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Prediction Model

LCK, survivin and PI-3K in the molecular biomarker profiling of oral lichen planus and oral squamous cell carcinoma

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Abstract:
T cell signaling is critical in oral lichen planus (OLP) based on the pathogenesis of this chronic inflammatory autoimmune mucocutaneous lesion. Lck plays a key role in T cell signaling; ultimately this signaling affects other targets such as PI-3K. Excessive activity in PI-3K inhibits apoptosis and promotes uncontrolled cell growth. Molecular biomarker profiling in OLP, Chronic Interface Mucosities (CIM), Epithelial Dysplasia (EpD) and Oral Squamous Cell Carcinoma (SCCA) with application of the principle of biomarker voting may represent a new frontier in the diagnosis, assessment and the arguable debate of OLP transformation to cancer. The presence of Lck, PI-3K and Survivin, a cancer specific anti-apoptotic protein was assessed, using immunohistochemistry and tissue micro-array on patient samples, in OLP, SCCA, CIM and EpD. Lck expression was very high in 78.6 % of OLP patients compared to 3.7% in SCCA; PI-3K was high in 63% of SCCA, 100% of EpD, and 35.7% OLP cases. Survivin was high in 64.3% of OLP cases, 96.3% of SCCA, and 100% of EpD. CIM cases may be slightly different molecularly to OLP. Taken together, our data suggest that biomarker protein voting can be effectively used to isolate high-risk OLP cases. Specifically, we show data with four remarkable cases demonstrating that molecular factors are predictive of histopathology. We conclude that it is safer to treat OLP as premalignant lesions, to adopt aggressive treatment measure in histopathologic described well and moderately differentiated SCCA, and to monitor progress of these diseases molecularly using individualized auto-proteomic approach. The use of Lck inhibitors in OLP management needs to be investigated in the future.

Keywords: oral carcinoma; biomarker; cancer; cellular immunity

Background:
Oral lichen planus (OLP) is an immune mediated chronic disease **[1, 2].** It usually affects muco-cutaneous tissues although it may affect any part of the oral cavity **[3].** It is a T cell mediated autoimmune disease that leads to destruction of the basal cell layer of the oral mucosa. Clinically, OLP may present in the mouth in reticular, erosive, papular, plaque-like, atrophic or bullous form **[1, 3].** The use of molecular approaches to study the pathogenesis of OLP is increasingly recognized diagnostic tool, and molecular approaches should further elucidate and characterize OLP pathogenesis. T cell signaling plays a key role in the pathogenesis of OLP [4]. The src family of kinases includes Lck and Fyn, that signal downstream of T cell receptors **[5, 6]** these molecules play a key role in T cell differentiation, survival and activation **[7].** Lck contributes actively to the phosphorylation of ZAP-70 **[6]** and may regulate the PI-3K/Akt pathway **[8, 9].**

Lck is considered pro-apoptotic **[10, 11]** and may be involved in the basal cell apoptosis associated with the pathogenesis of OLP. However, several studies found no apoptotic evidences in the basal cells of OLP cases **[12, 13].** Here, we hypothesize that a regulatory loop of T cell activation and anti-apoptotic forces are involved at the oral basal membrane and that may be associated, at the molecular level, with possible OLP transformation to squamous cell carcinomas (SCCA). To test this hypothesis, we studied Survivin, a critical cancer-specific protein **[14],** whose expression in tissues stimulates T cells. Survivin belongs to inhibitor of apoptosis family and is currently a key molecular target in anticancer therapy. Lck ultimately leads to activation of the PI-3K pathway in T cells. PI-3K/Akt pathway regulates cell growth and proliferation. Several studies have demonstrated the deregulation of this pathway in several cancers **[15, 16].** PI-3K is needed for normal T cell development **[17].** However, altered and unrestrained PI-3K signaling causes auto-immunity, an important determinant in OLP.

SCCA of the oral tissues makes up over 90% of the oral cancers **[18].** It may occur spontaneously particularly in the presence of risk factors such as tobacco, alcohol, and chronic inflammatory irritations **[19].** It may also develop from established pre-malignant lesions. OLP may in some cases be a pre-malignant lesion for SCCA **[20, 21],** but a full consensus about OLP potential for cancer transformation is still lacking. Issues complicating the understanding of OLP transformation to SCCA include the uncompleted definition of diagnostic criteria for OLP **[22],** and the current limits in understanding the biology of this disease. Taken together, we speculate that molecular profiling may be a promising approach to further investigate the pathogenesis of OLP and SCCA. The purpose of this study was to characterize, contrast and compare the molecular biomarker profiling of Lck, Survivin and PI-3K in OLP, chronic interface mucosities (CIM), epithelial dysplasia (EpD) and SCCA patients. Moreover, this study was aimed to achieve further molecular insights into the biology of these diseases, and specifically to provide additional clarification through molecular means on OLP – cancer transformation. The results shown suggest a molecular-driven approach to the management of OLP patients.

Methodology:
Patient Sample:
Paraffin blocks of fifty clinically diagnosed patients with OLP, EpD, CIM or SSCA were obtained from the University of California, Los Angeles (School or Dentistry, Oral Pathology archive), with IRB approval. Thirteen patients (fourteen samples) had OLP, six patients CIM, four patients epithelial dysplasia, and twenty-seven cases were diagnosed with SCCA **(Table1).**

Tissue micro-array:
The patient samples were coded to construct tissue micro-array (TMA; UCLA tissue array core facility). Core size was 0.6mm. Each sample has three panels on the TMA. The oral pathologist (RC) examined the patient samples, confirmed the diagnosis, and located the ideal segment for use in TMA construction, which was also verified by a second rater.

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Antibodies and other reagents:
Antibodies were from Abcam, USA (www.abcam.com). Rabbit anti-human Lck protein was used at the recommended dilution of 1:250. This Lck antibody does not cross react with any other Src family members. Rabbit anti-human Survivin protein was used at 0.5µg/ml. Rabbit anti-human PI-3K protein was used at the recommended dilution of 1:50. Biotinylated secondary goat anti-rabbit antibody was used with the Vectastatin avidin-biotin complex (ABC) kit (Vector Laboratories, USA www.vectorlabs.com). Color was developed with the Vector Nova Red Substrate kit.

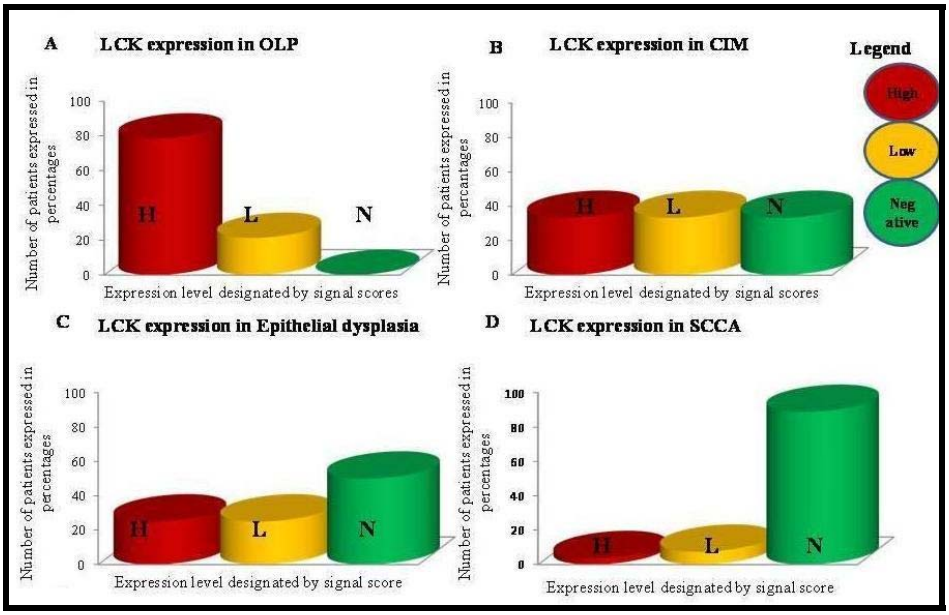

Figure 1: The expression levels of LCK in patients with Oral lichen planus (OLP), chronic interface mucosities (CIM), epithelial dysplasia (EpD), and Squamous cell carcinoma (SCCA) of the oral tissues are shown. The percentages of patient is plotted with the expression levels designated by signal score (Scale of 0-100, negative = ≤10, Low =11-24, high = ≥25). LCK is markedly expressed in OLP samples but low or lack of LCK expression is found in SCCA samples. LCK may be high or low in CIM cases as well as EpD, though CIM cases express more LCK than EpD.

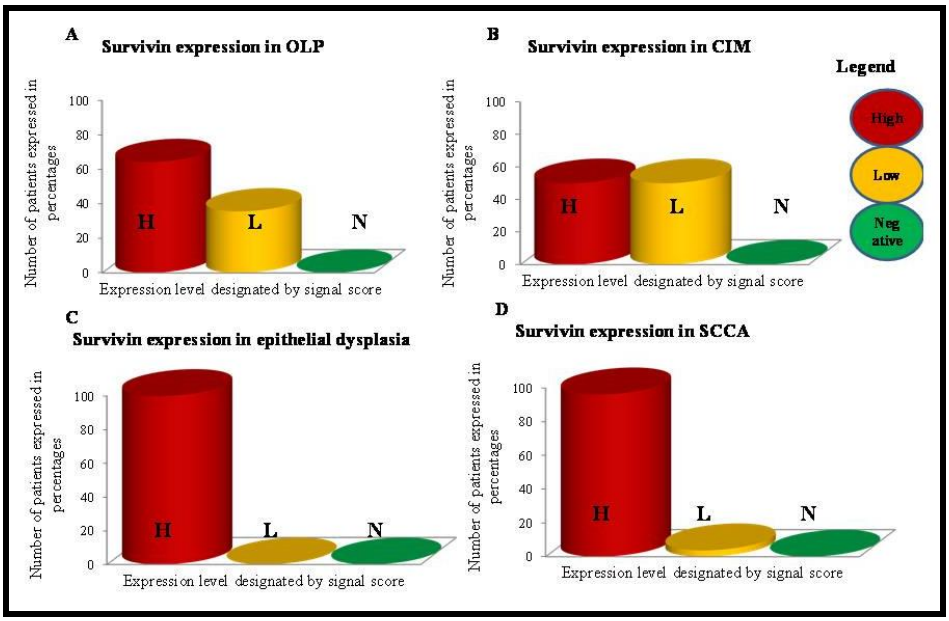

Figure 2: The expression levels of survivin in patients with OLP, CIM, EpD, and SCCA of the oral tissues are shown. The percentage of patients is plotted with the expression levels designated by signal score (same scale as Figure 1). Survivin expression is considerable high in OLP, CIM, EpD and SCCA. 100% SCCA and EpD cases exhibited high survivin expression; 70% and 50% of OLP and CIM samples, respectively, showed high expression of survivin.

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Immuno-histochemistry protocol:
Immuno-histochemistry (IHC) was performed as previously described **[4],** using the specific antibodies described above. Paraffin embedded TMA slides were deparaffinized (60º C, 30 min), xylene washed, re-hydrated by progressive washes in decreasing concentrations of ethanol, and rinsed copiously in double-distilled water. Endogenous peroxides were inhibited (H_2O_2 3%, room temp, 10 min), and antigen retrieval obtained with citrate buffer (0.01M, pH 6.0, microwave). Slides were blocked for 1 hour in blocking solution (2% normal goat serum [NGS] in Tween-20-supplemented Tris buffered saline [T-TBS]). Samples were incubated at 4°C overnight with the primary antibodies (see above) diluted in blocking solution. Following copious washes in T-TBS, the anti-rabbit specific secondary antibody diluted in blocking solution was applied for 30 min, and washed off with copious T-TBS, before treatment with the ABC kit (30 min). Following color development, the slides were counterstained with hematoxylin (Vector Laboratory), rinsed, dehydrated in graded alcohols, mounted with Vector Permanent mounting medium, sealed, and stored (room temp) until viewing and analysis.

Computer scanning, analysis and scoring:
Slides were scanned at 40x magnification. The aperio imagescope software (Version 9.1.19.1569) was used for scoring, additional analysis and subsequent magnification at 8X. Two independent assessors evaluated the expression (signals) of each epitope based on the nuclear and cytoplasmic stains. A circle of tissue on the TMA was scored from 0 to 100 numerical points, based on the extent of stain signals over the tissue. A score of 50 was interpreted as staining covering half of a circle of tissue, score of 75 means ¾ of the tissue were stained, while a score of 10 simply covers a small arc of a circle of tissue. Expressions of markers were ranked as low (signal scores: 11-24), negative (signal scores: 0-10), and high (signal scores ≥ 25) **[23].** Biopsy samples with signal scores for each marker ≥ 25 were identified for OLP, CIM, EpD and SCCA. The numbers of biopsy samples identified for each marker was transformed to percentage ofthe total number of biopsy samples per disease group.

Results:
High expression of Lck found in biopsies from OLP patients:
Lck expression was rated high in 78.6% of the OLP samples, compared to 3.7% of SCCA, 33.3% of the CIM samples and 25.0% of the EpD samples **(Figure 1, Table 2).** The pattern of Lck immunostaining in the OLP cases was peculiar in that a well distinct dense band-like appearance extended from the sub-epithelial boundary deeper into the mucosa **(Figure 4A-C).** This pattern of sub-epithelial band prominence staining was absent in SCCA biopsies **(Figure 4E-G)** and EpD cases. Evidence of this particular staining pattern in CIM was inconsistent.

Survivin expression levels high in OLP despite Lck pro-apoptotic activity:
Survivin expression was observed in 64.3% of biopsies from OLP patients **(Figures 2, 5A-C, 5I)** and 50.0 % of CIM cases showed high expression of survivin. All EpD biopsies (100%) and 96.3% of SCCA biopsies presented high Survivin levels **(Figures 2, 5E-G, 5J).** Regardless of how the Lck-T cell signaling apoptotic loop operates, the molecular signature of Survivin was clearly prominent in OLP.

PI-3K expression high in SCCA and some OLP cases:
Results revealed that 35.7% of the OLP biopsies **(Figure 3)** demonstrated high expression of PI-3K, which was observed respectively in 100% and 63.0% of the EpD and SCCA biopsies. None of the CIM biopsies showed PI-3K convincing immunostaining, as they were either low (≥ 11-24 expression signal scores) or negative (0-10 expression signal scores) **(Table 2).** OLP cases with negative, low and high expressions of PI-3K are shown in Figures 6A, 6B and 6C, respectively. Overall, five OLP biopsy samples **(Table 2)** showed high levels of PI-3K (Figure 6I). SCCA cases with negative, low and high expressions of PI-3K are shown in Figures 6E, 6F and 6G respectively.

Molecular Voting identifies four high risks OLP cases:
Identifying high-risk patients requires assessment of disease entities beyond the molecular signature of a single gene, RNA or protein. A panel of biomarkers must be used, whose votes (molecular signatures) are interpreted or counted together, and should be taken into consideration **[23].** Using this rationale we identified those samples that had high expressions across the three markers, particularly the samples that were high in PI-3K and Survivin. Based on this approach, four OLP samples were identified and are regarded as high risks **(Table 3).**

Molecular factors are suggested to be acting in advance of histopathology:
A few SCCA biopsy samples with well differentiated and moderately differentiated histomorphology also showed high expression levels of Survivin and PI-3K **(Table 2).** It is remarkable that all histomorphologically described EpD biopsy cases **(Table 2)** showed high expression levels of PI-3K and Survivin, a molecular signature similar to SCCA biopsy cases. All (100%) of the identified high-risk OLP biopsies were high in PI-3K, and Survivin, and suggested an OLP molecular signature similar to that of SCCA, despite the fact that the histomorphology of these lesions were characteristic of OLP.

Discussion:
Our current data reveal high (≥ 25 expression signal score) Lck expression in OLP biopsies. The expression follows a sub-epithelial band pattern of staining. Sub-epithelial dense mononuclear infiltrates of T cells have been previously described in OLP. By contrast, in CIM biopsy samples, Lck staining appears randomly distributed. The percentage of biopsy samples with high expression levels of Survivin in EpD and SCCA is greater than that of OLP and CIM biopsy samples. High PI-3K expression characterizes SCCA and EpD biopsies. Notably, all but one OLP samples with high expression levels of PI-3K also express high levels of Survivin and Lck. Taken together, our data suggest that concurrently elevated expression of PI-3K and Survivin in OLP lesions suggest an unfavorable molecular signature, one that appears typical for SCCA. By pooling the votes of the three markers together (biomarker voting), we identified four OLP biopsy samples that we concluded to be high-risk OLP lesions for transformation to cancer.

Molecular complementation of histopathology:
The identification of OLP biopsy samples that appears to have similar molecular signatures to SCCA biopsy samples may be highly suggestive, since these OLP lesions may represent heightened risk for transformation to SCCA. Histopathology remains a valued and viable diagnostic tool. However, in order to achieve a molecular targeted approach in diagnosis, prognosis and therapy, histopathology needs to be complemented with reliable molecular tools. Case in point, histopathology of breast cancer is complemented with molecular tool, and has become valuable in the selection of patients for appropriate therapy and management **[24, 25].** Taken together, our results lead us to propose that OLP lesions that are in the process of transforming to SCCA present histomorphologically as OLP, but possess a certain molecular signature that represents the specific factors that drive progression to cancer. Our results demonstrate that we now have the capability of reliably identifying this characteristic molecular signature. The four EpD biopsies tested as control in this study revealed molecular signatures similar to SCCA. Of note, EpD is currently recognized as a premalignant lesion **[26].** Therefore, and despite the limitations of a small sample size, our findings suggest that the above-mentioned molecular factors are preceding the phenotypic alterations in tissue histopathology. It follows that the histo-morphological changes that may be caused by high expression levels of Survivin and PI-3K may lag behind their established molecular

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impact, and may be predictive of OLP and EpD lesions as they progress to cancer.

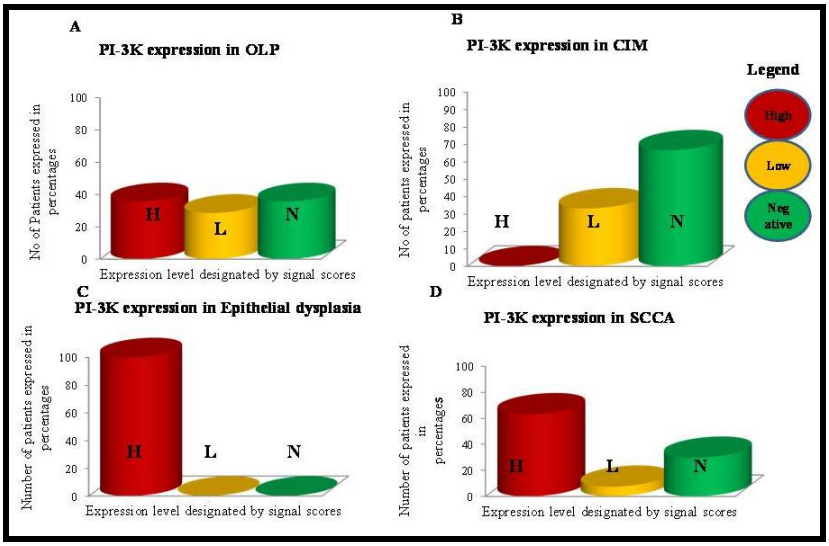

Figure 3: The expression levels of PI-3K in patients with OLP, CIM, EpD, and SCCA of the oral tissues are shown. The percentage of patient is plotted with the expression levels designated by signal score (same scale as Fig. 1). PI-3K expression is high in 63% of SCCA, all cases of epithelial dysplasia and about 35.7% of OLP cases.

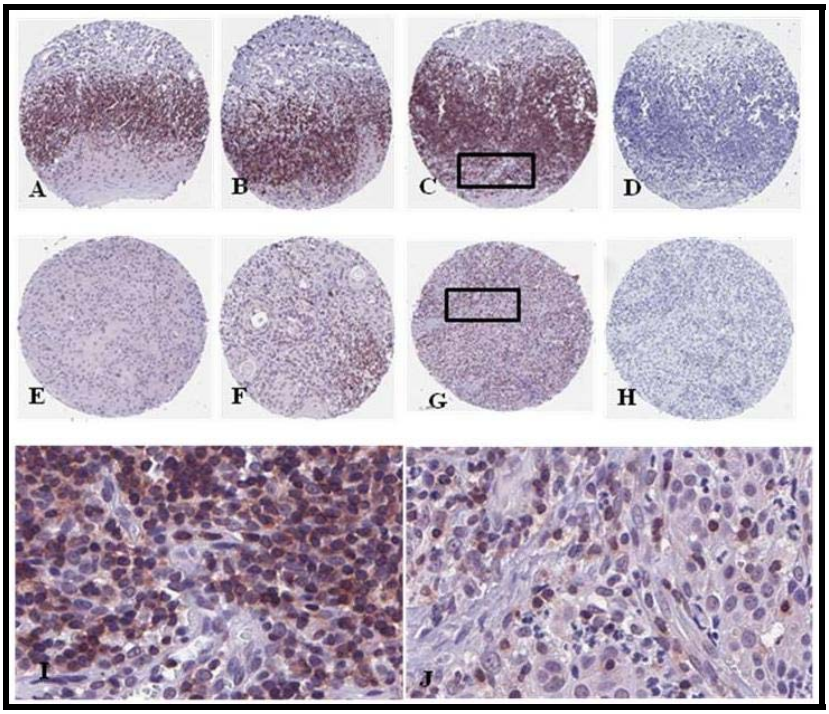

Figure 4: LCK tissue micro-array immunohistochemistry staining for OLP (panels A, B, C, D and I) and SCCA (panels E, F, G, H and J). All OLP cases show high expression of LCK (≥25). Panel A represents mild high LCK expression of the right lateral tongue, B moderate-high expression of LCK in left buccal mucosa, C marked high expression of LCK in right buccal mucosa. Panel D refers to OLP of the right buccal mucosa and is used as negative control (not stained with LCK primary antibody). Panel E shows mild or no expression of LCK in SCCA (moderately differentiated) of the left buccal mucosa F indicates moderate LCK expression in SCCA (poorly differentiated) of right lateral/ventral tongue while panel G is a case of marked LCK expression in SCCA (moderately differentiated) of right retromolar pad. Panel H is SCCA of right retromolar pad used as negative control. Panel I is 40x magnification of the box area in panel C; panel J is 40x magnification of the box area in panel G.

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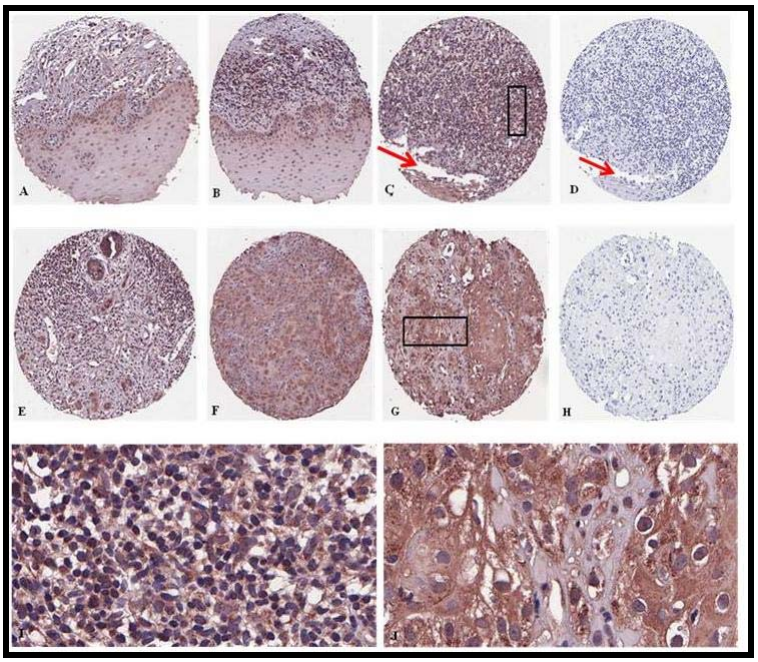

Figure 5: Survivin tissue micro-array immuno-histochemistry staining for OLP (panels A, B, C, D and I) and SCCA (panels E, F, G, H and J). High expressions of survivin further categorized as mild, moderate and marked in OLP and SCCA. Panel A shows mild expression in right buccal mucosa OLP; B indicates moderate survivin expression in OLP of left buccal mucosa. Panel C suggests marked expression in OLP of gingival tissues. Panel D shows a negative control. Red arrow in C and D indicates site of subepithelial tissue attachment to the surface epithelium. Panel E represents mild survivin expression in SCCA of right ventral lateral tongue; F shows moderate expression in SCCA of left buccal mucosa, while G indicated marked survivin expression in SCCA of left lateral tongue. Panel H shows a negative control. Panel I is 40x magnification of box area in C showing prominent nuclear and cytoplasmic survivin staining in OLP; panel J is 40x magnification of box area in G showing robust cytoplasmic survivin expression.

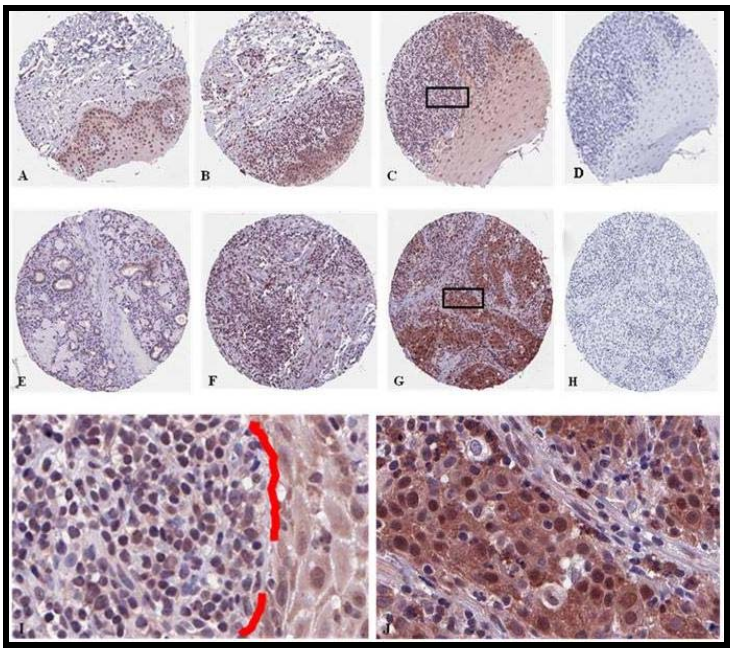

Figure 6: PI-3K tissue micro-array immunohistochemistry staining for OLP (panels A, B, C, D and I) and SCCA (panels E, F, G, H and J). Expression signal scores were used to identify negative, low and high expression (same scale as Fig. 1). Panel A indicates negative expression of PI-3K in OLP of posterior buccal mucosa; B shows low expression of PI-3K in OLP of the left vestibule, C represents OLP of the left buccal mucosa. Panel D shows a negative control. Panel E indicates negative PI-3K expression for SCCA of the floor of the mouth, F shows low expression in SCCA of right posterolateral tongue, while G is high expression in SCCA of right retromolar pad. Panel H presents a negative control. Panel I is 40x magnification of box area in C with red line showing the transition from the epithelium to the subepithelial area in OLP, while panel J is 40x magnification of the box area in G.

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Molecular differentiation between OLP and other lichenoid reactions:

To test the possibility that molecular tools may help characterize between OLP and other lichenoid reactions, we evaluated and compared six CIM and fourteen OLP samples. Our current preliminary findings with this small sample size suggest some degree of molecular differentiation between OLP cases and other lichenoid reactions. Case in point, Lck expression levels were high in 33.3% of the CIM samples compared to the 78.6% in OLP samples, 3.7% in SCCA and 25% in EpD biopsies. Lck expression revealed a random pattern in CIM lesions, compared to an almost seamless sub-epithelial band-like pattern in OLP biopsies. By contrast, none of the CIM biopsy samples showed high PI-3K expression levels. Further molecular analysis with more samples will possibly assist in a clearer description of these related inflammatory-driven entities.

Chronic inflammation, autoimmunity and cancer transformation: Is there any role for the Lck-PI-3K-Survivin molecular loop in OLP?

Recent studies have shown the role of chronic inflammation in the generation of cancer **[27].** At the molecular level, we need to determine at which stage inflammation ceases to be protective and becomes harmful, including the promotion of cancer transformation. Lck is a non-receptor src kinase, and studies have shown the role of non–receptor src kinases in cancer generation **[28].** PI-3K may be involved in the autoimmunity described in OLP since unrestrained PI-3K activation leads to auto-immunity and leukemia **[17].** In T cells, Lck ultimately activates PI-3K. The deregulation of the PI-3K/Akt pathway is responsible for several cancers **[29],** which is in part the rationale for current anti PI-3K clinical trials in cancer management **[30].** Acting downstream to PI-3K is Akt (protein kinase B). Akt functionally inhibits apoptosis and promotes cell growth. A modulatory switch at the PI-3K/Akt level may be hypothesized for this molecular loop. Therefore, it is critical to elucidate the role of Lck in OLP as a prototype for chronic inflammation with activated T cell signaling. In this context, our group has additional data-mining results that support the Lck-PI-3K-Akt link (Giacomelli et al.,-paper II in this series). Taken together, the Lck-PI-3K/Akt-Survivin molecular loop may play an important role in the spectrum of chronic inflammation, autoimmunity and cancer transformation in OLP. This loop may also act indirectly through activation of other pathways such as the mitogen activated protein kinase **[31].**

This model raises the next important question in OLP pathogenesis: Could the molecular imbalance of T cells and the local tissue environment in OLP itself determine whether chronic inflammation will be protective or lead to cancer? Interleukins, particularly interleukin 2 (IL-2), may be vital to the effects of chronic inflammation at the OLP tissue microenvironment. Our data-mining results supports such a possible role for IL-2 in this proposed molecular loop (Giacomelli et al.,-paper II in this series). This molecular loop involving IL-2 may further explain increase T cell proliferation with IL-2 **[32]** and subsequent continuous T cell signaling and activation.

Conclusions:

In conclusion, our data to date suggest that histopathological assessment of OLP, CIM, EpD and SCCA should be complemented and enhanced with molecular approaches. Taken together, our findings lend support to the proposition that OLP lesions may be pre-malignant, and that molecular assessments that involve an auto-proteomic approach **[30]** may provide novel and critical information on these disease entities and their natural history. This may help clarifying the complexities in diagnosis, further clarify the potential progression from OLP to SCCA, and, in the future, suggest a patient-driven, personalized, and molecular-based therapy. Future work based on the results shown here will elucidate the putative role of Lck inhibitors, such as A770041 **[33],** for the management of OLP.

References:

[1] F Chiappelli, OS Cajulis, *Quintessence Int.* (2004) **35:** 223 [PMID: 15119681]
[2] I Al-Hashimi *et al., Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* (2007) **103**: S25.e1 [PMID: 17261375]
[3] D Eisen., *J Am Acad Dermatol.* (2002) 46: 207 [PMID: 11807431]
[4] PB Sugerman *et al., Crit Rev Oral Biol Med.* (2002) **13**:350 [PMID: 12191961]
[5] M Lovatt et al., *Mol Cell Biol.* (2006) **26**: 8655 [PMID 16966372]
[6] RJ Salmond *et al.*, *Immunol Rev.* (2009) **228**:9 [PMID: 19290918]
[7] R Zamoyska *et al., Immunol Rev.* (2003) 191:107 [PMID: 12614355]
[8] R Taichman *et al., J Biol Chem.* (1993) **268**:20031 [PMID: 8397196]
[9] KV Prasad *et al., Mol Cell Biol.* (1993) **13**:7708 [PMID: 8246987]
[10] K Heyninck, R Beyaert, *Oncogene* (2006) **25**:186 [PMID: 16186791]
[11] AK Samraj *et al., Oncogene* (2006) **25**:1693 [PMID: 16116473]
[12] MA Gonzalez-Moles *et al., Arch Oral Biol.* (2006) **51**:1093 [PMID: 16914114]
[13] SI Tobon-Arroyave *et al., Oral Dis.* (2004) **10**:173 [PMID: 15089928]
[14] BM Ryan et al., *Cancer Treat Rev.* (2009) **35**:553 [PMID: 19559538]
[15] A Iamaroon, S Krisanaprakornkit *Oral Oncol.* (2009) **45**:e175 [PMID: 19628421]
[16] AA Molinolo *et al., Oral Oncol.* (2009) **45**:324 [PMID 18805044]
[17] K Okkenhaug, B. Vanhaesebroeck, *Nat Rev Immunol.* (2003) **3**:317 [PMID: 12669022]
[18] JR Montoro *et al., Braz J Otorhinolaryngol.* (2008) **74**:861 [PMID: 19582342]
[19] A Girod *et al., J Oral Maxillofac Surg.* (2009) **67**:1914 [PMID: 19686929]
[20] MA Gonzalez-Moles *et al., Oral Dis.* (2008) **14**:229 [PMID: 18298420]
[21] MM Bornstein *et al., Quintessence Int.* (2006) **37**:261 [PMID: 16594357]
[22] S Gandolfo *et al., Oral Oncol.* (2004) **40**:964 [PMID: 14662419]
[23] O Oluwadara, F. Chiappelli, *Bioinformation.* (2009) **3**:332 [PMID: 19707295]
[24] CI Sartor, *Semin Radiat Oncol.* (2002) **12**:341 [PMID: 12382192]
[25] P Tan, Yonsei *Med J.* (2009) **50**:464 [PMID: 19718393]
[26] H Jack et al., *N Z Med J.* (2009) **122**:89 [PMID: 19322259]
[27] M Khatami, *Cell Biochem Biophys.* (2009) **55**:55[PMID: 19672563]
[28] YM Chang et al., *Neoplasia.* (2007) **9**:90 [PMID: 17357254]
[29] BH Jiang, L.Z. Liu, *Adv Cancer Res.* (2009) **102**:19 [PMID: 19595306]
[30] R Marone et al., *Biochim Biophys Acta.* (2008) **1784**:159 [PMID: 17997386]
[31] M Li et al., *Mol Cell Biol.* (2008) **28**:630 [PMID 17998336]
[32] TA Fehniger et al., *Cytokine Growth Factor Rev.* (2002) **13**:169 [PMID: 11900992]
[33] RF Stachlewitz et al., *J Pharmacol Exp Ther.* (2005) **315**:36 [PMID: 16014572]

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Supplementary material

Table 1: Summary of the patient demographics

Patient	Diagnosis	Age at Diagnosis	Anatomical location	Sex
1	OLP	65	Left buccal mucosa	F
2	OLP	48	Right buccal Mucosa	F
3	OLP	59	Left buccal mucosa	F
4	OLP	54	Left Vestibule	M
5	*OLP	62	Right buccal mucosa	M
6	*OLP	62	Left buccal mucosa	M
7	OLP	77	Right buccal mucosa	F
8	OLP	61	Right lateral tongue	M
9	OLP	64	Gingiva area around teeth number 13 and 14	F
10	OLP	61	Left buccal mucosa	M
11	OLP	51	Gingiva area around teeth number 6 and 14	F
12	OLP	62	Right buccal gingival	F
13	OLP	68	Left buccal mucosa	F
14	OLP	45	Right attached gingival around teeth number 3 and 4	F
15	CIM	58	Posterior buccal mucosa	M
16	CIM	69	Right lateral tongue	F
17	CIM	58	Left buccal mucosa	F
18	CIM	21	Gingival area around teeth number 9 and 10	M
19	CIM	45	Right buccal mucosa	M
20	CIM	61	Right lateral tongue	F
21	EpD	57	Right postero-lateral tongue	F
22	EpD	85	Lingual area of teeth number 10 and 11	F
23	EpD	83	Right lateral border of tongue	F
24	EpD	41	Left buccal mucosa	F
25	SCCA	60	Gingiva area around teeth number 30 to 32	F
26	SCCA	57	Left posterior area of mandible	M
27	SCCA	91	Left maxilla	F
28	SCCA	63	Left lateral tongue	M
29	SCCA	54	Gingiva area around teeth 14	F
30	SCCA	82	Left gingival	F
31	SCCA	57	Gingiva around teeth 18 and 19	F
32	SCCA	81	Right posterior lateral tongue	M
33	SCCA	81	Right lateral/ventralTongue	F
34	SCCA	73	Right maxillary alveolar mass	M
35	SCCA	60	Left anterior floor of the mouth	M
36	SCCA	89	Left lateral tongue	M
37	SCCA	73	Left buccal mucosa	F
38	SCCA	51	Right floor of the mouth	M
39	SCCA	65	Right retromolar pad	M
40	SCCA	74	Left buccal mucosa	M
41	SCCA	68	Left lateral tongue	F
42	SCCA	81	Left posterior maxilla	F
43	SCCA	75	Left buccal mucosa	F
44	SCCA	80	Tongue	F
45	SCCA	42	Left lateral Tongue	F
46	SCCA	71	Right/ventral tongue	M
47	SCCA	57	Right posterior mandible	M
48	SCCA	30	Left lateral tongue	M
49	SCCA	36	Left lateral tongue	M
50	SCCA	63	Right tongue	M
51	SCCA	70	Left buccal mucosa	F

#5 and #6 are same patient but different anatomical location. OLP = Oral lichen planus; CIM = Chronic interface mucosities; EpD = Epithelial dysplasia and SCCA = Squamous cell carcinoma. M = Male; F = Female.

Table 2: Molecular expression of markers

No	Diagnosis	Expression of Markers		
		PI-3K	LCK	Survivin
1	OLP	N	H	H
2	OLP	N	L	L
3	OLP	H	H	L
4	OLP	L	H	H
5	*OLP	L	H	H

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6	*OLP	N	H	H
7	OLP	L	H	H
8	OLP	H	H	H
9	OLP	H	H	H
10	OLP	H	H	H
11	OLP	H	H	H
12	OLP	N	L	L
13	OLP	N	L	L
14	OLP	L	H	L
15	CIM	N	N	L
16	CIM	N	L	H
17	CIM	N	H	L
18	CIM	N	H	L
19	CIM	L	N	H
20	CIM	L	L	H
21	EpD	H	N	H
22	EpD	H	N	H
23	EpD	H	H	H
24	EpD	H	L	H
25	SCCA	N	L	H
26	SCCA	L	N	H
27	SCCA	N	N	H
28	SCCA	H	N	H
29	SCCA	H	N	H
30	SCCA	H	N	H
31	SCCA	H	N	H
32	SCCA	H	N	H
33	SCCA	H	H	H
34	SCCA	H	N	H
35	SCCA	N	N	L
36	SCCA	H	N	H
37	SCCA	H	N	H
38	SCCA	H	N	H
39	SCCA	H	L	H
40	SCCA	H	N	H
41	SCCA	H	N	H
42	SCCA	N	N	H
43	SCCA	N	N	H
44	SCCA	N	N	H
45	SCCA	N	N	H
46	SCCA	H	N	H
47	SCCA	H	N	H
48	SCCA	N	N	H
49	SCCA	L	N	H
50	SCCA	H	N	H
51	SCCA	H	N	H

*5 and 6 are same patient but different anatomical location. OLP = Oral lichen planus; CIM = Chronic interface mucosities; EpD = Epithelial dysplasia and SCCA = Squamous cell carcinoma; High (H) =≥ 25 signal score, Low (L) = 11-24 signal score, N (N) =< 10 signal score

Table 3: Data on OLP patients considered as high risks

Patient	Diagnosis	Age	Anatomical location	Sex	Expression of Markers			Summary of pathological report	History/clinical findings
					PI-3K	LCK	Survivin		
1	OLP	61	Right lateral tongue	M	**H**	**H**	**H**	Much lymphocytes, keratin layer with obvious focal thickening	Obvious white lacey lesion
2	OLP	64	Gingiva area around teeth number 13 and 14	F	**H**	**H**	**H**	Much lymphocytes, thick orthokeratin layer	-
3	OLP	61	Left buccal mucosa	M	**H**	**H**	**H**	Thickened parakeratin layer. Much lymphocytes	Asymptomatic white straie lesion
4	OLP	51	Gingiva area around teeth number 6 and 14	F	**H**	**H**	**H**	Much lymphocytes	3-4 months history of gingival inflammation

High (H) => 25 score; Low (L) = 11-24 signal score; Negative (N) =< 10 signal score

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