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MicroRNA-155, -185 and -193b as biomarkers in human papillomavirus positive and negative tonsillar and base of tongue squamous cell carcinoma

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ABSTRACT

Objective: Three-year disease-free survival (DFS) is 80% for human papillomavirus (HPV) positive tonsillar and base of tongue cancer (TSCC/BOTSCC) treated with radiotherapy alone, and today's intensified therapy does not improve prognosis. More markers are therefore needed to more accurately identify patients with good prognosis or in need of alternative therapy. Here, microRNAs (miRs) 155, 185 and 193b were examined as potential prognostic markers in TSCC/BOTSCC.

Material and methods: 168 TSCC/BOTSCC patients diagnosed 2000–2013, with known data on HPV-status, $CD8^+$ tumour infiltrating lymphocytes, tumour staging and survival were examined for expression of miR-155, -185 and -193b using Real-Time PCR. Associations between miR expression and patient and tumour characteristics were analysed using univariate testing and multivariate regression.

Results: Tumours compared to normal tonsils showed decreased miR-155 and increased miR-193b expression. miR-155 expression was associated with HPV-positivity, low T-stage, high $CD8^+$ TIL counts and improved survival. miR-185 expression was associated with HPV-negativity and a tendency towards decreased survival, while miR-193b expression was associated with higher T-stage, male gender and lower $CD8^+$ TIL counts, but not with outcome. Upon Cox regression, miR-185 was the only miR significantly associated with survival. Combining miR-155 and miR-185 to predict outcome in HPV^+ patients yielded an area under curve (AUC) of 71%.

Conclusion: Increased miR-155 expression was found as a positive predictor of survival, with the effect mainly due to its association with high $CD8^+$ TIL numbers, while miR-185 independently associated with decreased survival. Addition of these miRs to previously validated prognostic biomarkers could improve patient stratification accuracy.

Introduction

Human papillomavirus (HPV) is now acknowledged as a risk factor for oropharyngeal squamous cell carcinoma (OPSCC), where tonsillar and base of tongue cancer (TSCC/BOTSCC) make up most HPV-positive (HPV^+) cases, and as causative for the recent increase in the incidence of OPSCC [1–8]. Of note, HPV^+ TSCC/BOTSCC patients treated with radiotherapy alone have better disease-specific-survival (DSS) than those with corresponding HPV-negative (HPV^-) cancer, with 80% vs. 40% 3-year DSS [9–14]. Due to poor prognosis in the HPV^- group predominantly, head-neck cancer treatment has been intensified in the past years, with accelerated radiotherapy, induction/concomitant chemoradiotherapy, Erbitux and surgery. Nevertheless, most patients with HPV^+ TSCC and BOTSCC do not need intensified therapy and would benefit from treatment de-escalation. Before therapy de-escalation, the identification of patients with good prognosis is required [13]. Furthermore, today's intensified therapy does not improve survival for patients with HPV^+ tumours and therefore alternative therapies for patients with predicted poor prognosis, such as immunotherapy or novel targeted therapies, would be relevant options [15].

Others and we have, in these tumour types, identified age, stage, smoking, high $CD8^+$ tumour infiltrating lymphocyte (TIL) counts,

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absent/low HLA class I expression, CD44, LMP10 expression, high LRIG1 expression, absence of HLA-A*02, CD98 and presence of HPV16 E2 mRNA expression as potential prognostic markers [14,16–22]. In addition, we have employed different regression models using some of these markers to predict the risk of death/relapse in patients with HPV$^+$ TSCC and BOTSCC and found that, low age, low T-stage, high CD8$^+$ TIL counts and presence of HPV16 E2 mRNA expression were strong prognostic markers, followed to a lesser extent by low/absent HLA class I expression [15,23]. Using combinations of these markers, around 56% of patients with HPV$^+$ TSCC/BOTSCC with good prognosis could be identified [15,23]. However, in order to find additional patients with very good therapeutic response, or non-responders with a need for alternative therapies, the availability of more prognostic markers would be beneficial.

MicroRNAs (miRs) are 18–22 nucleotides long non-coding RNAs involved in many cellular processes, such as differentiation, proliferation and survival. An increasing number of studies have reported microRNA dysregulation in HNSCC [24–29], as well as association with patient survival [30–33] and a role in regulation of apoptotic and cancer development [34–36]. Importantly, Barker et al. [37], previously reported distinct tissue- and site-specific miR profiles in HNSCC, suggesting that aetiology, tissue type, and tumour site are important factors affecting the expression and distribution of these miRs. Therefore, when investigating differences in miR signatures as potential predictive or prognostic biomarkers in HNSCC, it is important to treat different tumour sites, as well as tumours with different aetiology (e.g. HPV$^+$ vs. HPV$^-$ TSCC/BOTSCC) as distinct entities. There are often discrepancies and poor consensus among reports on miR expression in HNSCC, which may be due to several reasons including limited sample numbers and inadequate differentiation between HPV$^+$ and HPV$^-$ samples [38–45].

After performing a screening of miR signatures in HPV$^+$ and HPV$^-$ OPSCC, data indicated that miR-155, -185 and -193b could be of interest with regard to clinical outcome. Therefore, in the present study, in order to identify potential prognostic markers for patient stratification, we explored the presence of miR-155, -185, and -193b, in tumour biopsies of patients with TSCC/BOTSCC, and analysed their association with survival and tumour characteristics.

Materials and methods

Patients, tumour characteristics, and definition of HPV status in the study

Patients diagnosed with TSCC (ICD-10 code C09.0-9) or BOTSCC (ICD-10 code C01.9) diagnosed 2000–2013 at the Karolinska University Hospital, with data on age, stage, presence of HPV DNA, p16 expression, and CD8$^+$ TIL counts obtained previously, were included in the study (Table 1) [20,22,23]. All patients were examined for their tumour miR-155, -185, and -193b expression. Tumours positive for both HPV DNA and p16^{INK4a} (p16) overexpression (> 70%) were regarded as being HPV-positive (HPV$^+$), while tumours negative for HPV DNA were considered as HPV-negative (HPV$^-$) regardless of p16 expression. Six HPV DNA positive p16 negative tumours were excluded from subgroup analyses based on HPV status. The study was done in compliance with permission 2009/1278-31/4 from the Regional Ethical Committee in Stockholm, Karolinska Institutet.

Extraction of mRNA

Total RNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissue biopsies using RecoverAll Total Nucleic Acid Isolation Kit for FFPE (Ambion) or Roche High Pure RNA Paraffin Kit (Roche Diagnostics) according to the manufacturer's instructions. RNA concentrations were measured by a Qubit 2.0 Fluorometer (Thermo Fisher Scientific) using the Qubit RNA BR Assay Kits (Thermo Fisher Scientific).

Table 1
Patient and TSCC/BOTSCC characteristics.

Patient and tumour characteristics		HPV + TSCC/BOTSCC (N = 110)		HPV − TSCC/BOTSCC (N = 52)		All TSCC/BOTSCC (N = 168)	
		N	%	N	%	N	%
Age	*Mean (years)*	59,5		64,5		61	
	Median (years)	58,5		64		61	
	Range (years)	30–91		44–85		30–91	
Diagnose	*Malignant neoplasm of the base of tongue (C01.9)*	17	15%	5	10%	22	13%
	Malignant neoplasm of the tonsil (C09.0–9)	93	85%	47	90%	146	87%
Gender	*Female*	28	25%	14	27%	42	25%
	Male	82	75%	38	73%	126	75%
Tumour differentiation	*Poorly*	66	60%	26	50%	94	56%
	Moderatley	32	29%	18	34%	58	35%
	Well	5	5%	3	6%	9	5%
	Undefined	7	6%	5	10%	7	4%
Tumour size	*T1*	33	30%	7	13%	40	24%
	T2	39	35%	16	31%	55	33%
	T3	26	24%	17	33%	46	27%
	T4	12	11%	12	23%	27	16%
Nodal disease	*N0*	7	6%	14	27%	22	13%
	N1	25	23%	7	13%	34	20%
	N2a	12	11%	2	4%	14	8%
	N2b	51	46%	17	33%	72	43%
	N2c	9	8%	7	13%	16	10%
	N3	4	4%	5	10%	9	5%
	NX	2	2%	0	0%	2	1%
Distant metastasis	*M0*	106	96%	51	98%	163	97%
	M1	3	3%	1	2%	4	2%
	MX	1	1%	0	0%	1	1%
Tumour Stage	*I*	2	2%	3	6%	5	3%
	II	7	6%	5	9%	12	7%
	III	24	22%	10	19%	36	21%
	IVa	67	61%	28	54%	99	59%
	IVb	6	5%	5	10%	11	7%
	IVc	3	3%	1	2%	4	2%
	Unknown	1	1%	0	0%	1	1%
	HPV16	90	81%	n.a.	n.a.	n.a.	n.a.
	HPV33	12	11%	n.a.	n.a.	n.a.	n.a.
	HPV35	3	3%	n.a.	n.a.	n.a.	n.a.
	Other	2	2%	n.a.	n.a.	n.a.	n.a.
	Non identified	3	3%	n.a.	n.a.	n.a.	n.a.
Treatment	*Induction chemotherapy and radiation*	44	40%	11	21%	57	34%
	Only Radiation	63	57%	38	73%	100	60%
	Untreated or no information available	3	3%	3	6%	11	7%
Brachytherapy boost	*Not administered*	81	74%	40	77%	120	71%
	Administered	26	23%	9	17%	37	22%
	Untreated or no information available	3	3%	3	6%	11	7%
Concomitant Erbitux	*Not administered*	82	74%	44	84%	125	74%
	Administered	25	23%	5	10%	32	19%
	Untreated or no information available	3	3%	3	6%	11	7%