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EXPRESSION OF CD44 AS A CANCER STEM CELL MARKER IN ORAL CANCER: A META-ANALYSIS STUDY

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ABSTRACT

Various researches have shown that *Cluster of Differentiation 44 (*CD44) is one of the valued markers. As it plays an important role in tumor growth and metastasis but studies also suggest it as a cancer stem cell (CSC) marker in oral cancer (OC). Therefore, we aimed to explore association between the expression of CD44 and clinicopathological characteristics along with the OC prognosis.We conducted literature search through PubMed database (till October 22, 2020) to determine and evaluate the clinical and prognostic significance of CD44 expression in OC patients. According to the inclusion criteria we finalized 9 studies with 867 OC cases. We found the positive expression of CD44 in advanced stages was prominently associated with reduced survival rate. Our analysis suggest that higher tumor expression of CD44 may predict poor survival in end staged OC patient.

KEYWORDS: Cancer Stem Cells, CD44, Oral Cancer, Tumor grade, Prognosis.

INTRODUCTION

OC is one of the major public health problem and the most common cancer which includes cancer of lip, mouth, tongue, salivary glands nasopharynx, oropharynx and hypopharynx. Thus, it comprises of numerous carcinomas with multiple etiologies globally (1).

According to GLOBOCAN 2018, OC is the second leading cause of incidence and mortality in India. In males, it accounts for 16·1% incident cases and 12·3% deaths as compared to the 4·8% new cases and 5·9% deaths in females. The estimated number of incident cases by 2040 will be 545,396 and deaths 275,164 in both sexes all over the world (2). According to cancer net statistics, about 29% of oral and oropharyngeal cancers are diagnosed at early stage. Overall 5 year survival rate is approximately 66%, if the cancer has spread to neighbouring tissues, organs and regional lymph nodes and the comprehensive 5 year survival rate is 39%, if the cancer has spread to a distant parts of the body (3). Included studies have reported various risk factors of OC such as tobacco smoking, alcohol consumption, and betel-quid chewing. The studies have also linked various other factors associated with the pathogenesis of OC such as human papillomavirus infection, exposure to ultraviolet rays, nutritional deficiency, poor oral cleanliness and oral lichen planus (4).

Almost 90% of all oral malignancies accounts for squamous cell carcinoma (5). It is a usual malignant tumor of head and neck and it accounts for the sixth largest cause of cancer death worldwide (6).

Evidences support the fact that recurrence and metastasis are linked with the existence and maintenance of CSCs (7). They are migrant and extremely metastatic cellular subpopulation inside tumor cells. CSCs have features of both cancerous cells and stem cells, as well as self-regeneration and anti-apoptotic characteristics. Studies have also shown that once the metastatic potential and migration in CSCs becomes higher, then they are liable to initiate tumor progression (8,9).

In 1982, CD44 was first discovered in lymphocytes and was referred to as P-glycoprotein 1 (8). It is the utmost reported CSC marker with the molecular weight of 85–200 kDa. CD44, a single-chain glycoprotein encoded by solitary gene is positioned on the short arm of chromosome 11 in human and chromosome 2 in mice. It is consists of 20 exons which comprise 10 variant exons (v1–v10) that can sustain alternative splicing to create multiple CD44 isoforms (Figure 1) (10, 11). CD44 is commonly linked to proliferation, migration, cell adhesion, and angiogenesis (12, 13,). These molecular functions attributed by CD44 are due to the fact that it acts as a hyaluronan receptor and relays the activation of factors involved in such processes.

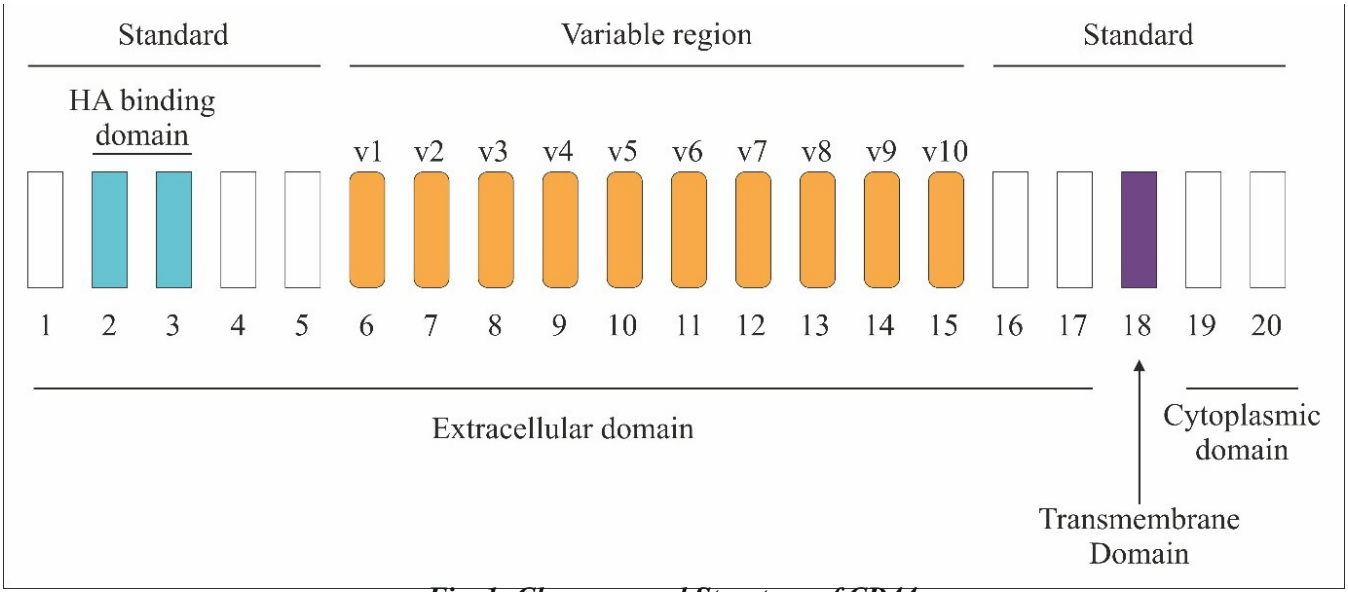

Fig. 1: Chromosomal Structure of CD44.

It may also bind to binding of different ligands, such as fibronectin, collagen type I and IV to initiate tumor matrix remodelling. Studies have proven a key significance of tumor development by facilitating cell motility and cell migration (10). Recurrence and tumour aggressiveness strongly correlates with the high frequency of CD44 positive cells in OC cases (16). Positivity of CD44 cell in association with poor outcome has also been reported in several OC cases and correlates to the clinical framework designation of a higher prevalent $CD44^{+}$ cells in patients with local recurrence and also in cervical metastatic lymph nodes (7).

For cancer patients, it was reported that prognostic value of CD44 is found in various tumors, such as cancer in lung (14), breast (15) and gastric tumor (17). As for OC, the relationship among expression of CD44, clinicopathological characteristics as well as prognosis remain debatable. Thus, we aimed to present a systematic review and meta-analysis of various research studies which evaluated the expression of CD44 in OC, as well as its correlation with histopathological parameters, tumor recurrence, metastasis and overall survival (OS).

METHOD

LITERATURE SEARCH

The PubMed electronic database was used for relevant studies using the key words “CD44”, “CD44v6”, and “oral cancer” through medical subject headings (MeSH) started from 22 September 2020 till 22 October 2020. The research was restricted to human studies published in English. The references of the original and review articles were also physically analyzed for content and statistical analysis.

ELIGIBILITY STANDARD

We have chosen those researches in which the object was to perform immunohistochemistry staining for CSC detection in human oral carcinomas biopsies. We analysed the articles according to the publication year, country, sample size, expression of CSC marker CD44/CD44v6 in human tissue samples. No time limit was applied.

EXCLUSION STANDARD

We have considered the following exclusion criteria:

(1) studies with invitro methods, (2) book chapters, (3) review articles, (4) abstracts, (5) studies that did not carry out immunohistochemistry for CD44 or CD44v6 expression analysis, (6) studies without clinicopathological data and statistical analysis.

INCLUSION STANDARD

The following inclusion criteria was considered: (1) studies which estimated the expression of CD44/CD44v6 in human tumor samples, (2) sample size should not be < 50, (3) a definite III and IV or T3 and T4 tumor stage was reported in case of the absence of stage III and IV, (4) the published articles should be in English language.

Hence, all the studies performed immunohistochemistry method to determine the expression of CD44/CD44v6. Among all the studies, some report the control sample collection from normal tissue sites of head and neck region.

STATISTICAL ANALYSIS

The statistical analysis were carried out by SPSS software version 23 according to the principles suggested by the Meta-Analysis of Observational

Studies in Epidemiology group. The heterogeneity among studies were determined through the p-value and I-square calculated by chi-square test and forest plot on Review Manager 5.4. This meta-analysis was performed while considering specific tumor stages of OC with CD44 expressions.

RESULTS

SEARCH OUTCOMES

Initially, we have retrieved 342 articles according to our search standard. After scrutinizing the irrelevant studies we have excluded 147 articles. These articles either included animal models or were not related to oral malignancies. During primary screening, the remaining 195 studies were evaluated to further exclude a total of 100 articles from the group that comprised either abstracts or review articles. Next, we performed secondary screening which included full text reading of the 95 studies and excluded 86 studies from this group. The eliminated studies lacked clinicopathological data and/or desired sample size. While, few amongst them did not employed the detection of CD44 expression by immunohistochemistry. Finally, a total of 9 studies with 867 OC cases were included for the meta-analysis.

DETAILS OF THE INCLUDED STUDIES

Table 1 comprehend all the characteristics of the selected studies. Amid the 9 examined studies, 2 were conducted in Brazil, 2 in India, 2 in Korea, 1 in China and the remaining 2 in Finland. These studies were published in English language in various journals either related to oncology or pathology during the period 2006 to 2018. A total of 867 patients with age ≥50 were considered in this study.The number of participants ranged from 50 to 231. Most of the patients with CD44/CD44v6 showed positive expression in oral squamous cell carcinoma and head and neck cell carcinoma cases. An immunochemistry staining technique was performed to identify CD44/CD44v6 expression. The tumour tissues were excised from patient's tongue, lip and oral floor.

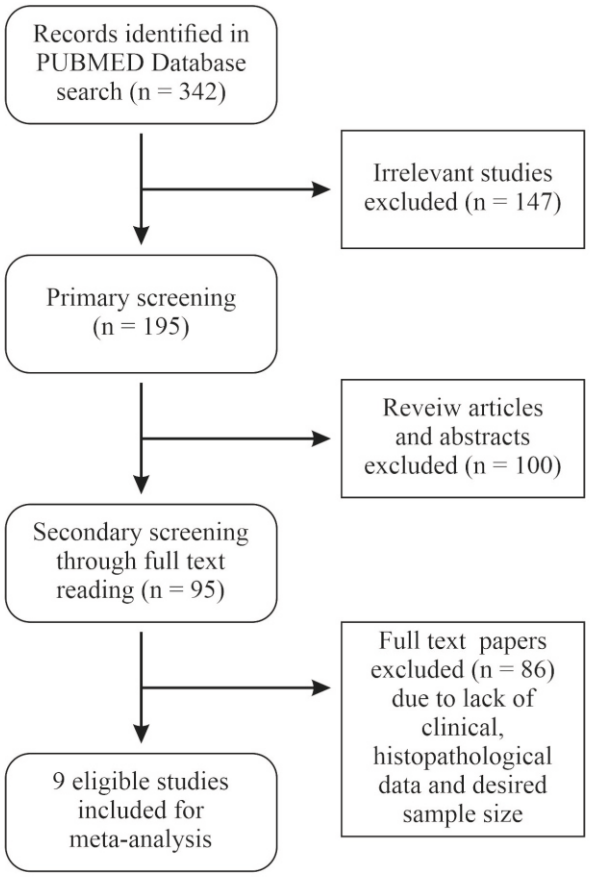

Fig. 2: Flow chart for the selection of inclusive studies

S. No.	Author/ Year	Country	No. of OC Cases	Gender (Male/ Female)	Age group studied/ range	Tumor site		Reference No.
						Oropharynx	Oral Cavity	
1	de Moraes et al. (2016)	Brazil	52	43/9	≤60 and >60	16	36	18
2	Lee et al. (2015)	Korea	57	36/21	17-90	–	57	19
3	Sawant et al. (2016)	India	87	75/12	≤50 and >50	–	87	20
4	Sawant et al. (2018)	India	64	48/16	<50 and ≥50	_	64	16
5	Ortiz et al. (2018)	Brazil	50	40/10	38-85	–	30	7
6	Kosunen et al. (2006)	Finland	138	65/73	10-88	–	138	5
7	Jiajia et al. (2017)	China	60	89/41	≥60 and <60	–	60	6
8	Lee et al. (2018)	Korea	231	144/87	23-88	–	231	21
9	Kokko et al. (2011)	Finland	135	87/48	–	67	68	22

Table 1: Characteristics of the Included Studies

S.No.	Author	Total no. of cases	Characteristics		CD44 /CD44v6 Expression		p-value	Reference No.
1	de Moraes et al. (2016)	45	**Histological grading**		**Positive**	**Negative**		18
			Well	29	21	8	**0.035**	
			Moderate+Poor	21	9	12		
			Tumor Stage		**Positive**	**Negative**	0.463	
			I+II	22	11	11		
			III+IV	23	14	9		
2	Lee et al. (2015)	57	**Histological grading**		**Positive**	**Negative**	0.474	19
			Well	22	18	4		
			Moderate+Poor	35	31	4		
			Tumor Stage		**Positive**	**Negative**	0.207	
			I+II	24	19	5		
			III+IV	33	30	3		
3	Sawant et al. (2016)	87	**Histological grading**		**High**	**Low**	0.672	20
			Well	2	2	0		
			Moderate+Poor	85	78	7		
			Tumor Stage		**High**	**Low**	0.061	
			I+II	15	12	3		
			III+IV	72	68	4		
4	Sawant et al. (2018)	64	**Histological grading**		**High**	**Low**	0.720	16
			Well	11	5	6		
			Moderate+Poor	53	21	32		
			Tumor Stage		**High**	**Low**	0.649	
			I+II	13	6	7		
			III+IV	51	20	31		
5	Ortiz et al. (2018)	50	**Histological grading**		**High**	**Low**	0.251	7
			Well	22	15	7		
			Moderate+Poor	28	23	5		
			Tumor Stage		**High**	**Low**	0.740	
			T1+T2	30	22	8		
			T3+T4	20	16	4		
6	Kosunen et al. (2006)	138	**Histological grading**		**High**	**Low**	**0.017**	5
			Well	82	40	42		
			Moderate+Poor	56	16	40		
			Tumor Stage		**High**	**Low**	**0.030**	
			I+II	88	43	45		
			III+IV	50	15	35		

Table 2: Table on Account of CD44 Expression on the basis of Histological Classification and Tumor Stage

S.No.	Author	Total no. of cases	Characteristics		CD44 /CD44v6 Expression		p-value	Reference No.
7	Jiajia et al. (2017)	60	**Histological grading**		**Positive**	**Negative**	**0.042**	6
			Well	20	11	9		
			Moderate+Poor	40	32	8		
			Tumor Stage		**Positive**	**Negative**	**0.038**	
			I+II	37	23	14		
			III+IV	23	20	3		
8	Lee et al. (2018)	231	**Histological grading**		**High**	**Low**	**0.016**	21
			Well	176	37	45		
			Moderate+Poor	55	9	12		
			Tumor Stage		**High**	**Low**	0.165	
			I+II	101	30	71		
			III+IV	130	50	80		
9	Kokko et al. (2011)	135	**Tumor Stage**		**Positive**	**Negative**	**0.008**	22
			I+II	64	27	37		
			III+IV	71	47	24		

Cont. Table 2: Table on Account of CD44 Expression on the basis of Histological Classification and Tumor Stage

STATISTICAL OUTCOMES

Meta-analysis of the data presented in these 9 investigations are summarized in the figure 1. For the experimental group we considered end staged OC cases (stage III+IV was taken into consideration from 8 studies while T3+T4 was considered from only 1 study due to lack of data). While the control group comprised only high/positive CD44 expression. The findings depicted no significant heterogeneity (p=0.25, I square= 0%) in our analysis. The pooled analysis using the random effect model was conducted. The odds ratio for developing OC in patients with CD44 positive expression was found to be 0.86 (95% confidence interval (CI): 0.68-1.10).

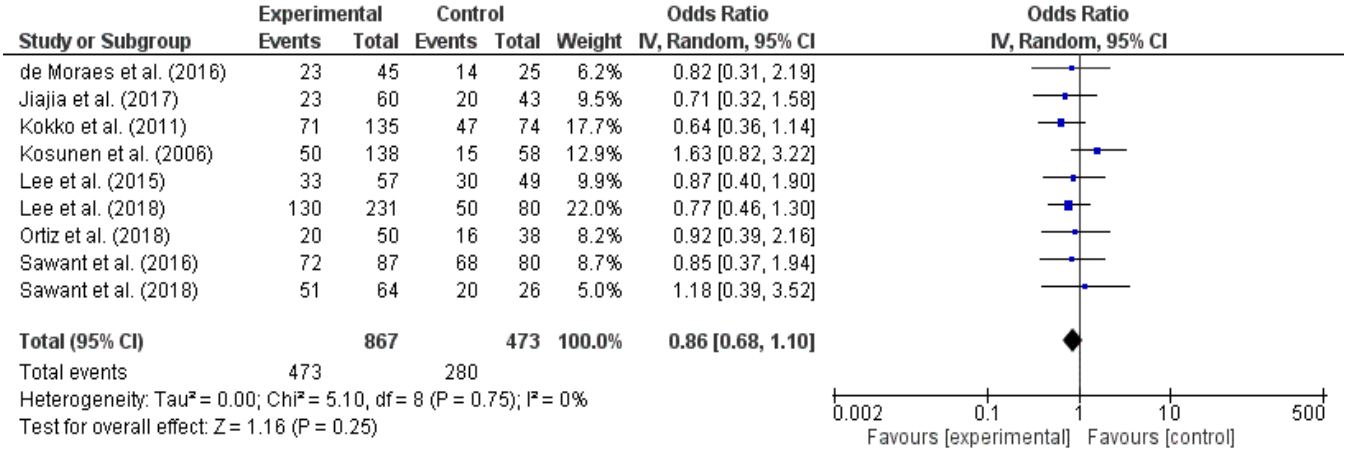

Fig. 1. Relation Between CD44 Expression and Tumor Stages via Forest Plot.

Author	Total no. of cases	Expression	Follow-up period (in months)	Type of regression analysis	Hazard Ratio (95% CI)	p-value	Refrence No.
de Moraes et al. (2016)	45	CD44	60	Univariate	2.32 (0.97-5.58)	0.052	18
Lee et al. (2015)	57	CD44	127	Univariate	2.31 (0.532-10.09)	0.263	19
Sawant et al. (2016)	87	$Oct4^{+}/CD44^{+}$	56	Univariate	6.27 (1.490-26.41)	**0.012**	20
Kosunen et al. (2006)	138	CD44	300	Multivariate	1.98 (1.06-3.70)	**0.03**	5
Lee et al. (2018)	231	CD44	113	Univariate	1.60 (1.10-2.35)	**0.014**	21
				Multivariate	1.65 (0.99-2.73)	0.052	

Table 3: Univariate/Multivariate Cox Regression Analysis of CD44 with Overall Survival.

DISCUSSION

CSCs are well known for initiation of tumor, maintenance, recurrence as well as metastasis, and its identification was also suggested as a therapeutic target against cancer (7, 9). Cell surface markers are used to isolate and enhance CSCs from various tumors (7, 9, 10). These markers are measured by immunohistochemistry in primary tumors, being generally associated with metastasis as well as poor prognosis in many cases (7). CD44 is a potential CSC marker in solid tumors, transmembrane glycoprotein and an adhesion molecule (10, 14, 15, 17). It has been reported in previous studies that CD44 is linked to numerous tumor biological behaviours, including progression, metastasis, recurrence and resistance to radiotherapy as well as chemotherapy (5, 10 , 16, 23).

Present meta-analysis have 867 participants in 9 different studies to estimate the expression, significance and CD44 prognosis in OC. According to previous study, the expression of biomarker CD44 was high in 54% of the samples, out of which 33% had poor result leading to metastasis, recurrence, death during the course of research (20). However, de Moraes and co-workers reported non-significant positive CD44 expression in 57% and 33% of the primary tumour cases and lymph nodes respectively (18). The authors also couldn't establish a significant association with OS, smoking, treatment modality, clinical stage and lymph node metastasis (18). Likewise, another study included in the present meta-analysis found no association between patient survival and CD44 expression pattern (19). Although, positive staining (moderate and weak) was reported in 49 OC cases but none of the cases showed strong CD44 staining intensity (19). On further evaluation of CD44 staining intensity with clinical parameters, the authors reported only weak staining pattern to be significantly associated with the higher frequency of negative lymph node metastasis (19). Sawant and colleagues analysed the significance of CD44v6 alone and in conjunction with other CSC markers in two separate studies conducted amongst Indian population (16, 20). Both serum and tumor CD44 expression was found to be significantly associated with tumor recurrence (16). Oliveira and co-workers found a total 68 cases comprising CD44 positive cases as well as cases with both CD44 and CD133 positivity (23). Kosunen A et al. stated that CD44 expression was present among 82 tumor tissues while 56 cases exhibited homogeneous staining pattern (5). Another study found a significant difference amongst CD44 expression in three groups i.e. normal oral mucosa (10/10), oral potentially malignant disorder (58/60) and OC (43/60) (6). Lee et al. and his companions found that high CD44 and cystine-glutamate transporter SLC7A11 expression levels were present in 80 (34.6%) and 133 patients (57.6%), respectively. While CD44 was found non-significant with OS in OC cases (21). Overall analysis shows that the high expression of CD44 in oral squamous cell carcinoma and head and neck cell carcinoma patients represent different clinical worth in the tongue, lip and oral floor. By the help of forest plot, we calculated the expression of CD44 in higher stages

(III+IV/T3+T4) and found insignificant heterogeneity in all the above studies.

CONCLUSION

In conclusion, CD44 is a proven CSC marker in OC progression. The expression of CD44 is elevated in end staged tumors leading to worse prognostic values and poor OS in OC patients. Collectively, the immunohistochemical expression pattern of CD44 could be used as a prognostic marker for OC.

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