의학석사 학위논문

Relationship Between Cancer Stem Cell Markers (CD24, 44, 133) and Postoperative Prognosis of Oral Squamous Cell Carcinoma

구강편평세포암에서 암줄기세포표시인자(CD24,44,133) 과 수술 후 예후의 관계 분석

2017 년 7 월

서울대학교 의과대학

의학과 이비인후과 전공

김 성 동

구강편평세포암에서
암줄기세포표시인자(CD24,44,133)
과
수술 후 예후의 관계 분석

지도교수 안 순 현

Relationship Between Cancer Stem Cell Markers (CD24, 44, 133) and Postoperative Prognosis of Oral Squamous Cell Carcinoma

July 2017

The Department of Clinical Medical Sciences,

Seoul National University

College of Medicine

Seong Dong Kim

Relationship Between Cancer Stem Cell Markers (CD24, 44, 133) and Postoperative Prognosis of Oral Squamous Cell Carcinoma

지도교수 안 순 현

이 논문을 김 성 동 석사학위논문으로 제출함

2017 년 7 월

서울대학교 대학원

의학과 이비인후과 전공

김 성 동

김 성 동 의 석사학위 논문을 인준함

2017 년 7 월

위 원 장 김 동 영 (인)

부 위 원 장 안 순 현 (인)

위 원 김 범 석 (인)

ABSTRACT

RELATIONSHIP BETWEEN CANCER STEM CELL MARKERS (CD24, 44, 133) AND POSTOPERATIVE PROGNOSIS OF ORAL SQUAMOUS CELL CARCINOMA

Seong Dong Kim

Department of Otorhinolaryngology,

The graduate school

Seoul National University

Introduction: Many studies have focused on the prognostic roles of cancer stem cell markers, but the results remain unclear. CD44 is the most well-known cancer stem cell marker in head and neck cancers, and CD24 and CD133 are representative cancer stem cell markers in many solid tumors. The aim of this study was to gain insight into the relationships between expression of CD24, CD44, and CD133, either alone or in combination, and prognostic

parameters of oral squamous cell carcinoma (OSCC).

Methods: Patients with OSCC who underwent successful surgical resection from January 2003 to December 2011 in a single tertiary hospital were included in this study. Tissue arrays composed of 67 primary tumor tissues were generated and used for immunohistochemistry (IHC) against CD24, CD44, and CD133. IHC was graded by a semiquantitative histologic scoring system (H score) that considered the extent and intensity of the staining. IHC results were correlated with clinicopathological characteristics and with clinical outcomes such as relapse-free, disease-free, and overall survivals.

Results: In the 67 cases, the oral tongue was the most frequently affected primary site (56.7%). In tumor-lymph node-metastasis (TNM) staging, stage IV (34.3%) was most frequent, followed by stages I (26.9%), II (25.4%), and III (13.4%). Despite successful resection, there was 28.3% recurrence. TNM stage IV was highly related with the recurrence rate ($p = 0.002$). None of the 3 cancer stem cell markers (CD24, CD44, and CD133) had a statistically significant relationship with lymph node metastasis, TNM stage, or microscopic invasion into adjacent tissues. High expression of CD44 alone was associated with relapse-free survival ($p=0.049$), as were combined high expression of CD44 and CD133 ($p=0.046$) and CD44 and CD24 ($p=0.015$). CD44 expression also tended towards correlation with disease-free survival;

however, this was not statistically significant (p=0.071).

Conclusions: Overall, the expression of CD44 had the strongest correlation with tumor recurrence. Additionally, when CD44 expression was combined with CD24 expression, CD24+CD44+ patients had the poorest chance of relapse-free survival. Thus, CD44 expression alone, and also in combination with CD24, should be considered when evaluating the prognosis for relapse-free survival of OSCC.

Keywords: Cancer stem cell marker, CD24, CD44, CD133, Immunohistochemistry, Oral Squamous Cell Carcinoma

Student number: 2015-22002

CONTENTS

Abstract ..... **i**

Contents ..... **iii**

List of tables and figures ..... **v**

List of abbreviations ..... **vii**

Introduction **1**

Materials and Methods **2**

Results **6**

Discussion **10**

Conclusion **15**

References **37**

Abstract in Korean **41**

LIST OF TABLES AND FIGURES

Table 1. Clinicopathological data of patients in this study

Table 2. H scores and mean comparison of each clinical index

Figure 1-1. Example of H scoring: weakly stained sample

Figure 1-2. Example of H scoring: strongly stained sample

Figure 2-1. Representative weakly stained sample: CD24

Figure 2-2. Representative strongly stained sample: CD24

Figure 2-3. Representative weakly stained sample: CD44

Figure 2-4. Representative strongly stained sample: CD44

Figure 2-5. Representative weakly stained sample: CD133

Figure 2-6. Representative strongly stained sample: CD133

Figure 3-1. Relapse-free survival curves according to CD24 expression

Figure 3-2. Relapse-free survival curves according to CD44 expression

Figure 3-3. Relapse-free survival curves according to CD133 expression

Figure 4-1. Relapse-free survival curves according to combined CD24/44 expression and associated P-values

Figure 4-2. Relapse-free survival curves according to combined CD44/133

expression and associated P-values

Figure 5-1. Disease-free survival curves according to CD24 expression

Figure 5-2. Disease-free survival curves according to CD44 expression

Figure 5-3. Disease-free survival curves according to CD133 expression

Figure 6-1. Overall survival curves according to CD24 expression

Figure 6-2. Overall survival curves according to CD44 expression

Figure 6-3. Overall survival curves according to CD133 expression

LIST OF ABBREVIATIONS

OSCC: oral squamous cell carcinoma

HNSCC: head and neck squamous cell carcinoma

CSC: cancer stem cell

IHC: immunohistochemistry

CD24: cluster of differentiation 24

CD44: cluster of differentiation 44

CD133: cluster of differentiation 133

Introduction

Cancer stem cells (CSCs) have gained much attention, due to their roles in the invasion and metastasis of numerous human cancer types. A CSC is defined as "a cell within a tumor that possesses the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor", according to the American Association of Cancer Research (1). Cluster of differentiation (CD) 24, CD44, and CD133 are transmembrane molecules and known cancer stem cell markers. CD24 is a mucin adhesion molecule expressed by pre-B lymphocytes and neutrophils, CD44 is a surface glycoprotein that is involved in cell migration and adhesion, and CD133 is a transmembrane glycoprotein characterized by its tendency to localize to cellular protrusions. Many studies have demonstrated that cells highly expressing these molecules having stem cell-like properties (2).

In several tumor sites, these cancer stem cell markers have prognostic value. In patients with breast cancer, a connection between CD44+/CD24− expression and aggressive clinical course and metastasis was determined (3). In colorectal cancer, CD133 expression is an independent prognostic marker that correlated with low survival in a stratified patient collective (4).

In oral squamous cell carcinoma (OSCC), many studies have focused on the prognostic roles of these cancer stem cell markers, but the results have been inconsistent. Some studies have suggested that CD44 is a suitable cancer

stem cell marker, as CD44+ head and neck squamous cell carcinoma (HNSCC) cell lines possessed the properties of CSCs (5, 6). However, no difference was detected in the invasiveness of $CD44^{high}$ HNSCC cells in comparison with $CD44^{low}$ cells (7). In OSCC, the correlation between expression of CD24 or CD133 and prognosis has not been sufficiently studied.

The aim of this study was to gain better insight into the relationships between expression of CD24, CD44, and CD133, alone or in combination, and prognostic parameters of OSCC.

Materials and methods

Subjects and tissue preparation

This study was designed as a retrospective cohort study. The study protocol was approved by the institutional review board at Seoul National University Bundang Hospital (No. B-1702/383-303). From July 2003 to December 2011, 71 OSCC patients who underwent surgery at the hospital were enrolled in this study. Paraffin blocks from 67 patients were selected to make a tissue array of primary tumor tissues. After review of the primary tumor tissues, core tissue sections were taken from the paraffin blocks and arranged in new tissue microarray blocks using a trephine apparatus (Superbiochips Laboratories, Seoul, Korea). A detailed description of the tissue array construction process can be found on the company homepage (http://www.tissue-array.com).

Immunohistochemistry (IHC)

The tissue array blocks were sectioned to 4-μm thickness for IHC and placed on slides. Using a Discovery XT automated IHC stainer (Ventana Medical Systems, Inc., Tucson, AZ, USA), slides were stained by the following procedure. Sections were deparaffinized using EZ Prep solution. CC1 standard (in Tris/Borate/EDTA buffer, pH 8.0) was used for antigen retrieval. Slides were blocked in Inhibitor D (3% H_2O_2, endogenous peroxidase) for 4

min at 37°C. Slides were incubated with primary antibodies for 32 min at 37°C, and secondary antibody (Omnimap anti-mouse) for 20 min at 37°C. Slides were incubated in 3,3'-diaminobenzidine (DAB)+ H_2O_2 substrate for 8 min at 37°C followed by counterstaining with Hematoxylin and Bluing reagent at 37°C. Reaction buffer (Tris pH 7.6) was used as washing solution. Slides were imaged using the Ventana Chromo Map Kit (Ventana Medical Systems).

Grading of the stained samples

The stained samples were evaluated based on the extent and intensity of the staining. A semi-quantitative histologic scoring system (H score) was adopted to compare the stained slides. The intensity was divided into 4 grades: none, weak, moderate, and strong. The extent of staining was scored from 0 to 100% according to the intensity under a lower power field (200× magnification); the sum of the extents of the four intensities became 100%. H score was calculated by the following formula:

H score = 1×(extent of weak staining) + 2×(extent of moderate staining) + 3×(extent of strong staining)

Examples of H scoring are presented in figure 1. In figure 1-1, 50% was weakly stained, 20% was moderately stained, and 20% was strongly stained. Thus, the calculated H score was 150. Another sample is shown in figure 1-2, which was relatively strongly stained. In that sample, 80% was regarded as

strongly stained, and 20% was moderately stained. Using the above formula, the final H score was 280.

Statistical analysis

Independent-sample t tests were used to analyze the relationship between the H scores and clinicopathological data. Correlations between relapse-free, disease-free, and overall survival and positive and negative IHC expression were investigated by univariate log rank testing of Kaplan-Meier survival analysis.

Results of all statistical tests were defined as statistically significant at $p < 0.05$ and marginally significant at $p < 0.10$. SPSS Statistics (Version 19.0; SPSS, Inc., Chicago, IL) was used for statistical analysis.

Results

Samples from a total of 67 patients, consisting of 45 males (64.4%) and 22 females (32.8%), were used for IHC. Age ranged from 23 to 84, with a mean age of 57.8 years. The most common primary site was the oral tongue (N=38, 56.7%); second was the buccal mucosa (N=13, 19.4%), followed by the floor of the mouth and the retromolar trigone. (N=8, 11.9% and N=7, 10.4%, respectively). There was one cancer of alveolar ridge origin. T stages consisted of 21 T1 (31.3%), 26 T2 (38.8%), 3 T3 (4.5%), and 17 T4 (25.4%). N stages consisted of 42 N0 (62.7%), 14 N1 (20.9%), and 11 N2 (16.4%). Tumor-lymph node-metastasis (TNM) stages included 18 stage I (26.9%), 17 stage II (25.4%), 9 stage III (13.4%), and 23 stage IV (34.3%). We accepted several parameters for evaluating tumor invasiveness. Angiolymphatic invasion was found in 19 cases (27.5%), perineural infiltration was detected in 19 cases (27.5%), and extracapsular spread was observed in 2 cases (3.7%). Finally, 19 cases (27.5%) had disease recurrence (Table 1).

The H scores of the cancer stem cell markers were variable, with means of 101.49 ± 34.07, 175.52 ± 46.33, and 68.81 ± 51.86 for CD24, CD44, and CD133, respectively. Representative samples of each cancer stem cell marker are shown in Figure 2.

Each H score was compared with various standards. When lymph node metastasis was divided into node-negative (N0) and node-positive (N1 and N2) groups, there were no significant differences in the mean H scores for CD24 (101.67 vs. 101.2, p=0.957), CD44 (175.0 vs. 176.4, p=0.906), or CD133 (70.24 vs. 66.40, p=0.772). There were also no statistically significant differences in cancer stem cell marker H scores when the N stages were grouped as N0 and N1 versus N2 (CD24, 102.22 vs 91.82, p=0.352; CD44, 175.93 vs. 173.64, p=0.884; CD133, 71.48 vs 57.27; p=0.419).

When the TNM stages were divided into early (stages I and II) and late (stages III and IV) groups, there were no significant differences in mean H score for CD24 (103.43 vs. 99.38, p=0.352), CD44 (178.18 vs. 171.88, p=0.546), or CD133 (67.71 vs. 70.0, p=0.861). Similarly, when we divided the TNM stages into one group comprising stages I, II, and III and another comprising stage IV, there was no statistically significant differences in cancer stem cell marker H scores (CD24, 105.45 vs. 93.91, p=0.190; CD44, 178.86 vs. 171.88, p=0.520; CD133, 67.05 vs. 72.17, p=0.737).

We observed similar results with regard to tumor invasiveness parameters. When we compared tumors positive and negative for angiolymphatic invasion, there were no significant differences in mean H score for CD24 (92.11 vs. 105.11, p=0.247), CD44 (168.42 vs. 178.30, p=0.441), or CD133 (54.74 vs. 75.96, p=0.131). There were also no significant differences in mean H scores between perineural invasion-positive and -negative groups (CD24, 98.42 vs. 102.55, p= 0.661; CD44, 184.21 vs. 171.91, p=0.336; CD133, 67.37 vs. 70.85,

p=0.806), or extracapsular spread-positive and -negative groups (CD24, 95.0 vs. 101.54, p=0.785; CD44, 225.0 vs. 173.46, p=0.120; CD133, 55.0 vs. 75.38, p=0.595; Table 2).

We compared each cancer stem cell marker with survival curves by univariate log rank test in Kaplan Meier survival analysis. We newly defined cancer stem cell marker-positive and -negative groups. A cut off values were calculated using ROC curve for recurrence rate which gave an appropriate combination of sensitivity and specificity. Calculated cut-off value of CD24 was 115 and those of CD 44 and CD133 were 215 and 115. Relapse-free period was defined as the period from first-diagnosed date to recurrence confirmed date after 1st treatment. Disease-free period was defined as the period until recurrence confirmed date after final treatment.

Relapse-free survival curves for each cancer stem cell marker are shown in Figure 1. High CD44 H score had a statistically significant relationship with poor relapse-free survival (p=0.049); however, the H scores for CD24 and CD133 displayed no such relationships (p=0.613 and p=0.391). Combining the expression patterns, we observed that when CD24 was combined with CD44, the CD24+/CD44+ group displayed significantly worse relapse-free survival than the CD24−/CD44− and CD24+/CD44− groups (p=0.04 and p=0.015, respectively). When CD44 was combined with CD133, the CD44+/CD133+ group showed significantly worse relapse-free survival than the CD44−/CD133+ group, but was not significantly different from the CD44−/CD133− group (Figures 3, 4).

Disease-free survival curves for the cancer stem cell markers are shown in Figure 5. The H score of CD44 displayed a marginal relationship with survival free survival (p=0.071); however, the H scores of CD24 and CD133 had no significant relationship with disease-free survival (p=0.618 and p=0.196, respectively). None of the H scores had a statistically significant relationship with overall survival (p=0.849, p=0.222, and p=0.371 for CD24, CD44, and CD133, respectively; Figure 6).

Discussion

When Bonnet et al. first isolated CSCs from acute myeloid leukemia samples, the tumor stem cell hypothesis was developed (8). Tumor stem cell markers in head and neck cancer was first identified by Prince et al. (5), who developed an immunodeficient mouse model to test the tumorigenic potential of different populations of cancer cells derived from primary, unmanipulated human HNSCC samples. They observed that the tumors that arose from purified CD44+ cells reproduced the original tumor heterogeneity and had two important properties of stem cells: the ability to self-renew and to differentiate. Since then, many studies on the cancer stem cell markers of head and neck cancers have been performed. The surface antigens CD24, CD44, and CD133 are representative cancer stem cell markers of the digestive tract.

CD44 is a single transmembrane protein with a comparably short intracellular domain (72 amino acids) (9). It is a known receptor of hyaluronic acid and interacts with other ligands, such as matrix metalloproteases (10). Kosunen et al. reported that irregular staining of CD44 in OSCC is associated with poor tumor differentiation, higher clinical stage, T3–4 tumor stages, and poor disease-free and overall survival (11). Some studies have revealed increased CD44 mRNA in patients with OSCC compared to healthy people (12, 13).

However, while in a meta-analysis of CD44 expression in head and neck cancers, Chen et al. concluded that CD44 is related to worse T and N categories, tumor grade, and prognosis in pharyngeal and laryngeal cancer, no clear association was revealed between CD44 expression and oral cancer (14).

In our study, similar results were observed. There were no statistically significant relationships between CD44 expression and T stage, N stage, clinical stage, or invasiveness. However, with Kaplan-Meier analysis we found that there were a significant relationship between relapse-free survival and CD44 expression. Andrew et al. reported that high CD44 expression is associated with high c-MET expression, p16-negative tumors, and EGFR-positive tumors. The combination of these markers is associated with poor prognosis in HNSCC patients treated with chemoradiation. It is intriguing to speculate that the correlation between CD44 and relapse-free survival could be caused by this chemoradiation-resistance.

CD24 is a comparably small and strongly glycosylated adhesion molecule that was first described in normal B and T cells. It is anchored into the plasma membrane via a glycosyl-phosphatidylinositol anchor and interacts with P-selectin. CD24 is implicated in T cell costimulation, regulation of the homeostatic proliferation of dendritic and T cells, growth and metastasis of cancer cells, and apoptosis (15). Soave et al. reported that CD24 expression is associated with advanced clinical stages, increased T stage, and lymph node invasion, and CD44/CD24 expression was associated with increased T stage and lymph node invasion in malignant salivary gland neoplasms (16).

Ghuwalewala et al. reported that $CD44^{high}CD24^{low}$ cells isolated from oral cancer cell lines not only express stem cell-related genes but also exhibit characteristics of epithelial-to-mesenchymal transition (EMT) (17). Todoroki et al. suggested that the CD44v3+/CD24− cell population in a human OSCC cell line displayed cancer stem cell properties. In their study, cases with CD44v3 expression in the invasive portion tended to show poor overall survival compared with those without CD44v3, and there was a significant difference in overall survival between CD44v3+/CD24− and CD44v3−/CD24− immunophenotypes in the invasive portion (18).

In our study, CD24 alone had no significant correlation with clinical stage, T stage, N stage, or invasiveness, but when CD24 and CD44 expression was combined, there was a correlation with relapse-free survival, as CD24+/CD44+ tumors had shorter relapse-free periods than CD24−/CD44− tumors. However, there was no significant difference between the CD24−/CD44+ and CD24−/CD44− groups, a finding not entirely consistent with previous studies. This may be due to differences in IHC protocols–we stained for total CD44, while Todoroki et al. stained specifically for CD44v3. We also found that the combination of increased CD24 and CD44 had no significant correlation with disease-free or overall survival.

CD133 (prominin-1) is a 120-kDa glycoprotein with an N-terminal extracellular domain, two large extracellular loops, which are strongly N-glycosylated, and an intracellular C-terminus (19). The AC133 antigen, which represents a hyper-glycosylated version of CD133, is primarily expressed in

stem and progenitor cells (20). Chiou et al. found that enriched oral cancer stem-like cells highly express CD133, and display differentiation ability and enhanced migration/invasion/malignancy capabilities *in vitro* and *in vivo*. In their study, elevated expression of CD133 was demonstrated in enriched oral cancer stem-like cells from OSCC tumors. Expression of CD133 correlated with poor overall survival for patients with OSCC (21). In xenograft models, cells with high expression of CD133 exhibit higher clonogenicity, sphere formation, and tumorigenic capacity compared with cells with low CD133 (22). In 2016, a study was conducted examining the relationship between CD133 expression and medullary thyroid carcinoma (23). However, in OSCC, CD133 has still only been studied in cell lines. Moon et al. found that CD133 promotes tumor invasion and metastasis in OSCC cells by inducing EMT (24). Yu et al. reported that side populations of cells with CSC properties exist in OSCCs, and that silencing CD133 has a prominent therapeutic effect, enhancing the sensitivity of OSCCs to chemotherapy through the elimination of CSCs (25).

While many studies have reported that CD133-positive cells have CSC characteristics, a clinical correlation between CD133 expression in OSCC and poor prognosis has not been proven. In our study, CD133 expression had no significant correlation with clinical stage (T, N, and TNM stages), or with indicators of tumor invasiveness. However, there was a significant correlation between CD133 expression and relapse-free survival. The CD44+/CD133+ group showed significantly better relapse-free survival than the

CD44−/CD133+ group, but had no significant relationship with the CD44−/CD133− group. This indicates that CD133 expression has clinical relevance when combined with CD44 expression.

In conclusion, in OSCC, the expression of CD44 is strongly correlated with tumor recurrence. Additionally, we have presented evidence that the analysis of CD44 expression, both alone and when combined with CD24 or CD133 expression, could provide prognostic information associated to relapse-free survival of OSCC.

Conclusion

Overall, the expression of CD44 was strongly correlated with tumor recurrence. We confirmed similar results when we analyzed CD44 expression, both alone and in combination with CD24 or CD133. When high CD44 expression was combined with high CD24 expression, patients had the poorest chance of relapse-free survival. Therefore, we suggest that CD44 expression, alone and in combination with CD24 expression, has value for evaluating the prognosis for relapse-free survival of OSCC.

Table 1. Clinicopathological data of patients in this study

	Patients	Percent
Number of patients	67	
Mean Age (± SD, range)	57.8 ± 15.02 (23–84)	
Gender		
Male	45	64.4
Female	22	32.8
Primary site		
Oral tongue	38	56.7
Buccal mucosa	13	19.4
Floor of mouth	8	11.9
Retromolar trigone	7	10.4
Alveolar ridge	1	1.5
T stage		
T1	21	31.3
T2	26	38.8
T3	3	4.5
T4	17	25.4
N stage		
N0	42	62.7
N1	14	20.9
N2	11	16.4
Clinical stage (TNM)		
I	18	26.9
II	17	25.4
III	9	13.4
IV	23	34.3
Angiolymphatic invasion		
negative	47	72.5
positive	19	27.5
Perineural infiltration		
negative	47	72.5
positive	19	27.5
Extracapsular spread		
negative	53	96.3
positive	2	3.7

SD, standard deviation; TNM, tumor-lymph node-metastasis.

Table 2. H scores of each cancer stem cell marker and mean comparison of each clinical index

		CD24		CD44		CD133	
		Mean	P-value	Mean	P-value	Mean	P-value
T stage	T1,2	102.34	**0.758**	177.02	**0.698**	67.02	**0.727**
	T3,4	99.50		172.0		73.00	
Lymph node metastasis	N0	101.67	**0.957**	175.00	**0.906**	70.24	**0.772**
	>N1	101.2		176.40		66.40	
Lymph node metastasis	<N1	102.22	**0.352**	175.93	**0.884**	71.48	**0.419**
	N2	91.82		173.64		57.27	
Clinical Stage	I.II	103.43	**0.630**	178.86	**0.546**	67.71	**0.861**
	III,IV	99.38		171.88		70.00	
Clinical Stage	I,II,III	105.45	**0.190**	178.18	**0.520**	67.05	**0.737**
	IV	93.91		170.43		72.17	
Angiolymphatic Spread	negative	105.11	**0.247**	178.30	**0.441**	75.96	**0.131**
	positive	92.11		168.42		54.74	
Perineural invasion	negative	102.55	**0.661**	171.91	**0.336**	70.85	**0.806**
	positive	98.42		184.21		67.37	
ECS	negative	101.54	**0.785**	173.46	**0.120**	75.38	**0.595**
	positive	95.00		225.00		55.00	

ECS, extracapsular spread

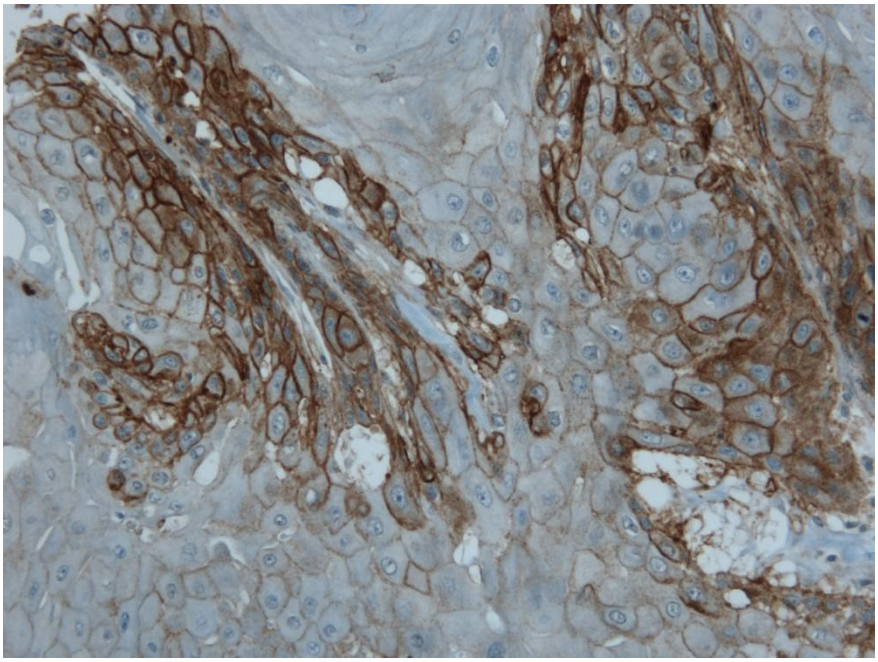


Figure 1-1.

Example of H scoring: weakly stained sample

H score = 1×(50%, weak) + 2×(20%, moderate) + 3×(20%, strong)

= 150

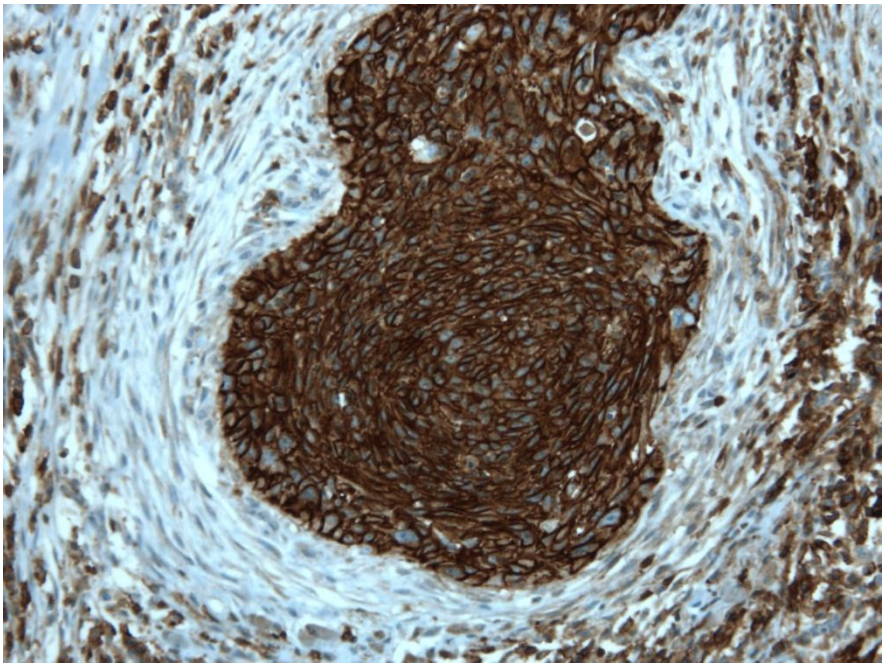


Figure 1-2.

Example of H scoring: Strongly stained sample

H score = 1×(0%, weak) + 2×(20%, moderate) + 3×(80%, strong)

= 280

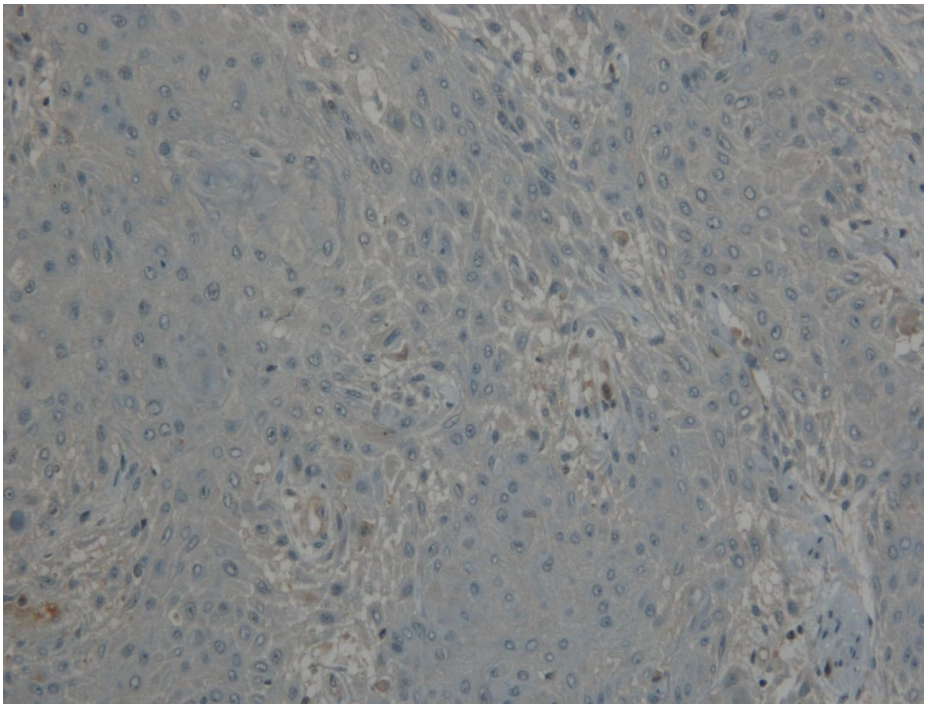


Figure 2-1. Representative weakly stained sample: CD24

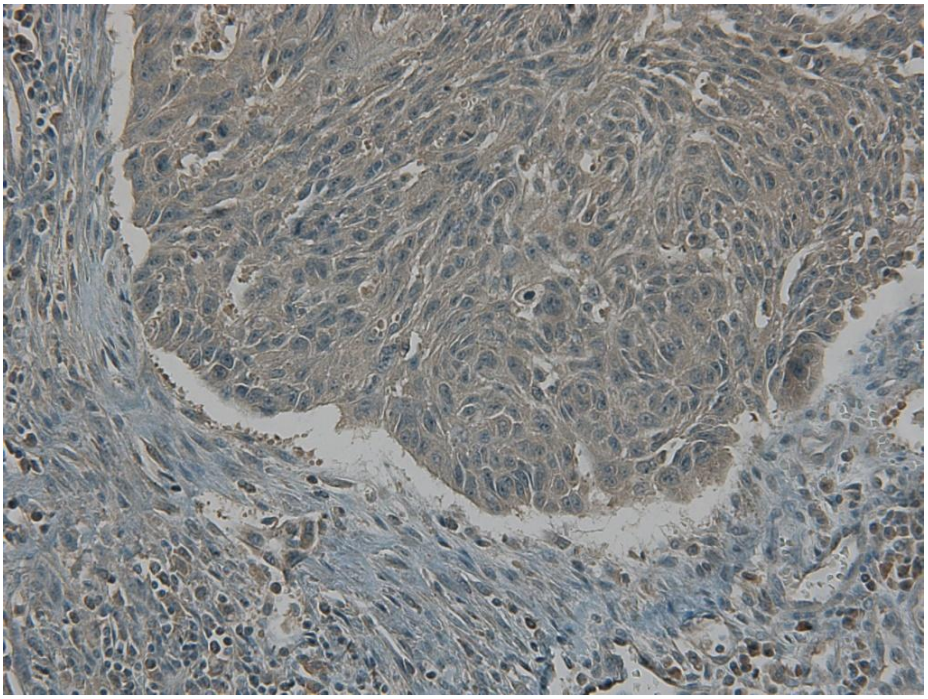


Figure 2-2. Representative strongly stained sample: CD24

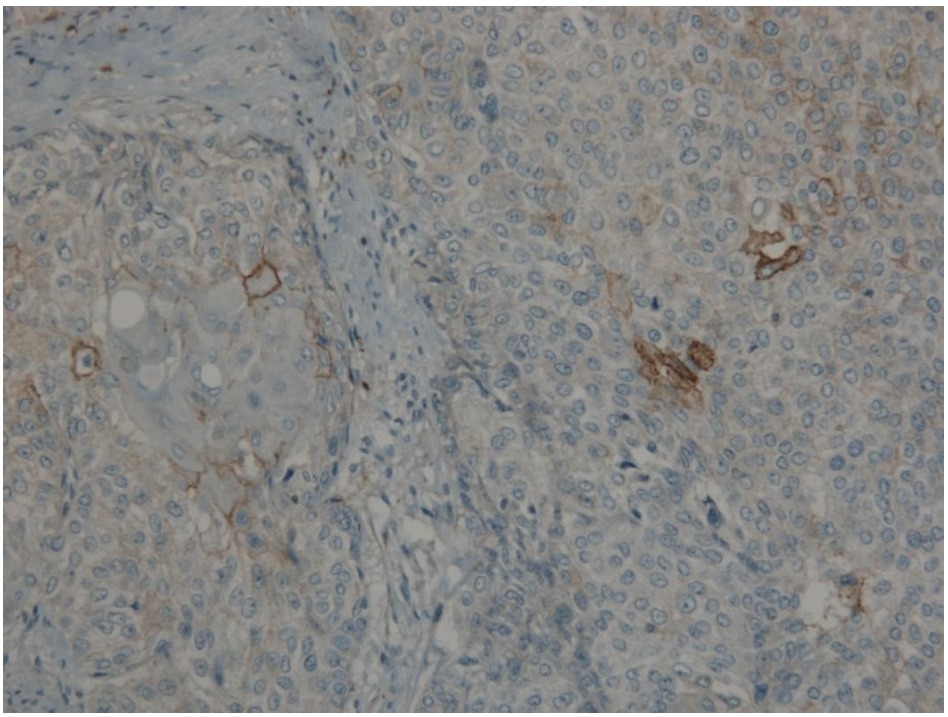


Figure 2-3. Representative weakly stained sample: CD44

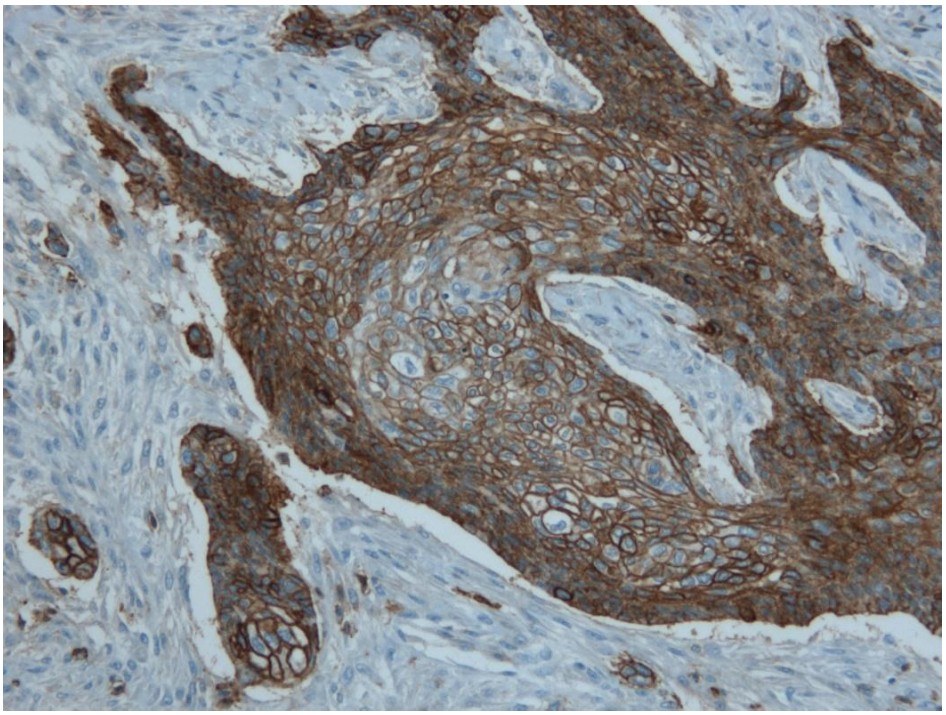


Figure 2-4. Representative strongly stained sample: CD44

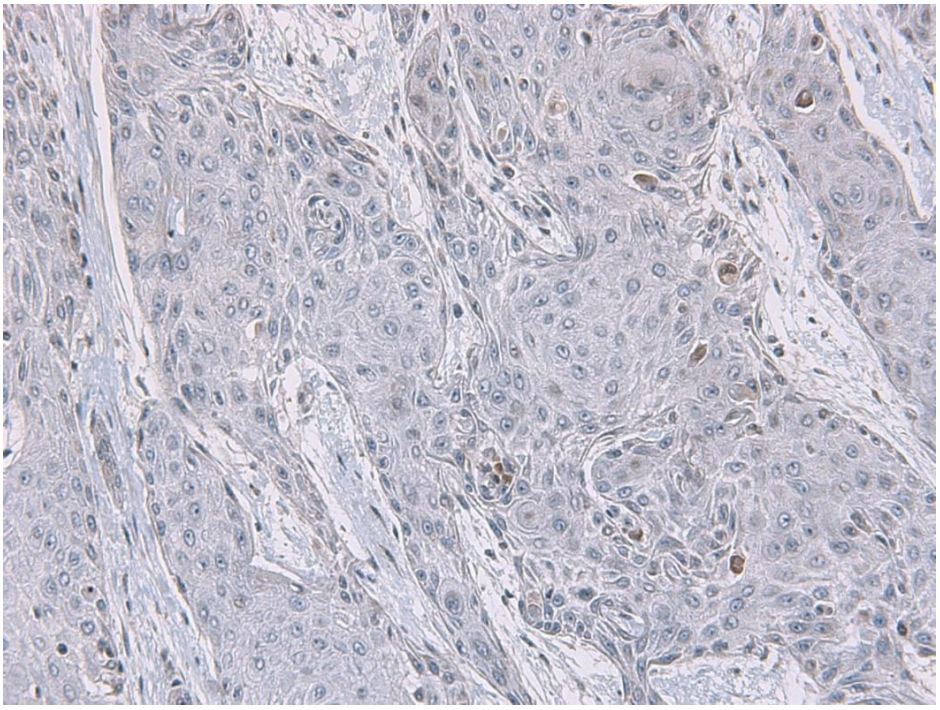


Figure 2-5. Representative weakly stained sample: CD133

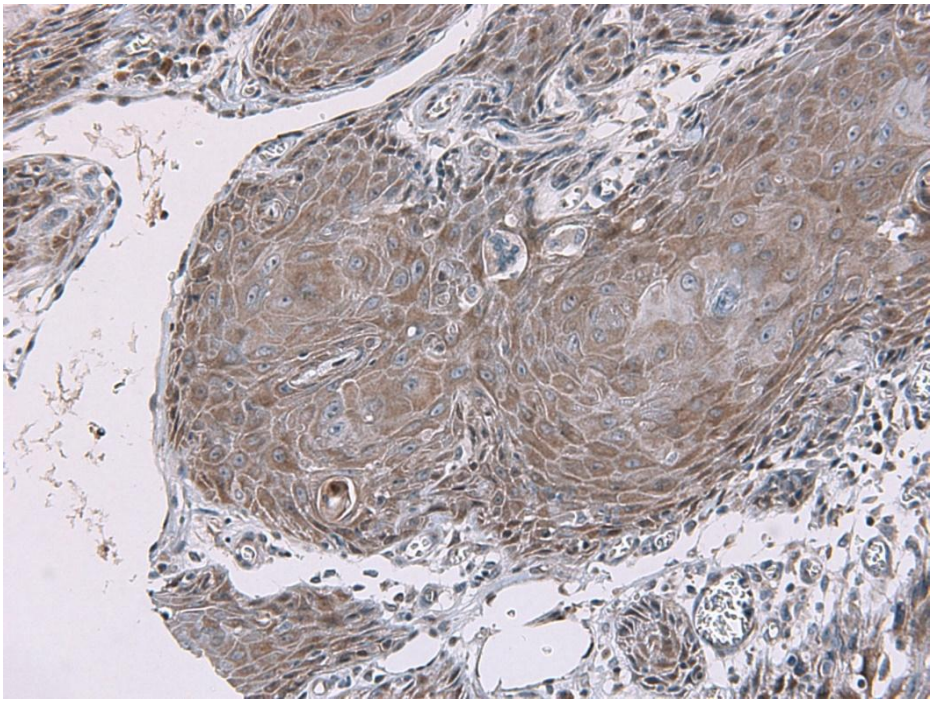


Figure 2-6. Representative strongly stained sample: CD133

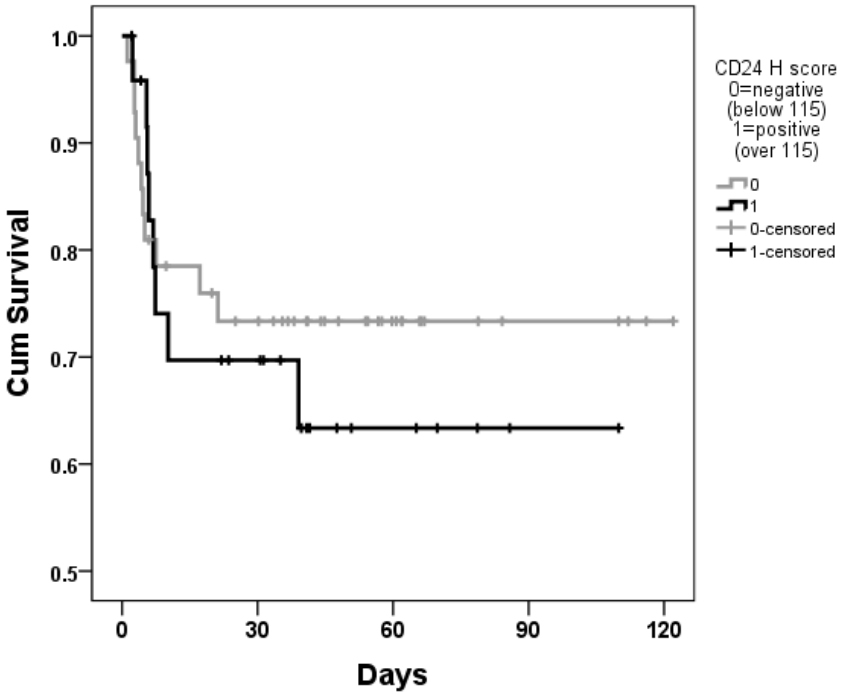


Figure 3-1. Relapse-free survival according to CD24 expression

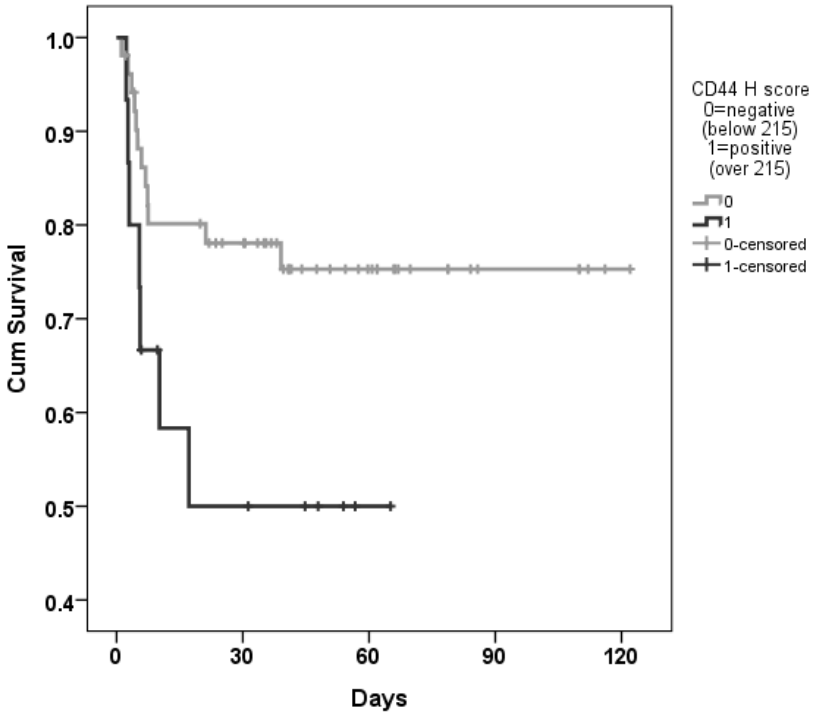


Figure 3-2. Relapse-free survival according to CD44 expression

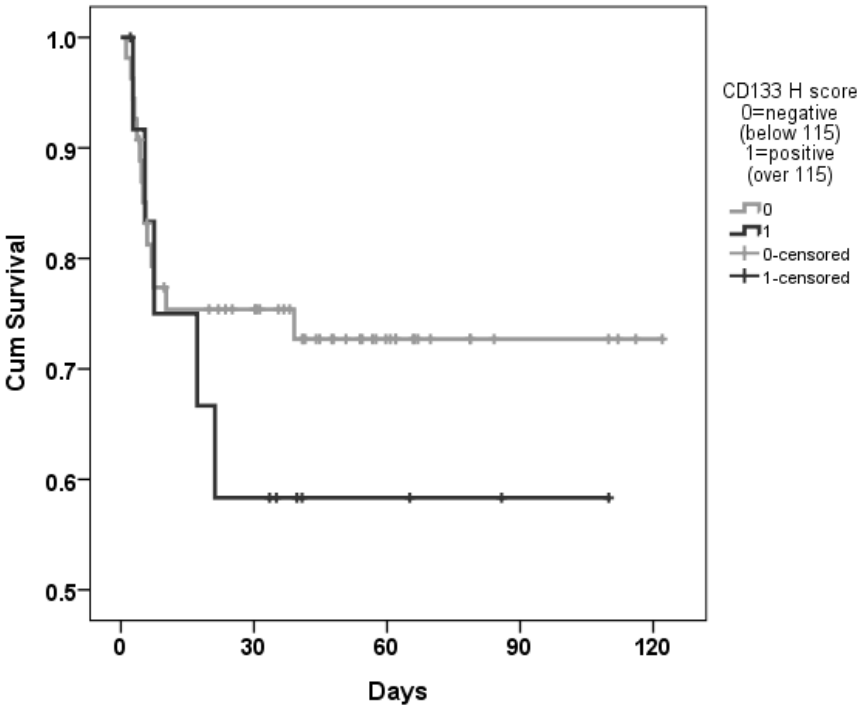
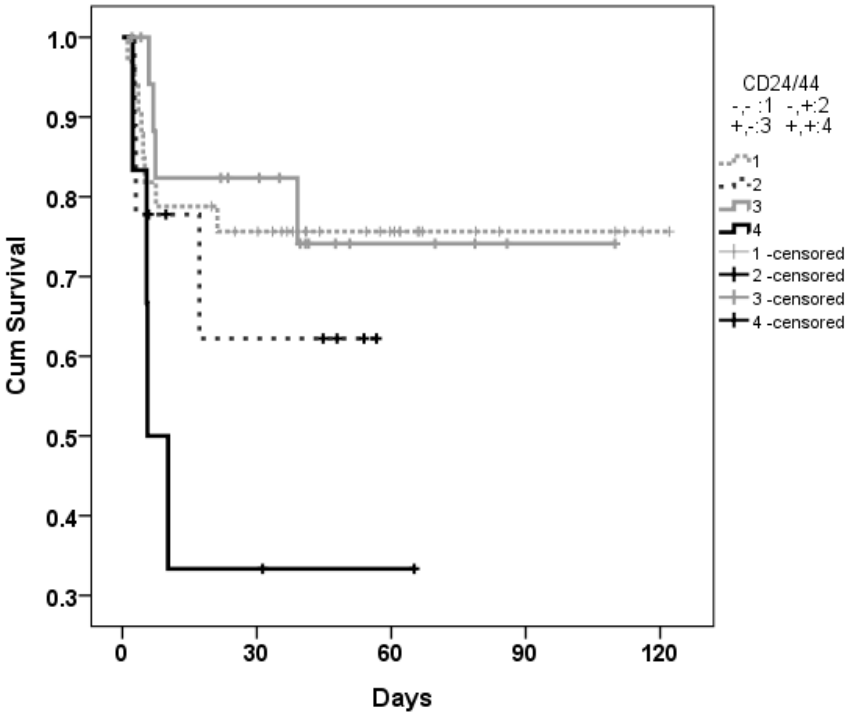
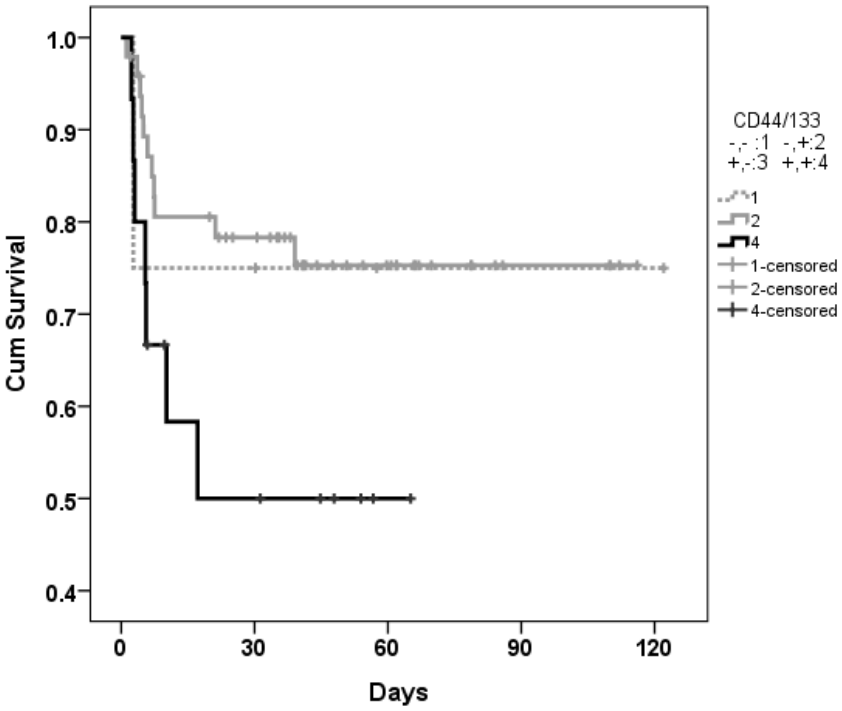


Figure 3-3. Relapse-free survival according to CD133 expression



P-value	**CD24-/44-**	CD24-/44+	**CD24+/44-**	**CD24+/44+**
CD24-/44-	.	0.492	0.822	0.04
CD24-/44+	0.492	.	0.407	0.265
CD24+/44-	0.822	0.407	.	**0.015**
CD24+/44+	**0.04**	0.265	**0.015**	.

Figure 4-1. Relapse-free survival according to combined CD24/44 expression and associated P-values



P-value	CD44-/133-	**CD44-/133+**	**CD44+/133+**
CD44-/133-	.	0.861	0.48
CD44-/133+	0.861	**.**	**0.046**
CD44+/133+	0.48	**0.046**	

Figure 4-2. Relapse-free survival according to combined CD44/133 expression and associated P-values

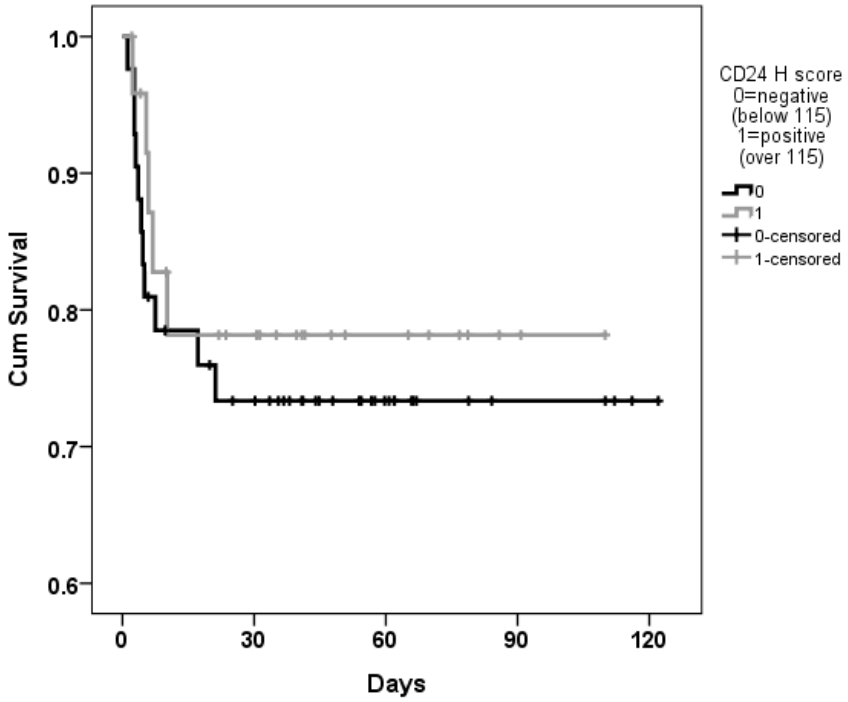


Figure 5-1. Disease-free survival according to CD24 expression

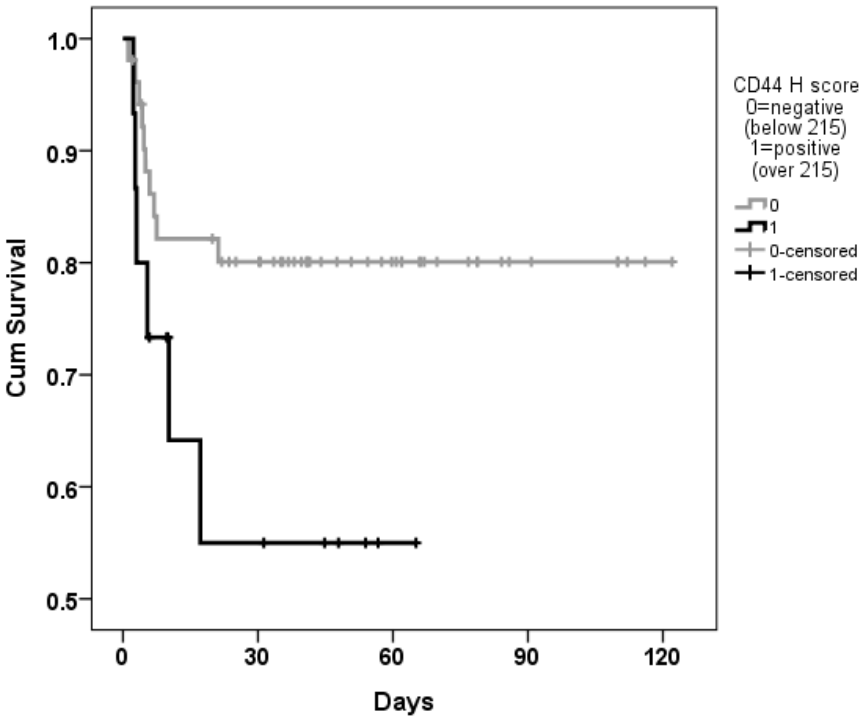


Figure 5-2. Disease-free survival according to CD44 expression

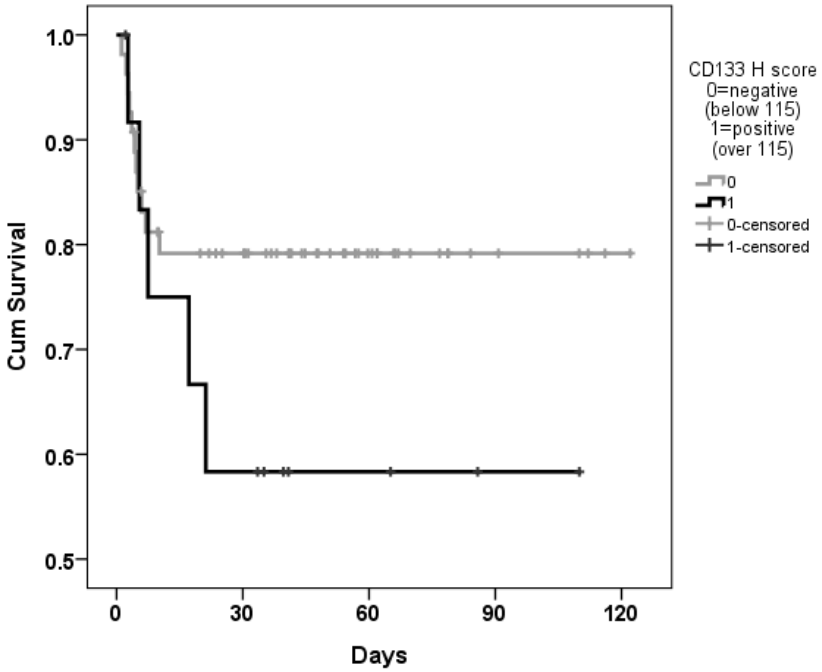


Figure 5-3. Disease-free survival according to CD133 expression

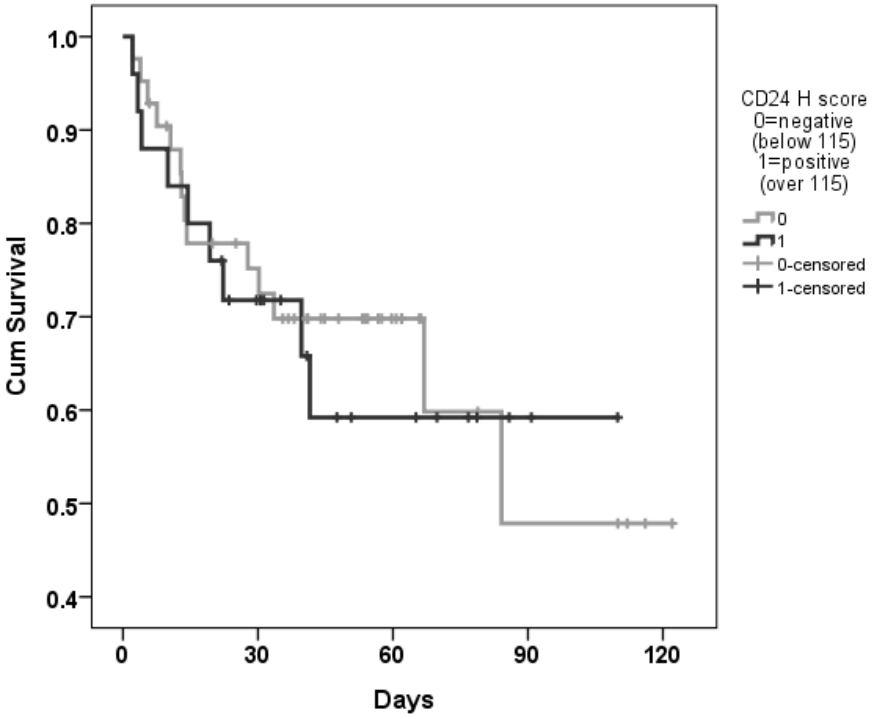


Figure 6-1. Overall survival according to CD24 expression

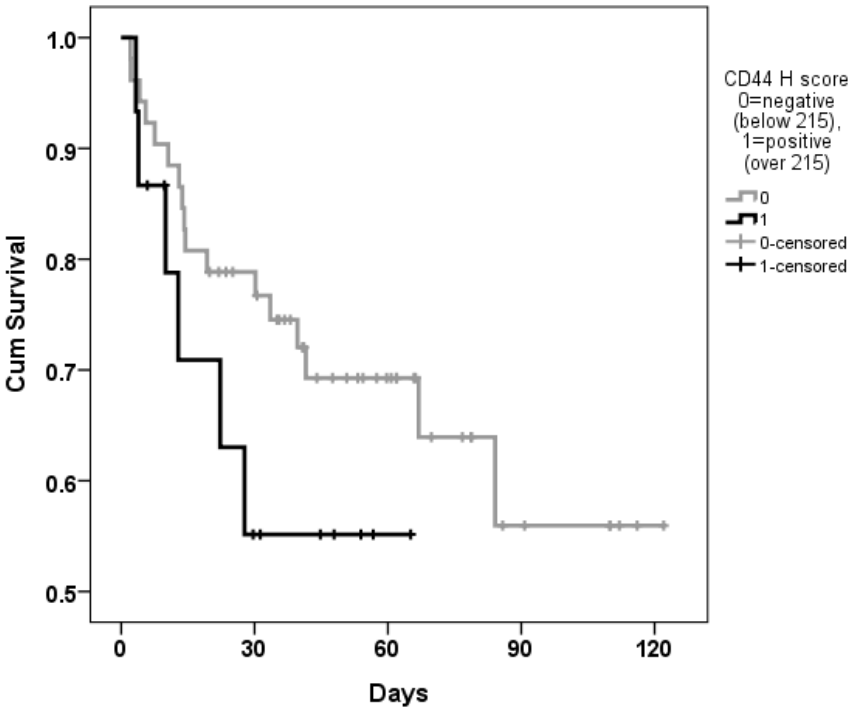


Figure 6-2. Overall survival according to CD44 expression

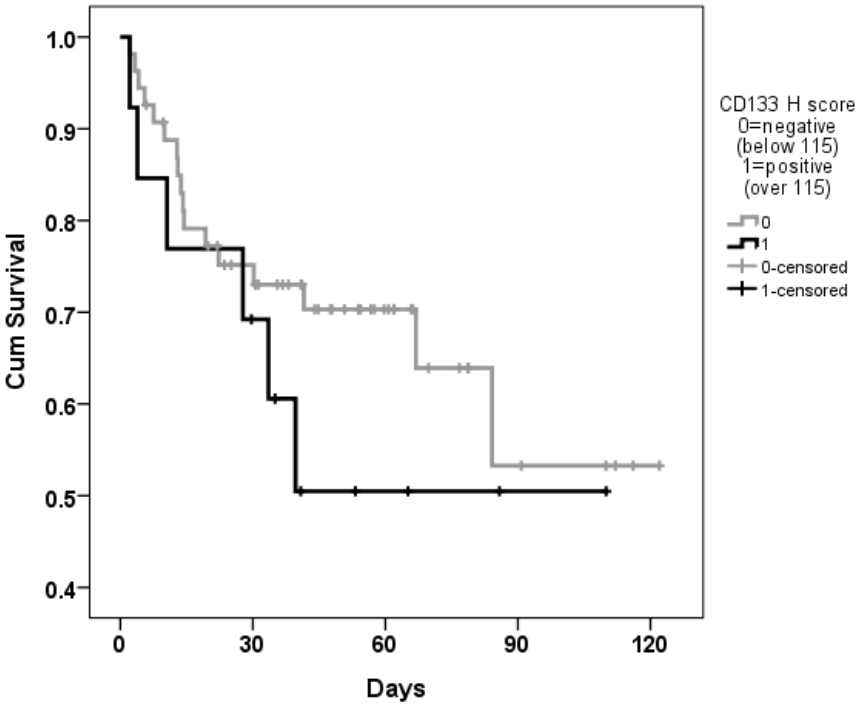


Figure 6-3. Overall survival according to CD133 expression

References

1. Clarke, M.F., et al., Cancer stem cells--perspectives on current status and future directions: AACR Workshop on cancer stem cells. Cancer Res, 2006. 66(19): p. 9339-44.

2. Satpute, P.S., et al., Cancer stem cells in head and neck squamous cell carcinoma: a review. Asian Pac J Cancer Prev, 2013. 14(10): p. 5579-87.

3. Zheng, Z., et al., Correlation between epidermal growth factor receptor and tumor stem cell markers CD44/CD24 and their relationship with prognosis in breast invasive ductal carcinoma. Med Oncol, 2015. 32(1): p. 275.

4. Horst, D., et al., CD133 expression is an independent prognostic marker for low survival in colorectal cancer. Br J Cancer, 2008. 99(8): p. 1285-9.

5. Prince, M.E., et al., Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. Proc Natl Acad Sci U S A, 2007. 104(3): p. 973-8.

6. Okamoto, A., et al., Expansion and characterization of cancer stem-like cells in squamous cell carcinoma of the head and neck. Oral Oncol, 2009. 45(7): p. 633-9.

7. Davis, S.J., et al., Metastatic potential of cancer stem cells in head and neck squamous cell carcinoma. Arch Otolaryngol Head Neck Surg, 2010.

136(12): p. 1260-6.

8. Bonnet, D. and J.E. Dick, Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med, 1997. 3(7): p. 730-7.

9. Wielenga, V.J., et al., Expression of CD44 in Apc and Tcf mutant mice implies regulation by the WNT pathway. Am J Pathol, 1999. 154(2): p. 515-23.

10. Kajita, M., et al., Membrane-type 1 matrix metalloproteinase cleaves CD44 and promotes cell migration. J Cell Biol, 2001. 153(5): p. 893-904.

11. Kosunen, A., et al., CD44 expression and its relationship with MMP-9, clinicopathological factors and survival in oral squamous cell carcinoma. Oral Oncol, 2007. 43(1): p. 51-9.

12. Rajarajan, A., et al., CD44 expression in oro-pharyngeal carcinoma tissues and cell lines. PLoS One, 2012. 7(1): p. e28776.

13. Lin, J.T., et al., Prognostic value of pretreatment CD44 mRNA in peripheral blood of patients with locally advanced head and neck cancer. Oral Oncol, 2010. 46(5): p. e29-33.

14. Chen, J., et al., Significance of CD44 expression in head and neck cancer: a systemic review and meta-analysis. BMC Cancer, 2014. 14: p. 15.

15. Liu, Y. and P. Zheng, CD24: a genetic checkpoint in T cell homeostasis and autoimmune diseases. Trends Immunol, 2007. 28(7): p. 315-

20.

16. Soave, D.F., et al., CD44/CD24 immunophenotypes on clinicopathologic features of salivary glands malignant neoplasms. Diagn Pathol, 2013. 8: p. 29.

17. Ghuwalewala, S., et al., CD44(high)CD24(low) molecular signature determines the Cancer Stem Cell and EMT phenotype in Oral Squamous Cell Carcinoma. Stem Cell Res, 2016. 16(2): p. 405-17.

18. Todoroki, K., et al., CD44v3+/CD24- cells possess cancer stem cell-like properties in human oral squamous cell carcinoma. Int J Oncol, 2016. 48(1): p. 99-109.

19. Corbeil, D., et al., Prominin: a story of cholesterol, plasma membrane protrusions and human pathology. Traffic, 2001. 2(2): p. 82-91.

20. Yin, A.H., et al., AC133, a novel marker for human hematopoietic stem and progenitor cells. Blood, 1997. 90(12): p. 5002-12.

21. Chiou, S.H., et al., Positive correlations of Oct-4 and Nanog in oral cancer stem-like cells and high-grade oral squamous cell carcinoma. Clin Cancer Res, 2008. 14(13): p. 4085-95.

22. Allegra, E. and S. Trapasso, Cancer stem cells in head and neck cancer. Onco Targets Ther, 2012. 5: p. 375-83.

23. Bi, Y., et al., Expression of the potential cancer stem cell markers CD133 and CD44 in medullary thyroid carcinoma: A ten-year follow-up and

prognostic analysis. J Surg Oncol, 2016. 113(2): p. 144-51.

24. Moon, Y., et al., Effect of CD133 overexpression on the epithelial-to-mesenchymal transition in oral cancer cell lines. Clin Exp Metastasis, 2016. 33(5): p. 487-96.

25. Yu, C.C., et al., Targeting CD133 in the enhancement of chemosensitivity in oral squamous cell carcinoma-derived side population cancer stem cells. Head Neck, 2016. 38 Suppl 1: p. E231-8.

국문초록

서론: 두경부암의 암줄기세포 표시인자 중 가장 잘 알려진 것은 CD44 이다. 또한 CD24와 CD133은 많은 고형암에서 대표적인 암줄기세포 표시인자로 알려져 있다. 종양줄기세포 표지자의 예후인자로서의 역할을 밝혀내기 위해 많은 연구가 이루어졌지만, 그 결과는 아직 명백하지 않다. 저자는 이 연구에서 CD24, CD44, CD133의 발현 정도를 단독 혹은 복합적으로 분석하여 구강편평세포암에서의 예후인자로서 의미가 있는 지 여부를 밝히려고 한다.

재료 및 방법: 2003년부터 2011년까지 단일 삼차병원에서 구강편평세포암으로 수술 받은 환자를 대상으로 원발부위에서 종양세포를 수집하였고, 이를 이용하여 파라핀 블록에 조직을 배열하여 보관하였다. 이후 3가지 암줄기세포 표시인자(CD24, CD44, CD133)을 통해 면역조직화학염색을 시행하여 환자의 임상정보와 비교 분석하였다.

결과: 총 67 례 중, 원발부위는 설암이 가장 많았다. (56.7%) TNM

병기는 4기가 가장 많았으며, (34.3%) 그 다음이 1기(26.9%), 2기(25.4%), 3기(13.4%) 순이었다. 종양은 모두 성공적으로 제거되었으나, 28.3%에서 재발하였다. 3가지 암줄기세포 표시인자 모두 환자의 TNM 병기, 림프절 전이여부, 주변조직으로의 미세 침범 여부와 통계적으로 유의한 상관관계는 없었다. CD44 단독으로 분석하였을 때, 재발 없는 생존기간과 CD44 발현은 유의한 상관관계가 있었다. 또한 CD44와 CD133을 조합하였을 때 역시 재발 없는 생존기간과 연관성이 있었으며,(p=0.046) CD24와 CD44를 조합하였을 때에는 가장 높은 상관관계를 보였다. (p=0.015) CD44의 발현이 높을 때 무병생존기간이 짧은 경향이 있었으나 통계학적으로 유의하지는 않았다. (p=0.071)

결론: 종합적으로 CD44의 과발현은 구강편평세포암에서 종양의 재발과 강한 상관관계를 보였다. 또한 CD44 단독으로도 의미 있는 결과를 보였으나 특히 CD24와 CD44를 조합하였을 때 재발 없는 생존기간과의 보다 강한 상관관계를 보였다. 이는 CD24, CD44 발현의 조합이 구강편평세포암 환자의 재발 없는 생존 기간을 예측할 수 있는 인자로 유용하게 이용될 수 있음을 시사한다.

주요어: 구강편평세포암, 암줄기세포표시인자, 면역조직화학염색

학번: 2015-22002