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Alterations of Smad expression and activation in defining 2 subtypes of human head and neck squamous cell carcinoma.

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Abstract

BACKGROUND: We postulated that disruptions of the canonical transforming growth factor-beta (TGF-β)/Smad signaling pathway might contribute to the development of head and neck squamous cell carcinoma (HNSCC).

METHODS: A cohort of 798 HNSCC tumor samples from 346 patients were analyzed by immunohistochemistry (IHC) to define the pattern of expression of (phospho)Smad2, (phospho)Smad3, and Smad4.

RESULTS: We found that 19%, 40%, and 12% of HNSCC specimens failed to express pSmad2, pSmad3, or Smad4, respectively. Loss of Smad2/3 activation was observed in 8.5% of specimens. In addition, 4% of specimens failed to express only Smad4. Moreover, patients with pSmad2/3-negative tumors had a significantly better overall survival than that of those whose tumors expressed activated Smad2/3. In contrast, loss of Smad4 expression did not have prognostic significance.

CONCLUSION: Our results indicate that HNSCC in which Smad2/3 are inactivated or in which Smad4 expression is lost represent 2 distinct tumor subtypes with different clinical outcomes.

HNSCC を Smad の発現のタイピングにより 2 種に分類

背景： HNSCC の発育に TGF-β/Smad シグナルパスの異常が関わっていると考えられている。

方法： 346 患者からの 798 サンプルを免疫組織染色にて(p)Smad2/(p)Smad3/Smad4 の発現パターンを分析。

結果： HNSCC では pSmad2/pSmad3/Smad4 の発現が 19%/40%/12%で欠損していた。Smad2/3 の不活性化は 8.5%で認められた。さらに、4%では Smad4 のみ発現していなかった。また、pSmad2/3 陰性の腫瘍群では、活性化 Smad2/3 の発現している腫瘍群に比べて生存率が有意に良かった。Smad4 は予後に関係なかった。

結論： Smad2/3 が不活性化されていたり、Smad4 発現が欠損している HNSCC は 2 種の臨床的予後の異なるサブタイプを示している。

SCC の発癌要因として、TGF-β/Smad シグナルパスの抑制の損傷が挙げられている。TGF-β・TβRI・TβRII のレセプターが共に働くことで Smad2・Smad3 がリン酸化され、Smad4 と複合体を作り、DNA に配列特異的な部分に働くことで、転写が活性化される。HNSCC の中には TβR-II レセプタや Smad4 に変異の生じている症例もある。また、Smad2 や Smad4 が発現していなかったり、Smad2/4 をコードしている 18q が欠失している例もある。実際の症例でどの程度なのかは正確に研究されていない。

症例期間は 1991 年から 2001 年、University Hospital, New Jersey Medical School/UMDNJ において HNSCC と診断され、病理部にホルマリン固定パラフィン包埋された検体を対象とし、346 症例 798 検体あった。免疫組織染色にて発現の有無を確認。

症例の分布はそれほど偏りは無かったが、Whites と African American は実際の人口分布よりやや多かった。

全症例中 Smad2 を発現していなかったのは 0.5%であった。pSmad2 を発現していないのは 18.5%だった。他のタンパクについては Figure 3 の通りである。Smad3 は細胞質より核で観察され、pSmad3 についても同様。

これらのパターンを分類すると、(1)Smad シグナル系は関係ない(2)Smad 活性化の問題(3)Smad 発現の問題(4)Smad 活性化か発現か特定できない、の 4 群になる。(1)は全部陽性、(2)は pSmad2 or 3 が陰性で Smad2 or 3 が陽性で Smad4 が陽性のもの、(3)は Smad2 or 3 も pSmad2 or 3 も陰性のもので判断できる。これらを表にすると Table 2 になる。pSmad2 陰性のうち 8 割が pSmad3 も陰性だったが、逆は 25%であった。Smad 発現のタイプと患者の臨床的背景については有意差を認めなかった。

生存率については、Smad 発現・抑制・Smad3/4 については有意差が無かったが、pSmad2/3 陰性の症例は比較的生存率が高かった。

TABLE 1. Patient demographics.

Feature	Present series	U.S. population	*p* value
Age, y ($n = 302$)	Range: 29–96; median: 65	Median: 64	N/A
Sex ($n = 260$)	Male: $n = 187$ (72%)	Male: 71%	1.00
	Female: $n = 73$ (28%)	Female: 29%	
Race ($n = 215$)	White: $n = 111$ (51%)	White: 24%	.002
	African-American: $n = 83$ (39%)	African-American: 29%	
	Hispanic: $n = 13$ (6%)	Hispanic: 14%	
	Asian/Pacific Islander: $n = 8$ (4%)	Asian/Pacific Islander: 19% Native American: 16%	
HNSCC site of origin ($n = 299$)	Lip and oral cavity: $n = 123$ (41%)	Lip and oral cavity: 46%	.4663
	Floor of mouth: $n = 43$		
	Tongue: $n = 41$		
	Retromolar trigone: $n = 15$		
	Gingiva: $n = 8$		
	Buccal mucosa: $n = 7$		
	Hard palate: $n = 7$		
	Lip: $n = 2$		
	Pharynx: $n = 67$ (22%)	Pharynx: 25%	
	Base of tongue: $n = 21$		
	Tonsil: $n = 21$		
	Pyriform sinus: $n = 11$		
	Soft palate: $n = 4$		
	Hypopharynx: $n = 4$		
	Oropharynx: $n = 3$		
	Pharynx: $n = 2$		
	Nasopharynx: $n = 1$		
	Larynx: $n = 90$ (30%)	Larynx: 25%	
	Supraglottis: $n = 35$		
	Larynx: $n = 30$		
	Glottis: $n = 25$		
	Salivary gland: $n = 4$ (1%)	Other: 4%	
	Unknown: $n = 15$ (5%)		

Abbreviation: HNSCC, head and neck squamous cell carcinoma.
Demographic characteristics and tumor sites of origin. The age and sex distribution of patients whose HNSCC specimens were used in our study were similar to the U.S. national averages (* from Jemal et al[2]). However, the ethnic origin of our patient population was significantly different from the national averages, with relative over-representation of Whites and African-Americans ($p = .002$, chi-square test for independence). The most common sites of HNSCC origin included floor of mouth, tongue, supraglottis, larynx, and tonsil. This is also consistent with the distribution of HNSCC in the U.S. population as a whole.

TABLE 2. Classification of HNSCC cases by Smad signaling phenotype.

Smad signaling	Smad2	pSmad2	Smad3	pSmad3	Smad4	No.	Subtotal: no. (%)
Intact Smad signaling	+	+	+	+	+	395	395 (51)
Smad activation defect	+	−	+	+	+	18	
	+	−	+	−	+	66	
	+	+	+	−	+	175	259 (33.5)
Smad expression defect	−	−	−	−	−	2	
	+	+	+	+	−	32	
	−	−	−	−	+	2	
	+	+	−	+	−	7	
	+	+	−	−	−	3	
	+	+	−	−	+	6	
	+	+	−	+	+	4	56 (7.2)
Smad signaling defect	+	−	+	−	−	17	
	+	−	+	−	−	17	
	+	−	−	−	−	11	
	+	−	−	+	+	3	
	+	−	−	−	+	13	
	+	+	+	−	−	11	
	+	−	−	+	−	4	64 (8.2)
All Smad signaling defects							379 (49) Total: 774

Abbreviations: HNSCC, head and neck squamous cell carcinoma; IHC, immunohistochemistry.
IHC was informative for all 5 Smads in 774 of the specimens (98%) in our tissue array. Of these, 395 specimens (51%) coexpressed Smad2, pSmad2, Smad3, pSmad3, and Smad4. Among the remaining 379 specimens, 259 were negative for pSmad2, pSmad3, or both (Smad activation defect); 56 specimens were negative for Smad2, Smad3, Smad4, or any combination of these (Smad expression defect); and 64 specimens had a mixed Smad signaling defect.

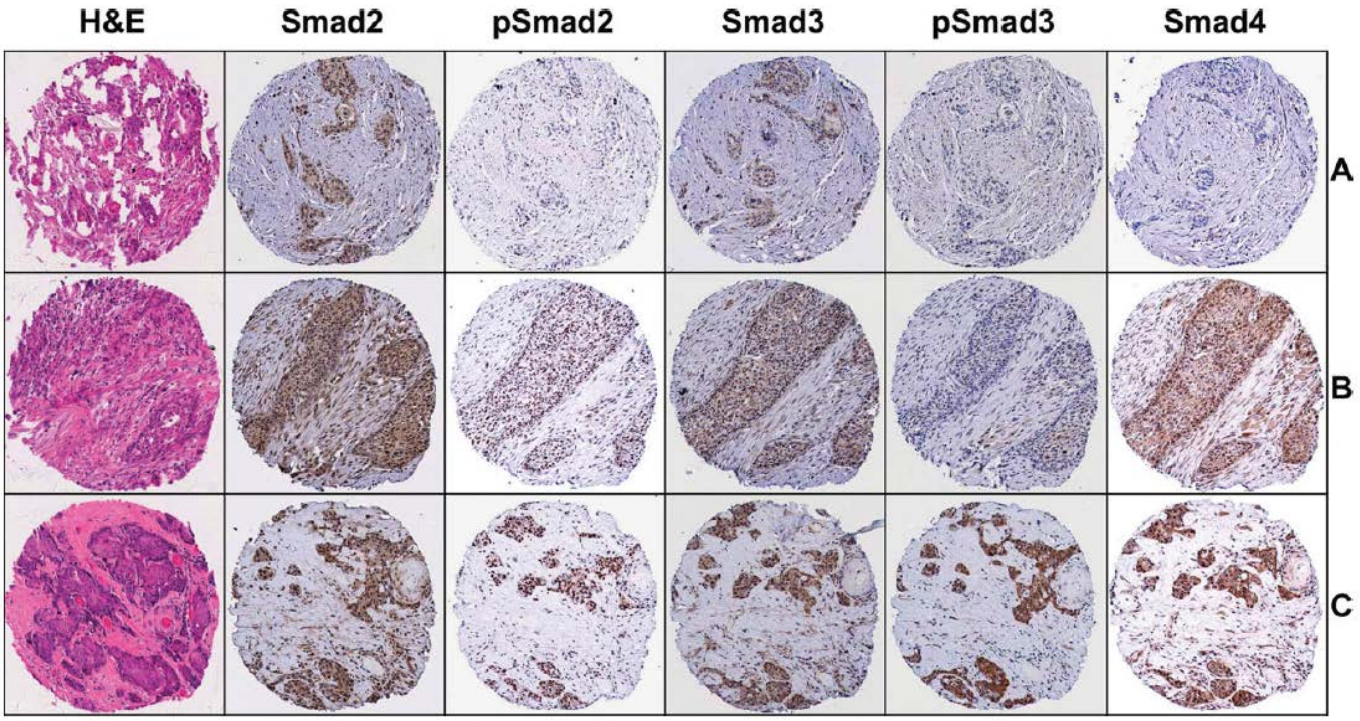


FIGURE. 2. Immunohistochemical (IHC) staining for Smads in HNSCC. Immunohistochemical staining of the HNSCC tissue array using antibodies against specific Smads. (A) A representative case that expressed Smad2 and Smad3, but failed to express pSmad2, pSmad3, and Smad4. Corresponding hematoxylin and eosin–stained sections were included in each panel. (B) A representative tumor that expressed Smad2, pSmad2, Smad3, and Smad4, but failed to express pSmad3. (C) Examples of positive Smad2, pSmad2, Smad3, pSmad3, and Smad4 expression (original magnification, ×100). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

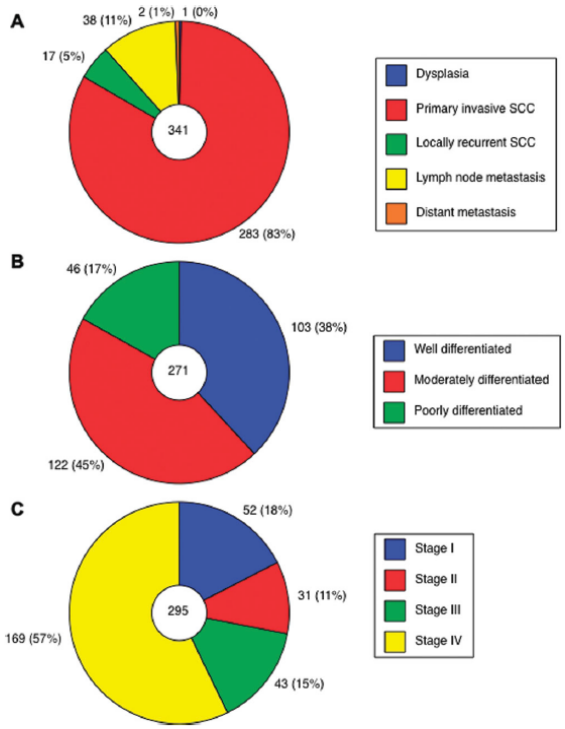
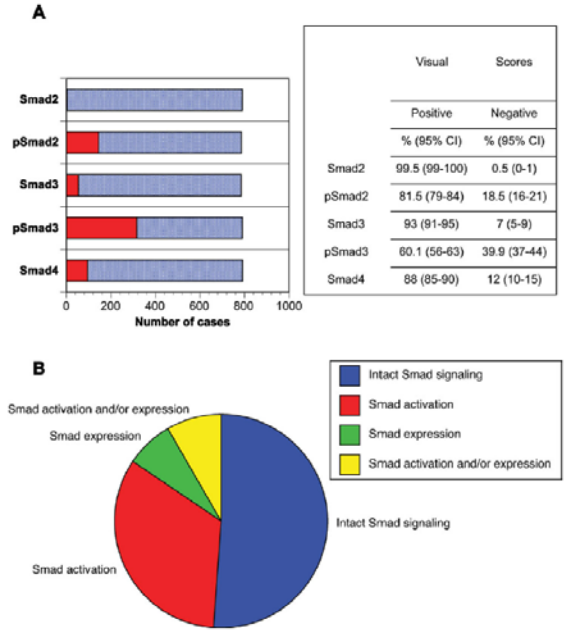


FIGURE. 1. Clinical and pathologic characteristics of HNSCCs. Distribution of the HNSCC in our series by stage of progression (A), histopathologic grade (B), and TNM stage (C) (AJCC Staging, 7th Edition, 2010). HNSCC, head and neck squamous cell carcinoma. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]



	Visual Scores	
	Positive	Negative
	% (95% CI)	% (95% CI)
Smad2	99.5 (99-100)	0.5 (0-1)
pSmad2	81.5 (79-84)	18.5 (16-21)
Smad3	93 (91-95)	7 (5-9)
pSmad3	60.1 (56-63)	39.9 (37-44)
Smad4	88 (85-90)	12 (10-15)

FIGURE. 3. Distribution of Smad signaling phenotypes. (A) Immunohistochemistry (IHC) was informative for all 5 Smads in 774 (98%) of the specimens in our tissue array. (B) Of these, 395 specimens (51%) coexpressed Smad2, pSmad2, Smad3, pSmad3, and Smad4. Among the remaining 379 specimens, 259 were negative for pSmad2, pSmad3, or both (Smad activation defect); 56 were negative for Smad2, Smad 3, Smad4, or any combination of these (Smad expression defect), and 64 had a mixed Smad signaling defect. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE 3. Classification of HNSCC cases by Smad signaling phenotype.

	pSmad2/pSmad3			Smad4		
Feature	Pos	Neg	*p* value	Pos	Neg	*p* value
Sex	217	13	0.75	216	15	1.00
Male	159	9		158	11	
Female	58	4		58	4	
Age, y	239	15	.59	240	15	.78
<60	85	4		83	6	
>60	154	11		157	9	
Race	184	11	.75	183	11	.37
White	95	7		97	5	
Black	71	3		69	4	
Hispanic	11	1		10	2	
Asian/Pacific Islander	7	0		7	0	
Smoking history	162	12	.55	163	10	.53
None or <50 pk/y	98	6		94	7	
>50 pk/y	64	6		69	3	
Tumor site	239	15	.56	241	14	.45
Lip and oral cavity	83	8		85	6	
Pharynx	69	2		70	1	
Larynx	72	4		71	6	
Major salivary gland	4	0		4	0	
Unknown	11	1		11	1	
Histologic grade	223	14	.49	227	13	.27
Poorly differentiated	51	4		54	2	
Moderately differentiated	100	4		100	4	
Well differentiated	72	6		73	7	
T classification	226	14	.36	228	13	.54
T1	52	6		57	2	
T2	51	3		51	3	
T3	38	1		38	1	
T4	85	4		82	7	
N classification	215	14	.84	217	13	.71
N0	100	6		99	7	
N1	21	2		21	2	
N2	86	6		89	4	
N3	8	0		8	0	
AJCC stage	234	15	.39	246	13	.81
I	30	4		34	1	
II	28	1		37	2	
III	35	1		34	1	
IV	141	9		141	9	

Abbreviation: AJCC, American Joint Committee on Cancer; HNSCC, head and neck squamous cell carcinoma.
Contingency table analyses using the Fisher's exact or chi-square test were used to determine the relationships between Smad expression and individual clinicopathologic features for the HNSCCs. None of the clinicopathologic features was found to be significantly associated with either of the 2 major Smad signaling defect phenotypes.

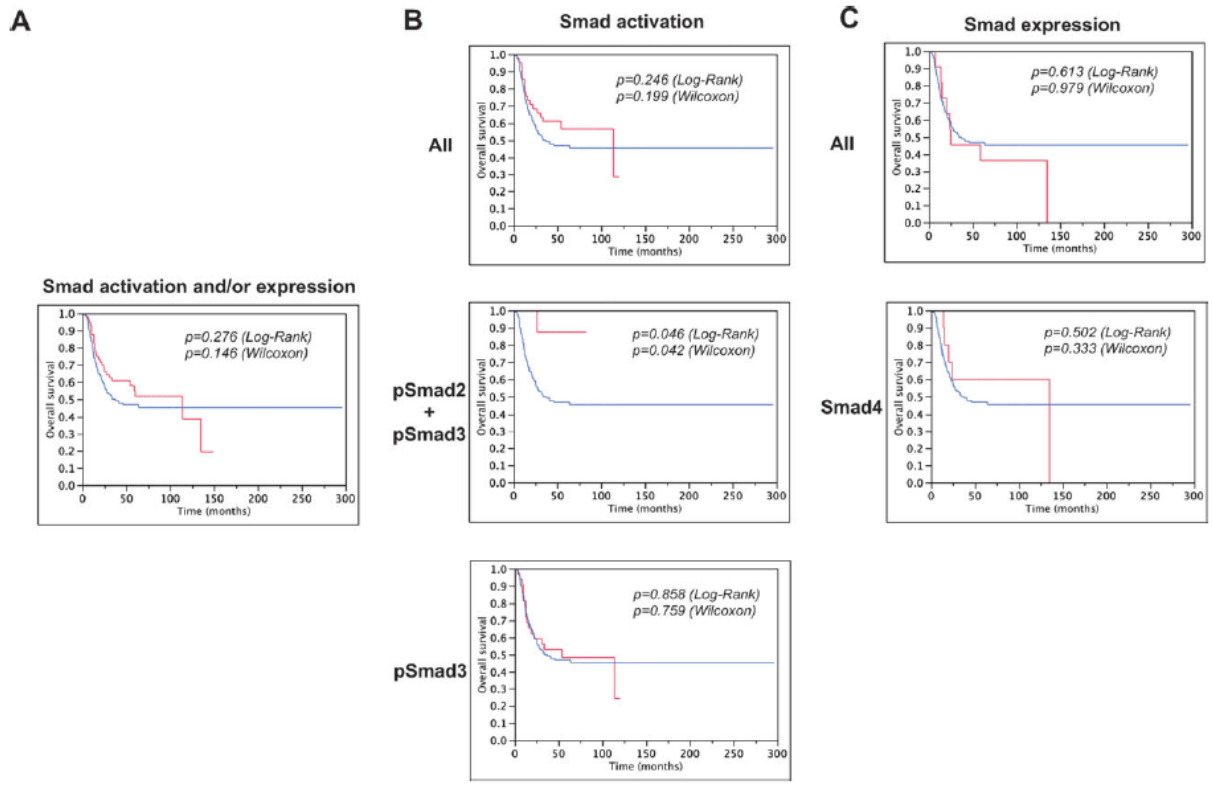


FIGURE. 4. Smad signaling phenotype and patient survival. Survival curves were estimated according to the method of Kaplan-Meier.[27] For each curve, the starting point was the date of surgical resection of HNSCC. Death from any cause was counted as an event in calculating survival time. For surviving patients, time was censored at the last available follow-up date. The median follow-up time in this series was 43 months (ranging from 1.7 to 296 months). The log-rank (Mantel-Cox) and Wilcoxon rank tests were used to compare outcomes of different groups. Major departures from proportional hazard assumptions were excluded by graphic checks. (A) The presence of any Smad signaling defect had no impact overall patient survival. (B) Patients with pSmad2/3-negative tumors had a significantly better overall survival rate than that of those with pSmad2/3-positive tumors. Moreover, pSmad3 negativity by itself was not associated with a favorable prognosis. This result indicates that the differences in patient survival can largely be attributed to loss of pSmad2 expression. (C) On the other hand, we found no significant association between loss of Smad4 expression and patient outcome. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]