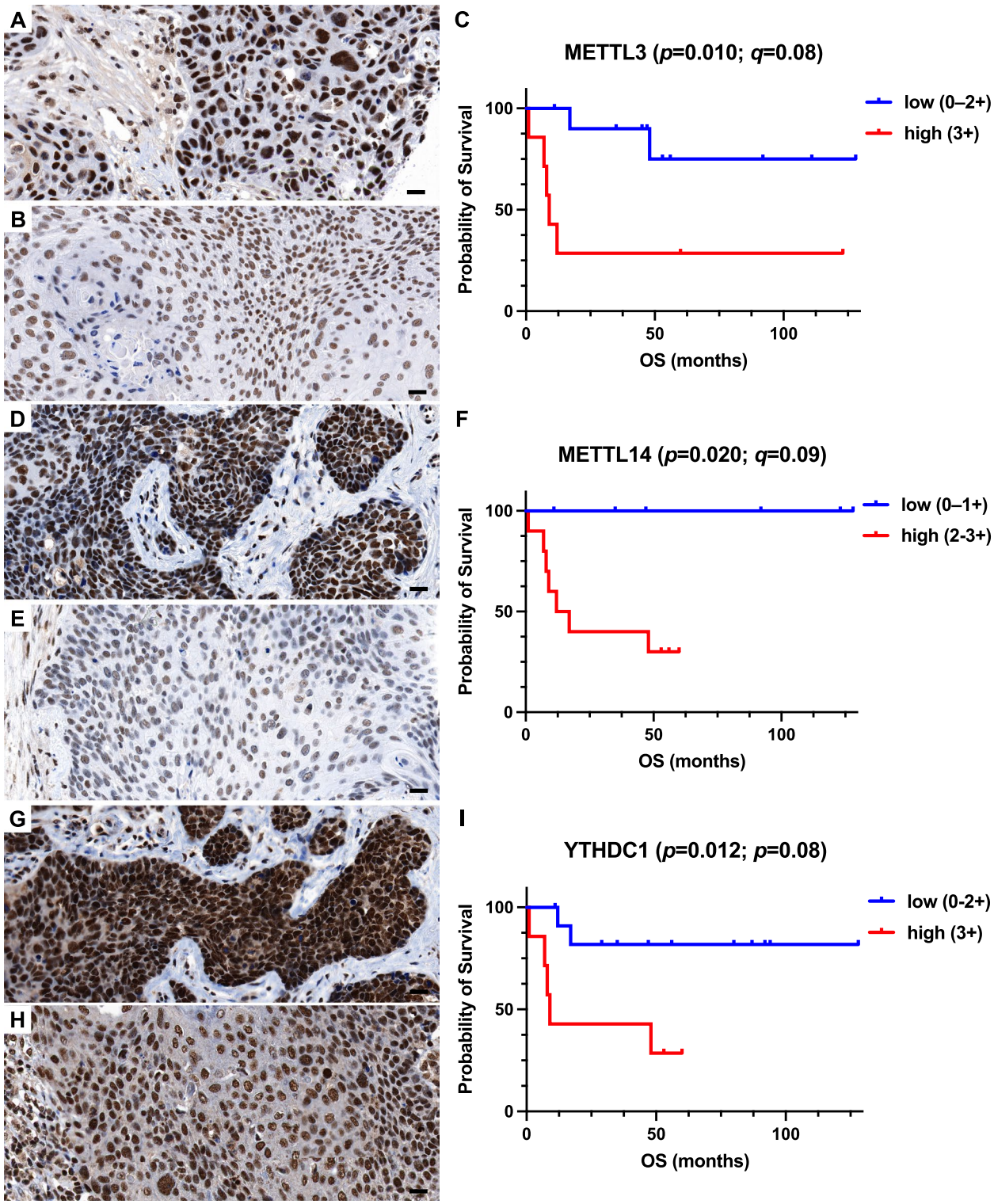

Fig. 2 Representative histology sections show high (**A**, **D**, **G**) and low (**B**, **E**, **H**) expression levels of METTL3, METTL14 and YTHDC1 visualized by immunohistochemistry; hematoxylin (blue) was used for nuclear staining (bright field image, 400xmagnification). Kaplan–Meier estimates show a significantly shorter 5-year survival ($p < 0.05$) in patients with high expression of METTL3, (**F**) METTL14, and (I) YTHDC1. Prognostic significance remained after correction for multiple testing ($q < 0.1$). Scale bar = 20 um

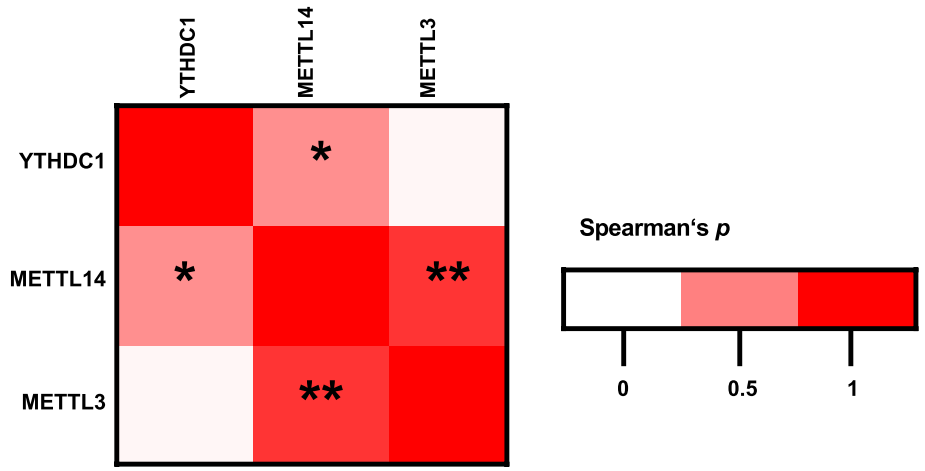

Fig. 3 Correlation heatmap visualizes Spearman's *p* correlation coefficients of METTL3, METTL14, and YTDHC1 in the HPV-dependent VSCC cohort. * $P < 0.05$; ** $P < 0.01$ (two-sided t-test)

metastatic spread. METTL3 was shown to be upregulated in CC cells and linked to lymph node metastasis and unfavorable outcomes [30]. Further, m6A dysregulation is linked to chemo- and radiotherapy-resistance and a more progressive CC phenotype [17]. In this context, especially the demethylase FTO was identified to be an important oncogenic driver by regulating proliferation and migration of CC cells [31]. Another study confirmed the importance of METTL14 in CC. Silencing METTL14 induced a cell cycle arrest in CC cells via the the PI3K/AKT/mTOR signaling pathway [32]. The interaction between m6A and the PI3K/AKT/mTor signaling pathway has also been described for endometrial cancer and further entities [16, 33]. The etiologic resemblance of CC and VSCC suggests dysregulated m6A modification to be involved in VSCC tumorigenesis. In head and neck SCC, which are frequently associated with HPV-infection, overexpression of METTL3 and METTL14 correlated with advanced T stage and poor OS [34]. Further, enhanced METTL3 expression was observed in oral SCC, that is also linked to HPV infection [35].

There is no data available regarding the precise biological mechanism of m6A modification and HPV-driven tumorigenesis. However, there is data on other oncogenic viruses like Kaposi's sarcoma-associated herpesvirus (KSHV): Research has shown, that depletion of METTL3 and YTHDF2 lead to lower expression levels of the lytic genes ORF50 and ORF57 as well as decreased virion production [36]. Lytic genes are required to enter the viral lytic replication cycle. These findings suggest m6A to promote a pro-viral environment for KSHV infection. Comparable data were obtained for simian virus 40. Here, overexpression of YTHDF2 was found to be associated with enhanced viral replication in BSC40 cells whereas depletion of YTHDF2 or METTLL3 lead to contrary effects [37].

Besides METTL3 and METTL14, our analysis also showed significant data for the reader YTHDC1 in the HPV-dependent VSCC subgroup. As YTHDF2, YTHDC1, is involved in mRNA splicing, nuclear export and translation. In the context of viral infection, research has shown, that YTHDC1 is involved in splicing of genes important for the lytic replication [36]. Given the involvement of m6A in HPV-dependent VSCC harbors the potential to be used therapeutically. 3-deazaadenosine (DAA) inhibits m6A modification and has exhibit antiviral effects in both, cell culture and mouse models of viral infection [38]. To date, it has not been studied whether there is also cytotoxic potential of DAA in HPV-dependent malignancies. In addition to direct drug targeting of methylation, inhibition of the PIK3/AKT/mTOR signaling pathway might be a promising therapeutic option, in particular due to the described interaction between m6A and this pathway. There are various therapeutic agents that could be considered, such as everolimus or the PIK3 inhibitor alpelisib. So far, these therapeutics have not been investigated in VSCC, but, however, might be of potential interest.

Our findings point towards the important role of m6A RNA modification in cancer and especially in HPV-dependent tumors. This is the first study implicating the relationship between HPV infection, m6A RNA modification, and carcinogenesis in VSCC. However, as a limitation of the present study, the relatively small cohort size of 23 HPV-dependent VSCC has to be mentioned. Consecutively, multivariate statistical analyses could not be

performed. A further limitation is the retrospective study design and the determination of protein expression based on a tissue microarray with sigle cores per sample. Hence, tumor heterogeneity might not be adequately reflected by our method approach. However, to the best of our knowledge, there is no evidence for intratumoral heterogeneity regarding m6A protein expression analysis. Of note this is also reflected by our own data regarding m6A protein expression in endometrial and cervical cancer [22, 39].

Dysregulation of m6A proteins might be used as biomarkers and indicators for poor prognosis but also as potential targets for novel therapeutic drugs. However, the specific mechanisms explaining the interaction of m6A modification and HPV infection remains to be elucidated in further studies.

Conclusion

High expression levels of proteins involved in m6A modification correlate with a poor OS in patients with HPV-dependent VSCC. Hence, m6A might serve as a prognostic biomarker in HPV-dependent VSCC.

Abbreviations

CC: Cervical cancer; FDR: False discovery rate; FFPE: Formalin-fixed paraffin-embedded; FIGO: International Federation of Gynecology and Obstetrics; HE: Hematoxylin and eosin; HPV: Human papillomavirus; KSHV: Kaposi's sarcoma-associated herpesvirus; m6A: N6-methyladenosine (m6A); mRNA: Messenger RNA; OS: Overall survival; SCC: Squamous cell carcinoma; TMA: Tissue microarray; UICC: Union for International Cancer Control; VSCC: Vulvar squamous cell carcinoma; WHO: World Health Organization (WHO).

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-022-10010-x.

Additional file 1.

Additional file 2.

Acknowledgements

The tissue examples were collected within the framework of the Biobank of the CIO Cologne-Bonn at the University Hospital Bonn. We thank Susanne Steiner for technical support.

Authors' contributions

M.C., T.T. and D.J.R. were involved in the study design and concept. M.C. and D.J.R. drafted the manuscript. M.C., T.T., C.S. and D.J.R. performed the experiments and statistical analysis. N.K., J.E., E.E., K.K., G.K. and A.M. revised the manuscript for critical intellectual content. All authors read and approved the final version of the manuscript.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available on request from the authors.

Declarations

Ethics approval and consent to participate

Tissue collection was performed within the framework of the Biobank initiative of the University Hospital Bonn. All patients provided written informed consent prior to the collection of biomaterials. The study was approved by the Ethics Committee of the Medical Faculty of the University of Bonn (vote: 208/21) and conducted in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no conflicts of interests.

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