Oral Cancer Screening on Oral Rinse Samples using Quantitative E6, E7 mRNA and Flow Cytometry

Amanda Chargin[1], Dinesh Gupta[2], Bruce Patterson[1]
[1] IncellDx Inc., Menlo Park CA, [2] CureHealth, New Delhi India

Objective

HPV (human papilloma virus) infects epithelial cells causing lesions with the potential to progress into carcinoma. This is most commonly seen in the area of the cervix where HPV infection leads to deregulation of the cell cycle through the expression of E6/E7 oncogenes. The same mechanism is functional in head and neck cancer. In addition to E6/E7, CD44 has been shown to have prognostic value in oral cancer. To that end, we report the development of a novel assay to determine the dual expression of these markers in oral cancer.

Materials & Methods

We screened for E6, E7 mRNA overexpression (HPV OncoTect, IncellDx Inc, Menlo Park, CA) by flow cytometry on oral rinse samples from 30 patients including 5 with oral cancer. Samples were collected by rinsing the mouth with 1-2 mL DPBS. Cells were pelleted and then fixed in 1 mL IncellFP to fix and permeabilize the cells for hybridization to mRNA protein markers. In a subset of patients, we stained for both CD44 protein (Cell Signaling Technologies) and E6, E7 mRNA.

Following the submission of this abstract, an additional 60 samples were collected and stained for E6, E7 mRNA. All samples were negative for oral cancer.

Results

In 25 individuals undergoing oral screening for various risks of oral cancer, 7/25 or 28% were positive for E6, E7 mRNA including 0/25 positive for CD44. In comparison, 3/5 (60%) of individuals with oral cancer were positive for E6, E7 mRNA. Positivity for E6, E7 mRNA was determined as >/= 3% in the P3 region.

A)

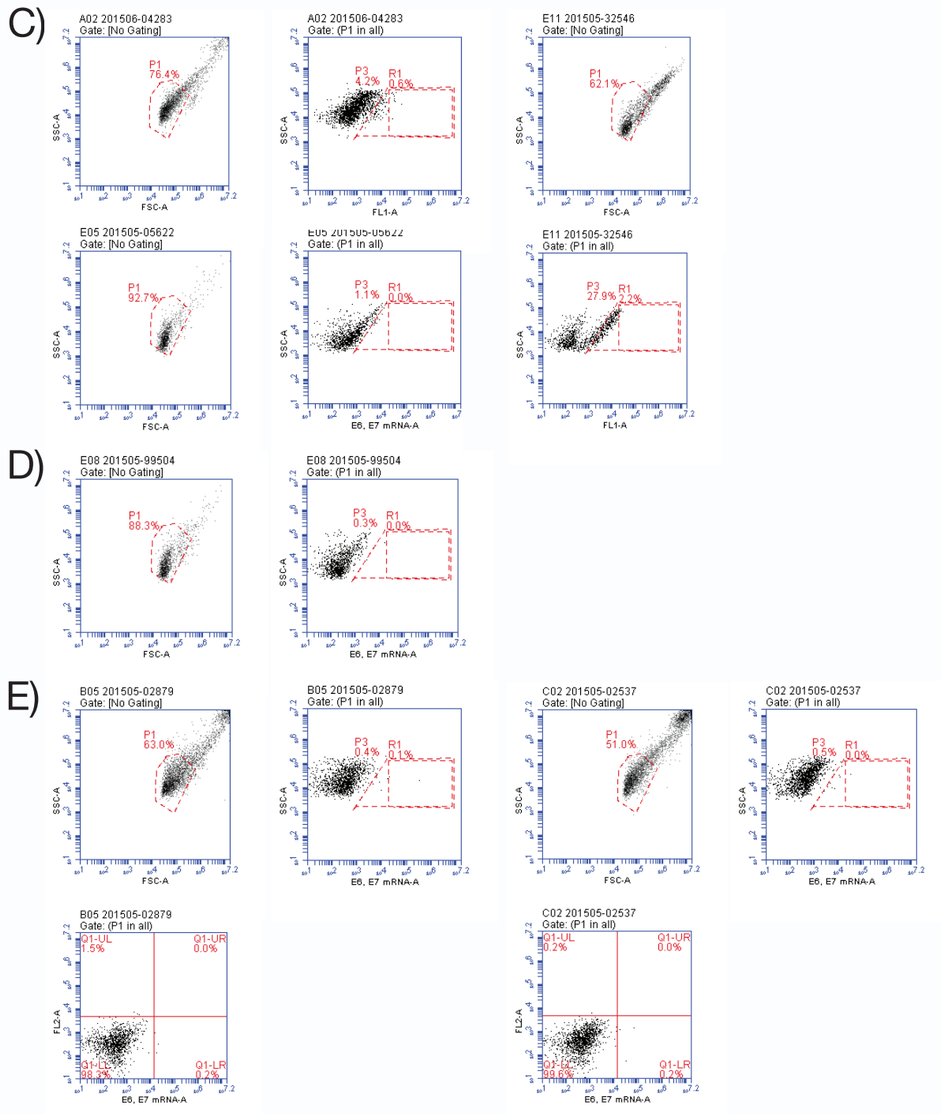
Sample ID	Patient Age	Gender	Diagnosis	P1 (Count)	% In P3
201506-04283	67	F	Hx of HPV 16 positive squamous cell carcinoma, soft palate	2,000	4.25%
201505-05622			Squamous cell carcinoma	1,016	1.1%
201505-04829			Squamous cell carcinoma	1,174	58.5%
201505-32546			Squamous cell carcinoma	1,049	27.9%
201505-99504			Squamous cell carcinoma	1,036	0.3%

Sample ID	Patient Age	Gender	Diagnosis	P1 (Count)	% In P3
201505-02879	49	M		2,000	2.70%
201505-02713	54	M		2,000	4.75%
201505-02715	34	M		2,000	1.85%
201505-02880	60	M		2,000	1.65%
201505-02881	39	F	lesion, floor of mouth	2,000	16.80%
201505-02883	20	M		690	2.90%
201505-02888	52	M	lesion, floor of mouth	1,145	11.70%
201505-02891	31	M		2,000	1.45%
201505-02892	61	M		2,461	0.49%
201505-02896	53	M		2,000	20.15%
201505-02898	27	M		2,000	18.20%
201505-02901	58	M		2,867	14.54%
201505-03170	36	M		2,000	0.50%
201505-02903	63	M		2,000	0.10%
201505-02904	25	M		2,000	0.35%
201505-02905	55	M		2,000	6.10%
201505-03077	28	M		2,000	1.70%
201505-03078	59	F		2,000	0.60%
201505-03080	52	M		2,000	1.35%
201505-03162	20	F		2,000	2.45%
201505-03163	49	F		2,000	0.90%
201505-03164	51	M		2,040	1.27%
201505-03167	32	M		2,000	0.30%
201505-03168	25	M		2,000	0.85%
201505-03169	30	M		2,000	0.05%

B)

Sample ID	Patient Age	Gender	Diagnosis	P1 (Count)	% In P3
201505-02537	58	M		1,394	4.16%
201505-02714	50	M		2,000	7.25%
201505-02716	50	M		2,000	11.80%
201505-02853	23	M		2,000	6.85%
201505-02857	43	F		2,000	29.75%
201505-02858	42	M		2,000	11.50%
201505-02865	54	M		2,000	1.05%
201505-02873	34	M		2,000	3.55%
201505-02877	54	F		2,000	3.90%
201505-03079	53	F		2,000	25.45%
201505-03081	44	M		2,000	13.40%
201505-03171	62	M		2,000	2.70%
201505-03172	27	M	lesion, back of tongue	2,000	6.90%
201505-03173	43	M		2,000	4.65%
201505-03174	22	M		2,000	7.70%
201505-03175	25	M		2,047	6.40%
201505-03176	37	M		2,000	2.60%
201505-03177	48	M		1,707	0.18%
201505-03178	23	M		2,000	1.35%
201505-03179	53	F		2,000	0.85%
201505-03522	56	F		2,000	2.95%
201505-03523	72	M		2,000	4.00%
201505-03524	26	M		2,000	8.65%
201505-03525	37	F		2,000	4.65%
201505-03526	53	F	lesion, soft palate	2,000	1.35%
201505-03527	44	F		2,000	1.00%
201505-03528	43	M		2,000	1.00%
201505-03529	45	F		2,000	1.20%
201505-03530	29	F		2,000	2.80%
201505-03531	41	F		2,000	2.60%
201505-03532	48	M		2,000	3.95%
201505-03533	41	F		2,000	2.40%
201505-03534	23	M		2,000	1.05%
201505-03535	49	F		2,000	1.75%
201505-03797	35	M		2,000	7.80%
201505-03798	59	F		2,000	6.90%
201505-03800	53	F		2,000	1.40%
201505-03804	56	F		2,000	0.50%
201505-03805	50	F		2,000	2.75%
201505-03799	28	M		2,000	9.40%
201505-03801	24	M		2,000	0.35%
201505-03802	44	F		2,000	0.10%
201505-03803	34	F		2,000	5.50%
201506-03880	65	F		2,000	0.00%
201506-03881	32	F		2,000	9.45%
201506-03967	48	M		2,000	0.35%
201506-03968	29	M		2,000	0.95%
201506-03969	53	M		2,000	24.05%
201506-03970	33	M		2,000	0.55%
201506-03971	47	M		2,000	0.05%
201506-03972	37	M		2,000	1.20%
201506-03973	30	M		2,000	0.65%
201506-04059	51	M		2,000	0.30%
201506-04060	38	M		2,000	0.95%
201506-04061	29	M		2,000	0.50%
201506-04062	36	F		2,000	0.30%
201506-04063	62	F		2,000	30.20%
201506-04281	33	M		2,000	2.10%
201506-04282	31	M		2,000	0.50%
201506-04284	39	M		2,000	2.65%

C) D) E)



A) Five oral cancer samples and 25 negative samples with E6, E7 percent positivity. B) Sixty additional oral rinse samples with E6, E7 percent positivity. Of these samples, 25/60 or 42% were positive for E6, E7 mRNA. C) Histograms for 3 oral cancer samples from table A. D) Histogram from a E6, E7 negative sample. E) Histograms from 2 E6, E7 negative samples with CD44 staining.

Conclusion

Quantitative intracellular E6, E7 mRNA determined by flow cytometry (HPV OncoTect) with or without CD44 is a viable method to screen for HPV related oral cancer. It remains to be determined what the prognostic value of E6, E7 mRNA expression is in this setting.