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REVIEW

Circulating tumour DNA alterations as biomarkers for head and neck cancer: a systematic review

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ABSTRACT

Background: Head and neck squamous cell carcinoma (HNSCC) is a significant global burden. The development of a diagnostic or recurrence monitoring test could evolve from the exploitation of molecular markers such as tumour-specific DNA alterations in plasma. The aim of this study was to report specific genetic alterations of DNA in plasma from HNSCC patients, report the diagnostic accuracy, and discuss potentials for a diagnostic or recurrence monitoring test based on circulating tumour DNA (ctDNA).

Methods: A systematic search was performed in PubMed, Embase, and Cochrane Library for articles published in English between 1 January 1980 and 24 October 2018. The search terms used were related to ctDNA methylations and mutations in HNSCC patients.

Results: We identified 16 studies from four countries (p = 1156 patients, c = 601 controls) examining ctDNA alterations of HNSCC patients. CtDNA methylations were significantly increased in HNSCC patients compared to controls. Five studies investigated ctDNA mutations in HNSCC. The most frequent examined gene mutation was TP53. Eleven studies investigated ctDNA methylations in HNSCC. Nine studies calculated the diagnostic accuracy of ctDNA methylations in HNSCC compared to controls. The most frequent examined gene methylations were CDKN2A, DAPK1, RASSF1, and P15.

Conclusion: We found that increasing the number of ctDNA genetic methylations resulted in an increase in diagnostic sensitivity accuracy. No studies investigating ctDNA mutations included a control group. A combination of multiple human ctDNA gene alterations with viral ctDNA are promising tools for developing a ctDNA biomarker for HNSCC.

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Introduction

Head and neck squamous cell carcinoma (HNSCC) is a significant global burden due to the annual increase in incidence accompanied by improved survival rates [1]. The estimated number of new annual cancer cases in 2018 for lip and oral cavity is 354,864, larynx 177,422, oropharynx 92,887, and nasopharynx 129,079 [2]. Today, the diagnostics of HNSCC is based on biopsy, imaging, and clinical investigation. The development of additional diagnostic and disease observing methods could improve the timeframe for detecting the primary disease or relapse. One such method might evolve from the exploitation of molecular markers such as tumour-specific DNA alterations found in plasma, e.g., cell-free DNA (cfDNA). cfDNA is extracellular DNA found in the blood. Patients with cancer have an increased amount of cfDNA. The increased levels of cfDNA for cancer patients are named (ctDNA) and can be illustrated either by the increased concentration of cfDNA in plasma from cancer patients, or by looking at specific genomic mutations in the cfDNA. The increased concentration of cfDNA is not specific for cancer. cfDNA levels are elevated in other situations with states with cell apoptosis or necrosis. The cfDNA concentration in plasma or serum is challenged because it is not specific enough to use as a biomarker [3]. One crucial challenge is to differentiate between non-cancerous free circulating DNA and tumour-specific DNA. Well-known tumour-specific alterations, such as methylations and mutations, are a potential strategy to differentiate between non-cancerous free circulating DNA and ctDNA [4].

ctDNA has been approved by the Food and Drug Administration for disease observing and tailored treatment of, e.g. non-small cell lung cancer for EGFR mutations [5] and hypermethylation of the SEPT5 gene approved as a screening marker for colorectal cancer [6].

In this systematic review, we report specific DNA alterations based on ctDNA in plasma of HNSCC patients. The aim of this study was to review the literature on known genetic alterations of DNA in plasma from HNSCC patients and to report the diagnostic accuracy of the genetic alternations. Furthermore, we wish to discuss the potentials for a diagnostic or recurrence monitoring test based on ctDNA.

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Methods

The systemic review was conducted with reference to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [7]. It was not possible to perform a meta-analysis due to the variance in genes investigated in the studies.

Systematic literature search and eligibility criteria

One author (AHP) systemically searched the Medline, Embase, and Cochrane databases. All included articles were evaluated by all authors in cases of doubt. The search was last updated on 24 October 2018. A search for ongoing clinical trials was performed on clinicaltrials.gov. The studies included were those with human DNA as a biomarker for HNSCC. Studies were excluded if they did not examine specific gene alterations (methylations or mutations), only described nonhuman DNA such as herpes, human papillomavirus (HPV) or Epstein-Barr virus (EBV), mitochondrial DNA, serum not derived from blood, or studies with too small a patient group including less than 10 patients. In *Medline*, the search consisted of phrases related to ctDNA alterations (ctDNA, cfDNA, DNA alterations, DNA mutations, genomic alterations, genomic mutations, NOTCH1, TP53, CDKN2A, DNA methylation, p16, RAS, cell-free nucleic acids, DNA), the media (plasma, serum, liquid biopsy), head and neck cancer (head and neck neoplasms, head and neck cancer, HNSCC, carcinoma squamous cell, oral cavity, larynx, pharynx, hypopharynx, oropharynx, nasopharynx, paranasal sinuses, upper aerodigestive tract, nasal cavity), and the specific settings (PCR, dPCR, non-invasive). In *Embase* and *Cochrane*, the search terms ctDNA, circulating tumour DNA, cfDNA, circulating fluid DNA, liquid biopsy, DNA alterations, DNA methylations, DNA mutations, and HNSCC were used. The search was limited to the English, Danish, Swedish, and Norwegian languages.

The following data were extracted from the included studies: country, year published, number of controls and patients, anatomic area/subtype of HNSCC, type of genomic alteration, gene examined, study design, focus of interest, sensitivity, and specificity.

Results

The literature search generated 1535 articles of which 15 studies were included (p = 1156 patients, c = 601 controls) (Figure 1). One additional study was identified through reference lists [8]. The studies were published between 2000 and 2017 and originate from four countries (France, Germany, USA, China) (Table 1). The studies varied in the technologies used to detect genetic alterations, which included quantitative polymerase chain reaction (qPCR), methylation-sensitive high-resolution melting PCR (MS-HRM PCR), combined bisulphite restriction analysis PCR (COBRA PCR), multiplex PCR, actin beta PCR (ACTB PCR), and next-generation sequencing PCR (NGS PCR). Two studies were based on the same dataset [6,9]. All liquid biopsies were taken prior to HNSCC treatment unless otherwise noted.

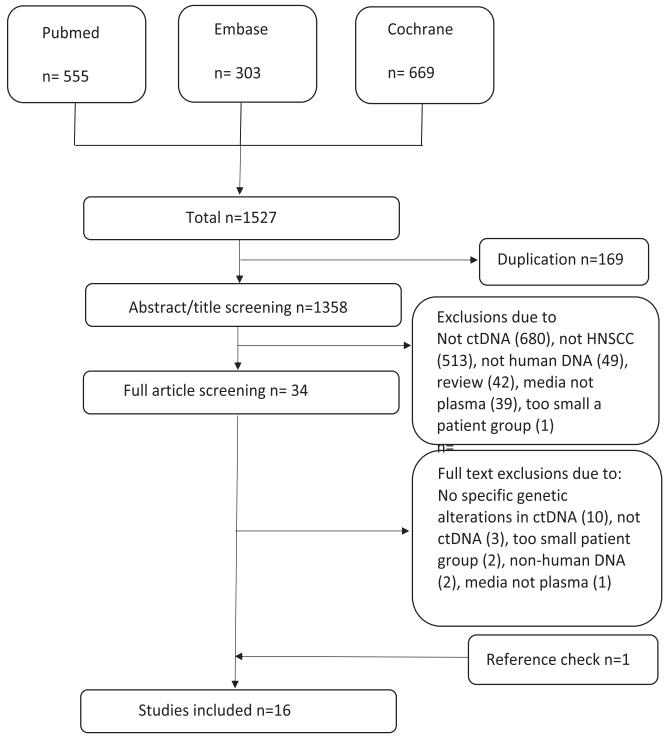

Figure 1. Flowchart. An overview of the selection process of studies. A total of 16 studies were included in the study. *n* = number of articles. Too small a patient group was defined as 10 patients or less.

Mutations

Five studies (p = 176) evaluated mutations found in ctDNA of HNSCC patients (Table 2).

One study, Schwaederle et al. [10], examined the frequency of genetic mutations of ctDNA in 670 cancer patients, of which 25 had HNSCC, and reported that HNSCC harbours the highest frequency of ctDNA mutations in plasma when compared to lung, gastrointestinal, brain, and breast cancers [10].

Three studies (p = 84) compared mutations in primary tumours to ctDNA mutations in plasma [11–13]. All three studies found the presence of ctDNA mutations in the plasma of HNSCC patients. The concordance in primary tumour mutations and ctDNA mutations varied significantly between studies, from 6 to 87% (Table 2).

Two studies examined a group of specific mutations [11,12] and two studies examined TP53, a single gene [11,13]. Perdomo et al. discovered a HNSCC patient found to have no TP53 mutation in primary tumour yet the patient was positive for TP53 mutation in ctDNA [11].

The study by Braig et al. reported that 33% of patients acquire ctDNA RAS mutations during the cetuximab treatment, which was also the subgroup with highest risk of disease progression. This illustrates that some cancers acquire ctDNA mutations during treatment [8].

Table 1. Sixteen included studies.

Study reference	Ref. nr.	Genes	Groups patients (n), controls (c)	Assay type	Type of alteration
de Vos et al. 2017	[6]	SHOX2 + SEPT9	p = 284, c = 22 p = 141, c = 102	ACTB triplex qPCR	Methylation
Braig et al. 2016	[8]	RAS	p = 20	NGS PCR	Mutation
Schröck et al. 2017	[9]	SHOX2 + SEPT9	p = 284, c = 122 p = 141, c = 102	ACTB triplex qPCR	Methylation
Schwaederle et al. 2017	[10]	EGFR + PIK3CA + BRECA2 + TP53 + APC + MET + BRAF + ERBB2 + MYC + NF1 + ARID1A + SMAD4 + BRCA2 + FGFR2 + BRCA1 + PDGFRA + ALK + AR + FGFR1	p = 25	NGS PCR	Mutation
Perdomo et al. 2017	[11]	TP53 + NOTCH1 + CDKN2A + CASP8 + PTEN	p = 37, p = 36	PCR	Mutation
Wang et al. 2015	[12]	TP53 + PIK3CA + CDKN2A + FBXW7 + HRAS + NRAS	p = 47	multiplex PCR	Mutation
Coulet et al. 2000	[13]	TP53	p = 11	PCR	Mutation
Wong et al. 2002	[14]	DAPK1 (Death-associated kinase)	p = 12	qMSP	Methylation
Sanchez-Cespedes et al. 2000	[15]	CDKN2A (p16), DAPK1 Death-associated kinase), MGMT, GSTP1	p = 42	qMSP	Methylation
Chang et al. 2003	[16]	Fn 2 RIZ1 (PRDM2)	p = 30, c = 29	qMSP	Methylation
Tian et al. 2013	[17]	RASSF1, CDKN2A (p16), DLEC1, DAPK1 Death-associated kinase), UCHL1	p = 40, c = 41	qMSP	Methylation
Yang et al. 2015	[18]	DAPK1 (Death-associated kinase), RARβ2, WIF1, RASSF1A	p = 220, c = 50	MS-HRM PCR	Methylation
Tiwawech et al. 2014	[19]	Alu elements	p = 50, c = 140	PCR qCOBRA-Alu	Methylation
Wong et al. 2003	[20]	p16, p15	p = 20, c = 24	qMSP	Methylation
Mydlarz et al. 2016	[21]	p16, EDNRB, DCC	p = 100, c = 50	qMSP	Methylation
Wong et al. 2004	[22]	RASSF1, CDKN2A (p16), DAPK1 Death-associated kinase), p15, MLH1, CDH1	p = 41, c = 43	qPCR	Methylation

An overview of the genes investigated, number of patients and controls investigated, the assay type used, and the type of genetic alteration investigated.
p: patients.
c: control group.

Table 2. Studies evaluating HNSCC ctDNA mutations.

Ref. Nr.	Genes	Cancer	Groups	Focus of interest	Results
[8]	RAS	HNSCC	p = 20	Responses and acquired mutations in the RAS gene of ctDNA of HNSCC patients treated with cetuximab plus chemotherapy	Patients with progression in disease showed acquired RAS mutations. Patients with no disease progression showed no mutation for any of the RAS genes
[10]	EGFR + PIK3CA + BRECA2 + TP53 + APC + MET + BRAF + ERBB2 + MYC + NF1 + ARID1A + SMAD4 + BRCA2 + FGFR2 + BRCA1 + PDGFRA + ALK + AR + FGFR1	HNSCC	p = 25	The study explores specific mutations in ctDNA of diverse cancers. The study included 25 HNSCC patients	88% had a mutation. 40% has 1–3 alterations. 48% has >3 mutations
[11]	TP53	HNSCC	p = 37	Are mutations found in primary tumour and in plasma	2 (6%) found mutation in ctDNA
[11]	TP53 + NOTCH1 + CDKN2A + CASP8 + PTEN	HNSCC	p = 36	Are the mutations in primary tumour to be found in plasma	17 (42%) found mutation in ctDNA
[12]	TP53 + PIK3CA + CDKN2A + FBXW7 + HRAS + NRAS	HNSCC	p = 47	Are mutations in primary tumour to be found in plasma	41 (87%) found mutation in ctDNA
[13]	TP53	HNSCC	p = 11	Is TP53 mutation in primary tumour to be found in plasma	2 (18%) found mutation in ctDNA

Five studies included. An overview of cancer type, number of patients included, focus of interest and results.
HNSCC: head and neck cancer squamous cell carcinoma.
p: patients.
c: control group.

Methylations

Eleven studies (p = 980; c = 601) investigated methylation as a diagnostic marker for HNSCC (Table 3). Six studies (p = 393, c = 303) investigated nasopharyngeal squamous cell carcinoma (NPSCC), and five studies (p = 587, c = 298) investigated all HNSCC sites.

Three studies (p = 72) compared gene hypermethylation found in primary tumours to the ctDNA gene promotor hypermethylation in plasma [14–16] (Table 3). The studies showed a correlation between primary tumour gene methylation and ctDNA gene methylation. The gene methylation was found in primary tumours but not always in ctDNA plasma.

Nine studies (p = 926, c = 601) performed a comparison of specific gene methylation in patients and controls [6,9,16–22]. Eight of the nine studies (p = 876, c = 461) found increased gene methylation levels. The ninth study investigated hypomethylation of Alu elements and showed a

Table 3. Studies evaluating HNSCC ctDNA methylations.

Ref.	Genes	Cancer type	Groups	Focus of interest
[6]	SHOX2 + SEPT9	HNSCC	p = 284, c = 122 p = 141, c = 102	Improve clinical performance of methylated ctDNA as a biomarker with SEPT9 and SHOX2 genes as examples
[9]	SHOX2 + SEPT9	HNSCC	p = 284, c = 122 p = 141, c = 102	Diagnostics and disease monitoring in ctDNA gene methylation of HNSCC patients compared to controls
[14]	DAPK1	NPSCC	p = 12	Hypermethylation of gene promotor regions of ctDNA genes compared to primary tumour in patients and controls
[15]	CDKN2A (p16), DAPK1, MGMT, GSTP1	HNSCC	p = 42	Hypermethylation of gene promotor regions of ctDNA genes compared to controls
[16]	Fn 2 RIZ1 (PRDM2)	NPSCC	p = 30, c = 29	Hypermethylation of gene in ctDNA compared to primary tumour NPSCC and controls in cell lines M1 and CNE-2
[17]	RASSF1, CDKN2A (p16), DLEC1, DAPK1, UCHL1	NPSCC	p = 40, c = 41	Hypermethylation of gene promotor regions of ctDNA genes compared to controls
[18]	DAPK1, RARβ2, WIF1, RASSF1A	NPSCC	p = 220, c = 50	Hypermethylation of gene promotor regions of ctDNA genes compared to controls. EBV was also investigated
[19]	Alu elements	NPSCC	p = 50, c = 140	Alu methylation levels in ctDNA of patients compared to controls
[20]	p16, p15	HNSCC	p = 20, c = 24	Hypermethylation of gene promotor regions of ctDNA genes compared to controls
[21]	p16, EDNRB, DCC	HNSCC	p = 100, c = 50	Hypermethylation of gene promotor regions of ctDNA genes compared to controls
[22]	RASSF1, CDKN2A (p16), DAPK1, p15, MLH1, CDH1	NPSCC	p = 41, c = 43	Hypermethylation of gene promotor regions of ctDNA genes compared to controls

Eleven studies included. An overview of cancer type, number of patients included and focus of interest.
NPSCC: nasopharyngeal squamous cell carcinoma.
HNSCC: head and neck squamous cell carcinoma.
p: patients.
c: control group.

significant hypomethylation of overall Alu element methylation levels of ctDNA in patients (p = 50, c = 140) [19]. Common to the nine studies was the examination of diagnostic power (sensitivity and specificity) by comparing the ctDNA in plasma of HNSCC patients to controls (Table 4).

One study by de Vos et al. is not included in Table 4, as the same data were used by A. Schröck et al. All studies investigating one or several different genomic methylations found an increase in sensitivity as a result of an increase in the number of different genomic methylations in one liquid biopsy [9,17,19,22] (Table 4).

The most frequently examined gene methylations were CDKN2A, DAPK1, RASSF1, and p15. Four studies (p = 201, c = 158) evaluated CDKN2A (gene coding for p16 protein) gene methylation in patients compared to controls. The sensitivity ranged between 1 and 65% and the specificity ranged between 80% and 100% (Table 4).

Three studies (p = 301, c = 84) evaluated DAPK1 gene methylation. The sensitivity varied between 20% and 51% and the specificity between 90% and 100% (Table 4).

For calculating the best diagnostic power the studies used different cut-off values to calculate sensitivity and specificity. One study created a cut-off value based on control patients [9]. Most studies exclusively compared methylation positive individuals to unmethylated individuals, thus as categorical variables [17,18,22].

Tian et al. suggested a diagnostic test where two different genes of the multiple investigated must be methylated, thus increasing the specificity of the test. At the same time, the opposite concerns the sensitivity [17].

Methylation of the SHOX2 and SEPT9 genes in plasma were examined by Schröck et al. (p = 425, c = 224). The SHOX2 and SEPT9 genes' mean value resulted in an increased sensitivity [9]. de Vos et al. used the same data to analyse different quantification algorithms in order to apply the biomarkers with the best reliability in regard to sensitivity and specificity [6].

Two studies (p = 260, c = 91) compared human DNA methylations in plasma to viral DNA of EBV and found an increase in diagnostic power when combining the viral DNA measurement with the ctDNA gene methylations [17,18].

Discussion

This review investigated the use of DNA gene mutations and methylations in plasma of HNSCC patients. The aim was to emphasise the current knowledge of HNSCC ctDNA alterations in plasma as a diagnostic or disease observing test, and to improve the outcome for HNSCC patients. Six studies compared DNA methylation and mutations in primary tumours to plasma (p = 156) [11–16]. The mutational studies did not compare ctDNA in patients to controls, but instead compared mutations in primary tumours to plasma. This may not reflect the best way to validate the use of ctDNA as a biomarker because the relation between primary tumour mutations and plasma mutations cannot be used to calculate the diagnostic accuracy of ctDNA mutations. A possible way to select the genes could be based on genomic characterisation of HNSCC in primary tumours. This characterisation of HNSCC genomic alterations in primary tumours has recently been described [23]. The analysis of mutation status showed that an increase in the number of investigated mutations resulted in an increase in patients with ctDNA mutations found in plasma [11–13] (Table 2).

Perdomo et al. showed that a mutation found in plasma was not found in primary tumour HNSCC. This could be due to intra-tumour heterogeneity as the blood test does not represent one specific area of the tumour, as does a biopsy, but various genetic tumour area alterations of the cancer. Development of a new mutation in blood is also a possibility

Table 4. The diagnostic accuracy of gene methylations in eight HNSCC ctDNA studies.

Genes	Ref	Cancer	Sensitivity (%)	Specificity (%)	*p* Value	Groups	Assay	Cut-off value	Statistics
SHOX2	[9]	HNSCC	50	95	<.001	p = 137, c = 122	qPCR	Methylation levels in control patients, 95% controls methylation levels below the cut-off	Log rank test and Wald test
SEPT9	[9]	HNSCC	57	95	<.001	p = 137, c = 122	qPCR	Methylation levels in control patients, 95% controls methylation levels below the cut-off	Log rank test and Wald test
SHOX2 + SEPT9	[9]	HNSCC	59	95	<.001	p = 137, c = 122	qPCR	Methylation levels in control patients, 95% controls methylation levels below the cut-off	Log rank test and Wald test
RIZ1	[16]	NPSCC	23	100	–	p = 30, c = 18	MSP PCR	Detectable in plasma	–
UCHL1	[17]	NPSCC	64.90	81	<0	p = 40, c = 41	qPCR	Detectable in plasma	Fisher's exact test
CDKN2A + DLEC1 + DAPK1 + UCHL1	[17]	NPSCC	85	66	–	p = 40, c = 41	qPCR	Detectable in plasma	Fisher's exact test
DLEC1	[17]	NPSCC	25	93	.037	p = 40, c = 41	qPCR	Detectable in plasma	Fisher's exact test
DAPK1 (Death-associated kinase)	[17]	NPSCC	51	90	<0	p = 40, c = 41	qPCR	Detectable in plasma	Fisher's exact test
	[18]	NPSCC	27	98	–	p = 220, c = 50	MS-HRM PCR	Detectable in plasma	–
	[22]	NPSCC	20.00	100	.0002	p = 41, c = 43	qPCR	Detectable in plasma	Fisher's exact test
CDKN2A (p16)	[17]	NPSCC	22	98	.007	p = 40, c = 41	qPCR	Detectable in plasma	Fisher's exact test
	[20]	HNSCC	65	80	.016	p = 20, c = 24	MSP PCR	Detectable in plasma	*t*-test
	[21]	HNSCC	1	100	.48	p = 100, c = 50	MSP PCR	Detectable in plasma	Fisher's exact test
	[22]	NPSCC	42	100	<0	p = 41, c = 43	qPCR	Detectable in plasma	Fisher's exact test
RASSF1	[17]	NPSCC	18	95	.088	p = 40, c = 41	qPCR	Detectable in plasma	Fisher's exact test
	[22]	NPSCC	5	100	.235	p = 41, c = 43	qPCR	Detectable in plasma	Fisher's exact test
RASSF1A + WIF1 + DAPK1 + RARβ2	[18]	NPSCC	73	96	–	p = 220, c = 50	MS-HRM PCR	Detectable in plasma	–
RARβ2	[18]	NPSCC	16	98	–	p = 220, c = 50	MS-HRM PCR	Detectable in plasma	–
RASSF1A	[18]	NPSCC	24	100	–	p = 220, c = 50	MS-HRM PCR	Detectable in plasma	–
WIF1	[18]	NPSCC	52	100	–	p = 220, c = 50	MS-HRM PCR	Detectable in plasma	–
Alu overall methylation levels	[19]	NPSCC	94	43	.0002	p = 50, c = 140	qCOBRA-alu PCR	No cut-off, ROC analysis	–
p15	[20]	HNSCC	60	50	.0037	p = 20, c = 24	MSP PCR	Detectable in plasma	*t*-test
	[22]	NPSCC	20	100	.002	p = 41, c = 43	qPCR	Detectable in plasma	Fisher's exact test
EDNRB	[21]	HNSCC	10	100	.03	p = 100, c = 50	MSP PCR	Detectable in plasma	Fisher's exact test
DCC	[21]	HNSCC	2	100	.32	p = 100, c = 50	MSP PCR	Detectable in plasma	Fisher's exact test
MLH1	[22]	NPSCC	0	100	–	p = 41, c = 43	qPCR	Detectable in plasma	Fisher's exact test
MLH1 + RASSF1A + p15 + p16 + DAP–kinase + CH1	[22]	NPSCC	71	91	–	p = 41, c = 43	qPCR	Detectable in plasma	Fisher's exact test
CDH1	[22]	NPSCC	46	91	<.000	p = 41, c = 43	qPCR	Detectable in plasma	Fisher's exact test

NPSCC: nasopharyngeal squamous cell carcinoma.
HNSCC: head and neck squamous cell carcinoma.
p: patients.
c: control group.

[11]. Schwaederle et al. showed that HNSCCs hold a large heterogenicity in plasma DNA mutations compared to other cancers. This also advocates for a biomarker consisting of multiple different ctDNA genetic alterations, as a conceivable option for a HNSCC ctDNA biomarker [10].

Of the 11 studies investigating methylation status, nine studies explore the diagnostic power of the ctDNA gene methylations. All these studies imply that the low sensitivity and specificity of the individual gene biomarkers make it impossible to currently use as a diagnostic tool for HNSCC. The methylations investigated by more than one study were CDKN2A, DAPK1, RASSF1, and p15, though the diagnostic accuracy of the calculated methylations varied significantly between studies, thus it is not possible to estimate the best methylations for a ctDNA HNSCC biomarker from current knowledge (Table 4). This indicates the need for further investigation in order to reach a conclusive result. In addition, a correlation between a high sensitivity, low specificity, and vice versa, was shown.

Some studies investigated other possibilities for increasing the diagnostic accuracy. The study by Tian et al. set criteria for the number of different genes that need to be methylated. It is an important consideration when developing a diagnostic test to set a specific level for how many different genes must be altered in order to test with the best diagnostic power [17]. The use of different quantification algorithms is also something that should be taken into consideration when selecting the method with the best diagnostic power [6,9]. Two studies (p = 260, c = 91) revealed an increase in diagnostic accuracy when combining human DNA alterations with viral EBV DNA [17,18]. A previous study (p = 411) has shown promising results for identifying HPV ctDNA in patients with HPV + HNSCC [24]. Combining different viral and human ctDNA mutations with methylations united in developing a biomarker is also an option, though it has not yet been investigated.

Ongoing clinical trials are currently being performed regarding HNSCC ctDNA genetic alterations as a biomarker and the monitoring of treatment responses. The findings of these studies might reveal new inventive results for ctDNA alterations of HNSCC [25,26].

Many of the investigated methylation studies concerned NPSSC. It is essential that these examinations should be applied to cancers in the entire head and neck squamous cell area.

This review reveals a significantly greater amount of ctDNA methylations in HNSCC patients compared to controls. A greater number of different ctDNA genetic alterations investigated resulted, to some extent, in an increase in diagnostic accuracy. The importance of identifying the ctDNA gene alterations with the greatest diagnostic accuracy has been presented. Further investigations of additional gene alterations are needed. The results of this study suggest several ctDNA gene mutations and methylations in combination with viral ctDNA, as well as the use of mathematical algorithms, are prospects to develop a ctDNA biomarker for HNSCC.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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