Early Detection of Oral Lesions with Dysplasia at High-Risk of Cancer Development Using a Proteomic Signature Image Analysis Based Straticyte™

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Background

Detection of oral lesions with epithelial dysplasia at high-risk of cancer development yet remains an unmet clinical challenge. Biomarkers that allow identification of high-risk oral lesions are urgently needed. Using tissue proteomics and bioinformatics, we identified and verified a biomarker signature comprising of a panel of proteins that distinguishes oral lesions with dysplasia from oral cancers and from normal tissues. The potential of this molecular signature for identification of oral dysplastic lesions at high-risk of cancer development was evaluated using immunohistochemistry and correlation with clinical outcome. Based on these findings, we developed a protein biomarker-based prognostic test, Straticyte, to better stratify oral lesions for predicting the risk of cancer development.

Methods

Oral dysplasia cases with long term follow up from Mount Sinai Hospital were immunostained for various candidate potential cancer marker (PCM) proteins identified by Liquid chromatography-Tandem Mass spectrometry and bioinformatics using specific antibodies, evaluated by two observers blinded to clinical outcomes, image analysis performed and risk prediction algorithm developed from data analysis using the R package.

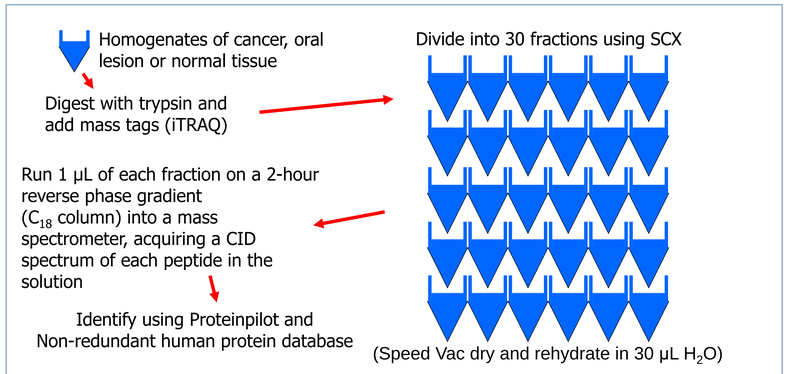

Figure 1. Multidimensional LC-MS/MS has been used for the analysis of biological samples labeled with isobaric mass tags for relative and absolute quantitation (iTRAQ) to identify proteins that are differentially expressed in human oral squamous cell carcinomas (OSCCs) in relation to oral lesions with dysplasia and non-cancerous oral tissues for cancer biomarker discovery.

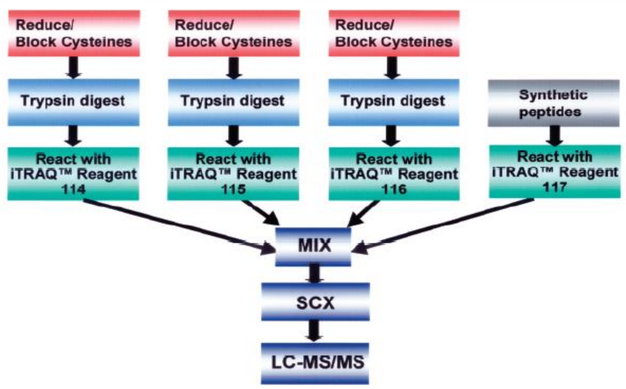

Figure 2. Work flow of iTRAQ-LC-MS/MS analysis of normal oral tissues and OSCC or oral lesions with dysplasia.

Results

Protein /	D1	D2	D3	D4	D5	D6	N1	N2	N3	N4*	N5*	N6*
PCM 69	0.74	2.15	1.94	1.53	1.77	1.63	0.88	0.74	0.75	0.84	0.78	1.03
PCM 70	1.02	3.07	1.19	1.88	0.81	1.5	1.18	0.84	0.99	1	0.8	0.97
PCM 72	9.86	1.14	1.13	1.71	1.25	1.5	1.04	1.24	1.1	1.23	0.9	
PCM 10	2.86	1.65	1.46	3.38	0.8	0.99	1.16	1.19	0.61	0.98	1.05	1.02
PCM 12	0.64	0.61	1.19	1.53	3.01	2.54	0.69	0.91	1.57	0.88	1.16	1.66
PCM 28	NQ	NQ	NQ	3.87	2.35	2.89	NQ	NQ	0.81			
PCM 31	2.97	1.25	1.73	0.87	1.96	1.52	1.2	0.82	1.83	1.2	1.38	1.5
PCM 74	1.2	0.96	1.56	1.66			0.77	0.97		1.1721	1.05	
PCM 76	1.22	1.55	0.86	1.35	0.92	1.11	1.15	0.78	1.11	0.94	0.71	0.99
PCM 78	NQ	1.2			1.15	1.12	NQ			0.96	1.24	
PCM 11	0.78	1.14	0.36	0.39	0.54	0.78	0.96	0.29	0.82	0.87	0.77	0.73
PCM 73	0.67	0.5	0.57	0.61	0.72	0.6	0.56	0.57	0.83		0.54	0.83
PCM 75	1.63	0.29			0.5	0.53	0.43		0.75		0.68	0.99
PCM 81	0.45	0.57	0.67	0.85	1.36	0.99	0.92	0.77	0.79	0.81	0.69	1.06
PCM 77	0.89	0.72	0.62	0.63	0.64	0.47	0.99	0.7	0.69	0.6	0.75	
PCM 84	0.78	1.43	0.79	0.67	0.58	0.66	0.89	0.97	0.87	0.83	0.93	0.93
PCM 79	1.13	0.58	1.15	0.67			1.11	1.24				
PCM 80	0.48	0.45			0.84	1.18	0.51		1.19	1.23	1.19	
PCM 81					4.09	1.77			0.95			
PCM 82	7.77	4.35					0.97					
PCM 83					1.34	1.51			1.23		0.81	1.13
PCM 85	1.04	1.37	1.26	1.66	1.09	1.12	1.12	0.94	1.09	1.01	0.87	0.97
PCM 86	1.3	1.7	1.16	1.25	0.9	1.87	0.81	1.33	1.04	1.23	0.88	1.07
PCM 87	1.17	1.88	0.78	1.27	1.13	2.35	0.96	1.17	0.83	0.89	1.23	0.95
PCM 88	1.13	1.27	1.45	2.06	1.17	1.17	0.73	0.93	1.05	1.04	0.9	
PCM 89	1.37	1.55	0.75	1.5	1.13	1.12	1.39	0.62	1.36	1.17	0.71	0.97
PCM 90	0.93	1.7	1.35	1.17	1.1	1.25	0.93	1.06	1.24	1.09		0.77
PCM 91	1.26	1.47			1.35	1.28	1.03		1.34	1.22	1.07	0.93
PCM 92	NQ	NQ	1.3	1.4			NQ	0.88		0.81	1.03	1.09
PCM 93					1.4	1.57			1.05			
PCM 94	1.25	0.97	1.15	1.15	4.55	1.08	0.89	1	0.89	0.51	1.02	1.06

Figure 3. iTRAQ ratios of oral lesions with dysplasia and normal tissues. D, dysplasia; N, normal oral tissue

<0.4	<0.5	<0.67	<0.8

>1.25	>1.5	>2.0	>2.5

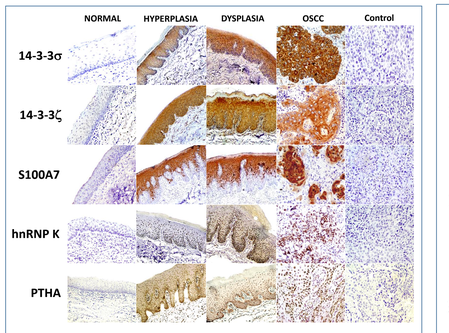

Figure 4. IHC verification of the panel of PCMs in 50 OSCC, 30 oral lesions with dysplasia and 50 non-malignant oral tissues.

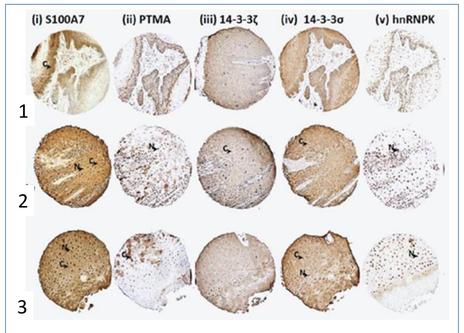

Figure 5. IHC-TMA of 5 protein biomarkers in independent cohort of oral lesions with dysplasia. 1: mild; 2: moderate; 3: severe dysplasia.

Characteristics		Transformed N (%)	Untransformed N (%)	p-Value
Dysplasia (n = 110)		39	71	–
p16 expression	Nuclear positive	32 (82.1)	53 (74.6)	0.375
HPV 16/18+		0	0	–
S100A7+	Overexpression (cytoplasm/nuclear)	36 (92.3)	51 (71.8)	0.014
	Cytoplasm	32 (82.1)	38 (53.5)	0.003
	Nuclear	35 (89.7)	49 (69.0)	0.018
PTMA+	Overexpression (cytoplasm/nuclear)	37 (94.9)	67 (94.4)	0.911
	Cytoplasm	25 (64.1)	36 (50.7)	0.176
	Nuclear	36 (92.3)	63 (88.7)	0.743
14-3-3ζ+	Overexpression (cytoplasm/nuclear)	37 (94.9)	69 (97.2)	0.536
	Cytoplasm	31 (79.5)	62 (87.3)	0.277
	Nuclear	21 (53.8)	38 (53.5)	0.974
14-3-3σ+	Overexpression (cytoplasm/nuclear)	27 (69.2)	62 (87.3)	0.040
	Cytoplasm	25 (64.1)	57 (80.3)	0.062
	Nuclear	18 (46.2)	46 (64.8)	0.071
hnRNP K+	Nuclear[1]	39 (100)	70 (98.6)	1.000

Figure 6. Correlation of candidate protein biomarkers expression with transformation in oral dysplasia.

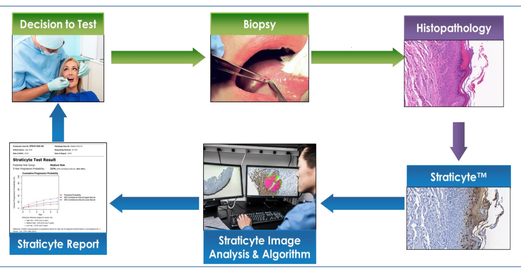

Figure 7. Incorporating Straticyte into clinical practice

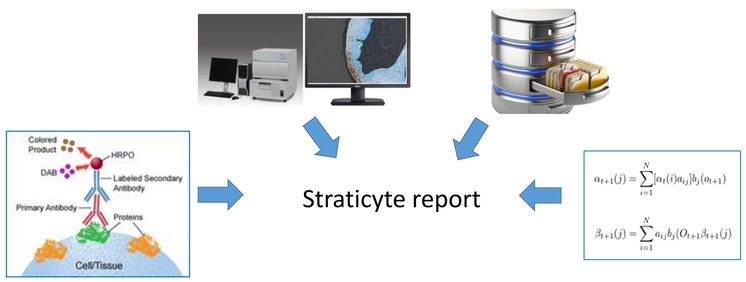

Figure 8. Components of Straticyte. From left to right: Patented biomarker: immunohistochemistry; Image analysis APPs; Reference database; Proprietary prediction algorithm.

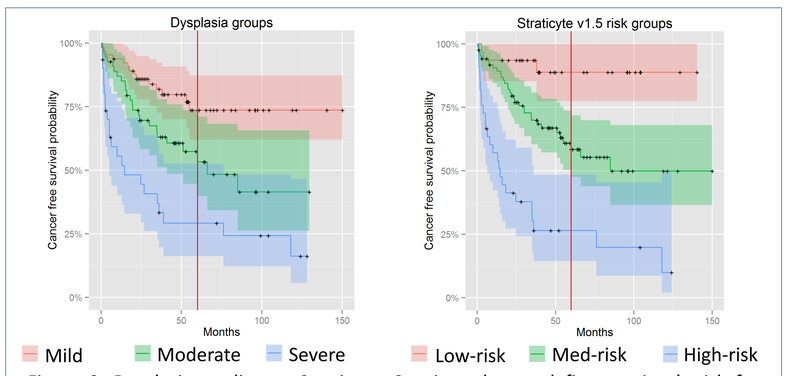

Figure 9. Dysplasia grading vs Straticyte. Straticyte better defines patient's risk for developing oral cancer by separating the lesions into risk categories with very little overlap.

Straticyte Compared to Dysplasia Outcome: cancer within 5 years			
Assessment	**Categories**	**Sensitivity (%)**	**False Negative Rate (FNR %)**
Straticyte	Low versus Medium/High	92	8
Dysplasia	Mild vs Moderate/Severe	74	26

Figure 10. Straticyte is more sensitive than dysplasia grading with a much lower False Negative Rate.

Conclusions

- Straticyte improves risk prediction beyond the current histopathologic grading and allows better prognostic assessment of oral dysplastic lesions.
- Straticyte provides confidence to clinicians in monitoring the low-risk oral lesions over long intervals, while high-risk oral lesions can be surgically excised with greater confidence
- Straticyte is likely to find utility in clinical practice for predicting patients with oral lesions at high-risk of cancer development.